Vascular adrenergic tone and structural narrowing constrain reactive hyperemia in skeletal muscle of obese Zucker rats

Jefferson C. Frisbee

Center for Interdisciplinary Research in Cardiovascular Sciences, Department of Physiology and Pharmacology, West Virginia University School of Medicine, Morgantown, West Virginia

Submitted 28 November 2005; accepted in final form 20 December 2005

Frisbee, Jefferson C. Vascular adrenergic tone and structural narrowing constrain reactive hyperemia in skeletal muscle of obese Zucker rats. Am J Physiol Heart Circ Physiol 290: H2066–H2074, 2006. First published December 22, 2005; doi:10.1152/ajpheart.01251.2005.—Previous studies have demonstrated that skeletal muscle perfusion is impaired in obese Zucker rats (OZR) under control conditions and with elevated metabolic demand versus responses in lean Zucker rats (LZR). To further our understanding of processes contributing to impaired perfusion, we determined whether hyperemic responses following periods of occlusion were altered in skeletal muscle of OZR versus LZR. In isolated hindlimbs, basal blood flow in OZR was less than in LZR, and total perfusion responses after 30, 90, and 180 s of occlusion were reduced. Treatment of animals with an antioxidant (polyethyleneglycol-superoxide dismutase) had no effect on reactive hyperemia, although blockade of α-adrenoceptors (α1 > α2) improved responses to 30 and 90 s of occlusion; responses to 180 s of occlusion were unaltered. Pump perfusion of a diluted distal hindlimb demonstrated that increased volume flow elicited a greater increase in perfusion pressure in OZR versus LZR, suggesting structural contributions to an increased vascular resistance. Responses were comparable for in situ cremaster muscle because reactive hyperemia following serial arterial occlusion was attenuated in OZR versus LZR, treatment with polyethyleneglycol-superoxide dismutase was ineffective, and hyperemic responses were improved following inhibition of α-adrenoceptors (α1 > α2). Treatment of cremaster muscle with adenosine (10−3 M) caused flow to increase to a level comparable to that following 180 s of occlusion in both strains, although this level was reduced in OZR versus LZR. These results suggest that increased adrenergic tone may constrain reactive hyperemia in OZR with brief occlusion, although structural increases in vascular resistance can contribute to constrained perfusion after longer periods of occlusion.

The metabolic syndrome is, in part, defined as the combined presentation of obesity, hypertension, insulin resistance/Type II diabetes mellitus, and dyslipidemia. This constellation of pathologies is a source of significant concern for public health policy, because it not only currently impacts ~47 million American adults (1, 5), but the prevalence of this syndrome is increasing rapidly among both adult (1, 5, 26) and pediatric populations (7, 18). From the standpoint of cardiovascular health, the most profound implication of evolution of the metabolic syndrome is an increased likelihood for the development of peripheral vascular disease (1, 26), a condition that is associated with compromised perfusion of the affected limbs and tissues, leading to impaired function and a progressive deterioration in the viability of those tissues.

From a clinical perspective, one of the commonly determined markers of vascular dysfunction in the metabolic syndrome is that of an impaired perfusion response following occlusion (29, 39, 42, 46). Although frequently referred to as flow- or shear-induced dilation, or endothelial dysfunction, the response is actually one of reactive hyperemia and can represent a more integrated and multifactorial perfusion response than one that is wholly localized to the vessel itself. Whereas specific mechanisms underlying this impaired perfusion response remain elusive, previous results suggest that oxidant stress-based reductions in dilator reactivity (2, 20), adrenergic constraints on vascular reactivity (25, 31), and altered passive mechanical characteristics of vessels (i.e., structural remodeling; Ref; 19, 22) have been implicated as potential contributing mechanisms to alterations in reactive hyperemia in patients suffering with either the full metabolic syndrome or with specific contributing pathologies (i.e., dyslipidemia or Type II diabetes mellitus).

