What governs skeletal muscle $\dot{V}O_{2max}$? New evidence

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ABSTRACT

RICHARDSON, R. S. What governs skeletal muscle $\dot{V}O_{2max}$? New evidence. *Med. Sci. Sports Exerc.*, Vol. 32, No. 1, pp. 100–107, 2000. Recent investigations into the determinants of skeletal muscle maximal oxygen consumption ($\dot{V}O_2$) have provided further evidence regarding the role of O_2 supply and demand in governing exercise metabolism. Specifically, four studies utilizing both animal and human exercise models are highlighted here: 1) the role of the diffusive O_2 component was examined in the exercising canine gastrocnemius muscle by a rightward shift in the O_2 dissociation curve while maintaining O_2 delivery constant; 2) the role of peripheral and central components was examined by studying the human quadriceps muscle, already recognized to have a very high mass specific O_2 delivery, under conditions of increased (hyperoxia) and reduced O_2 availability (hypoxia); 3) the role of intracellular PO_2 in the progressive increase in lactate efflux from skeletal muscle from submaximal to maximal effort; and finally 4) the role of intracellular PO_2 itself as a determinant of maximal mitochondrial O_2 consumption. In summary, these investigations illustrate 1) the importance of the diffusion gradient from blood to muscle cell; 2) illustrate that even in functionally isolated trained skeletal muscle the highest recorded metabolic rates can be increased by increasing O_2 supply; 3) that a constant intracellular PO_2 during graded exercise is therefore unrelated to increasing lactate efflux; and 4) that only in hyperoxia does trained human skeletal muscle approaching very high mitochondrial metabolic limits, as shown by a disproportionate increase in intracellular PO_2 for the recorded change in $\dot{V}O_{2max}$. **Key Words:** EXERCISE, MYOGLOBIN, INTRACELLULAR PO_2, LACTATE, HYPEROXIA, HYPOXIA

Maximal oxygen consumption ($\dot{V}O_{2max}$), can be calculated at the muscular level by: $\dot{Q}$ (CaO_2 - C $\dot{V}O_2$), where $\dot{Q}$ is skeletal muscle blood flow and CaO_2 and C $\dot{V}O_2$ are arterial and venous O_2 content, respectively. The purpose of this paper is to highlight recent data that specifically address the question of whether skeletal muscle $\dot{V}O_{2max}$ is governed by O_2 supply or O_2 demand. This series of investigations utilized both *in situ* animal and *in vivo* human skeletal muscle models to further elucidate the factors that influence $\dot{V}O_{2max}$. Specifically, data are presented that examine 1) the role of O_2 diffusion by studying the effect of a decreased hemoglobin (Hb) affinity produced by an infusion of 2-(4{[(3,5-dimethylanilino)carbonyl]methyl}phenoxy)-2-methyl-proprionic acid (RSR13, Allos Therapeutics, Denver, CO.) on skeletal muscle $\dot{V}O_{2max}$, while ensuring constant O_2 delivery by controlling muscle blood flow and arterial O_2 content (35); 2) the effect of an elevated and reduced O_2 delivery in a functionally isolated skeletal muscle that has previously been documented to receive double the mass specific O_2 delivery measured during conventional whole body exercise (30,31); 3) the relationship between lactate efflux and intracellular partial pressure of O_2 (PO_2) measured by magnetic resonance spectroscopy (MRS) from moderate to maximum work rates (32,33); and finally 4) the effect of hypoxia and hyperoxia on intracellular PO_2 and the resulting relationship between $\dot{V}O_{2max}$ and intracellular PO_2 in exercise trained human skeletal muscle (32).

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ANIMAL STUDY METHODS

Twelve adult mongrel dogs with a weight range of 14–26 kg were anesthetized with pentobarbital sodium (30 mg·kg) and kept under a suitable level of anesthesia by maintenance doses as required. The dogs were intubated with a cuffed endotracheal tube and were ventilated (Harvard 613) to maintain arterial PO_2, PCO_2, and pH in the normal range. Esophageal temperature was monitored by a thermistor and maintained at 36–38°C by heating pads.

Exercise model: The functional and vascular isolation of the left gastrocnemius-flexor digitorum superficialis muscle complex (referred to as the gastrocnemius) was achieved as described previously (42). When surgically isolated, the achilles tendon was attached to an isometric myograph (Interface MFG, Scottsdale, AZ) to measure tension development. Isometric muscle contractions were elicited by electrical stimulation of the sciatic nerve (tetanic train:6–8 V, 0.2 msec stimuli for 200 msec duration at 50 Hz, once per second). The muscle was stimulated in this fashion for 3 to 3.5 min. Before each exercise period the blood supply to the isolated muscle was switched from self-perfusion to pump-perfusion.

