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Oxygen availability and PCr recovery rate in untrained human calf muscle: evidence of metabolic limitation in normoxia

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[1]*Department of Medicine, University of California, San Diego La Jolla, California;* [2]*School of Physiotherapy and Exercise Science, Griffith University, Queensland, Australia;* [3]*Huntington Medical Research Institutes, Pasadena, California;* [4]*Department of Medicine and Department of Exercise and Sport Science, University of Utah, Salt Lake City, Utah;* [5]*Department of Medicine, Norwegian University of Science and Technology, Trondheim, Norway; and* [6]*Geriatric Research Education and Clinical Center, Salt Lake Veterans Affairs Medical Center, Salt Lake City, Utah*

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Haseler LJ, Lin A, Hoff J, Richardson RS. Oxygen availability and PCr recovery rate in untrained human calf muscle: evidence of metabolic limitation in normoxia. *Am J Physiol Regul Integr Comp Physiol* 293: R2046–R2051, 2007. First published August 22, 2007; doi:10.1152/ajpregu.00039.2007.—In contrast to their exercise-trained counterparts, the maximal oxidative rate of skeletal muscle in sedentary humans appears not to benefit from supplemental O_2 availability but is impacted by severe hypoxia, suggesting a metabolic limitation either at or below ambient O_2 levels. However, the critical level of O_2 availability at which maximal metabolic rate is reduced in sedentary humans is unknown. Using ^{31}P magnetic resonance spectroscopy and arterial oximetry, phosphocreatine (PCr) recovery kinetics and arterial oxygenation were assessed in six sedentary subjects performing 5-min bouts of plantar flexion exercise followed by 6 min of recovery. Each trial was repeated while breathing one of four different fractions of inspired O_2 ($F_{I_{O_2}}$) (0.10, 0.12, 0.15, and 0.21). The PCr recovery rate constant (a marker of oxidative capacity) was unaffected by reductions in $F_{I_{O_2}}$, remaining at a value of 1.5 ± 0.2 min^{-1} until arterial O_2 saturation (Sa_{O_2}) fell to less than ~92%, the average value reached breathing an $F_{I_{O_2}}$ of 0.15. Below this Sa_{O_2}, the PCr rate constant fell significantly by 13 and 31% to 1.3 ± 0.2 and 1.0 ± 0.2 min^{-1} ($P < 0.05$) as Sa_{O_2} was reduced to 82 ± 3 and 77 ± 2%, respectively. In conclusion, this study has revealed that O_2 availability does not impact maximal oxidative rate in sedentary humans until the O_2 level falls well below that of ambient air, indicating a metabolic limitation in normoxia.

oxidative capacity; ^{31}P-magnetic resonance spectroscopy; exercise

THE REDUCED OXYGEN AVAILABILITY of severe hypoxia uniformly attenuates human maximal oxidative metabolic rate; however, limitations to maximal oxidative rate in normoxia and the impact of moderate hypoxia appear to be dependent on the exercise training status of the population studied (5, 16, 24). The clear dependence between O_2 supply and skeletal muscle maximal oxidative rate assessed during maximal exercise in trained human muscle has been previously recognized (24). In fact, these in vivo studies revealed that under hyperoxic conditions the maximal metabolic rate of these well-trained subjects increased beyond ambient levels, revealing O_2 supply limitation in normoxia (24).

Phosphocreatine (PCr) recovery measurements, as an index of maximal oxidative rate, have provided further evidence of this O_2 supply limited maximal metabolic rate in normoxia in the muscle of exercise-trained humans but have failed to identify the level of O_2 availability that creates an O_2 surplus as this appears to fall beyond that achieved by delivering 100% O_2 (9). While a similar hyperoxic approach failed to alter the metabolic rate in the untrained muscle (Fig. 1), a unifying result is observed in both trained and untrained human skeletal muscle when severe hypoxia compromises maximal metabolic rate. Taken together, these data suggest that in untrained subjects the level of O_2 availability under normoxic conditions may be either perfectly matched or in excess of metabolic capacity, but this "critical" level of O_2 availability is currently unknown (10)(Fig. 1). It is upon the later unanswered question that we now focus our experiments to initially provide a benchmark for the normal healthy balance between O_2 supply and demand in ambient O_2 in untrained skeletal muscle and ultimately better understand the numerous pathologies that alter O_2 availability and metabolism either individually or in unison. Unlike maximal exercise, an advantage of assessing maximal muscle oxidative metabolism by PCr recovery from submaximal exercise is the low level of stress involved, making it suitable for studying oxidative rate during exercise under conditions of severe hypoxia, and in subjects who are unaccustomed to strenuous exercise due to inactivity, aging, or debilitating pathologies (10, 14, 18).

