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 control (CONT) 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 PmvO2 profile to muscle contractions, which reflects an imbalance between O2 delivery (QO2) and utilization (VO2) [type I (3); type 2 (28)]. How-ever, these altered dynamics do not ipso facto mean that diabetic muscle cannot modulate its steady-state blood flow (and thus VO2 and PmvO2) appropriately either at rest or in response to altered metabolic demands (8).
To date, the response of diabetic muscle PmvO₂ to HBO therapy has never been determined. Therefore, we sought to characterize the temporal profile of muscle PmvO₂ 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 PmvO₂ 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 Uriesu Ga, Terumo, Japan) 2 days after STZ injection with the onset of diabetes raising glucose concentrations above 500 mg/dl. These measurements of urine glucose were continued each week for 4 wk. After 4 wk post-STZ, blood was collected from a tail vein puncture to confirm that the blood glucose concentrations within the microvasculature up to over 0.5 mm deep were maintained at a level of 350–500 mg/dl.

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 baseline measurements. Subsequently, the HBO protocol shown in Fig. 1 was fit to a straight line by least-squares regression analysis. The resultant lifetime of the phosphorescence (700 nm) and were repeated four times in different individual rats. Curve fitting (HBO pressurization phase) was accomplished using KaleidaGraph software (Synergy 2.5). 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.

Measurement Protocol 1: PmvO₂ 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 PmvO₂. After the overlying skin was reflected and the fascia was removed, the muscle surface was superfused with Krebs-Henseleit solution equilibrated with 5% CO₂-95% N₂ 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.

PmvO₂ measurements. PmvO₂ 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 O₂ dependence of the phosphorescent probe. R2 is a nontoxic dendrimer (18) that binds commonly with albumin, the net negative charge of R2 helps facilitate restriction of R2 to the intravascular space (31). To determine PmvO₂, a PMOD 2000 frequency domain phosphorometer (Oxygen Enterprises, Philadelphia, PA) was used; the common end of the bifurcated light guide was placed ~2–3 mm above the medial region of the tibialis anterior 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 PmvO₂ profiles. Curve fitting (HBO pressurization phase) was accomplished using KaleidaGraph software (Synergy 2.5). 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.
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 τ 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 placed on the nerve to measure LSNA. After the optimal nerve signal was confirmed using auditory monitoring, 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 Kaleida Graph software (Synergy Software) and was performed on HR and LSNA data using a one-component model:

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

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

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.

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 were 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 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 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 τ 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, \( \tau \) 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-
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 PmvO₂ in Type 1 diabetic rats to levels commensurate with those found in their healthy CONT counterparts. Moreover, the PmvO₂ 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 PmvO₂ 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 PmvO<sub>2</sub> by Phosphorescence Quenching During HBO

Tissue O<sub>2</sub> measurements during HBO have historically employed micro-O<sub>2</sub> electrodes (7) and percutaneous PO<sub>2</sub> 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 PO<sub>2</sub> 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 PmvO<sub>2</sub> even at the most extreme HBO condition (i.e., 100% O<sub>2</sub>, 3.0 ATA).

PmvO<sub>2</sub> 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, PmvO<sub>2</sub> reflects the balance between Q<sub>O2</sub> and V<sub>O2</sub> 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 Q<sub>O2</sub> (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 PmvO<sub>2</sub>, this response was not evident herein (Fig. 2).

The hyperoxia/HBO response. Certainly under resting conditions in CONT muscle no supply dependency of metabolism (V<sub>O2</sub>) is expected, and so any increase in PmvO<sub>2</sub> will result directly from an elevated arterial O<sub>2</sub> content and any changes in blood flow (i.e., Q<sub>O2</sub>) to the anterior tibialis muscle (Fig. 2). Using the ideal alveolar gas equation for an alveolar and arterial Pco<sub>2</sub> of 40 Torr and assuming the respiratory exchange ratio is 0.8, the pulmonary end-capillary PO<sub>2</sub> 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 O<sub>2</sub> in normobaric normoxia and 100% in normobaric hyperoxia, at a Hb concentration of 15 g/100 ml, this constitutes an additional 0.4 ml O<sub>2</sub>/100 ml to arterial content. According to Henry’s law, the portion dissolved in plasma will be the presiding PO<sub>2</sub> x 0.003 ml·Torr<sup>-1</sup>·100 ml<sup>-1</sup>. This dissolved O<sub>2</sub> 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 PmvO<sub>2</sub> increased systematically to 1.2 ATA, reflecting the hyperoxic arterial PO<sub>2</sub> loading more O<sub>2</sub> 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 PmvO<sub>2</sub> 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 system responses.

Vasomotor control. The vasoconstrictive response to hyperoxia (41) has been attributed to inhibition of prostaglandin production by elevated reactive O<sub>2</sub> species (35) and also downregulation of the adenosinergic pathway (increased plasma adenosine deaminase) as well as T cell surface CD26 activity (6). The precise interplay between these controllers in HBO remains to be characterized in health (or DIA). Notwithstanding, it is extremely likely that the DIA state, with its proinflammatory nature, impacts these aspects of vasomotor control and, in particular, compromises the ability to respond rapidly to altered muscle metabolic demands. In contrast, one aspect of vasomotor control that appears to be enhanced in Type 1 DIA is the elevated arteriolar myogenic tone that is conferred, in part, by smooth muscle voltage-dependent Ca<sup>2+</sup> channels and protein kinase C (43). Although no formal kinetics analysis was undertaken in that investigation, the rat gracilis arterioles ~100 μm examined demonstrated a greater response to increased luminal pressure that was at least as rapid in DIA as CONT. In these same vessels there was impaired endothelial function in the presence of heightened norepinephrine-induced vasoconstriction (43). Thus, at present, there is no clear mechanistic answer to why the time course of HBO-induced vasoconstriction, as inferred from the PmvO<sub>2</sub> 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 \( P_{\text{mvO}_2} \) in the dynamic phase after exercise/contraction onset [decreased \( P_{\text{mvO}_2} \) (3, 28); increased deoxy[Hb + myoglobin] (2, 47)], causing, or at least contributing to, the slowed \( V'O_2 \) kinetics in DIA (26, 33, 47).

**Extramuscular influences on the \( P_{\text{mvO}_2} \) 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 \( P_{\text{CO}_2} \) 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 \( P_{\text{O}_2} \) 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 \( P_{\text{O}_2} \) by only ~20 mmHg. Thus, the RR response to HBO could not account for a sizeable portion of the DIA – CONT difference in \( P_{\text{mvO}_2} \). Despite the apparent similarity in the averaged time courses of HR (Fig. 3A) and LSN A (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 \( Q'O_2 \) 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 \( Q'O_2 \) 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 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 \( P_{\text{mvO}_2} \) 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 \( P_{\text{mvO}_2} \) 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**

Author contributions: K. Yamakoshi, K. Yagishita, D.C.P., and Y.K. conceived and designed the research; K. Yamakoshi, H.T., T.I., and Y.K. performed the experiments; K. Yamakoshi, K. Yagishita, M.S., D.C.P., and Y.K. analyzed the data; K. Yamakoshi, K. Yagishita, M.S., D.C.P., and Y.K. contributed to writing, drafting, and revising the manuscript; K. Yamakoshi, H.T., T.I., and Y.K. contributed to funding acquisition; K. Yamakoshi, K. Yagishita, M.S., D.C.P., and Y.K. edited and revised the manuscript.

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