Experimental protocol: We studied the gastrocnemius electrically stimulated to elicit maximal exercise in 8 dogs at a normal P_{50} and then again with the oxygen dissociation curve (ODC) shifted to the right by the allosteric modifier of Hb (methylpropionic acid, RSR13, Allos Therap. Inc., Denver, CO). Before each exercise period arterial and venous blood samples were analyzed at varied levels of inspired O_2 (12, 16, 21, 30, and 100% O_2) and the data used to calculate the P_{50} and Hill coefficient "n" with the Hill equation. Due to the reduction in arterial O_2 saturation produced by the rightward shift in the ODC, all dogs breathed 30% O_2 between contraction periods and 100% O_2 during each contraction sequence. This successfully maintained arterial O_2 saturation at 99.6% during exercise both before and after the P_{50} change. O_2 delivery during each bout of exercise was kept constant by alterations in the blood flow to the maximally exercising isolated muscle as necessary. Due to the 1.5 to 2 h half-life of RSR13, we were unable to produce the preferred balanced experimental design in which half the animals received the RSR13 treatment first and normal Hb in the second exercise period. To address this potential ordering effect we evaluated the effect of time and repeated exercise bouts on 4 control dogs who did not receive the RSR13, but did experience exactly the same protocol (including a sham infusion of saline) and performed two exercise bouts over the same period of time as the RSR13 treated animals.

Measurements: Arterial and venous samples were drawn anaerobically during the last 20 s of each contraction. Samples were analyzed immediately for PO_2, PCO_2, pH, O_2 saturation, and [Hb]. Between taking of blood samples blood flow measurements of the venous outflow were made by an in-line ultrasonic flow probe (Transonic Systems Inc. NY).

Blood O_2 concentration was calculated as (1.39 mL·O_2 × [Hb] g/100 mL × measured O_2 saturation) + 0.003 mL·O_2/100 mL × measured PO_2. Arterial-venous [O_2] difference was calculated from the difference in carotid artery and popliteal venous O_2 concentration. This difference was then divided by arterial concentration to give O_2 extraction. Gastrocnemius $\dot{V}O_2$ was calculated as the product of arterial-venous O_2 concentration difference and blood flow. The standard P_{50} of the blood was calculated before each exercise bout by varying the inspired O_2 concentration. The Hill equation was then used to calculate the P_{50} and Hill coefficient (n) for each ODC in both the normal and right shifted conditions.

HUMAN STUDY METHODS

For all subjects Informed consent was obtained according to both the University of California San Diego and/or the University of Pennsylvania. All subjects were young healthy nonsmoking male volunteers, each competitive bicycle racers, regularly riding 200–400 miles per week.

Exercise model: Exercise was performed on a knee-extensor ergometer constructed from nonmetallic materials to allow its use in both the human physiology lab in San Diego and the MRS facility in Philadelphia. An illustration of the apparatus is presented in reference 32.

Experimental Protocol: These studies were performed on the same subjects in both San Diego and Philadelphia. In San Diego, two catheters (radial artery and left femoral vein) and a thermocouple (left femoral vein) were emplaced using sterile technique as previously reported (27,32,34). Allowing arterial and venous blood samples in conjunction with muscle blood flow measured by the thermodilution technique (32,40). Three 8–12 min bouts of incremental exercise to maximum were then performed: 1) left leg quadriceps exercise breathing room air (21% O_2), 2) left leg quadriceps exercise breathing 12% O_2 and 3) left leg quadriceps exercise breathing 100% O_2.

The samples of arterial and venous blood were used to measure PO_2, PCO_2, pH, O_2 saturation, and Hb. Blood lactate concentration was determined using a blood lactate analyzer (Yellow Springs Instrument model 1500). Net muscle lactate efflux was calculated as the product of blood flow and venous-arterial lactate concentration difference. Plasma epinephrine and NE were assayed in duplicate by the method of Kennedy and Zeigler (21). Oxygen transport variables were calculated as described above. Using the intracellular PO_2 values measured by MRS, mean capillary PO_2 ($capPO_2$) and muscle O_2 conductance were calculated as described previously (32,43).

In Philadelphia the incremental exercise was reproduced in a 2.0 Tesla Oxford imaging magnet (Figure in Ref. 32). Spectra were collected from the muscle region below the 7 cm diameter surface coil double-tuned to proton (85.45 MHz) and phosphorous (34.59 MHz) placed over the rectus femoris portion of the quadriceps group (38). Details of the theory behind oxygen-sensitive Mb signals have been published previously (4,32). Fraction deoxy-Mb (^{f}deoxy-Mb) was determined by the normalizing the plateaued signals obtained during the last two min of cuff occlusion (270 mm Hg) which represent complete deoxygenation of Mb and thus may be used to estimate total Mb content within the muscle (45). Conversion to PO_2 values were then calculated from the oxygen binding curve for Mb:

$$PO_2 = ((1\text{-}f)/f) * P_{50}$$

where 1-f is the fraction of myoglobin that is oxygenated, f is the fraction of myoglobin that is not oxygenated, and P_{50} is the oxygen pressure where 50% of the myoglobin binding sites are bound with oxygen. The temperature-dependent Mb half saturation (P_{50}) of 3.2 mm Hg was used (37).

Statistical analyses. Least-squares regression, repeated measures ANOVA (Tukey *post hoc*), and *t*-test analyses were computed using a commercially available software package (Graphpad Instat, Graphpad Software). Variables were considered significantly different when the p-value was 0.05 or less. Data are presented as the mean ± SE throughout the manuscript.

