IMPAIRED SKELETAL MUSCLE PERFUSION IN OBESE ZUCKER RATS

Jefferson C. Frisbee
Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, 53226

Subject Codes: Skeletal muscle blood flow and Syndrome X

Send Correspondence to:
Jefferson C. Frisbee, Ph.D.
Department of Physiology
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee, WI 53226
Phone: (414) 456-8785
Fax: (414) 456-6546
Email: jfrisbee@mcw.edu
ABSTRACT

Skeletal muscle arterioles from obese Zucker rats (OZR) exhibit oxidant stress-based alterations in reactivity, enhanced α-adrenergic constriction, and reduced distensibility versus microvessels of lean Zucker rats (LZR). The present study determined the impact of these alterations for perfusion and performance of in situ skeletal muscle during periods of elevated metabolic demand. During bouts of isometric tetanic contractions, fatigue of in situ gastrocnemius muscle of OZR was increased vs. LZR; this was associated with impaired active hyperemia. In OZR, vasoactive responses of skeletal muscle arterioles from the contralateral gracilis muscle were impaired, due in part to elevated oxidant tone; reactivity was improved following treatment with polyethylene glycol-superoxide dismutase (PEG-SOD). Arterioles of OZR also exhibited increased α-adrenergic sensitivity, which was abolished by treatment with phentolamine (10^{-5} M). Intravenous infusion of phentolamine (10 mg/kg) or PEG-SOD (2000 U/kg) in OZR altered neither fatigue rates nor active hyperemia from untreated levels, however combined infusion improved performance and hyperemia, although not to levels in LZR. Microvessel density in the contralateral gastrocnemius muscle, determined via histological analyses was reduced by ~25% in OZR versus LZR, while individual arterioles from the contralateral gracilis muscle demonstrated reduced distensibility. These data suggest that altered arteriolar reactivity contributes to reduced muscle performance and active hyperemia in OZR. Further, despite pharmacological improvements in arteriolar reactivity, reduced skeletal muscle microvessel density and arteriolar distensibility also contribute substantially to reduced active hyperemia, and potentially to impaired muscle performance.

Key Words: skeletal muscle microcirculation, skeletal muscle fatigue, obesity, models of Syndrome X.
INTRODUCTION

Ongoing epidemiological study has demonstrated that the incidence of obesity, type II diabetes mellitus, dyslipidemia and hypertension continues to rise in Western society (1, 2, 32, 34). Taken together, combined expression of these pathologies creates a condition referred to as metabolic Syndrome X (33). Due to a deficient leptin receptor gene (5, 6, 20, 28), the obese Zucker rat (OZR) experiences chronic hyperphagia (5, 6, 28), with the ensuing development of obesity, type II diabetes mellitus, hypertension and dyslipidemia versus its control strain, the lean Zucker rat (5, 6, 20, 28). As such, the OZR represents an appropriate animal model for Syndrome X.

We have demonstrated that development of Syndrome X in OZR is associated with altered skeletal muscle arteriolar reactivity; including impaired dilator responses to hypoxia (13) and elevated wall shear rate (14), and increased myogenic activation (15) and α-adrenergic vasoconstriction (38). The altered vascular reactivity in response to hypoxia, shear and pressure is partly a function of elevated vascular oxidant tone, as normalization of oxidant stress improved arteriolar reactivity to these stimuli (13-15). Other investigators have demonstrated impaired endothelium-dependent dilator reactivity within OZR versus control animals (4, 24-27) and an elevation in plasma levels of 8-epi-prostaglandin F2α, a lipid peroxidation product indicating elevated oxidant stress (26). These alterations were also correctable following reductions in oxidant tone (25-27). OZR also manifest a significant increase in not only the sensitivity of skeletal muscle arterioles in response to α-adrenergic stimulation (38), but also a significant increase in sympathetic nerve activity (9). This generates a substantial increase in the basal tone of in vivo skeletal muscle resistance arteries of OZR versus LZR (38), which may impair perfusion of skeletal muscle, both at rest and during elevated metabolic demand.
A recent study in our laboratory has suggested that remodeling of the skeletal muscle microcirculation develops in OZR with the progression of Syndrome X. In OZR, the stress versus strain curve of passive resistance arterioles is left-shifted, indicating a reduced distensibility of the arteriolar wall, while the density of microvessels within skeletal muscle (gastrocnemius) was reduced compared to values in LZR (11). Further, these structural alterations to the microcirculation increased vascular resistance to perfusion of the maximally dilated microcirculation within the gastrocnemius muscle of OZR versus LZR (11).