Owing to a dysfunctional leptin receptor gene, the obese Zucker rat demonstrates an impaired satiety reflex, resulting in chronic hyperphagia (24). As a result, obese Zucker rats rapidly develop profound obesity, associated with significant insulin resistance, dyslipidemia, and following sexual maturity, a moderate hypertension (24, 38, 41). Previously, we have demonstrated that the reactivity of peripheral microvessels is impaired in response to both pharmacological (14, 15) and physiological stimuli (11, 13, 37), and these data suggest that a complex interaction of oxidant stress, vascular adrenergic tone, and structural alterations all combine to impair both resting perfusion and active hyperemia (9–13, 36, 37). To more fully investigate the effects of the development of the metabolic syndrome and the processes that are associated with its evolution on the regulation of skeletal muscle perfusion, we employed two distinct preparations to examine the effects of the metabolic syndrome on the reactive hyperemic responses to skeletal muscle. The present study tested the hypothesis that the hyperemic responses in skeletal muscle of obese Zucker rats after periods of occlusion are reduced compared with responses in lean Zucker rats. Furthermore, the present study tested the secondary hypotheses that an elevated vascular oxidant stress, an enhanced vascular adrenergic tone, and a structural remodeling of the microcirculation [including previously demonstrated reductions in the passive diameter of skeletal muscle arterioles (12) and in skeletal muscle microvessel...
density (10, 12) contribute to this constrained perfusion response.

**MATERIALS AND METHODS**

**Animals.** Male lean and obese Zucker rats (LZR and OZR, respectively) of 17 wk in age were used for all experiments. Rats were housed in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility and were fed standard rodent chow and tap water ad libitum. All protocols received prior IACUC approval. After an overnight (12 h) fast, rats were anesthetized with injections of pentobarbital sodium (50 mg/kg ip) and received tracheal intubation to facilitate maintenance of a patent airway. In all rats, a carotid artery and an external jugular vein were cannulated to facilitate determination of arterial pressure and for intravenous infusion of supplemental anesthetic, if necessary. Additionally, an aliquot of blood was drawn from the jugular vein to be used for the determination of plasma glucose (Freestyle, Abbott, Abbott Park, IL) and insulin concentrations (Linco Research, St. Charles, MO) as well as an evaluation of the plasma lipid profile (LiquiColor, Stambio, Boerne, TX).

**Preparation of in situ cremaster muscle and distal hindlimb.** After the initial surgical preparation, the right cremaster muscle from each rat was prepared for in situ investigation (15), with special attention paid to prevent disruption of the deferential feed vessels (17). Once completed, the cremaster muscle was continuously bathed in warm (35°C) physiological salt solution (PSS) while the remainder of the surgical procedure was completed. The ionic composition of the PSS was as follows (in mM): 119.0 NaCl, 4.7 KCl, 1.6 CaCl2, 1.18 NaH2PO4, 1.17 MgSO4, and 24.0 NaHCO3. These rats then received subsequent surgical procedures to isolate the left femoral artery immediately proximal to the knee, and this vessel was cleaned to its proximal origin at the external iliac artery. All branches arising from the femoral artery, proximal to the knee, were either ligated or cauterized, depending on size. After this procedure, a loose ligature was placed on the proximal end of the femoral artery, and a microcirculation blood flow probe (0.7 PSB, Transonic, Ithaca, NY) was placed around the artery, proximal to the knee and distal to the ligature, to monitor distal hindlimb perfusion during subsequent procedures. At the conclusion of these steps, rats were placed into a transilluminated video microscope such that the cremaster muscle microcirculation could be visualized. After all surgical procedures, each animal received an intravenous infusion of 1,000 IU/kg heparin (Elkins-Sinn, Cherry Hill, NJ), and all exposed surgical areas were covered in PSS-soaked gauze to minimize evaporative water loss.

After the animal was placed within the video microscope and a postsurgical equilibration period of 30 min was allowed, a second-order arteriole (~60 μm diameter) was identified in the cremaster muscle. Arterioles chosen for study had walls that were clearly visible, a brisk flow velocity, and active tone, as indicated by the occurrence of significant dilation in response to topical application of 10^{-3} M adenosine to determine passive arteriolar diameter. Subsequently, the left femoral artery was cannulated at the iliac artery, and this line was connected to a syringe infusion pump and contained a side branch for monitoring perfusion pressure. At this time, the carotid artery and jugular vein cannulas were opened and Ca^2+-free PSS containing 10^{-3} M sodium nitroprusside (Sigma-Aldrich) and 10^{-4} M papaverine (Sigma-Aldrich) was infused at 1.5 ml/min via the femoral artery cannula, procedures that caused the death of the anesthetized rat. Subsequently, Ca^2+-free PSS containing 10^{-3} M sodium nitroprusside was infused via the femoral arterial cannula at 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml/min for 5 min each, and perfusion pressure was continuously monitored. Distal hindlimb mass was not different between LZR (39.8 ± 3.1 g) and OZR (44.6 ± 2.9 g).