RESULTS AND DISCUSSION

Role of O_2 diffusion in determining $\dot{V}O_{2max}$. It has been demonstrated that an increase in O_2 delivery can increase $\dot{V}O_{2max}$ (3,22,29,48) which suggests that O_2 supply

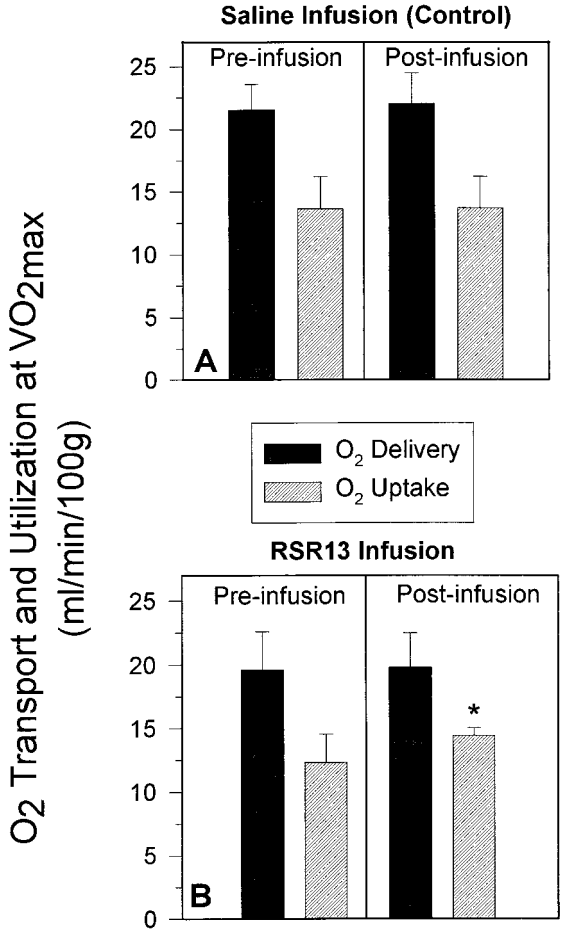

Figure 1—The effect of RSR13 infusion (P_{50} = 53.2 mm Hg) and the sham saline infusion (P_{50} = 34 mm Hg) on O_2 delivery and O_2 uptake. * Significantly different from preinfusion value ($P < 0.05$). Reproduced with permission of the American Physiological Society.

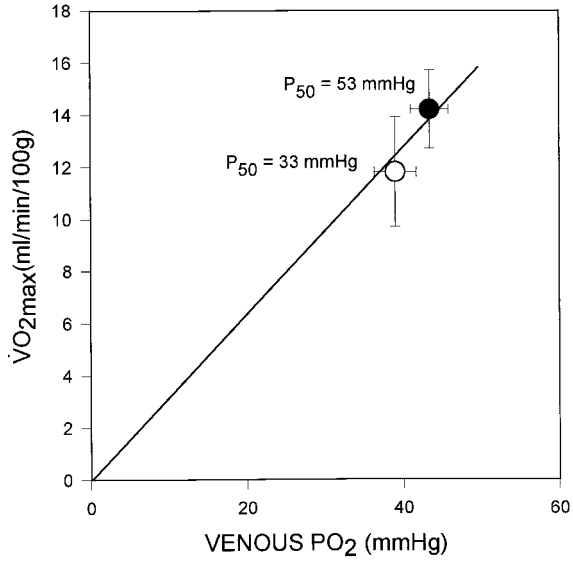

Figure 2—Assuming venous PO_2 represents end capillary PO_2 (15), the unchanged relationship between $\dot{V}O_{2max}$ and venous PO_2 after RSR13 administration illustrates that O_2 conductance (DmO_2) has remained unchanged. Reproduced with permission of the American Physiological Society.

limitation does exist. However, it has also been shown that this is not the unique determinant of $\dot{V}O_{2max}$ (43). The principal observation in this animal study is that under conditions of constant convective arterial O_2 delivery, an increase in P_{50} allowed exercising skeletal muscle to achieve a greater $\dot{V}O_{2max}$ (Fig. 1). This provides evidence that $\dot{V}O_{2max}$ at a normal P_{50} is not determined by mitochondrial metabolic limits, but rather by O_2 supply: an increase in P_{50} producing a steeper O_2 gradient (driving force) from capillary-to-tissue, providing more O_2 and allowing tissue $\dot{V}O_{2max}$ to increase (Fig. 1). Thus, the present experimental findings support the concept that for a given O_2 delivery the amount of O_2 that can be extracted and used by the working muscle is determined by the DmO_2 and the PO_2 gradient from the red cell to the mitochondria (Fick's Law of Diffusion). Theoretically, if the O_2 conductance is held constant and DmO_2 does not change, a right shifted ODC should decrease the rate at which the capillary PO_2 declines as O_2 is removed by the working muscle, thereby increasing the capillary-to-tissue PO_2 driving gradient along the capillary length. This rightward shift in the ODC should then increase $\dot{V}O_{2max}$, if DmO_2 is an important determinant of $\dot{V}O_{2max}$. Due to the lack of intracellular PO_2 measurements in this particular study we were unable to calculate these variables with confidence so after RSR13 we utilized venous PO_2 as our best approximation of end capillary PO_2 (15) (Fig. 2). Such a substitution of venous PO_2 for calculated $CapPO_2$ has previously produced qualitatively interchangeable conclusions about DmO_2 (32). This analysis suggests that there was an increase in $\dot{V}O_{2max}$ and no substantial change in DmO_2 (Fig. 2).