The purpose of the present study was to determine if these alterations to skeletal muscle arteriolar structure and reactivity impact the ability of skeletal muscle to increase perfusion and to maintain performance (i.e., to resist the development of fatigue) during periods of high metabolic rate muscle contraction. Specifically, these studies tested the hypotheses that: 1) the active hyperemic response and ability of blood perfused skeletal muscle to resist fatigue during periods of elevated metabolic demand are impaired in OZR versus LZR; 2) the elevation in vascular oxidant stress and/or the increased α-adrenergic reactivity of skeletal muscle arterioles of OZR contribute to demonstrated impairments in active hyperemia and skeletal muscle performance; and 3) that alterations in microvessel and microvascular network structure will contribute to the impaired active hyperemic response of in situ contracting skeletal muscle of OZR.

**MATERIALS AND METHODS**

**Animals:** 15 week old male lean (LZR; Harlan; n=15) and obese (OZR; Harlan; n=15) Zucker rats maintained on standard rat chow and tap water *ad libitum* were used for all experiments. Rats were housed in an AAALAC-accredited animal care facility at the Medical College of Wisconsin, and all protocols received prior IACUC approval. All rats were anesthetized with an injection of sodium
pentobarbital (60 mg·kg⁻¹ i.p.), and received tracheal intubation to facilitate maintenance of a patent airway. In all rats a carotid artery and an external jugular vein were cannulated for determination of arterial pressure and for intravenous infusion of additional substances as necessary (e.g., anesthetic, heparin, physiological salt solution).

**Investigation of Isolated Vessels:** Following the initial surgery, the intramuscular continuation of the right gracilis artery was surgically removed from the anesthetized rat, as described previously (12). Arterioles were placed in a heated chamber (37°C) that allowed the lumen and exterior of the vessel to be perfused and superfused, respectively, with physiological salt solution (PSS; equilibrated with 21% O₂, 5% CO₂; 74% N₂) from separate reservoirs. Vessels were cannulated at both ends with glass micropipettes and were secured to the inflow and outflow pipettes which were connected to a reservoir perfusion system that allowed intraluminal pressure and lumenal gas concentrations to be controlled. Vessel diameter was measured using television microscopy and an on-screen video micrometer. Arterioles were extended to their *in situ* length and were equilibrated at 80% of the animal's mean arterial pressure (84±4 mmHg for LZR, n=10; 107±5 mmHg for OZR, n=10). Active tone for vessels in the present study, calculated as \( \frac{\Delta D}{D_{\text{max}}} \)·100, where \( \Delta D \) is the diameter increase from rest in response to Ca²⁺-free PSS, and \( D_{\text{max}} \) is the maximum diameter measured at the equilibration pressure in Ca²⁺-free PSS, averaged 33.8±2.7% in LZR and 30.1±3.1% in OZR. The reactivity of isolated arterioles was assessed in response: 1) hypoxia (ΔPO₂ from ~140 mmHg to ~35 mmHg; Ref. 12), 2) altered intravascular pressure (myogenic activation; 40 mmHg to 160 mmHg in randomized 20 mmHg increments; Ref. 15), 3) norepinephrine (10⁻¹⁰ M – 10⁻⁶ M; Sigma), 4) iloprost (10⁻¹⁵ g/ml – 10⁻⁹ g/ml; Berlex), and 5) acetylcholine (10⁻⁹ M – 10⁻⁶ M; Sigma).
To determine the contribution of α-adrenoreceptors to alterations in arteriolar reactivity during challenge with norepinephrine, and to determine the capacity for blocking α-adrenergic reactivity of vessels, the constrictor responses of isolated arterioles to norepinephrine was assessed under untreated conditions and in the presence of 10⁻⁵ M phentolamine (Sigma). To determine the extent to which increased vascular oxidant stress contributes to alterations in arteriolar reactivity between LZR and OZR, and to assess the ability to correct these alterations, vessels from both OZR and LZR were treated with the oxidative free radical scavenger polyethylene glycol-superoxide dismutase (PEG-SOD; 200 U⋅ml⁻¹; Sigma). Previous studies in our laboratory have demonstrated that application of catalase has no effect on the levels of oxidant stress or arteriolar reactivity in OZR (13).

Following completion of the above procedures, the perfusate and superfusate were replaced with Ca²⁺-free PSS and vessels were repeatedly challenged with 10⁻⁷ M norepinephrine until all reactivity and tone were abolished. At this time, intralumenal pressure within the isolated vessel was altered, in 20 mmHg increments, between 0 mmHg and 160 mmHg and the inner and outer diameter of arterioles was determined at each pressure. To ensure that a negative intralumenal pressure was not exerted on the vessel, 5 mmHg was used as the “0 mmHg” intralumenal pressure point; all other values of intralumenal pressure were multiples of 20 mmHg up to 160 mmHg. Specific pressures were randomized to prevent the occurrence of ordering effects. All calculations of passive arteriolar wall mechanics (used as indicators of structural alterations to individual microvessels) are based on those described previously (3, 11):

Vessel wall thickness was calculated as:

\[ WT = \frac{(OD - ID)}{2} \]
where WT represents wall thickness (µm) and OD and ID represent arteriolar outer and inner diameter, respectively (µm).

