Microvascular oxygen partial pressure during hyperbaric oxygen in diabetic rat skeletal muscle

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Yamakoshi K, Yagishita K, Tsuchimochi H, Inagaki T, Shirai M, Poole DC, Kano Y. Microvascular oxygen partial pressure during hyperbaric oxygen in diabetic rat skeletal muscle. Am J Physiol Regul Integr Comp Physiol 309: R1512–R1520, 2015.—Hyperbaric oxygen (HBO) is a major therapeutic treatment for ischemic ulcerations that perforate skin and underlying muscle in diabetic patients. These lesions do not heal effectively, in part, because of the hypoxic microvascular O2 partial pressures (PmvO2) resulting from diabetes-induced cardiovascular dysfunction, which alters the dynamic balance between O2 delivery (QO2) and utilization (VO2) rates. We tested the hypothesis that HBO in diabetic muscle would exacerbate the hyperoxic PmvO2 dynamics due, in part, to a reduction or slowing of the cardiovascular, sympathetic nervous, and respiratory system responses to acute HBO exposure. Adult male Wistar rats were divided randomly into diabetic (DIA: streptozotocin ip) and healthy (control) groups. A small animal hyperbaric chamber was pressurized with oxygen (100% O2) to 3.0 atmospheres absolute (ATA) at 0.2 ATA/min. Phosphorescence quenching techniques were used to measure PmvO2 in tibialis anterior muscle of anesthetized rats during HBO. Lumbar sympathetic nerve activity (LSNA), heart rate (HR), and respiratory rate (RR) were measured electrophysiologically. During the normobaric hyperoxia and HBO, DIA tibialis anterior PmvO2 increased faster (mean response time, CONT 78 ± 8 s, DIA 55 ± 8 s, P < 0.05) than CONT. Subsequently, PmvO2 remained elevated at similar levels in CONT and DIA muscles until normobaric normoxic recovery where the DIA PmvO2 retained its hyperoxic level longer than CONT. Sympathetic nervous system and cardiac and respiratory responses to HBO were slower in DIA vs. CONT. Specifically the mean response times for RR (CONT: 6 ± 1 s, DIA: 29 ± 4 s, P < 0.05), HR (CONT: 16 ± 1 s, DIA: 45 ± 5 s, P < 0.05), and LSNA (CONT: 140 ± 16 s, DIA: 247 ± 34 s, P < 0.05) were greater following HBO onset in DIA than CONT. HBO treatment increases tibialis anterior muscle PmvO2 more rapidly and for a longer duration in DIA than CONT, but not to a greater level. Whereas respiratory, cardiovascular, and LSNA responses to HBO are profoundly slowed in DIA, only the cardiovascular arm (via HR) may contribute to the muscle vascular incompetence and these faster PmvO2 kinetics.

HYPERBARIC OXYGEN (HBO) therapy is used widely in diabetic patients to alleviate tissue hypoxia [i.e., raise microvascular oxygen partial pressures (PmvO2)] and promote healing of chronic ischemic ulcerations that perforate skin and underlying muscle in diabetic patients (42). HBO raises arterial O2 by increasing hemoglobin (Hb) O2 saturation to 100% (from ~97% or lower) and enhancing dissolved O2 in the plasma, which acts to alleviate tissue hypoxia and may initiate crucial angiogenic and tissue restorative pathways (21). With HBO arterial P2O2 should increase roughly in proportion to inspired O2 pressure. However, HBO is also associated with altered breathing [i.e., transient hypopnea (46)] and cardiovascular responses such as vasocostriction (41), bradycardia, and decreased cardiac output (15, 45). These effects of HBO are coordinated through chemoreflex (reviewed in Ref. 46)- and baroreflex (10)-mediated mechanisms, and it is pertinent that chemoreflex (11) and baroreflex control in experimental diabetes is impaired (9, 39). Thus, HBO-induced respiratory, cardiovascular, and baroreflex dysfunction in diabetes can, in addition to altered muscle vascular control, affect the dynamic balance between O2 delivery (QO2) and utilization (VO2) [i.e., PmvO2 (31, 32)] in skeletal muscle of diabetic patients. An important perspective here is that PmvO2 constitutes the sole driving force for blood-tissue O2 flux, and therefore the beneficial effects of HBO are contingent on PmvO2 rising appropriately with HBO. Thus, in understanding the therapeutic potential of HBO, it is important to resolve the time course(s) of PmvO2 and these processes (i.e., their kinetics) and gain some insight into their potential to impact tissue PmvO2 responses to HBO.