Consequently, we sought to identify the critical Sa_{O_2} below which maximal oxidative rate would be reduced in sedentary humans by using arterial oximetry, serial reductions in O_2 availability from normoxia ($F_{I_{O_2}}$ = 0.21, 0.15, 0.12, and 0.1), and ^{31}P magnetic resonance spectroscopy (MRS) to measure PCr recovery from plantar flexion exercise. The specific hypothesis tested was that metabolic capacity in untrained human skeletal muscle is perfectly matched to ambient O_2 availability, and hence even a modest reduction in Sa_{O_2} will significantly impact the maximal mitochondrial oxidative rate, as measured by PCr recovery.

METHODS

Subjects. Six sedentary subjects (equally divided by sex and means of 26.5 ± 0.8 yr of age, 78.9 ± 19.1 kg body wt, and 172.7 ± 11.7 cm height) volunteered to participate in this study and gave written informed consent. Although not expressly performed for this investigation, the majority (5/6) of the subjects had previously performed a

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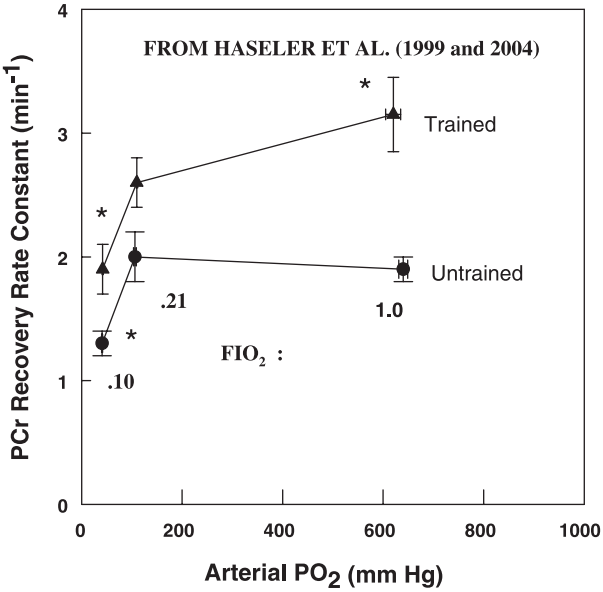

Fig. 1. Phosphocreatine (PCr) recovery rate constants, an index of maximal oxidative ATP synthesis, in exercise-trained and sedentary humans following submaximal plantar flexion exercise under varying levels of O_2 availability. Note: exercise-trained subjects exhibit O_2 supply dependence even in normoxia (9), although O_2 supply dependence in sedentary subjects has been documented in severe hypoxia (10), it remains unknown whether the level of O_2 availability under normoxic conditions is either perfectly matched or in excess of metabolic capacity in untrained muscle. $F_{I_{O_2}}$, fraction of inspired O_2. *Significantly different from normoxic PCr recovery rate constants ($P < 0.05$).

$\dot{V}_{O_{2max}}$ test either within our or another laboratory and were documented to be below 40 ml·kg^{-1}·min^{-1} within the last 6 mo. The study was approved by the University of California, San Diego, Human Subjects Protection Program and was in accordance with the declaration of Helsinki. The subjects were screened to assess their level of physical activity by using a modified version of the Minnesota Leisure Time Physical Activity questionnaire that correlates well with exercise testing (7).