**Contribution of α1- versus α2-adrenoreceptors to reactive hyperemia.** In a separate group of animals (n = 10 LZR and n = 10 OZR), the relative contribution of α1- and α2-adrenoreceptors to differences in reactive hyperemic responses were assessed. After assessment of reactive hyperemia to 30, 90, or 180 s of occlusion as described above, individual rats received intravenous infusions of either the α1-adrenoreceptor antagonist prazosin (1 mg/kg, Sigma-Aldrich) or the α2-adrenoreceptor antagonist yohimbine (5 mg/kg, Sigma-Aldrich), and the assessment of reactive hyperemia following the three occlusion periods was performed as described above. In the group of animals, the control blood flow was measured in the absence of any intervention and was assumed to be a baseline measurement.

**Data and statistical analyses.** Within the cremaster muscle preparation, blood flow through the artery of interest was calculated from erythrocyte velocity and vessel radius (3):

\[ F = \frac{(V \cdot 1.6) \cdot (\pi r^2)}{(0.001)} \]

where F represents the volume of blood flow (in nl/s), V represents centerline erythrocyte velocity (in mm/s), and r represents vessel radius (in μm).

Reactive hyperemic responses (i.e., volume blood flow vs. time) were fit with polynomial regression equations (\( y = a_1 x^2 + a_2 x + a_3 \)) where y represents blood flow and x represents time following removal of occlusion). This equation was then integrated with respect to time, and the resulting equation was used to evaluate...
the area under the curve (i.e., the total perfusion response; Refs. 16 and 30) between the immediate removal of the occlusion (t = 0), and the conclusion of the hyperemic response (varies with duration of occlusion).

Active tone in situ arterioles was calculated as (ΔD/Dmax) × 100, where ΔD is the diameter increase from control in response to Ca(2+)-free PSS containing 10^{-3} M adenosine, and Dmax is the maximum diameter measured under superfusion with Ca(2+)-free PSS containing 10^{-3} M adenosine. Minimum vascular resistance across the perfused distal hindlimb was calculated as the quotient of perfusion pressure and perfusate flow.

All data are presented as means ± SE. Statistically significant differences in basic characteristics and total perfusion responses between LZR and OZR were determined using Student’s t-test or ANOVA, as appropriate. ANOVA was used to determine statistically significant differences in the minimum vascular resistance between LZR and OZR across the different levels of perfusion and maximum cremasteric arteriolar blood flow. In all cases, Tukey’s test was used post hoc, and P < 0.05 was taken to be statistically significant.

**RESULTS**

**Baseline animal characteristics.** Data describing the baseline characteristics of 17-wk-old LZR and OZR utilized in the present study are presented in Table 1. OZR were significantly heavier than LZR at this age and, while exhibiting a moderate hypertension, demonstrated significant insulin resistance and hypertriglyceridemia, as well as elevated fasting hyperglycemia and hypercholesterolemia. Under control conditions, both cremasteric second-order arteriolar diameter and blood flow within those vessels were significantly reduced in OZR versus LZR. Additionally, distal hindlimb blood flow, normalized to distal hindlimb mass, was also reduced in OZR compared with LZR.

**Reactive hyperemia in situ hindlimb preparation.** Alterations in hindlimb perfusion in OZR and LZR with increasing duration of arterial occlusion are presented in Fig. 1. After 30 s (Fig. 1A), 90 s (Fig. 1B), or 180 s (Fig. 1C) of occlusion, perfusion responses following restoration of blood flow were significantly reduced in the hindlimb of OZR compared with responses in LZR. However, the extent of the perfusion impairment in response to occlusion was variable, because the total perfusion response in OZR was 65.1 ± 3.4% of that in LZR after 30 s of occlusion, 80.8 ± 2.9% of the LZR response after 90 s of occlusion, and 67.2 ± 3.7% of that in LZR after 180 s of occlusion.