$\dot{V}O_{2max}$ rose by 20% with an increase in P_{50} from 33 to 53 mm Hg (Fig. 3), similar in magnitude to the reduction reported by Hogan et al. with a decrease in P_{50} from 32 to 23 mm Hg (−17%, 13). Here, it is also pertinent to note that in addition to the work of Hogan et al. (13), Schumacker et al. (39) assessed the effect of a reduced P_{50} (by sodium cyanate infusions) on exercise performance in dogs on a treadmill and found no effect on O_2 extraction and exercise performance. In this study (39) because the animals were only minimally instrumented it was not possible to control O_2 delivery to the exercising muscles at maximum exercise. However, Schumacker et al. (39) did demonstrate that for the same O_2 delivery less O_2 was extracted during exercise when the ODC was shifted to the left, consistent with Hogan et al. (13) and the present study which suggest an important role of P_{50} in determining O_2 extraction.

Role of central and peripheral limits in determining $\dot{V}O_{2max}$. The major observation in this isolated human skeletal muscle study is that even in an exercise paradigm where O_2 delivery per unit of muscle mass is very high, an elevated O_2 delivery afforded by breathing 100% O_2 results in an increase in $\dot{V}O_{2max}$ in trained skeletal muscle. This provides evidence that in trained subjects normoxic knee-extensor exercise, which has demonstrated the highest mass specific skeletal muscle $\dot{V}O_2$ in man (Fig. 4), is limited by O_2 supply, not O_2 demand. Additionally, these data indicate that factors which determine both O_2 supply and DO_2 from blood to skeletal muscle play a key role in determining

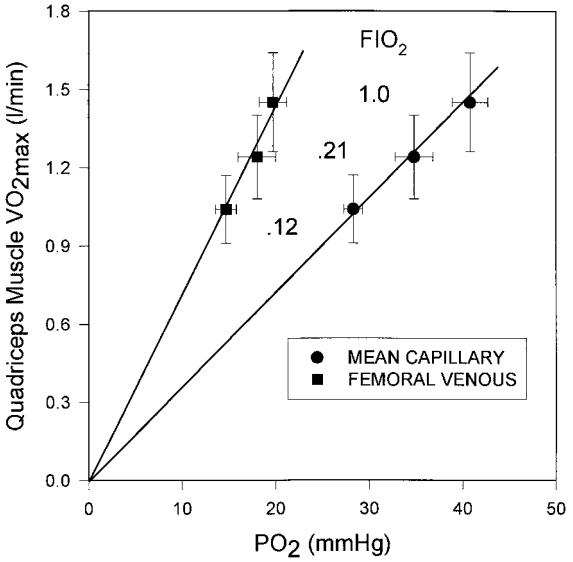

Figure 3—Figure 2: The relationship between $\dot{V}O_{2max}$ and mean capillary and femoral venous PO_2 in hypoxia, normoxia, and hyperoxia during maximal knee-extensor exercise. Reproduced with permission of the American Physiological Society.

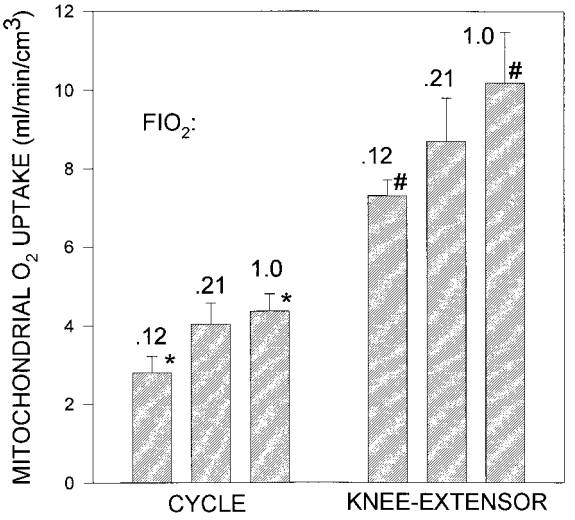

Figure 4—The large increase in mitochondrial O_2 uptake facilitated by changing the exercise paradigm from cycling (22) to KE (30) where cardiac output and muscle O_2 delivery are not limiting. As both sets of data were collected in endurance-trained subjects, mitochondrial $\dot{V}O_2$ was calculated based on an assumed mitochondrial fiber volume of 7.5%, a myofibril volume of 80%, and a muscle density of 1.06 g·cm^{-1} (16,17,28). Active muscle mass used in the normalization was 7.5 kg for cycle exercise and 2.5 kg for KE. * Significantly greater than normoxic $\dot{V}O_2$ in the same exercise modality ($P < 0.05$).

$\dot{V}O_{2max}$ (Fig. 3). Specifically, during maximal single leg knee-extensor exercise the proportional relationship between both PvO_2 and $PcapO_2$ and maximal $\dot{V}O_2$ accompanying elevations and reductions in the fraction of inspired O_2 are consistent with the concept of tissue diffusion limitation of $\dot{V}O_{2max}$ in normal humans (Fig. 3) (43).