Exercise protocol. Subjects performed plantar flexion exercise in a supine position. The ergometer consisted of a footplate against which the subject worked and a weight and pulley system that offered variable resistance. Range of motion was controlled by a fixed distance that the footplate could be moved during flexion and by the distance the weight could move during relaxation. Thus, range of motion was fixed at 10 cm of plantar flexion with the foot starting perpendicular to horizontal. A simple force × distance calculation provided an estimate of the measurable work that, divided by the frequency of contraction, gave a measure of power. Subjects were familiarized with plantar flexion exercise in the confines of a whole body MRS system. At this time a level of work was determined for each subject that would result in a PCr depletion of between 30–40% under normoxic conditions. Subjects performed constant-load submaximal plantar flexion at this intensity (6.0 ± 0.5 W) (frequency of 1 Hz, maintained with the aid of an electronic metronome), while breathing serial reductions in $F_{I_{O_2}}$ (0.21, 0.15, 0.12, and 0.1). The order of the four treatments was randomized to minimize any ordering effects, and the treatment order was not disclosed to the subjects. Subjects breathed each gas mixture until their Sa_{O_2} had equilibrated before 2 min of resting baseline data collection and the commencement of exercise. In each $F_{I_{O_2}}$, subjects performed 5 min of exercise followed by 6 min of recovery, and MRS data were acquired continuously. Throughout each exercise bout subjects breathed through a low resistance two-way breathing valve (model 2700; Hans-Rudolph, Kansas City, MO) connected to a 150-liter reservoir bag containing the appropriate $F_{I_{O_2}}$. Subjects were allowed 30 min of rest between each exercise bout.

Heart rate, arterial O_2 saturation, and arterial P_{O_2}. Heart rate and Sa_{O_2} were monitored continuously throughout the experiment with a finger probe oximeter (Omni-Trak; In Vivo Research). To provide an estimate of oxygen tension, Sa_{O_2} data were used to calculate Pa_{O_2} using the Hill equation, assuming a normal P_{50}. Previously, during exercise with the same mode of ergometry and across the range of $F_{I_{O_2}}$ employed in this study, we have documented a high correlation between Sa_{O_2} measured with this analyzer and the subsequent estimation of Pa_{O_2} with end-tidal O_2 gas measurements (9, 11).

^{31}P MRS. MRS was performed using a clinical 1.5T General Electric Signal system (5.4.2 version) operating at 25.86 MHz for ^{31}P. The ^{31}P MRS data were acquired with a dual-frequency flexible array spectroscopy coil (Medical Advances) placed around the calf at its maximum diameter. The centering of the coil around the working muscles of the lower leg was confirmed by T_1-weighted ^1H localizing images obtained in the axial plane. For all subjects, a similar ratio between the volumes of gastrocnemius/soleus muscles was maintained within the coil. Shimming on the proton signal from tissue water, optimized magnetic field homogeneity, and the ^{31}P MRS signal was optimized by prescan transmitter gain adjustment. A 500-μs hard pulse was used for signal excitation. The spectral width was 2,500 Hz, and data were acquired continuously for 13 min, with a single free-induction decay (FID) acquired every 4 s. Thus, a total of 195 FIDs were acquired during the 2-min rest period, 5 min of exercise, and 6 min of recovery.

^{31}P data analysis. Data were processed using SAGE/IDL software on a Silicon Graphics INDIGO workstation. Each FID consisted of 1,024 complex points and was processed with 5 Hz exponential line broadening prior to zero filling and Fourier transformation. All spectra were manually phased using zero and first-order phase corrections. There were no phase variations between rest, exercise, and recovery data acquisition during the experiment. The levels of PCr determined from the intensity of that peak were normalized to 100% using the average value obtained from the last 40 s of preexercise rest acquired for each subject as a reference. Muscle intracellular pH was calculated from the chemical shift difference (δ) of the Pi peak relative to the PCr peak using the following equation (32): $pH = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$.

Signal-to-noise ratios (~30:1) were sufficient to allow PCr levels to be determined with a temporal resolution of 4 s during exercise and recovery. Changes in PCr during recovery were fit to a monoexponential function: $PCr(t) = PCr_0 + PCr_1 [1 - e^{-(t - TD)/\tau}]$, where PCr_0 is the baseline value, PCr_1 is the difference between the baseline and the recovery value, t is time, TD is the time delay, and τ is the time constant. The PCr recovery rate constant was calculated as $1/\tau$.