Underlying the physical and metabolic dysfunction in diabetes is the induction of profound structural and functional alterations that impact the capability of the oxygen (O2) transport system to deliver sufficient O2 (QO2) to meet the metabolic requirements (VO2) especially of skeletal muscle at rest and during exercise. Thus, Type 1 diabetes induces muscle atrophy, capillary rarefaction, and impaired vascular and capillary hemodynamics as well as reduced muscle oxidative capacity (3, 16, 38). Type 2 diabetes also presents a severe impairment of arterial (19, 34) and microcirculatory (27, 28) hemodynamics in skeletal muscle at rest and during exercise. Key contributors to this condition are thought to include upregulation of endothelin-1 (37), prostaglandin (1), and myogenic (43) vasoconstrictor pathways combined with downregulation of vasodilatory endothelial function and nitric oxide bioavailability (17, 24, 48). Given the above, it is not surprising that the dynamics of QO2, following exercise onset, for example, may be deficient as evidenced by the aberrant PmvO2 profile to muscle contractions, which reflects an imbalance between QO2 and VO2 responses [type I (3); type 2 (28)]. However, these altered dynamics do not ipso facto mean that diabetic muscle cannot modulate its steady-state blood flow (and thus QO2 and PmvO2) appropriately either at rest or in response to altered metabolic demands (8).
To date, the response of diabetic muscle P\textsubscript{mvO\textsubscript{2}} to HBO therapy has never been determined. Therefore, we sought to characterize the temporal profile of muscle P\textsubscript{mvO\textsubscript{2}} together with respiratory and cardiovascular responses to HBO [1.0 –3.0 atmospheres absolute (ATA)] in healthy and diabetic rats. Specifically, we tested the hypothesis that HBO in diabetic muscle would: 1) speed and accentuate the hyperoxic P\textsubscript{mvO\textsubscript{2}} dynamics and that 2) these responses would relate temporally to a reduction or slowing of the cardiovascular, sympathetic nervous [assessed via lumbar sympathetic nerve activity (LSNA)], and respiratory system responses to acute HBO exposure.

**MATERIALS AND METHODS**

**Animals**

Male Wistar rats (total n = 36, 10 wk of age; Japan SLC, Shizuoka, Japan) were used in this study. Rats were maintained on a 12:12-h light-dark cycle and received food and water ad libitum. Rats were divided into the following two groups: healthy control (CONT) and diabetic (DIA) rats. Rats were anesthetized using isoflurane and given intraperitoneal injection of 45 mg/kg body wt of streptozotocin (STZ; Sigma Aldrich, St. Louis, MO) prepared fresh in saline solution. CONT animals were injected with saline vehicle. Urine glucose levels of rats were measured (New Orieu Ga, Terumo, Japan) 2 days after STZ injection with the onset of diabetes raising glucose concentrations above 300 mg/dl. All experiments were conducted under the guidelines established by the Physiological Society of Japan and were approved by the University of Electro-Communications Institutional Animal Care and Use Committee. The rats were anesthetized using pentobarbital sodium (60 mg/kg ip), and supplemental doses of anesthesia were administered as needed. At the end of experimental protocols, animals were killed by pentobarbital sodium overdose.

**HBO Exposures**

Rats were placed in the small animal hyperbaric chamber in the prone position. During measurement of oxygen pressures in the tibialis anterior muscle the ankle joint was fixed at 90°. To maintain body temperature a 37°C thermal pad (Deltaphase isothermal pad; Braintree Scientific, Braintree, MA) was placed over the abdominal region. Each rat was instrumented and stabilized before collection of the normoxic normobaric baseline measurements. Subsequently, the HBO protocol shown in Fig. 1 was initiated. Following return to normobaric normoxia a further 5 min of monitoring were performed (i.e., Post 1–4).