Although it is likely that there will always be disagreement on factors that limit muscle $\dot{V}O_{2max}$, in addition to the present findings, several recent studies have provided evidence supporting the concept that O_2 supply rather than biochemical limitation (10,41) sets $\dot{V}O_{2max}$. Specifically, despite using a similar optical technique to Stainsby et al. (41), Duhaylongsod et al. (9) reported contrasting results in the canine gracilis muscle where maximal exercise resulted in near-complete reduction of cytochrome aa_3. This was interpreted to reflect deficient O_2 provision to this muscle (9). In man, Richardson et al. (32) measured *in vivo* myoglobin desaturation at maximal exercise, as an endogenous probe of intracellular PO_2, and found a proportional fall in muscle $\dot{V}O_{2max}$ with a hypoxically induced reduction in intracellular PO_2. These data provide support for the concept that maximal respiratory rate ($\dot{V}O_{2max}$) is limited by O_2 supply (32). During whole body exercise indirect pulmonary gas exchange measurements have continued to support the importance of O_2 supply in determining muscle $\dot{V}O_{2max}$ (1,26), while more direct evidence attained by blood gas and blood flow measurements during cycle exercise have also recently been provided by Knight et al. (22). Here, normoxic leg $\dot{V}O_{2max}$ was increased by 8% in hyperoxia (100% O_2) and reduced by 30% in hypoxia (12% O_2). As illustrated in Figure 4, another clear indication that O_2 supply governs muscle $\dot{V}O_{2max}$ became apparent with the introduction of the functionally isolated knee-extensor model by Andersen and Saltin (2). The comparison that we make here between data collected from human quadriceps acting as part of whole body (cycle) exercise (22) and the knee-extensors in isolation confirms much higher specific mitochondrial $\dot{V}O_2$ when central limitations to O_2 delivery are not present (Fig. 4).

Role of intracellular PO_2 in determining lactate efflux. In the first quarter of this century Hill et al. (12) postulated that blood lactate concentration increased with progressive muscular work because of the inadequacy of O_2 supply to support the metabolic requirements of the contracting muscles. In 1964 the term "anaerobic threshold" was coined by Wasserman and Mclroy (47) to describe this concept. However, the major finding of the present human skeletal muscle study is that intracellular PO_2 remains constant during graded incremental exercise in man (from 50–100% of muscle $\dot{V}O_{2max}$) and is unrelated to the linear fall in intracellular pH and concomitant linear rise in net muscle lactate efflux (Fig. 5). In addition, we found that a reduction in the fraction of inspired O_2 (despite the same O_2 delivery at any given muscle $\dot{V}O_2$) resulted in a significant reduction in intracellular PO_2 which again remained constant during graded incremental exercise (Fig. 5). Under these hypoxic conditions the rate of fall in intracellular pH and the rate of muscle lactate efflux, both in relation to absolute $\dot{V}O_2$, were significantly increased. With respect to the concept of the "anaerobic" threshold these data demonstrate that during incremental exercise skeletal muscle cells do not become "anaerobic" as lactate levels suddenly rise, since intracellular PO_2 is well preserved at a constant level, even at maximal exercise. Thus, our data illustrate the lack of a relationship between intracellular PO_2, lactate efflux and muscle pH. However, the observation that in hypoxia intracellular PO_2 and muscle $\dot{V}O_{2max}$ are reduced and muscle lactate efflux is accelerated leaves open the possibility that

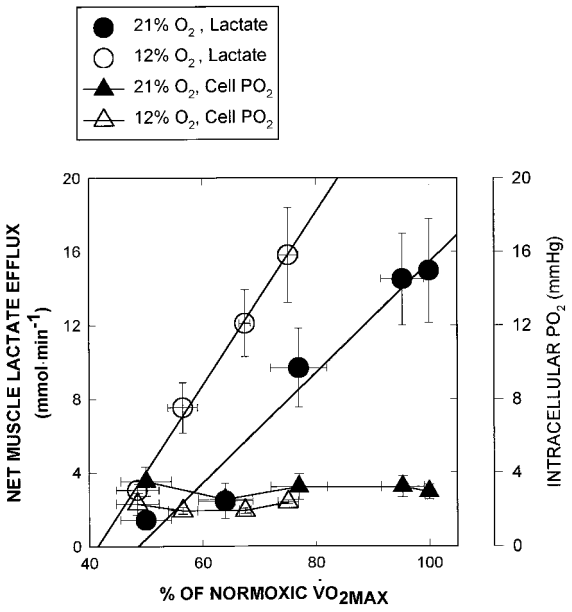

Figure 5—Net muscle lactate efflux and intracellular PO_2 as a function of $\dot{V}O_2$ in normoxia and hypoxia in group 1 subjects (for lactate efflux: r = 0.97 N; r = 0.99 H, $P < 0.05$). Reproduced with permission of the American Physiological Society.

intracellular PO_2 may still play a role in modulating muscle metabolism and ultimately muscle fatigue.