After treatment with the oxidative radical scavenger PEG-SOD, reactive hyperemia following any period of occlusion in OZR was not significantly altered from that determined in the control condition. In OZR under control conditions, the total perfusion response following alleviation of the occlusion was 2.74 ± 0.45, 4.84 ± 0.61, and 7.31 ± 0.88 (ml • g^{-1} • min^{-1}) s in response to 30, 60, and 90 s of occlusion, respectively. After treatment of OZR with PEG-SOD, these responses were 2.80 ± 0.48, 4.78 ± 0.58, and 7.68 ± 0.67 (ml • g^{-1} • min^{-1}) s for the same occlusion durations. Data from LZR following treatment with PEG-SOD demonstrated a similar lack of any significant effect compared with that in untreated control conditions.

Data describing the effects of treating OZR with the α₁ and α₂-adrenergic antagonist phentolamine on perfusion responses after occlusion are presented in Fig. 2. In response to 30 (Fig. 2A) or 90 s (Fig. 2B) of occlusion, adrenergic blockade increased total perfusion responses in the OZR hindlimb compared with control conditions. However, this effect was reduced with occlusion duration; after 30 s of occlusion, phentolamine treatment increased the total perfusion response in OZR by 27.6 ± 3.4% and after 60 s of occlusion, this improvement in total perfusion was reduced to 14.4 ± 2.8% above that in untreated OZR. In response to 180 s of occlusion (Fig. 2C), adrenergic blockade had a statistically insignificant effect on restoring perfusion responses (3.6 ± 3.8% above control OZR), and the total perfusion response remained significantly reduced compared with that in untreated LZR.

The results from determining adrenoreceptor subtypes contributing to the impaired reactive hyperemic responses of OZR hindlimb are presented in Fig. 3. Treatment with the α₁-adrenoreceptor antagonist prazosin improved hyperemic responses following occlusion in a manner that was similar to that determined with phenolamine in response to 30 (Fig. 3A), 90 (Fig. 3B), and 180 s of occlusion in OZR (Fig. 3C). Treatment with prazosin did not significantly alter the total reactive hyperemic response following any period of occlusion in LZR (Fig. 3), whereas treatment with the α₂-adrenoreceptor antagonist yohimbine did not significantly improve reactive hyperemic responses following any occlusion period in either rat strain (data not shown).

Figure 4 presents the results of perfusion of the maximally dilated hindlimb vasculature of OZR and LZR with PSS at constant rates. At perfusion rates below 1.5 ml/min (~0.035 ml • g^{-1} • min^{-1}), perfusion pressure was comparable between LZR and OZR. However, above this level, these relationships diverged and the resistance of the hindlimb circulation to perfusion within OZR was elevated compared with LZR, suggesting that the structure of the hindlimb circulation begins

<table>
<thead>
<tr>
<th>Pathophysiology</th>
<th>LZR</th>
<th>OZR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, weeks</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Mass, g</td>
<td>361±11</td>
<td>598±10*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>101±3</td>
<td>125±4*</td>
</tr>
<tr>
<td>[Glucose]plasma, mg/dl</td>
<td>106±8</td>
<td>180±11*</td>
</tr>
<tr>
<td>[Insulin]plasma, ng/ml</td>
<td>1.40±0.3</td>
<td>9.2±0.6*</td>
</tr>
<tr>
<td>[Cholesterol]plasma, mg/dl</td>
<td>103±8</td>
<td>140±12*</td>
</tr>
<tr>
<td>[Triglycerides]plasma, mg/dl</td>
<td>122±10</td>
<td>303±13*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. MAP, mean arterial pressure; LZR, lean Zucker rats; OZR, obese Zucker rats. *P < 0.05 vs. LZR.
Fig. 1. Hindlimb perfusion patterns in lean Zucker (LZR) and obese Zucker rats (OZR) after removal of prolonged femoral artery occlusion (i.e., reactive hyperemia). Data describing changes in perfusion are presented in response to 30 (A), 90 (B), and 180 s (C) of blood flow occlusion. Inset, total perfusion response after removal of occlusion (i.e., area under blood flow vs. time curve) between LZR (open bars) and OZR (solid bars). Please see text for details. *P < 0.05 vs. LZR.

Fig. 2. Hindlimb perfusion patterns in LZR and OZR after removal of prolonged femoral artery occlusion under control conditions and after treatment of the rat with the adrenoreceptor antagonist phentolamine. Data describing changes in perfusion are presented in response to 30 (A), 90 (B), and 180 s (C) of blood flow occlusion. Inset, total perfusion response after removal of occlusion (i.e., area under blood flow vs. time curve) between LZR and OZR. Please see text for details. Open bars, LZR under control conditions; hatched bars, LZR treated with phentolamine; solid bars, OZR under control conditions; crosshatched bars, OZR treated with phentolamine. *P < 0.05 vs. LZR; †P < 0.05 vs. OZR.
to act as a physical constraint on further increases in perfusion.