O\textsubscript{2} (100%) was instilled in the small animal hyperbaric chamber from an oxygen tank by regulating the flow control valve, and the chamber was pressurized to 3.0 ATA at 0.2 ATA/min. Subsequently, the chamber was depressurized at 0.2 ATA/min. This protocol is more gradual than rat models of decompression sickness (30, 44) where peak pressure is set at 10.0 ATA and compression and decompression rate at 1.0 ATA/min and does not lead to gas bubbles.

**Measurement Protocol 1: P\textsubscript{mvO\textsubscript{2}} Measurement (n = 13)**

*Surgical preparation for phosphorescence quenching*. Before the surgical procedures, the animals were anesthetized with pentobarbital sodium. The rat was placed on a heating pad (37°C) to maintain body temperature. The left carotid artery was cannulated (PE-50) for infusion of the phosphorescent probe [palladium meso-tetra(4-carboxyphenyl)porphyrin dendrimer (R2)] at 15 mg/kg body wt. The tibialis anterior muscle was exposed to provide measurement of P\textsubscript{mvO\textsubscript{2}}. After the overlying skin was reflected and the fascia was removed, the muscle surface was superﬁshed with Krebs-Henseleit solution equilibrated with 5% CO\textsubscript{2}-95% N\textsubscript{2} at 38°C and adjusted to pH 7.4. The phosphor R2 was infused via the arterial cannula ~15 min before initiation of the experiments, which were conducted in a darkened room to prevent contamination from ambient light.

P\textsubscript{mvO\textsubscript{2}} measurements. P\textsubscript{mvO\textsubscript{2}} was determined at 1-s intervals at rest and during HBO. The theoretical basis for phosphorescence quenching has been detailed previously (4, 5, 36). Briefly, the Stern-Volmer relationship (36) describes quantitatively the O\textsubscript{2} dependence of the phosphorescence probe. R2 is a nontoxic dendrimer (18) that binds completely to albumin at 38°C and pH 7.4, with a quenching constant of 409 Torr\textsuperscript{−1}/s and lifetime of decay in the absence of O\textsubscript{2} of 601 ms under the physiological conditions extant herein (20, 29). In addition to binding with albumin, the net negative charge of R2 helps facilitate restriction of R2 to the intravascular space (31). To determine P\textsubscript{mvO\textsubscript{2}}, a PMOD 2000 frequency domain phosphorometer (Oxygen Enterprises, Philadelphia, PA) was used; the common end of the bifurcated light guide was placed over the muscle, and blood was sampled within the microvasculature up to 0.5 mm deep within a circular region ~2 mm in diameter.

The phosphorometer employs a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows for phosphorescence lifetime measurements from 10 μs to ~2.5 ms. In the single-frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and were repeated every 1 s. To obtain the phosphorescence lifetime, the logarithm of the intensity values was taken at each time point, and the linearized decay was fit to a straight line by least-squares regression analysis.

*Modeling of P\textsubscript{mvO\textsubscript{2}} profiles*. Curve fitting (HBO pressurization phase) was accomplished using KaleidaGraph software (Synergy...

Fig. 1. Hyperbaric oxygen (HBO) protocol. The pressurization and depressurization were controlled in 0.2 atmospheres absolute (ATA)/min. **Experiment 1** [microvascular O\textsubscript{2} (P\textsubscript{mvO\textsubscript{2}})], **experiment 2** [heart rate (HR), respiration rate (RR), lumbar sympathetic nerve activity (LSNA)], and **experiment 3** [blood pressure (BP)] were performed in different individual rats.
Software, Reading, PA) and was performed on the PmvO\textsubscript{2} data using a one-component model:

\[
P_{\text{mvO}_2(i)} = P_{\text{mvO}_2(\text{baseline})} + \Delta P_{\text{mvO}_2} \left[1 - e^{-(T/D)\tau}\right]
\]

where \(P_{\text{mvO}_2(i)}\) is a given time point, \(P_{\text{mvO}_2(\text{baseline})}\) is baseline (i.e., pre-HBO), and \(\Delta P_{\text{mvO}_2}\) is the increase in \(P_{\text{mvO}_2}\), from baseline to the peak O\textsubscript{2} inhalation condition (i.e., 3.0 ATA, 100% O\textsubscript{2}) values. TD is the time delay, and \(\tau\) is the time constant. To determine whether the one component would adequately describe the \(P_{\text{mvO}_2}\), response goodness-of-fit for the model was determined via the following three criteria: 1) the coefficient of determination (i.e., \(r^2\), 2) the sum of the squared residuals term (i.e., \(\chi^2\), and 3) visual inspection of the model fit to the data.