Incremental arteriolar distensibility (DISTINC; % change in arteriolar diameter/mmHg) was calculated as:

\[ \text{DIST}_{\text{INC}} = \frac{\Delta ID}{(ID \times \Delta P_{\text{IL}})} \times 100 \]

where \( \Delta ID \) represents the change in internal arteriolar diameter for each incremental change in intralumenal pressure (\( \Delta P_{\text{IL}} \)).

For the calculation of circumferential stress, intralumenal pressure was converted from mmHg to N/m\(^2\), where 1 mmHg = 1.334×10\(^{2}\) N/m\(^2\). Circumferential stress (\( \sigma \)) was then calculated as:

\[ \sigma = \frac{(P_{\text{IL}} \times ID)}{(2WT)} \]

Circumferential strain (\( \varepsilon \)) was calculated as:

\[ \varepsilon = \frac{(ID - ID_5)}{ID_5} \]

where ID\(_5\) represents the internal arteriolar diameter at the lowest intralumenal pressure (i.e., 5 mmHg).

**Histological Determination of Microvessel Density:** Following removal of the resistance arteriole from the right gracilis muscle, the right femoral artery from each rat was ligated and the right gastrocnemius muscle was removed, rinsed in PSS and fixed in 1% formalin. Muscles were embedded in paraffin and cut into 5 µm cross sections. Sections were then treated with proteinase K (50:50, Dako Corp, Carpinteria, CA), and were stained with *Griffonia simplicifolia* I lectin (Sigma), as described previously (16, 21). This procedure selectively stains all
microvessels with a diameter \( \leq 20 \) \( \mu m \), preferentially arterioles and capillaries versus venules, regardless of perfusion status (16). Sections were rinsed in PSS and were mounted on microscope slides with a water-soluble medium (SP, ACCU-MOUNT 280, Baxter). Using epifluorescence microscopy, microvessel localization was performed with a Nikon E600 upright microscope with a 20x objective lens (Plan Fluo phase NA 0.5). Excitation was provided by a 75 watt Xenon Arc lamp through a Lambda 10-2 optical filter changer (Sutter Instrument Company, Novato, CA) controlling a 595nm excitation filter and a 615 nm emission filter. The microscope was coupled to cooled CCD camera (Micromax; Princeton Instruments Inc, Trenton, NJ). From each gastrocnemius muscle, 6 individual cross sections were used for analysis, with 6 randomly selected regions within an individual cross section chosen for study. Each region of study had an area of \( \sim 1.47 \times 10^5 \) \( \mu m^2 \). Within each region studied, all labeled microvessels were counted. All acquired images from individual sections were analyzed for microvessel and skeletal muscle fiber number using MetaMorph Imaging software (Universal Imaging Co., Downingtown, PA). Since the procedures used in the present study label microvessels up to 20 \( \mu m \) in diameter, the present results are somewhat different from those from other established techniques such as the myosin adenosinetriphosphatase staining (23, 36) and the perfusion fixation (22, 29) methods which are used for capillary identification.

**Preparation of the In Situ Gastrocnemius/Plantaris Muscle:** The surgical preparation employed was based on that used for previous investigation (35), with minor modification. Following removal of the right gastrocnemius muscle, the left leg of the anesthetized rat (n=15 LZR and 15 OZR) received a medial incision running from the calcaneus to the femoral triangle, with removal of saphenous artery, vein and nerve. Subsequently, the semitendinosus, semimembranosus, gracilis and caudo-femoralis muscles were removed from the leg, exposing the gastrocnemius muscle,
sciatic nerve (overlaying the biceps femoris muscle) and the vascular supply to the gastrocnemius muscle. The sciatic nerve was double-ligated as proximally as possible and was sectioned between the ties, thus creating a ~1 inch length of the sciatic nerve to facilitate stimulation of muscle contraction. All branches from the femoral/popliteal artery that did not perfuse the gastrocnemius muscle were ligated or cauterized, depending on size and location. A full pictorial and textual description of the anatomy of the rat hindlimb is described elsewhere (19). Subsequently, the tendon of insertion for the gastrocnemius muscle was isolated, clamped and sectioned, distal to the clamp. The gastrocnemius muscle was reflected toward its origin with all continuances of the sciatic nerve and vascular supply to the underlying muscles being cauterized upon exposure. The distal stump of the sciatic nerve was inserted into a stimulating electrode and tied in place using 4-0 silk suture. The clamp on the gastrocnemius muscle tendon was attached to a force transducer (Grass; FT03) and the muscle was extended to its optimal length for tension development in response to a single isometric twitch contraction (L0). Finally, a microcirculation flow probe (Transonic) was placed around the femoral artery, immediately distal to its origin from the iliac artery, in order to measure the volume flow of blood to the gastrocnemius muscle. The blood flow probe was initially calibrated according to the manufacturer’s instructions. Following placement around the femoral artery, the probe was calibrated to “zero-flow” by briefly clamping the iliac artery upstream from the origin of the femoral artery. The entire preparation was then covered in physiological salt solution-soaked gauze and plastic film to minimize evaporative water loss and was placed under a heat lamp to maintain temperature at 37°C. At this time, heparin (1500 IU/kg) was infused via the jugular vein to prevent blood coagulation.