When taken as a whole, the slope of the relationship between volume perfusion and perfusion pressure \[(\text{pressure}) / (\text{flow})\] was significantly greater in the hindlimb circulation of OZR versus LZR, as shown in Fig. 4.

**Reactive hyperemia for in situ cremaster muscle preparation.** Alterations in cremasteric arteriolar blood flow in response to increasing periods of serial occlusion in OZR and LZR are presented in Fig. 5. Comparable to observations in the hindlimb circulation, hyperemic responses to arteriolar occlusion were significantly impaired in OZR versus LZR after 30 (Fig. 5A), 90 (Fig. 5B), and 180 (Fig. 5C) s of interrupted blood flow.

In response to treatment of the cremaster muscle of OZR with PEG-SOD, reactive hyperemic responses following serial occlusion were not altered from the control, untreated condition. Under control conditions, the total perfusion response following removal of the occlusion was 902 ± 102, 2,110 ± 231, and 4,100 ± 498 nl in response to 30, 60, and 90 s of occlusion, respectively. After treatment of the cremaster muscle of OZR with PEG-SOD, these responses were 931 ± 79, 2,203 ± 254, and 4,312 ± 504 nl for the same occlusion durations. Data from LZR following treatment with PEG-SOD demonstrated a similar lack of any significant effect compared with that in untreated control conditions.

**Fig. 6** presents data describing the effects of adrenergic blockade on reactive hyperemia in cremasteric arterioles of OZR. Application of phentolamine significantly improved the total perfusion responses in arterioles of OZR following 30 (Fig. 6A) and 90 (Fig. 6B) s of occlusion. In contrast, adrenergic blockade with phentolamine did not alter reactive hyperemia in response to 180 s of occlusion (Fig. 6C).

Data describing the effects of treating OZR with prazosin or yohimbine on perfusion responses following serial occlusion are presented in Fig. 7. The beneficial impact of blockade of specific adrenergic receptor subtypes on total perfusion responses was significant with \(\alpha_1\)-adrenoreceptor blockade only and only after 30 (Fig. 7A) and 90 (Fig. 7B) s of occlusion. In contrast, \(\alpha_2\)-adrenoreceptor blockade did not result in a statistically significant improvement in reactive hyperemic re-

---

**Fig. 3.** Hindlimb perfusion patterns in LZR and OZR following removal of prolonged femoral artery occlusion under control conditions and after treatment of the animal with the \(\alpha_1\)-adrenoreceptor antagonist prazosin. Data describing changes in perfusion are presented in response to 30 (A), 90 (B), and 180 s (C) of blood flow occlusion. Inset, total perfusion response after removal of occlusion (i.e., area under blood flow vs. time curve) between LZR and OZR. Please see text for details. Open bars, LZR under control conditions; hatched bars, LZR treated with prazosin; solid bars, OZR under control conditions; crosshatched bars, OZR treated with prazosin. *\(P < 0.05\) vs. LZR; †\(P < 0.05\) vs. OZR.

**Fig. 4.** Data describing change in perfusion pressure in maximally dilated hindlimb circulation of LZR and OZR during pump perfusion at incrementally increasing volume flow rates. Slope of this relationship \[(\text{pressure}) / (\text{flow})\] in OZR \((68.4 \pm 5.1 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min})\] was significantly greater than that determined in LZR \((32.4 \pm 4.4 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min}; P < 0.05)\). *\(P < 0.05\) vs. LZR.
Fig. 5. Cremasteric arteriolar blood flow in LZR and OZR following upstream serial occlusion under control conditions. Data describing changes in blood flow are presented in response to 30 (A), 90 (B), and 180 s (C) of serial occlusion. Inset, total perfusion response after removal of occlusion (i.e., area under blood flow vs. time curve) between LZR (open bars) and OZR (solid bars). Please see text for details. *P < 0.05 vs. LZR.