**Measurement Protocol 2: Electrophysiological Measurement** \((n = 9)\)

Heart rate and respiration rate. Electrical signals were recorded using Teflon-coated stainless steel needle electrodes (A-M Systems, Carlsborg, WA) inserted into an intercostal muscle. Heart rate (HR) and respiration rate (RR) were obtained by filtering the recorded electrical signal. For RR measurement, the respiratory electromyogram was recorded, and RR was extracted by band pass filter and integrated within a \(\tau\) increment of 0.3 s. The electrocardiogram (HR) was isolated from the electromyogram by frequency analysis.

Lumbar sympathetic nerve activity. The lumbar sympathetic nerve was exposed through an abdominal incision, and the nerve was dissected free of surrounding connective tissue. A thin film was placed under the nerve, and silver recording electrodes were glued to the nerve with silicon gel (Kwik-Sil; World Precision Instruments, Sarasota, FL). The raw nerve signal was filtered, amplified, rectified, and then integrated online, and the integrated nerve signal was displayed in real time. Data were recorded using signal-processing software and analyzed with LabChart (PowerLab; ADInstruments; Colorado Springs, CO).

**Modeling of HR, RR, and LSNA profiles.** Curve fitting was accomplished using KaleidaGraph software (Synergy Software) and was performed on HR and LSNA data using a one-component model:

\[
HR(t) = HR(\text{baseline}) - \Delta HR \left[1 - e^{-(T/D)\tau}\right]
\]

\[
LSNA(t) = LSNA(\text{baseline}) - \Delta LSNA \left[1 - e^{-(T/D)\tau}\right]
\]

The assessment of goodness-of-fit for the model was performed as detailed above for \(P_{\text{mvO}_2}\).

**Measurement Protocol 3: BP Measurement** \((n = 14)\)

A PE-50 catheter filled with heparinized saline was inserted in the right carotid artery to record arterial blood pressure via a pressure transducer (DX-100, Nihon Kohden). The pressure signals were continuously sampled at 1 kHz with a PowerLab (PowerLab; ADInstruments) and recorded on a computer using Chart software. HR was derived from the arterial systolic peaks, and mean arterial pressure (MAP) was calculated online. The BP was calibrated accounting for the pressure changes in the chamber. The range of this measurement system was 1.0–1.4 ATA, and hence no measurements were collected in the pressure interval above 1.4 ATA.

**Statistical Analysis**

All experimental data are expressed as means ± SE. All statistical analyses were performed in Prism version 6.01 (GraphPad Software, San Diego, CA). Significant differences were identified by a two-way repeated-measures ANOVA and Bonferroni post hoc test, and significant main effects of diabetes or significant interaction between diabetes and each pressure condition are shown for \(P_{\text{mvO}_2}\), HR, RR, LSNA, and BP. Differences between model parameter estimates [i.e., TD, \(\tau\), mean response time (MRT), etc.] were determined by two-tailed paired t-test. Significance was accepted at \(P \leq 0.05\).

**RESULTS**

Blood glucose concentration was 83 ± 4 (range of 65–97) and 408 ± 19 (range of 302–600) mg/dl in CONT and DIA, respectively (\(P < 0.01\)). DIA rats evidenced a significant decrease in body weight at 4–6 wk post-STZ injection compared with CONT (CONT: 263.9 ± 6.2, DIA: 217.5 ± 4.5 g, \(P < 0.01\)).