Upon completion of the surgical preparation, the gastrocnemius muscle was stimulated to perform, via the sciatic nerve, bouts of isometric tetanic contractions (60/min, 50Hz, 200 ms
duration, 5V) lasting for 4 minutes followed by 30 minutes of self-perfused recovery time. Arterial pressure and femoral artery blood flow was continuously monitored. In separate experiments, the muscle contraction protocol was performed following intravenous infusion of the α-adrenergic antagonist phentolamine (10 mg/kg), the oxidative radical scavenger polyethylene glycol-superoxide dismutase (PEG-SOD; 2,000 U/kg) or both agents.

**Data and Statistical Analyses:**

**Arteriolar Reactivity Experiments:** Arteriolar reactivity data to all stimuli except hypoxia were fit with linear ($y = \alpha_0 + \beta x$; for myogenic activation) or semi-logarithmic ($y = \alpha_0 + \beta \log x$; for acetylcholine, iloprost, and norepinephrine concentration-response curves) regression equations. In all cases, $y$ represents the change in arteriolar diameter in response to imposition of a specific stimulus $x$, $\alpha_0$ represents an intercept term and $\beta$ represents the rate of change in arteriolar diameter with either linear or logarithmic changes in $x$, as appropriate. All fitting of regression equations employed ordinary least squares analysis with $r^2 > 0.79$. Statistically significant differences between slope coefficients were determined using analysis of variance (ANOVA; [n=10 for both LZR and OZR]). Statistically significant differences in arteriolar responses to hypoxia were assessed using ANOVA only. Student-Newman-Keuls-test post-hoc was employed as appropriate.

**Microvessel Remodeling Experiments:** Statistically significant differences in passive mechanical characteristics (diameter, incremental distensibility) of isolated arteries were assessed using repeated measures ANOVA (n=10 for LZR and OZR). Circumferential stress versus strain curves were fit with an exponential regression equation: $y = \alpha_0 e^{\beta x}$; where $y$ represents circumferential wall stress at a given wall strain $x$, $\alpha_0$ represents an intercept term, and $\beta$ represents a constant
related to the rate of increase of the stress versus strain curve. All fitting of regression equations employed ordinary least squares analysis with $r^2 > 0.87$). Statistically significant differences between slope coefficients and differences in microvessel density between LZR and OZR were evaluated using Student’s $t$-test.

**Muscle Perfusion/Performance Experiments:** Muscle performance and blood flow data were normalized to gastrocnemius muscle mass, which did not differ between LZR ($2.28 \pm 0.08$ g) and OZR ($2.31 \pm 0.10$ g). Vascular resistance within the gastrocnemius muscle, at rest and during muscle contraction, was calculated as the quotient of mean arterial pressure and muscle blood flow. Muscle fatigue curves from individual rats were fit with a semi-logarithmic regression equation (see above). For these analyses, $y$ represents developed tension by the muscle at time $x$, $a_0$ represents an intercept term and $\beta$ represents the rate of change in developed tension by the gastrocnemius with logarithmic changes in time $x$, measured in seconds. All fitting of regression equations employed ordinary least squares analysis with $r^2 > 0.85$. Differences between slope coefficients were determined using ANOVA. Differences in muscle blood flow and average vascular resistance during muscle contraction were determined using ANOVA with repeated measures and ANOVA, respectively, with Student-Newman-Keuls-test post-hoc employed as appropriate.

In all cases, $p < 0.05$ was taken to reflect statistical significance. All data are presented as mean $\pm$ SEM.

**RESULTS**

Baseline data describing the characteristics of LZR and OZR used in the present study are summarized in Table 1. In addition to being significantly heavier than their age-matched LZR counterparts, OZR also demonstrated moderate hypertension, as well as significant hyperglycemia.
and hyperinsulinemia.

**Muscle Perfusion/Performance Experiments:** Table 2 presents summary data describing mean arterial pressure, femoral artery blood flow (gastrocnemius at rest), vascular resistance within the gastrocnemius muscle (at rest) and peak developed tension by the muscle in LZR and OZR under control conditions and in response to intravenous infusion of phentolamine, PEG-SOD, or both. Treatment of OZR with phentolamine normalized arterial pressure as well as resting blood flow to, and vascular resistance within, gastrocnemius muscle to levels that were not different from those in LZR. In contrast, treatment of OZR with PEG-SOD had minimal impact on these parameters versus untreated conditions. Peak developed tension by the gastrocnemius muscle of OZR was not altered by application of PEG-SOD and/or phentolamine.