Fig. 6. Cremasteric arteriolar blood flow in LZR and OZR following upstream serial occlusion under control conditions and in response to treatment of the animal with phentolamine. Data describing changes in blood flow are presented in response to 30 (A), 90 (B), and 180 s (C) of serial occlusion. Inset, total perfusion response after removal of occlusion (i.e., area under blood flow vs. time curve) between LZR and OZR. Please see text for details. Open bars, LZR under control conditions; solid bars, OZR under control conditions; hatched bars, OZR treated with phentolamine. *P < 0.05 vs. LZR; †P < 0.05 vs. OZR.
sponses within cremasteric arterioles of OZR. Finally, neither prazosin nor yohimbine treatment had a significant impact on reactive hyperemia in cremasteric arterioles of OZR after 180 s of occlusion (Fig. 7C).

As presented in Table 2, following superfusion with Ca\(^{2+}\)-free PSS containing 10^{-3} M adenosine, second-order arteriolar blood flow in the cremaster muscle of LZR and OZR increased significantly compared with resting values. Interestingly, although the maximum arteriolar perfusion was significantly decreased in OZR versus LZR, the percent increase in perfusion in response to superfusion with Ca\(^{2+}\)-free PSS containing 10^{-3} M adenosine from baseline levels was very similar between LZR and OZR, averaging 150–155% in both strains. These data also demonstrated that the maximum perfusion attained in cremasteric arterioles of OZR was approximately equal to that determined following the restoration of perfusion subsequent to 180 s of occlusion, suggesting a structural constraint on reactive hyperemic responses following the longest period of imposed perfusion restriction.

**DISCUSSION**

Previous studies from our group (9–13) and by others (37, 44, 45) have demonstrated that skeletal muscle perfusion in the obese Zucker rat model of the metabolic syndrome is impaired relative to that in the control strain the lean Zucker rat, although one recent study has suggested that functional hyperemia in the hindlimb of OZR may be intact (43). Whereas these studies have generally focused on alterations to either basal perfusion or the increases in perfusion that accompany elevated metabolic demand, the purpose of the present study was to determine the impact of the development of the metabolic syndrome in OZR on hyperemic responses in skeletal muscle following brief periods of vascular occlusion (i.e., reactive hyperemia). The results of the present study suggest that evolution of the metabolic syndrome in OZR is associated with an impaired perfusion response in skeletal muscle following removal of vascular occlusion. Additionally, whereas it is apparent that this impairment reflects the combined impact of different processes, the contribution of which is partially dependent on the duration of the original occlusion, the present results contribute to a growing body of literature that suggests that the regulation of skeletal muscle perfusion in OZR, although strongly impaired, may experience this impairment primarily through the actions of a limited number of factors.

We (10, 15) and others (6, 23) have determined that OZR manifest a significant increase in vascular oxidant stress, and that this increase can be determined in both individual blood vessels (dihydroethidine staining for superoxide levels and immunohistochemistry for nitrotyrosine residues) and in the plasma (biochemical analyses for 8-epi-prostaglandin F\(_{2\alpha}\) levels). However, whereas we have found that elevated vascular oxidant tone contributes to impaired dilator reactivity in reduced preparations (e.g., isolated arterioles), we have been unable to identify a significant role for elevated oxidant tone in contributing to alterations to the moment to moment regulation of skeletal muscle perfusion (11). Whereas the concept of a limited role for oxidant stress in regulating perfusion is in contrast with previous work examining reactive hyperemia in the canine myocardium (40), results from the present study support this hypothesis because acute reductions in vascular...
oxidant stress (through intravenous infusion of PEG-SOD) had no impact on reactive hyperemia in OZR, regardless of either occlusion duration or level of resolution (i.e., cremasteric arteriole vs. hindlimb). Rather, based on other recent studies from our laboratory, it seems that the chronic elevations in oxidant tone may contribute to the regulation of skeletal muscle blood flow through long-term alterations in vascular network structure, increasing the resistance to perfusion at higher flow rates (10).