\(P_{\text{mvO}_2}\) Kinetics

There was no significant difference in baseline \(P_{\text{mvO}_2}\) at normal atmospheric pressure between CONT and DIA rats (CONT: 22.9 ± 4.2, DIA: 27.8 ± 3.3 Torr, \(P > 0.05\) (Fig. 2). However, the profile of \(P_{\text{mvO}_2}\) at the onset of HBO (from Pre to hyperoxia condition) was substantially different between CONT and DIA rats. Specifically, on exposure to the 100% \(O_2\), \(P_{\text{mvO}_2}\) in DIA rats increased more rapidly (i.e., mean response time, CONT 78 ± 8, DIA 55 ± 8 s, \(P < 0.05\)) and to a higher level at 1.0 (CONT: 45.5 ± 10.4, DIA: 70.4 ± 12.0 Torr), 1.2 (CONT: 68.6 ± 10.0, DIA: 96.5 ± 20.8 Torr), and 1.4 (CONT: 75.2 ± 12.0, DIA: 112.9 ± 24.7 Torr) ATA. In the hyperbaric normoxia (21% oxygen) condition, \(P_{\text{mvO}_2}\) increased in a close-to-linear fashion (1.0 ATA: 24.0 ± 2.7 Torr, 3.0 ATA: 64.8 ± 12.4 Torr) in healthy control rats (data not shown in Fig. 2, \(n = 4\)).

Model parameters for CONT and DIA rats are presented in Fig. 2, right. From 1.4 to Post 3, \(P_{\text{mvO}_2}\) of DIA rats was maintained between 112.9 ± 24.7 and 98.3 ± 27.0 Torr such that \(P_{\text{mvO}_2}\) at 3.0 ATA, while apparently higher, was not significantly different between CONT (120.8 ± 17.6 Torr) and DIA (99.4 ± 15.7 Torr) rats. However, the dynamics of \(P_{\text{mvO}_2}\) during the return to normobaric normoxia differed markedly, with DIA displaying slower dynamics. This effect produced a significantly higher \(P_{\text{mvO}_2}\) in DIA from Post 1 (100.8 ± 16.4 Torr) to Post 3 (98.3 ± 27.0 Torr) than baseline (Pre).

**HR Response to HBO**

Resting HR in DIA rats was decreased at 4 wk after STZ injection (\(P < 0.01\) vs. CONT). As shown in Fig. 3B, both CONT and DIA rats decreased HR during the switch from room air to the pressurization phase. However, the observed bradycardia occurred far faster in CONT than DIA (i.e., the TD and MRT were −500 and −300% longer, respectively, for DIA). Upon resumption of normobaric normoxia, the bradycardia was relieved, and HR recovered to pre-HBO levels in CONT rats (Pre: 370 ± 189, Post 4: 380 ± 18 beats/min, \(P > 0.05\)). On the other hand, the bradycardic response of DIA rats persisted at Post 4 (Pre: 325 ± 20, Post: 314 ± 18 beats/min, \(P < 0.05\)).

**RR Response to HBO**

A rapid decrease of RR was observed concomitant with the onset of hyperoxia in both CONT (Pre/normoxia: 84.9 ± 4.8 breaths/min, hyperoxia: 56.9 ± 6.0 breaths/min) and DIA (Pre/normoxia: 91.7 ± 2.6 breaths/min, hyperoxia: 79.2 ± 2.1 breaths/min) rats, TD and \(\tau\) were extended in DIA rats compared with CONT rats such that the mean response time increased ~500% (\(P < 0.05\), Fig. 4B). CONT RR fell to a lower value under Pre/hyperoxia conditions. However, by the 1.4 ATA condition RR was identical in CONT and DIA groups and subsequently stabilized 15–20% below the normoxic normobaric baseline and was not significantly different between groups thereafter.
LSNA Response to HBO

The proportional change in LSNA from Pre/normoxia to HBO was not significantly different between CONT and DIA rats (CONT: 62.3 ± 3.0%, DIA: 53.0 ± 11.4% at 3.0 ATA, Fig. 5A). However, as evident in Fig. 5B, the dynamic profile of LSNA from Pre to the 3.0 ATA condition was substantially different between CONT and DIA rats. Specifically, τ was far longer in DIA rats (CONT: 34.5 ± 12.5 s, DIA: 141.8 ± 22.0 s, P < 0.05) such that the mean response time increased by ~75% (P < 0.05). During the depressurization phase, LSNA in CONT rats increased and returned to approximately baseline levels (Post 4: 90.0 ± 6.0%), whereas LSNA in DIA rats evidenced a robust elevation that was not resolved by Post 4 (Post: 166.0 ± 45.5%).