There is considerable circumstantial evidence to support the notion that lactate production is related to inadequate O_2 availability during exercise (20,46). However, until the present study only limited evidence has indicated that this relationship may be spurious: Jobsis and Stainsby (19) studied the oxidation of $NADH/NAD^+$ at rest and in lactate-producing muscle and found no difference. One would expect a reduction in the members of the respiratory chain (including the $NADH/NAD^+$ pair) to coincide with increased lactate production if lactate output was caused by O_2-limited oxidative phosphorylation. With another approach, Mb cryomicrospectroscopy in dog gracilis muscle, Connett et al. (6–8) were unable to find loci with a PO_2 of less than 2 mm Hg. As previous investigations (5) suggested that the critical PO_2 (PO_{2crit}), below which maximal mitochondrial rate is compromised (between 0.1 and 0.5 mm Hg), Connett et al. (8) concluded the elevated lactate concentration must be caused by factors other than simply O_2-limited mitochondrial ATP synthesis rate. The present data support and extend these latter observations by providing *in vivo* data in man and suggest that average intracellular PO_2 remains above mitochondrial PO_{2crit} even at maximal exercise in hypoxia. However the present data do differ from the findings of Connett et al. (8) in two important respects: 1, our data revealed lower mean intracellular PO_2 values (3 mm Hg vs 5.5 mm Hg) which suggests more loci may be in the realm of the PO_{2crit} and 2, their data clearly indicates that intracellular PO_2 in contracting muscle declines as exercise intensity increases (9 to 5.5 mm Hg from 50% to 100% of $\dot{V}O_{2max}$) whereas our measured intracellular PO_2 remained consistently low across the same relative work rates (approximately 3 mm Hg). Until the present observations there has been a general agreement that PO_2 in contracting muscles declines as exercise intensity increases (11). These differences could be methodological or could reflect species differences, but since there are no comparable data in intact man, their resolution must await further investigation.

A discussion of lactate efflux from exercising muscle would not be complete without recognizing the role of catecholamines in the stimulation of glycolysis (predominantly epinephrine via cyclic AMP) and the subsequent relation to lactate production. There is strong positive correlation between blood [La], [epinephrine], exercise intensity (18,25), and arterial oxygen saturation (23) and thus the role of increased sympathetic drive in the progressive increase in net muscle lactate efflux in both hypoxia and normoxia should not be overlooked. The upper panel of Figure 6 illustrates the elevated arterial epinephrine and net muscle lactate efflux in hypoxia in comparison to normoxia at a given quadriceps $\dot{V}O_2$. In the lower panel it is evident that the level of net muscle lactate efflux is closely related to arterial epinephrine levels and that this relationship is independent of percentage of inspired O_2. Additionally, it has been documented that β-adrenergic blockade results in a profound reduction in arterial blood lactate concentration (approximately 50%) during exercise at altitude; however it should be recognized that in this case similar patterns of lactate production were recorded with and without blockade indicating that sympathoadrenal response, although important, does not entirely account for lactate changes during exercise at altitude (24). These observations taken in conjunction with the present lack of a relationship between intracellular PO_2 and lactate efflux add credence to the hypothesis that increased blood lactate levels may, to some extent, be influenced by elevated sympathetic drive during exercise, more so in hypoxia, rather than due to a lower intracellular PO_2 *per se*.

Role of intracellular PO_2 in determining $\dot{V}O_{2max}$. It has recently become apparent that in exercising skeletal muscle there is a substantial vascular to intracellular PO_2 gradient which may be manipulated by altering the fraction of inspired O_2 (32). The present data collected in human skeletal muscle *in* vivo support this observation with the smallest PO_2 gradient of 26.7 mm Hg in hypoxia, 32.2 mm Hg in normoxia, and the largest gradient of 36.9 mm Hg in hyperoxia. Additionally, the significant increase in $\dot{V}O_{2max}$ associated with both an increase in O_2 delivery and the O_2 gradient from blood to cell support the theory that O_2 supply plays an important role in determining $\dot{V}O_{2max}$ in trained skeletal muscle. However, perhaps the most novel observation here is that unlike the linear increase in $\dot{V}O_{2max}$ with an increase in $MbPO_2$ from hypoxia to normoxia (with the origin as an initial point), hyperoxia increased $\dot{V}O_{2max}$ relatively less than $MbPO_2$ suggesting an approach to maximal mitochondrial capacity in this condition (Fig. 7B.)

It is apparent that the degree of myoglobin saturation and therefore intracellular PO_2 is linearly dependent upon the capillary PO_2 (Fig. 7). An extrapolation of this relationship also yields the inference that even with a substantial mean