Recent studies from our laboratory (9, 11) and from others (28, 35, 37) have provided evidence suggesting that an enhanced arteriolar adrenergic tone can contribute to an under-perfusion of skeletal muscle in OZR, both under resting conditions and with mild-to-moderate elevations in metabolic demand. Additionally, other studies have clearly demonstrated that an increased α-adrenergic tone can readily blunt reactive hyperemic responses in other organs (4, 34). However, as metabolic demand increases, the ability of the adrenergic influences to impact perfusion becomes progressively diminished (9). The results from the present study suggest that a comparable effect may contribute to impairments in reactive hyperemia, because adrenoreceptor blockade significantly improved the total perfusion response of OZR following 30 and 90 s of occlusion only, although the effectiveness of adrenoreceptor blockade in improving reactive hyperemia was reduced following the 90-s occlusion period. With 180 s of vascular occlusion, adrenoreceptor blockade had no significant impact on reactive hyperemia, regardless of muscle preparation. This pattern of decreasing effectiveness of adrenergic blockade on reactive hyperemia in OZR may reflect an encroachment of perfusion on a maximum level owing to an increasing physical constraint on perfusion as a result of structural alterations to individual microvessels and vascular networks (12). These results may be analogous to that determined in our previous study of active hyperemia, where despite an increased vascular adrenergic tone, a sufficient accumulation of metabolic dilator stimuli was sufficient to override the constrictor influences, although the sensitivity of this relationship was reduced (11).

With the use of both the hindlimb and cremaster preparation, the effects of α1-adrenoreceptor blockade were nearly identical to that for combined α1/α2-adrenoreceptor antagonism, whereas infusion of the α2-adrenoreceptor antagonist yohimbine was without statistically significant effect. Whereas previous studies have demonstrated a longitudinal heterogeneity with regard to the adrenergic control of vascular tone in rat skeletal muscle, where adrenergic constriction of larger arterioles occurs primarily through α1-adrenoceptor activity, and distal arterioles experience a more balanced effect mediated through both α1- and α2-adrenoreceptors (8, 32, 32), the results of the present study suggest that the preponderance of the adrenergic influence over reactive hyperemia in skeletal muscle of OZR is mediated via the α1-receptor alone. Given that the results of the present study do not address alterations in α-adrenoreceptor expression pattern or adrenergic receptor sensitivity within the skeletal muscle microcirculation of OZR, further investigation into the cellular mechanisms through which an adrenergic constraint on perfusion is manifested appears to be warranted.

One other significant contributor to the impaired reactive hyperemia in skeletal muscle of OZR following vascular occlusion appears to be alterations in microvessel and microvascular network structure. We and others have previously demonstrated that OZR exhibit a significant loss in both the distensibility of individual skeletal muscle resistance arteriole (12, 36) and the density of skeletal muscle microvessels (10, 12), alterations that will dramatically increase resistance to perfusion above a critical level of flow (27). These observations are applicable to the current study because in the perfused hindlimb this significant increase in resistance occurred at a perfusion rate above ~2.0 ml/min (~0.047 ml·g⁻¹·min⁻¹) and hindlimb perfusion in the OZR rarely exceeded 0.05–0.06 ml·g⁻¹·min⁻¹ under any conditions of the current study. Additionally, when the maximum perfusion through the cremasteric arterioles was determined, OZR experienced a significant reduction in this increased flow rate compared with that determined in LZR, and the maximum flow rate determined in the cremasteric arterioles following superfusion with adenosine in the absence of calcium approximated that of the maximum level of perfusion identified during the reactive hyperemic response. Thus it seems plausible that structural alterations to the skeletal muscle microcirculation may have contributed to this constraining “upper bound” on physiological levels of perfusion in OZR. It is important to note, however, that the results from the present study do not allow for the determination of the relative contribution of reduced microvessel density and structural narrowing of individual microvessel to the increased vascular resistance identified in OZR.

In summary, the results of the present study demonstrate that, following occlusive periods of increasing duration, reactive hyperemic responses in skeletal muscle of obese Zucker rats manifesting the metabolic syndrome are significantly reduced compared with those determined in control, lean Zucker rats. Whereas no identified role for elevated vascular oxidant stress was demonstrated in contributing to this impaired perfusion response, additional studies suggest that increased adrenergic vascular tone (mediated primarily via the α1-adreno-receptor) and a remodeling of the skeletal muscle microcirculation, likely including both structural narrowing of individual vessels and a reduced microvessel density, may both contribute to these reductions in reactive hyperemia.

ACKNOWLEDGMENTS

The author thanks Milinda E. James for expert technical assistance. The author thanks Dr. Julian H. Lombard from the Medical College of Wisconsin for helpful suggestions and support during the performance of some of the experiments in the present study.

GRANTS

This work was supported by National Institutes of Health Grant R01 DK-64668 and the American Heart Association Grant SDG 0330194N.

REFERENCES