BP Response to HBO

MAP of the DIA rats was significantly lower at Pre/normoxic baseline (CONT: 121 ± 3 mmHg, DIA: 103 ± 6 s, P < 0.05). MAP rose significantly in DIA at 1.4 ATA HBO but remained substantially ~20 mmHg below that of CONT rats for all mea-

Fig. 2. Microvascular PO2 (PmvO2) responses to HBO. A: PmvO2 was averaged under each pressure condition. Where no main effect of diabetes was detected (P > 0.05), a significant interaction between diabetes and each pressure condition was identified with a 2-way ANOVA test (P < 0.01). *P < 0.05 vs. Pre. B: dynamic PmvO2 profiles for representative response from healthy control (CONT) and diabetic (DIA) rats. MRT, mean response time. *P < 0.05 vs. CONT. Values shown are means ± SE (CON, n = 6; DIA, n = 7). Arrow represents the start of 100% oxygen administration.

Fig. 3. HR responses to HBO. A: HR was averaged under each pressure condition. Significant main effects of diabetes and pressure condition were identified with a 2-way ANOVA test. HR was lower for DIA for all time points and conditions (P < 0.05). B: dynamic HR profiles for representative response from CONT and DIA rats. *P < 0.05 vs. CONT. Values shown are means ± SE (CONT, n = 5; DIA, n = 4). Arrow represents the start of 100% oxygen administration.
measurements up to 1.4 ATA. As stated in MATERIALS AND METHODS it was not feasible to measure MAP > 1.4 ATA HBO.

DISCUSSION

The major original findings of this investigation are that HBO raises muscle PmvO$_2$ in Type 1 diabetic rats to levels commensurate with those found in their healthy CONT counterparts. Moreover, the PmvO$_2$ response to normobaric hyperoxia and HBO in DIA is significantly faster at the onset of hyperoxia and slower on the return to normobaric normoxia actually increasing muscle exposure to the hyperoxic environment. The differences between CONT and DIA PmvO$_2$ corre-

Fig. 4. Respiratory rate (RR) responses to HBO. A: RR was averaged under each pressure condition. There was no significant main effect (diabetes) and interaction between diabetes or each pressure condition. B: dynamic RR profiles for representative response from CONT and DIA rats. *P < 0.05 vs. CONT. Values shown are means ± SE (CONT, n = 5; DIA, n = 4). Arrow represents the start of 100% oxygen administration.

Fig. 5. Lumbar sympathetic nerve activity (LSNA) responses to HBO. A: LSNA was averaged under each pressure condition. Where no main effect of diabetes was detected (P > 0.05), a significant interaction between diabetes and each pressure condition was identified with a 2-way ANOVA test (P < 0.01). *P < 0.05 vs. CONT. B: dynamic LSNA profiles for representative response from CONT and DIA. *P < 0.05 vs. CONT rats. Values shown are means ± SE (CON, n = 5; DIA, n = 4). Arrow represents the start of 100% oxygen administration.
spond temporally to sluggish respiratory and sympathetic nerve responses operating in concert with the presiding DIA-induced bradycardia and hypotension. These latter effects presumably combine with any aberrant vascular control mechanisms that may alter the vasoconstrictive response to hyperoxia and HBO.

**Measurement of Pmvo2 by Phosphorescence Quenching During HBO**

Tissue O2 measurements during HBO have historically employed micro-O2 electrodes (7) and percutaneous PO2 techniques (13). The advantages of the present phosphorescence quenching technique (36) are powerful. 1) As the R2 phosphor is restricted to the vascular compartment consequent to its binding to albumin and negative charge, the signal is not contaminated by what may be extremely low intracellular PO2 values (31). 2) Other than infusing R2 in the blood and exposing the surface of the muscle, this method is noninvasive, and its calibration is absolute in living tissue (12). 3) The measurements have high temporal fidelity and do not compromise hemodynamic stability even in small animals. One putative concern was that, in a hyperoxic environment, the phosphorescent signal intensity may be too low for effective measurement. However, at no time was the signal intensity inadequate to measure Pmvo2 even at the most extreme HBO condition (i.e., 100% O2, 3.0 ATA).