The responses of blood perfused gastrocnemius muscle of LZR and OZR during a bout of isometric tetanic contractions are presented in Figure 1. During contraction, *in situ* muscle of OZR fatigued at a significantly greater rate than did muscle of LZR (Panel A), as indicated by a more negative β (slope) coefficient describing the developed tension versus time relationship. The impaired muscle performance was associated with a reduced hyperemic response of the skeletal muscle microcirculation, as gastrocnemius muscle blood flow (Panel B) was significantly lower in OZR, while vascular resistance (Panel C) was elevated, versus responses in LZR throughout the duration of muscle contraction.

In response to infusion of norepinephrine (5 μg/kg; i.v.), mean arterial pressure in LZR increased by 39±10 mmHg. This response was enhanced in OZR, as the pressor response to norepinephrine infusion was 88±12 mmHg. Following treatment with phentolamine (10 mg/kg; i.v.), pressor responses to norepinephrine were severely attenuated in both LZR (6±5 mmHg) and OZR (11±6 mmHg), indicating an effective blockade of vascular α-adrenoreceptors with this
Intravenous infusion of methacholine (5 μg/kg) caused a transient decrease in arterial pressure of 26±6 mmHg in LZR. A depressor response to infused methacholine was not present in OZR, as mean arterial pressure fell by only 2±2 mmHg. Following infusion of PEG-SOD into OZR (2000 U/kg, i.v.), the depressor response to infused methacholine was increased to 19±4 mmHg. This improved reactivity to the endothelium-dependent dilator agonist methacholine suggests that vascular oxidant stress levels were lowered in OZR following treatment with PEG-SOD. Treatment of LZR with PEG-SOD did not alter this response from control conditions.

The effect of blockade of α-adrenoreceptors in OZR and LZR on muscle performance and perfusion are presented in Figure 2. Although improving skeletal muscle blood flow under rest conditions, treatment of OZR with phentolamine had no effect on the development of muscle fatigue (Panel A) or blood flow during contraction (Panel B). Decreases in calculated resistance during muscle contraction in OZR treated with phentolamine (Panel C) reflect the change in mean arterial pressure (Table 1) rather than muscle blood flow.

Data describing the effects of infusion of PEG-SOD on gastrocnemius muscle fatigue and perfusion during contraction in LZR and OZR are presented in Figure 3. Infusion of PEG-SOD alone, although improving vasodilation in response to endothelium-dependent stimuli in OZR (methacholine) had no significant effect on muscle performance (Panel A), muscle blood flow (Panel B) or vascular resistance in the gastrocnemius muscle (Panel C) during tetanic contractions, compared to responses determined in untreated rats.

The effects of combined infusion of phentolamine and PEG-SOD into LZR and OZR on muscle performance and perfusion are presented in Figure 4. Treatment of OZR with both agents significantly improved muscle performance versus responses in untreated OZR, as evidenced by
a reduction (i.e., less negative) in the \( \beta \) coefficient describing the fatigue curve (Panel A). However, muscle performance in OZR treated with phentolamine and PEG-SOD remained significantly impaired versus responses in LZR. Associated with improved muscle performance was an increased hyperemic response (Panel B) and a reduced vascular resistance (Panel C) within contracting gastrocnemius muscle of OZR treated with both agents versus responses in untreated animals; however, both blood flow and resistance remained significantly reduced and elevated, respectively, from that determined in LZR.

**Arteriolar Reactivity Experiments:** Figure 5 presents data describing reactivity of isolated gracilis muscle resistance arterioles in the contralateral leg of the LZR and OZR in the present study. In response to reduced oxygen tension (Panel A), increasing iloprost (Panel B) and acetylcholine (Panel C) concentration, arterioles from OZR demonstrated impaired dilator reactivity versus that from LZR. In each case, reactivity of vessels from OZR in response to these stimuli was improved by treatment of arterioles with PEG-SOD; although reactivity did not increase to that determined in LZR. Additionally, pressure-induced constriction of arterioles from OZR was significantly increased versus this response in vessels of LZR (Panel D). This increased myogenic sensitivity of arterioles from OZR was normalized following application of PEG-SOD. Finally, arterioles of OZR exhibited an enhanced reactivity to application of norepinephrine as compared to vessels of LZR (Panel E). Application of the \( \alpha \)-adrenergic antagonist phentolamine eliminated reactivity of vessels from both strains in response to challenge with norepinephrine.

**Microvessel Remodeling Experiments:** The results from studies investigating the mechanical properties of the wall of gracilis muscle resistance arterioles from the LZR and OZR used in the present study are presented in Figure 6. Under \( \text{Ca}^{2+} \)-free conditions, arterioles of LZR increased their diameter to a significantly greater extent with increased intralumenal pressure, as compared to
responses determined in OZR (Panel A). This restricted expansion of passive arterioles from OZR with increasing intralumenal pressure resulted in a reduced incremental distensibility at intralumenal pressures \( \leq 60 \) mmHg (Panel B) and a significant left-shift of the circumferential stress-strain relation (Panel C) as compared to responses calculated for passive arterioles of LZR.