Statistical analysis. Recognizing that our interest was in the physiological response to alterations in $F_{I_{O_2}}$ (which can vary widely) the data were instead grouped according to Sa_{O_2} achieved by each $F_{I_{O_2}}$. Thus, the data were considered categorical in nature, and nonparametric statistics were employed. Specifically, data grouped according to Sa_{O_2} were analyzed with a Friedman repeated-measures test (post hoc: Wilcoxon ranked sign test with Bonferroni correction) using a commercially available software package (Instat, San Diego). Additionally, because of this grouping strategy, not all comparisons had equal numbers ($n = 5$ at both the lowest and Sa_{O_2} levels), and in these two cases, the means of the existing data were substituted for the missing values. Although this approach artificially preserves the degrees of freedom and may diminish the variance, it allows the use of repeated-measures analyses, does not alter the mean values, and is an accepted methodological approach. The impact of this approach in the current case was tested by running the statistical analyses twice, once with this approach to handling the missing data and once with the alternative method of dropping this subject and data and reducing the subject number in the study. Power analyses revealed that in all major variables the statistical power was ≥ 0.8. Significance was

established at $P < 0.05$. The results are presented as means ± SD throughout this manuscript.

RESULTS

Subject activity level. The physical activity assessment revealed that the subjects performed no regular or occasional physical exercise above that required for daily activities and reported no previous history of physical training or recreational sports participation.

Missing data. As not all subjects responded consistently in terms of their Sa_{O_2} response to varied FI_{O_2}, there were inevitably missing data in this study. However, in all variables, whether these data were excluded from the analysis (decreased *n*) or the mean was substituted for these data, the statistical results were unaltered.

PCr recovery, rate constant, and depletion. The data were fit to a monoexponential function for the calculation of PCr recovery (τ), in a similar manner to that published previously (Fig. 2) (9, 10). As can been seen from Fig. 2, the quality of curve fitting from a single set of PCr data was excellent, supported by an average confidence interval of 7 ± 1 s with respect to an average τ across all FI_{O_2} conditions of 47 ± 9 s. There was no evidence of a time delay in this fitting process, as determined by no difference between the start of the exponential upon recovery, whether fixed mathematically by timing of the event or allowed to fit freely. PCr τ was unaffected by the reduction in Sa_{O_2} from the room air value of 97 to 92%, but was significantly and progressively elongated by the reduction in Sa_{O_2} to 82 and 77%, respectively (Table 1). As illustrated in Fig. 3*A*, the rate constant of PCr recovery revealed the same changes as a function of O_2 availability, with no impact of a reduction in Sa_{O_2} to 92%, but falling significantly by 13 and 31% as Sa_{O_2} was reduced to 82 and 77%, respectively (Table 1, Fig. 3*A*). Figure 3*B* shows the individual variation in Sa_{O_2} as the FI_{O_2} is reduced with the eventual reduction in PCr recovery rate. An interesting exception to this group response was *subject A* (open triangles and broken line) who defended their Sa_{O_2} as FI_{O_2} was reduced. For this subject, the Sa_{O_2} was only reduced to 92% under the most severe hypoxic condition (0.1 FI_{O_2}), which was still greater than the critical Sa_{O_2} revealed by the rest of the group, and consequently, in this subject there was no effect of serial reductions in FI_{O_2} on the rate of PCr recovery. Although, on average PCr depletion tended to become greater with progressive hypoxia and attaining significance in the lowest Sa_{O_2} achieved (Table 1), there was no significant relationship between PCr depletion and the rate of PCr recovery (Fig. 4).

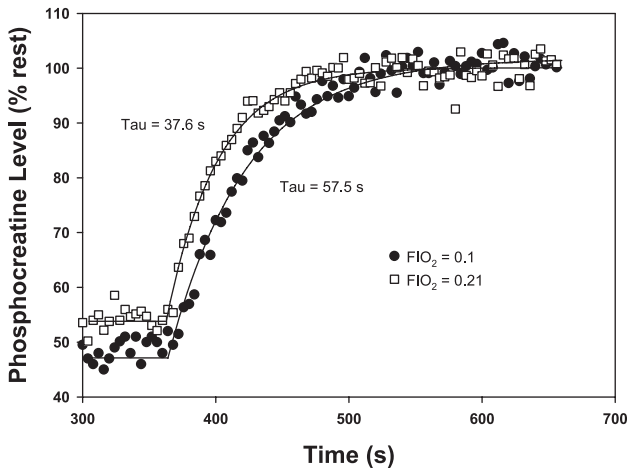

Fig. 2. Raw data and model monoexponential fit for PCr recovery from exercise in a representative subject in normoxia (FI_{O_2} = 0.21) and the most severe hypoxic condition (FI_{O_2} = 0.1).