**Pmvo2 Measurements**

**Baseline (Pre/normobaric normoxia).** As presented in the Introduction, previous evidence supports that Type 1 diabetes causes extreme structural and functional alterations in the muscle microcirculation. Specifically, the reduction of capillary internal diameter observed in DIA impairs the normal capillary hemodynamics (16, 38). However, Pmvo2 reflects the balance between Qo2 and VO2 per unit volume of muscle. Thus, the substantial muscle atrophy incurred by DIA acts to increase capillary density and preserve capillary volume density (38) and VO2 (8, 40), even in the presence of a greater proportion of non- or low-red blood cell-flowing capillaries. Consequently, although DIA can reduce resting muscle Pmvo2, this response was not evident herein (Fig. 2).

**The hyperoxia/HBO response.** Certainly under resting conditions in CONT muscle no supply dependency of metabolism (VO2) is expected, and so any increase in Pmvo2 will result directly from an elevated arterial O2 content and any changes in blood flow (i.e., QO2) to the anterior tibialis muscle (Fig. 2). Using the ideal alveolar gas equation for an alveolar and arterial PCO2 of 40 Torr and assuming the respiratory exchange ratio is 0.8, the pulmonary end-capillary PO2 would be 99 Torr in normobaric normoxia (Pre condition). This value would rise to 663 Torr for normobaric hyperoxia and, in the extreme, to 2,183 Torr at 3 ATA HBO. Thus, considering that Hb is 98% saturated with O2 in normobaric normoxia and 100% in normobaric hyperoxia, at a Hb concentration of 15 g/100 ml, this constitutes an additional 0.4 ml O2/100 ml arterial content. According to Henry's law, the portion dissolved in plasma will be the presiding PO2 × 0.003 ml-Torr−1·100 ml−1. This dissolved O2 will rise from 0.3 ml in normobaric normoxia to 2.0 ml in normobaric hyperoxia and then sequentially to 6.5 ml/100 ml at 3 ATA HBO.

In CONT anterior tibialis muscle Pmvo2 increased systematically to 1.2 ATA, reflecting the hyperoxic arterial PO2 loading more O2 on Hb and also in the plasma. Beyond that, there was a more gentle and approximately linear rise with increased pressure to 2.6 ATA. This overall profile contrasted sharply with the sharp and continued Pmvo2 increase to ~113 Torr at 1.4 ATA in DIA muscle. We surmise that the difference in these two profiles is likely to be the combination of differential vasomotor control within DIA muscle (i.e., slowed rate of vasoconstriction) with additional contributions from the impaired vasomotor control within DIA muscle (13). Thus, at present, there is no clear mechanistic answer to why the time course of HBO-induced vasoconstriction, as inferred from the Pmvo2 response
herein, may be impaired in DIA. On the other hand, the vascular adaptation dynamics in response to increased muscle energetic demands in DIA are slowed (2), and microcirculatory function is compromised (16, 27). These effects reduce PmvO₂ in the dynamic phase after exercise/contraction onset [decreased PmvO₂ (3, 28); increased deoxyHb + myoglobin (2, 47)], causing, or at least contributing to, the slowed VO₂ kinetics in DIA (26, 33, 47).