Figure 7 summarizes data describing microvessel density within the gastrocnemius muscle of LZR and OZR used in the present study. Both microvessel density, expressed as the number of microvessels per unit cross sectional area (Panel A) and the microvessel:muscle fiber ratio (Panel B) was significantly reduced in OZR versus levels determined in LZR.

**DISCUSSION**

The central observation of the present study was that, although peak developed tension was similar between the two strains of rats, the rate of fatigue development (i.e., the fall in developed tension over time) of in situ, blood perfused gastrocnemius muscle of OZR was significantly greater than that determined in LZR (Figure 1). Further, this impaired performance was associated with a reduced muscle blood flow and an elevated vascular resistance within the gastrocnemius muscle at rest and during high metabolic rate muscle contractions. As previous investigations have indicated that reactivity of skeletal muscle resistance and distal arterioles of OZR is altered due to elevated oxidative stress (13-15, 25-27) and potentially, through a hyper-reactivity in response to \( \alpha \)-adrenergic activation increasing vascular tone (9, 38), the present study tested the hypothesis that these processes negatively impact skeletal muscle perfusion in OZR.

To minimize any contribution of enhanced arteriolar \( \alpha \)-adrenergic sensitivity imposing an increased vascular tone on the skeletal muscle microcirculation of OZR, animals were treated with the \( \alpha \)-adrenergic antagonist phentolamine. Treatment with phentolamine was highly
effective in abolishing arteriolar α-adrenergic reactivity in both LZR and OZR, as the constrictor response of isolated vessels (Figure 5, Panel E) and the pressor response in the anesthetized rat was eliminated in both strains following challenge (addition to the vessel chamber or intravenous infusion, respectively) with norepinephrine. *In vivo* blockade of α-adrenoreceptors improved blood flow to the gastrocnemius muscle at rest, but had minimal impact on the rate of muscle fatigue, active hyperemia or vascular resistance within contracting gastrocnemius muscle. These observations suggest that, although the hyper-reactivity of skeletal muscle arterioles to α-adrenergic stimuli contributes to a relative ischemia under resting conditions, with severe elevations in metabolic demand, this hyper-reactivity is not a critical determinant of the blunted active hyperemia determined in OZR versus LZR.

In agreement with our previous studies (13-15), treatment of isolated arterioles from OZR with PEG-SOD improved reactivity in response to reduced oxygen tension, the prostacyclin analog iloprost, and acetylcholine, while normalizing responses to elevated intralumenal pressure. Further, intravenous infusion of PEG-SOD into anesthetized OZR increased the depressor response to subsequent infusion of methacholine versus the minimal response identified in untreated rats. These observations suggest that skeletal muscle arteriolar reactivity was improved in OZR following treatment with PEG-SOD and that this may have been due to a reduced vascular oxidant tone. However, despite this improved vascular reactivity demonstrated using either a reduced vascular preparation, or in response to infused methacholine, the perfusion and performance of *in situ* gastrocnemius muscle of OZR treated with PEG-SOD alone was not altered from levels determined in untreated OZR.

However, when OZR were treated with both phentolamine and PEG-SOD, the fatigue-resistance of *in situ* gastrocnemius muscle was improved during contractions versus untreated
OZR, and this was associated with an enhanced active hyperemic response and a reduced vascular resistance (Figure 4). That this improvement in muscle performance was demonstrated only with combined $\alpha$-adrenergic blockade and a reduced oxidative stress suggests that each treatment, acting alone, although able to impact arteriolar reactivity may be insufficient in terms of improving muscle perfusion during periods of extreme metabolic demand. Alternately, it may be that each treatment exerts its most pronounced effect at different levels of the skeletal muscle microcirculation (resistance arterioles versus distal arterioles) and that combined treatment will improve function within the skeletal muscle microcirculation across multiple levels. Further investigation is warranted to more clearly elucidate the respective roles of enhanced adrenergic sensitivity and increased vascular oxidant tone as they relate to skeletal muscle perfusion in OZR, both at rest and during periods of increased activity.

Despite improved arteriolar reactivity, muscle perfusion and muscle performance, the fatigue rate and level of vascular resistance in OZR treated with phentolamine and PEG-SOD remained elevated versus LZR, with a persistent reduction in active hyperemia. Previous study in our laboratory has determined that, due to structural alterations to individual microvessels and to the microvascular network, resistance to perfusion of a maximally dilated (passive) skeletal muscle microcirculation is increased in OZR versus LZR (11). In the present study, the imposed contraction regimen creates an extreme metabolic demand on the skeletal muscle (8) and should cause a near maximum dilation of the gastrocnemius muscle microcirculation, thus minimizing vascular resistance. The presence of reduced blood flow under these conditions suggests that structural remodeling of the skeletal muscle microcirculation may contribute to a “residual resistance” to perfusion during severe elevations in metabolic demand. This resistance to perfusion can manifest itself through two primary mechanisms, a decreased radius of individual
arterioles (structural narrowing) and a reduced number of pathways in parallel (microvessel rarefaction).