Table 1. *PCr recovery kinetics and physiological data averaged across the final 40 s of submaximal exercise, highlighting the effect of serial reductions in arterial saturation*

	Hypoxia		Normoxia	
Arterial O_2 Saturation, %	77±4*	82±7*	92±5*	97±0.5
PCr recovery (τ), s	59±13*	47±7*	41±5	40.±5
PCr recovery rate (1/τ), min^{-1}	1.0±0.2*	1.3±0.2*	1.5±0.2	1.5±0.2
Arterial P_{O_2}, mmHg	47±2*	53±5*	73±3*	107±5
Heart rate, beats/min	106±9*	97±7*	89±12*	81±15
PCr depletion, %	55±20*	43±13	45±15	37±13
End-exercise pH	6.95±0.09	6.97±0.07	6.98±0.07	6.99±0.05

Values are reported as means ± SD. Data were grouped according to arterial O_2 saturation as alterations in FI_{O_2} did not reflect O_2 availability due to variations in the subjects' ability to defend arterial O_2 saturation; $n = 6$ all data collected at the two highest Sa_{O_2} levels, while in the lowest two Sa_{O_2} columns $n = 5$. PCr, phosphocreatine; τ, time constant; P_{O_2}, partial pressure of oxygen. *Significantly different from normoxia and from each other ($P < 0.5$).

Arterial O_2 saturation and arterial P_{O_2}, heart rate, and pH. Arterial O_2 saturation and Pa_{O_2}, PCr depletion, heart rate, and pH during the last 40 s of submaximal plantar flexion exercise are displayed in Table 1. By experimental design, as FI_{O_2} was manipulated, Sa_{O_2} and, therefore, estimated Pa_{O_2} were altered in a reasonably uniform manor across most subjects allowing the analyses to be performed as a function of Sa_{O_2} and not FI_{O_2} (Table 1 and Fig. 3). The exception to this was the single subject described above whose arterial O_2 levels did not decline during severe hypoxia (Fig. 3*B*). At each level of altered Sa_{O_2}, heart rate was significantly altered, with the tachycardia being inversely related to Pa_{O_2} ($r = -0.64$) (Table 1). Alterations in Sa_{O_2} had no effect on resting levels of PCr, while end-exercise PCr level was also unaffected until the lowest Sa_{O_2} (Table 1). The most severe hypoxic condition resulted in a slight, but not significant, fall in intracellular pH (Table 1).

DISCUSSION

Theoretically, the level at which O_2 availability limits maximal metabolic rate depends upon the oxidative capacity of the subject. Studies that have utilized hyperoxic breathing to enhance metabolic rate in exercise-trained skeletal muscle have concluded that O_2 availability in normoxia limits metabolic capacity (15, 28). The failure of untrained skeletal muscle to increase maximal metabolic rate under these circumstances leaves uncertainty as to whether O_2 supply is perfectly matched with, or is in excess of, the metabolic capacity of these subjects in normoxia (10). The present study has disproved our original hypothesis that metabolic capacity in untrained human skeletal muscle is perfectly matched to ambient O_2 availability as an initial reduction in Sa_{O_2} had no significant impact upon maximal metabolic rate, as measured by PCr recovery. Therefore, the major conclusion from our results is that maximal oxidative