**Extramuscular influences on the PmvO₂ response.** One of the most rapid responses to inspired hyperoxia is the carotid body-mediated hypopnea (Fig. 4, reviewed in Ref. 46). However, the impact of this will be relatively modest and will depend on the degree to which the decline in RR influences alveolar ventilation allowing alveolar Pco₂ to rise. Thus, if tidal volume remains unchanged and the ~40% decrease of RR seen in CONT lowers alveolar ventilation by a similar amount, this would reduce the normobaric hyperoxic end-pulmonary capillary Po₂ from ~660 to ~590 mmHg. In DIA this response had a delayed onset (TD, CONT 3 ± 1 vs. DIA 18 ± 3 s) and was overall slowed (MRT, CONT 6 ± 1 vs. DIA 29 ± 4 s) and less pronounced (~20%). This effect results from a desensitization of the carotid bodies by hyperglycemia (9, 14) and would act to reduce the fall in Po₂ by only ~20 mmHg. Thus, the RR response to HBO could not account for a sizeable portion of the DIA − CONT difference in PmvO₂. Despite the apparent similarity in the averaged time courses of HR (Fig. 3A) and LSNA (Fig. 5A), inspection of the precise time course in Figs. 3B (MRT, CONT 16 ± 1 vs. DIA 45 ± 5 s) and 5B (MRT, CONT 140 ± 16 vs. DIA 247 ± 34 s), respectively, reveals a profound sluggishness in their responses to the normobaric hyperoxia and HBO. These effects will impact muscle QO₂ in opposite ways. Thus, following initiation of the hyperoxic conditions, and, unless stroke volume falls, which is unlikely, HR and cardiac output will have decreased less for DIA and thus may be contributing to the elevation of tibialis anterior QO₂ in DIA vs. CONT. It is pertinent that this response occurs against a background of hypotension in DIA (Fig. 6), which will act to reduce inflowing muscle arterial pressure and flow. HBO does relieve this hypotension slightly with the increased MAP during HBO (Fig. 6) that results presumably from the elevated systemic vascular resistance mediated partially through arterial baroreceptor activation (10). In contrast to the above, the slowed withdrawal of LSNA (Fig. 5), which constitutes the primary sympathetic innervation of the limb vasculature (25), will certainly not act to elevate QO₂, and therefore PmvO₂ in DIA vs. CONT, and cannot therefore explain the divergent PmvO₂ response seen in DIA (Fig. 2). It was interesting that, upon resumption of normobaric normoxia in DIA, the LSNA was substantially increased, and this corresponded temporally with continued elevation of PmvO₂. It is pertinent that dysfunctional baroreflex control has been reported in animal models of diabetes mellitus (9, 39). Thus, it is possible that this LSNA hyperactivity in DIA following termination of HBO exposure may be associated with hyposensitivity of the baroreceptors.

In summary, either the small impact of the respiratory response or the directionally opposed nature of the LSNA differences disqualify these mechanisms from contributing substantially or at all to the elevated PmvO₂ seen in DIA vs. CONT during the initial hyperoxic/HBO exposure. In contrast, the slowing of the HBO-induced reduction in HR could potentially account for some of the observed transient elevation of PmvO₂ in DIA vs. CONT. However, the modest size of this effect suggests a primary intramuscular vasomotor etiology.

**Perspectives and Significance**

Our expectation that the impaired vasomotor control in DIA muscle would lead to a faster increase of PmvO₂ during HBO was substantiated. However, beyond the transient, and under conditions from normobaric hyperoxia up to 3 ATA, HBO PmvO₂ levels were certainly no lower (or higher) for DIA than CONT. Interestingly, during recovery in normobaric normoxia, PmvO₂ remained elevated longer in DIA than CONT. These muscle oxygenation differences likely relate primarily to altered vasomotor control within the muscle, since they could not be explained adequately by the respiratory, cardiovascular, or sympathetic responses. As with many therapeutic approaches, HBO therapy in DIA is a double-edged sword, with the occurrence of both harmful oxidative stress (22, 23) and the initiation of palliative cell signaling cascades that help restore tissue integrity (21, 42). A greater understanding of the relationship between HBO-induced PmvO₂ elevations (i.e., signal) and both pernicious and helpful oxidative stress/signaling pathway activation (i.e., response) will help define optimal HBO treatment regimens for reducing morbidity in DIA patients.

In conclusion, despite severe structural and functional pathology, HBO raises PmvO₂ at least as effectively, and possibly more so, in DIA muscle compared with that of CONT animals. Therefore, HBO therapy certainly has the potential to initiate cell signaling cascades that promote angiogenesis and wound healing within skeletal muscle.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