Skeletal muscle (gracilis) resistance arterioles from the contralateral leg of LZR and OZR used in the present study demonstrated that distensibility of these arterioles was reduced, as evidenced by a left-shifted stress-strain relationship (Figure 6) and that the density of microvessels within the contralateral gastrocnemius muscle was also reduced in OZR versus LZR (Figure 7). The left-shifted stress-strain curve for resistance arterioles of OZR would result in a structural narrowing of these vessels which, when combined with the reduced numbers of parallel pathways within the skeletal muscle (due to the microvessel rarefaction), would increase the resistance to perfusion of the gastrocnemius muscle, regardless of the improved reactivity of individual arterioles. The contribution of these structural alterations to the resistance to perfusion has been mathematically modeled using a theoretical network based on the hamster cheek pouch microcirculation (17). The authors demonstrated that, although the effects of structural narrowing dominate over that of microvessel rarefaction, both processes contribute significantly to elevate resistance to perfusion within a network. It is highly likely that these processes contribute to the “residual resistance” identified in the gastrocnemius muscle microcirculation of OZR treated with phentolamine and PEG-SOD versus LZR demonstrated in the present study. Additionally, reduced microvessel density within the contracting gastrocnemius can have substantial implications for the transport and exchange of oxygen and metabolic substrate within the muscle, and consequently on the ability of the skeletal muscle to maintain performance (39). As has previously been modeled (17), a reduction in microvessel density on the order of that identified in the gastrocnemius muscle of OZR in the present study can cause regions of the skeletal muscle to experience extremely low values of PO₂ during elevated metabolic demand,
such that metabolic activity within the tissue becomes O₂-limited.

Previous studies have demonstrated that the development of diabetes and/or obesity can significantly impact the metabolic capacity of skeletal muscle fibers *per se*, which could have contributed to the impaired skeletal muscle performance identified in OZR in the present study independent of alterations to the microcirculation. Simoneau *et al.* (37) demonstrated a reduced oxidative capacity of skeletal muscle of obese, diabetic women, with a shift in metabolic capacity towards anaerobic and glycolytic sources of energy. Further, using streptozotocin-treated diabetic rats, previous investigators have demonstrated reductions in oxidative enzyme capacity (10), abnormal fatty acid metabolism (7) and alterations in Ca²⁺ handling in skeletal muscle, each of which contributed to compromised skeletal muscle performance (30, 31). If these structural and functional alterations to the skeletal muscle microcirculation demonstrated in the present study contribute to regions in the tissue which become hypoxic relative to conditions in LZR, this process could exacerbate demonstrated insufficiencies in the metabolic capacity of the skeletal muscle, further impairing skeletal muscle performance. Further study will be necessary to explore the extent to which regions of low oxygen tension within the skeletal muscle of OZR contributes to the compromised ability of the blood perfused gastrocnemius muscle to maintain performance.

**ACKNOWLEDGEMENTS**

The author thanks Mr. G.R. Slocum, Ms. C.A. Bobrowitz and Ms. D.N. Schippers for their expert technical assistance. This work was supported by Scientist Development Grant #0330194N from the American Heart Association, a Faculty Development Grant from the Medical College of Wisconsin and National Institutes of Health HL65289 and HL29587.
REFERENCES

1. American Heart Association; Statistical Fact Sheets: “Risk Factors”.


Table 1. Characteristics of LZR and OZR used in the present study. * p<0.05 vs. LZR.

<table>
<thead>
<tr>
<th></th>
<th>LZR</th>
<th>OZR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (g)</td>
<td>344±9</td>
<td>622±18*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>105±4</td>
<td>134±5*</td>
</tr>
<tr>
<td>[glucose]_{blood} (mg/dl)</td>
<td>128±14</td>
<td>286±40*</td>
</tr>
<tr>
<td>[insulin]_{plasma} (ng/ml)</td>
<td>3.4±0.5</td>
<td>18.4±1.8*</td>
</tr>
</tbody>
</table>
Table 2. Baseline data describing mean arterial pressure, femoral artery blood flow, vascular resistance within the gastrocnemius muscle and the peak developed tension of the muscle in LZR and OZR under control conditions, following treatment with PEG-SOD or phentolamine, or following both treatments. * p<0.05 vs. LZR Control; † p<0.05 vs. OZR Control.