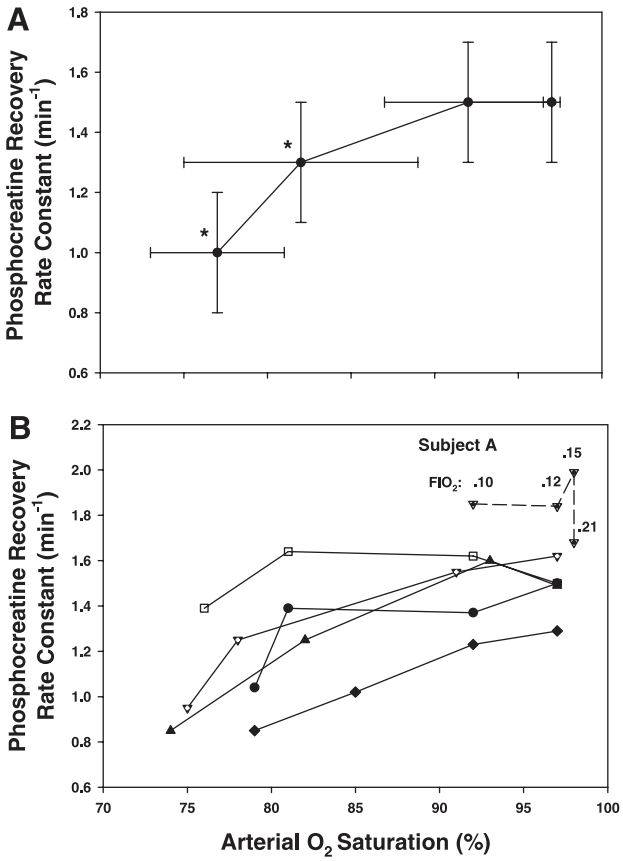

Fig. 3. *A*: mean PCr recovery rate constants as a function of Sa_{O_2} in the muscle of sedentary human subjects. The failure to impact PCr recovery rate until Sa_{O_2} fell well below normoxic conditions revealed metabolically limited skeletal muscle in these subjects even in mild hypoxia. *Significantly different from PCr recovery rate in ambient conditions. *B*: individual variability in Sa_{O_2} with manipulations in FI_{O_2} and the consequences in terms of PCr recovery rate following submaximal plantar flexion exercise. Most subjects demonstrated a decline in Sa_{O_2} with successive reductions in FI_{O_2} and a subsequent attenuation of maximal oxidative rate. In contrast, *subject A* (open triangles) appeared to defend their alveolar P_{O_2}, presumably due to a strong hypoxic ventilatory response, and thus maintain an adequate Sa_{O_2} despite breathing 10% O_2. The maximal metabolic rate of this subject was unaffected.

rate in the calf muscles of untrained humans performing small muscle-mass exercise is metabolically limited in normoxic conditions and continues to reveal this characteristic, even when exposed to mild hypoxia. Indeed, it was not until Sa_{O_2} had fallen to 82% that maximal oxidative rate was attenuated. This relatively low critical Sa_{O_2} (in comparison with everyday levels) in sedentary subjects contrasts, in a conceptually acceptable fashion, with the previously reported data in exercise-trained human skeletal muscle that appears to have a metabolic capacity in excess of ambient O_2 availability (9, 24) (Fig. 1).

PCr recovery to titrate the role of O_2 in limiting oxidative capacity. The recovery rate constant for PCr recovery is a function of the maximum rate of oxidative ATP synthesis (Q_{max}), which can be estimated as $Q_{max} = (1/\tau)[PCr_{rest}]$, where $[PCr_{rest}]$ is PCr concentration in resting muscle (13). Therefore, both the recovery rate constant for PCr recovery and $\dot{V}O_{2max}$ are indices of the maximal rate of oxidative ATP synthesis and are considered to be linearly dependent on muscle oxidative capacity (18–20).

The low level of stress involved in PCr recovery measurements from submaximal exercise make it suitable to study interventions that may be too severe systemically for maximal exercise testing and for populations that have difficulty with such strenuous testing (9, 10, 14, 33). A further advantage of the measure is that PCr recovery measurements do not require a correction for active muscle mass (13, 18) and are independent of the work level (19), provided that muscle intracellular pH does not fall severely (2).

The combination of PCr recovery measurements under conditions of altered O_2 availability has previously demonstrated that under normoxic conditions, O_2 availability limits maximal metabolic rate in the skeletal muscle of exercise-trained humans (9) and does not in their untrained counterparts (10). Therefore, it should be clear that PCr recovery assessed only in normoxia must be interpreted with caution as differences in PCr recovery between subjects may be due not only to metabolic limitations (1, 6, 22) but also to inherent limitations in O_2 supply (33). The current investigation and our previous work has taken this into account by assessing PCr recovery kinetics under conditions of varying O_2 availability and not simply relying upon the normoxic assessment of maximal metabolic rate, allowing the titration of O_2's role in limiting metabolism (9, 10). Such unique observations demonstrate that PCr recovery data coupled with manipulations in O_2 availability are capable of noninvasively distinguishing between O_2 supply and metabolic limitations, providing a powerful addition to the study of muscle pathophysiology.