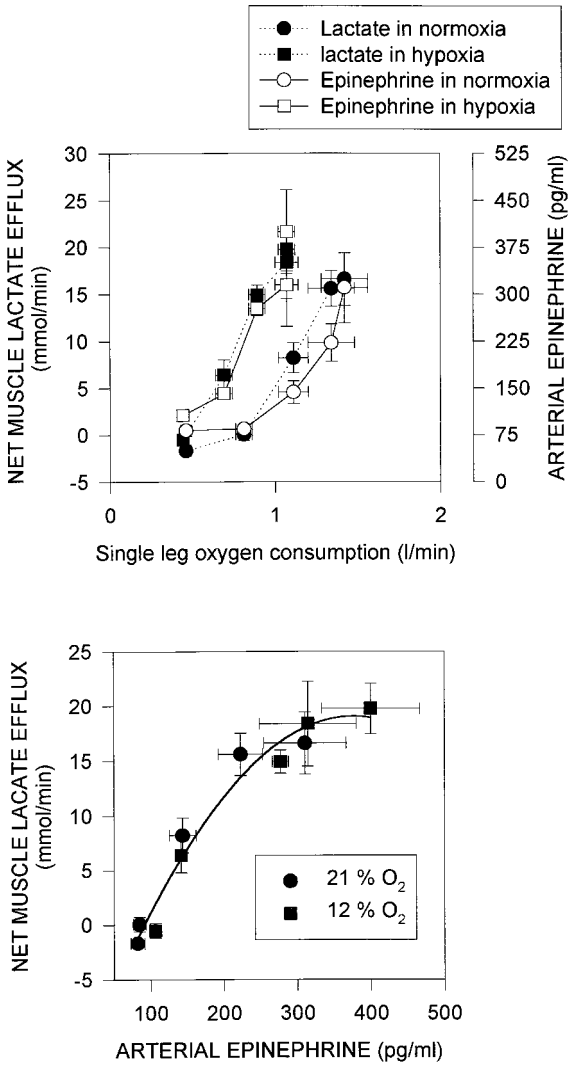

Figure 6—Upper panel: Relationships of net muscle lactate efflux and arterial epinephrine levels to $\dot{V}O_2$ in hypoxia and normoxia in group 2 subjects. Lower panel: The relationship between net muscle lactate efflux and arterial epinephrine level is independent of inspired oxygen concentration. Reproduced with permission of the American Physiological Society.

capillary PO_2 (approximately 15 mm Hg) intracellular PO_2 would fall to zero (Fig. 8). This interpretation supports the importance of muscle DO_2 which determines both the slope of this relationship and the intercept or "critical vascular PO_2 " at which intracellular PO_2 would equal zero resulting in zero $\dot{V}O_2$ (Fig. 8). It also suggests that when mean capillary PO_2 and $\dot{V}O_{2max}$ data are plotted with the origin as a hypothetical data point (31,32,43), this may be incorrect because beyond the critical vascular PO_2 (mean capillary), $\dot{V}O_2$ will no longer fall in a linear fashion as intracellular PO_2 has already reached zero.

There is a contrast between the linear relationship between mean capillary and intracellular PO_2 described above (Fig. 8) and the hyperbolic relationship between intracellular PO_2 and $\dot{V}O_{2max}$ (Fig. 7). This suggests that in hyperoxia there is the expected rise in intracellular PO_2 (due to increased mean capillary PO_2), but this elevated O_2 availability is now in excess of mitochondrial capacity (Fig. 7B).

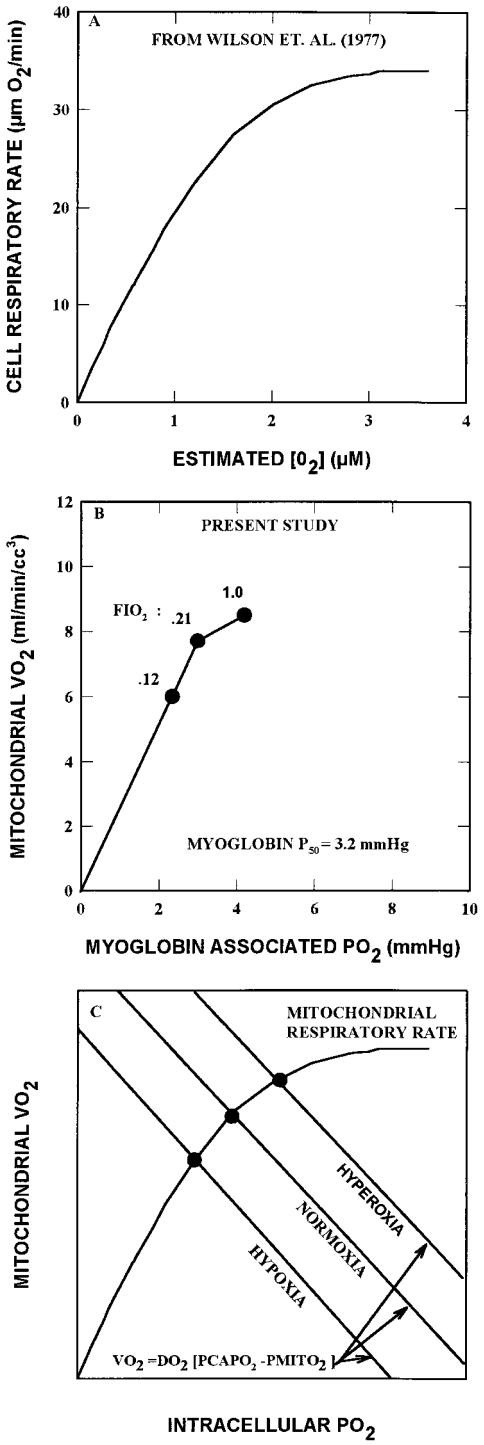

Figure 7—A comparison of the relationship between mitochondrial respiratory rate and O_2 availability *in vitro* made by Wilson and colleagues (49)(A) with the present *in vivo* measurements of the relationship between mitochondrial $\dot{V}O_2$ and intracellular PO_2 (B). Panel C theoretically combines the previous relationship described by Wilson et al. with the present observations to illustrate how the myoglobin associated PO_2 data fit with the O_2 supply dependence of $\dot{V}O_{2max}$ in intact normal man.