<table>
<thead>
<tr>
<th></th>
<th>MAP (mmHg)</th>
<th>Blood Flow (ml/100g/min)</th>
<th>Vascular Resistance (mmHg/[ml/100g/min])</th>
<th>Peak Tension (g·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LZR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>116±3</td>
<td>11.3±0.7</td>
<td>10.7±0.8</td>
<td>978±42</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>113±5</td>
<td>12.3±1.0</td>
<td>9.4±0.6</td>
<td>980±58</td>
</tr>
<tr>
<td>PEG-SOD</td>
<td>117±4</td>
<td>11.1±0.4</td>
<td>10.5±0.4</td>
<td>996±70</td>
</tr>
<tr>
<td>Phent./PEG-SOD</td>
<td>113±4</td>
<td>11.9±0.6</td>
<td>9.7±0.5</td>
<td>957±48</td>
</tr>
<tr>
<td><strong>OZR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>145±3*</td>
<td>7.0±0.4*</td>
<td>21.7±1.5*</td>
<td>948±39</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>123±4†</td>
<td>12.6±0.7†</td>
<td>9.7±0.5†</td>
<td>938±60</td>
</tr>
<tr>
<td>PEG-SOD</td>
<td>137±6*</td>
<td>8.0±0.7*</td>
<td>17.6±1.9*</td>
<td>980±71</td>
</tr>
<tr>
<td>Phent./PEG-SOD</td>
<td>117±3†</td>
<td>12.8±0.4†</td>
<td>8.9±0.3†</td>
<td>956±41</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. The change in developed tension (Panel A), muscle blood flow (Panel B) and mean vascular resistance during contraction (Panel C) of in situ gastrocnemius muscle of LZR (n=5) and OZR (n=5). Data are presented as mean±SEM. * p<0.05 vs. LZR Control.

Figure 2. The change in developed tension (Panel A), muscle blood flow (Panel B) and mean vascular resistance during contraction (Panel C) of in situ gastrocnemius muscle of LZR (n=5) and OZR (n=5). Data (mean±SEM) are presented for rats under untreated control conditions and following intravenous infusion of phentolamine (10 mg/kg). * p<0.05 vs. LZR Control; † p<0.05 vs. OZR Control.

Figure 3. The change in developed tension (Panel A), muscle blood flow (Panel B) and mean vascular resistance during contraction (Panel C) of in situ gastrocnemius muscle of LZR (n=5) and OZR (n=5). Data (mean±SEM) are presented for rats under untreated control conditions and following intravenous infusion of PEG-SOD (2000 U/kg). * p<0.05 vs. LZR Control; † p<0.05 vs. OZR Control.

Figure 4. The change in development tension (Panel A), muscle blood flow (Panel B) and mean vascular resistance during contraction (Panel C) of in situ gastrocnemius muscle of LZR (n=10) and OZR (n=10). Data (mean±SEM) are presented for rats under untreated control conditions and following intravenous infusion of both phentolamine (10 mg/kg) and PEG-SOD (2000 U/kg). * p<0.05 vs. LZR Control; † p<0.05 vs. OZR Control.
**Figure 5.** The change in diameter of isolated skeletal muscle resistance arterioles from LZR (n=10) and OZR (n=10) in response to reduced oxygen tension (Panel A), increasing iloprost concentration (Panel B), increasing acetylcholine concentration (Panel C), increasing intraluminal pressure (Panel D) and increasing norepinephrine concentration (Panel E). Data are presented for arteries from LZR and OZR under control conditions and following treatment of vessels with 200 U/ml PEG-SOD (Panels A-D) or 10^{-5} M phentolamine (Panel E). * p<0.05 vs. LZR Control; † p<0.05 vs. OZR Control.

**Figure 6.** The passive mechanical properties of the wall of isolated skeletal muscle resistance arterioles from LZR (n=10) and OZR (n=10) in response to increasing intraluminal pressure under Ca^{2+}-free conditions. Data (mean±SEM) are presented for changes in arteriolar internal diameter (Panel A), the incremental distensibility of the arteriolar wall (Panel B) and for the circumferential stress versus strain relation of the arteriolar wall (Panel C). * p<0.05 versus LZR.

**Figure 7.** Data (mean±SEM) describing the density of microvessels within the gastrocnemius muscle of LZR (n=5) and OZR (n=5). *Panel A:* microvessel density within the gastrocnemius expressed as number of microvessels/mm² cross sectional area of the gastrocnemius muscle. *Panel B:* microvessel:muscle fiber ratio within the gastrocnemius muscle of LZR and OZR. * p<0.05 versus LZR.
**Figure 1.**

Frisbee
Figure 2

Frisbee
Figure 3.
Frisbee
Figure 4.
Frisbee
A: Hypoxia

B: Iloprost

C: Acetylcholine

D: Myogenic

E: Norepinephrine

Figure 5.
Frisbee
Figure 6.
Frisbee
Figure 7.
Frisbee