A potentially confounding issue with the current use of PCr recovery rate as an index of metabolic capacity in the face of somewhat variant PCr depletion levels is the disagreement about the association of these two variables. Although, there are data that suggest PCr recovery rate is linked to PCr depletion (30), in our hands (10) and in older (19) and very recent publications (17) it is more commonly recognized that these variables are independent of each other (especially if pH is relatively unperturbed, as it was in the current study). The

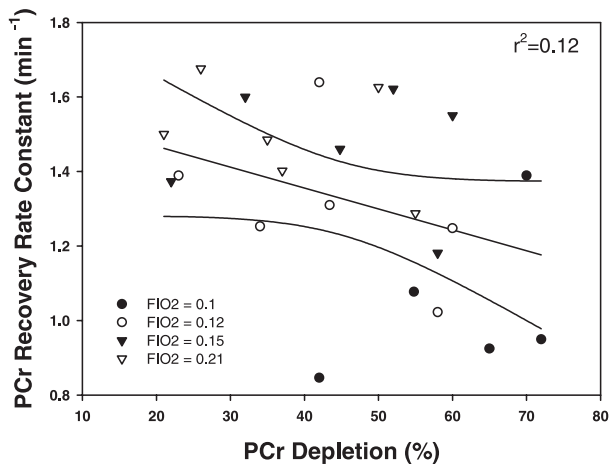

Fig. 4. The weak relationship exhibited between PCr depletion and the subsequent PCr recovery rate constant following exercise across all levels of FI_{O_2}. With the confidence intervals at 95% > 60% of the data lie outside of these boundaries. This observation supports the interpretation of the current data with regard to a metabolic O_2 supply dependence that relies upon PCr recovery rate to be independent of PCr depletion, which varied within this study.

current data support this observation with a weak, nonsignificant, relationship being displayed between PCr depletion and the PCr recovery rate constant following exercise at all levels of $F_{I_{O_2}}$ employed (Fig. 4). Thus, adding credence to the O_2 supply dependence-related interpretation of the current data.

Metabolic and O_2 supply limited maximal metabolic rate. Several earlier studies have illustrated a strong relationship between O_2 supply and skeletal muscle oxidative capacity during maximal exercise in exercise-trained subjects (15, 25). However, the limited research performed in sedentary subjects suggests maximal oxidative rate appears to be determined by mitochondrial capacity and not O_2 supply (5). As already indicated, our previous PCr recovery assessments in trained and untrained subjects contrast the consequences of supplemental O_2 as a function of exercise training, with the untrained unable to take advantage of the increased O_2 supply but revealed a similar negative impact of severe hypoxia in both groups (9, 10).

However, these studies failed to determine whether the metabolic capacity of untrained human skeletal muscle is perfectly matched to ambient O_2 availability (9, 10). The current data suggest that this not the case, with the initial $F_{I_{O_2}}$-induced reductions in Sa_{O_2}, having no impact on maximal metabolic rate, as measured by PCr recovery (Fig. 3*A*). Conceptually, the critical level of O_2 availability that affects maximal oxidative rate is dependent upon metabolic capacity. This is supported by the prior observation that the PCr recovery rate of exercise-trained skeletal muscle was impacted with a reduction in Pa_{O_2} from ~600 mmHg induced by hyperoxia (9), while in the current sedentary subjects, estimated Pa_{O_2} had to fall to $\leq$ 50 mmHg to impact metabolism (Table 1). With this apparent link between mitochondrial capacity and O_2 availability, the current technique (^{31}P MRS coupled with altered O_2 availability) may have a clinical role in distinguishing between O_2 supply and demand limitations in the study of muscle pathophysiology (e.g., aging and mitochondrial myopathy).

It would also be useful to bring this isolated small skeletal muscle mass study back into the realm of whole body exercise, such as walking, running, or cycling and provide implications for the relationship between untrained muscle and O_2 availability in this type of activity. However, the translation of these data to a situation where a much greater muscle mass is recruited is actually quite complex and now introduces the potential for central limitations (e.g., cardiac output and or blood flow distribution) that may lead to differing results. Thus, although the current data are in line with the concepts that untrained subjects are probably not O_2 supply limited when studied during whole body exercise breathing ambient air (34), there is actually a continuum of responses (21) and that exercise-trained individuals are more susceptible to reductions in O_2 availability (16), this translation between paradigms should be viewed with caution.

