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

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Yamakoshi K, Yagishita K, Tsuchimoichi 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. First published October 14, 2015; doi:10.1152/ajpregu.00380.2015.—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 (Q˙O2) 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 ± 0.05) than CONT. Subsequently, PmvO2 remained elevated at similar levels in CONT and DIA muscles until normobaric normoxic recovery where the phosphorescence quenching; streptozotocin; tibialis anterior muscle; 5-methyltetrahydrofolate; antioxidants; hyperbaric oxygen 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 PO2 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 vasoconstriction (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 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 (Q˙O2) 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 (Q˙O2) 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 Q˙O2, following exercise onset, for example, may be deficient as evidenced by the aberrant PmvO2 profile to muscle contractions, which reflects an imbalance between Q˙O2 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 Q˙O2 and PmvO2) appropriately either at rest or in response to altered metabolic demands (8).

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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; Sol130; 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 sampled within the microvasculature up to over 0.5 mm deep by 10.220.33.4 on July 9, 2017 http://ajpregu.physiology.org/ Downloaded from http://ajpregu.physiology.org/ by 10.220.33.4 on July 9, 2017
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).

PmvO2 Kinetics

There was no significant difference in baseline PmvO2 at normal atmospheric pressure between CONT and DIA rats (CONT: 22.9 ± 4.2, DIA: 27.8 ± 3.3 Torr, P > 0.05). However, the profile of PmvO2 at the onset of HBO (from Pre to hyperoxia condition) was substantially different between CONT and DIA rats. Specifically, on exposure to the 100% O2, PmvO2 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, PmvO2 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, PmvO2 of DIA rats was maintained between 112.9 ± 24.7 and 98.3 ± 27.0 Torr such that PmvO2 at 3.0 ATA, while apparently higher, was not significantly different between CONT and DIA rats. 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).

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 MRT 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.

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**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, 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-

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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 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-
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 QO₂ (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 to 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 and sympathetic nervous system responses.

**Vasomotor control.** The vasoconstrictive response to hyperoxia (41) has been attributed to inhibition of prostaglandin production by elevated reactive O₂ 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 Ca²⁺ 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 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 deoxy[Hb + 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 P0₂ 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 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**

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**REFERENCES**