Thus indicating that intracellular PO_2 is a determinant of $\dot{V}O_{2max}$ in 12, 21, and 100% O_2, but that in the latter case the increased intracellular PO_2 results in diminishing returns with respect to an increase in $\dot{V}O_{2max}$. These observations are consistent with cellular metabolism that is moving

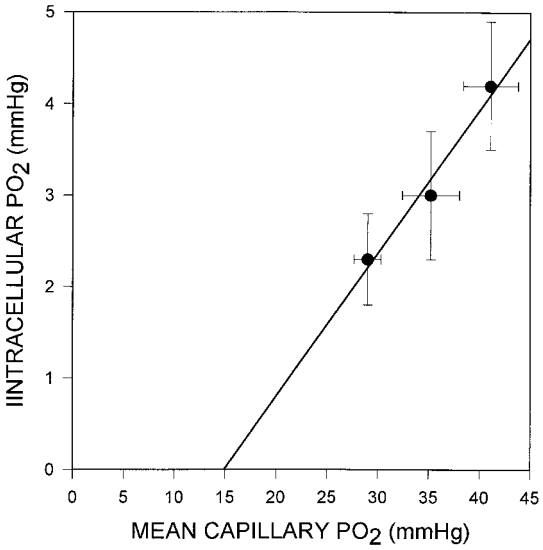

Figure 8—The proportional relationship between mean capillary PO_2 and intracellular PO_2 at $\dot{V}O_{2max}$. Note that the intersection of this line with the x-axis may indicate the capillary PO_2, which must be exceeded to achieve gas exchange into the cell.

toward a transition between O_2 supply as a determinant of $\dot{V}O_{2max}$ and O_2 demand as a determinant of $\dot{V}O_{2max}$. This is illustrated in Figure 7C where further increases in intracellular PO_2, beyond those recorded in hyperoxia, have smaller effects upon $\dot{V}O_{2max}$ until a plateau is reached and $\dot{V}O_{2max}$ becomes invariant with intracellular PO_2. From this point intracellular PO_2 is no longer a determinant of skeletal muscle $\dot{V}O_{2max}$. This hyperbolic relationship, originating from the origin, between O_2 tension and cellular respiration is in agreement with data previously described by Wilson et al.. in kidney cells (Fig. 7A; 49). We again (32), although now with more conclusive data, suggest that these findings may represent the hyperbolic relationship between *in vivo* muscle $\dot{V}O_2$ and intracellular PO_2, supporting the concept that maximal respiratory rate ($\dot{V}O_{2max}$) is limited by O_2 supply. In fact, we suggest that our data may describe a similar relationship to those of Wilson et al. (49) by reconciling the hyperbolic expression of O_2 utilization in Figure 7A with the linear expression of O_2 transport (equation in Fig. 7C), as theoretically illustrated in Figure 7C. Thus, this equation, when O_2 is plotted against intracellular PO_2, is a straight line of similar slope (DO_2) in hypoxia, normoxia and hyperoxia, but with a lower and higher intercept in hypoxia and hyperoxia due to the lower and higher mean capillary PO_2, respectively, at $\dot{V}O_{2max}$. The intersection of these lines with the intrinsic mitochondrial $\dot{V}O_2/PO_2$ hyperbolic relationship shows how the present myoglobin-associated PO_2 data fit with O_2 supply dependence of $\dot{V}O_{2max}$ in intact normal man. The conclusions are identical, but the data essentially independent of those relating $\dot{V}O_{2max}$ to mean capillary PO_2 supported by both the present and previous studies (14,31,36).

The present data once again raise an interesting issue: Why at normoxic or hyperoxic $\dot{V}O_{2max}$ did the Mb-associated PO_2 not fall to the level reached in hypoxia and why in all three conditions the desaturation was far less at maximum work rate than under conditions of cuff occlusion? A possible explanation may be found by attempting to reconcile our Mb-associated data with the recent measurements of cytochrome a a_3 oxidation-reduction state in exercising skeletal muscle (9). These data illustrated a progressive decrease in the concentration of oxidized cytochrome a a_3 (which correlated highly with rising muscle lactate efflux), with increasing muscle O_2 extraction. At $\dot{V}O_{2max}$ the magnitude of this redox response was equivalent to that observed at death or complete anoxia, suggesting that near depletion of O_2 at the mitochondrial level accompanies maximal exercise intensities (9). These findings are in contrast to our Mb-associated PO_2 data which revealed a constant intracellular PO_2, which did not correlate with rising muscle lactate efflux (33), with increasing work intensity. However, a reconciliation of these data is possible by using similar logic employed to explain the observation that there is a large gradient from blood to cell and venous PO_2 (representative of end capillary PO_2) does not fall to zero even at $\dot{V}O_{2max}$ (32,44). The concept being that there is a finite O_2 conductance (DO_2) and that this may limit O_2 transport (43). Thus a mitochondrial DO_2, which limits O_2 conductance from the cytosol to mitochondria, may explain both the difference in oxygen availability outside and within the mitochondria as well as the inability for Mb-PO_2 to fall to a greater extent before the cessation of high intensity exercise. In this scenario a gradient exists from capillary to cytosol (approximately 30 mm Hg) and from cytosol to mitochondria (approximately 2 mm Hg). It should be noted that although the gradient is vastly different in each case the physiological significance may well be of equal magnitude.

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