Role of diffusion limitation in determining maximal metabolic rate. The diffusion of O_2 from blood to cell has been thought of as a somewhat discrete and potentially independent limitation to O_2 transport and ultimately maximal metabolic rate (12, 29, 34). According to Fick's law of diffusion, muscle O_2 diffusing capacity (D_{O_2}) in conjunction with the P_{O_2} gradient from capillary ($P_{cap}O_2$) to mitochondria ($P_{mito}O_2$) plays a critical role in determining the maximal rate of O_2 consumption ($\dot{V}O_{2max}$): $\dot{V}O_{2max} = D_{O_2} (P_{cap}O_2 - P_{mito}O_2)$.

Studies have shown that muscle blood flow is elevated in hypoxia and decreased in hyperoxia across a range of submaximal power outputs (4, 8, 31). Thus, alterations in muscle blood flow during submaximal exercise either partially or totally compensate, in terms of O_2 delivery, for the changes in arterial O_2 content (Ca_{O_2}) induced by breathing hypoxic or hyperoxic gas mixes. Under such conditions, convective O_2 delivery would remain relatively constant, while the diffusive component of O_2 transport would be altered. Data supporting this concept have been reported previously (27), where submaximally both the D_{O_2} and the convective delivery of O_2 were unchanged between normoxia and hypoxia, but the P_{O_2} gradient from capillary ($P_{cap}O_2$) to mitochondria ($P_{mito}O_2$) in hypoxia was reduced, significantly decreasing both $P_{mito}O_2$ and skeletal muscle $\dot{V}O_{2max}$.

In the current study, the more severe levels of hypoxia resulted in an estimated $Pa_{O_2} \leq 50$ mmHg ($Sa_{O_2} \leq 82\%$) and significantly slowed PCr recovery, despite the likely increase in muscle blood flow that compensates for reduced arterial O_2 content (8, 24, 31). Therefore, these data may be used to highlight the importance of the diffusive component of O_2 transport in determining maximal metabolic rate, in this case, under conditions of reduced O_2 availability.

$F_{I_{O_2}}$, hypoxic ventilatory response, and in vivo O_2 availability. Because of the O_2 cascade from blood to tissue, graded reductions in inspired O_2 would be expected to ultimately alter in vivo O_2 availability all the way to the myocyte itself (23, 26). However, although convenient, it is not appropriate to assume that alterations in $F_{I_{O_2}}$ reflect in vivo O_2 availability. On an individual basis, the defense of alveolar P_{O_2} by an increase in alveolar ventilation can markedly influence this chain of events. This hypoxic ventilatory response (HVR) varies widely between individuals and has been used to distinguish between those who will thrive and those who will perish at high altitude (3).

The importance of recognizing this phenomenon and its impact upon manipulations in O_2 availability became apparent in the current investigation. This is highlighted in Fig. 3*B*, which contrasts the dramatically attenuated fall in Pa_{O_2} of a single subject (*subject A*) compared with the other subjects exposed to the same array of hypoxic gases. Clearly, a failure to relate this subject's response in terms of both PCr recovery rate constant and Sa_{O_2} would have resulted in the incorrect conclusion that O_2 availability does not impact muscle oxidative capacity. As illustrated (Fig. 3, *A* and *B*), the data for this subject are, to a point, consistent with the group data, but due to this subject's vigorous HVR they maintained their Sa_{O_2} above the threshold necessary to impact maximal metabolic rate, despite breathing a 10% O_2 gas mixture (our ethical limit for this study). In addition, this observation adds credence to the concept that a vigorous HVR has consequences, not only at the level of arterial oxygenation but also at the level of the myocyte, for in this subject, maximal metabolic rate was not compromised despite exposure to relatively severe hypoxia.

Summary. In conclusion, this study has identified a critical level of Sa_{O_2} in sedentary subjects, below which maximal metabolic rate is compromised. This critical O_2 level is lower than a Sa_{O_2} of 92% and therefore lies much below typical normoxic values. Thus, the maximal oxidative rate in the skeletal muscle of these subjects would be classified as metabolically limited in normoxic and mildly hypoxic conditions.

However, even in these untrained subjects moderate-to-severe hypoxia ($Sa_{O_2} \leq 82\%$) ultimately leads to an O_2 supply-limited scenario in which skeletal muscle maximal metabolic rate is attenuated, presumably due to a reduction in the O_2 driving gradient from air to blood to cell that reduces intracellular P_{O_2} and limits mitochondrial function.

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