Remodeling of the skeletal muscle microcirculation increases resistance to perfusion in obese Zucker rats

Jefferson C. Frisbee

Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Submitted 10 February 2003; accepted in final form 18 March 2003

Remodeling of the skeletal muscle microcirculation increases resistance to perfusion in obese Zucker rats. Am J Physiol Heart Circ Physiol 285: H104–H111, 2003. First published March 20, 2003; 10.1152/ajpheart.00118.2003.—Whereas previous studies have demonstrated that the development of syndrome X in obese Zucker rats (OZR) is associated with impaired arteriolar reactivity to vasoactive stimuli, additional results from these studies indicate that the passive diameter of skeletal muscle arterioles is reduced in OZR versus lean Zucker rats (LZR). On the basis of these prior observations, the present study evaluated structural alterations to the skeletal muscle microcirculation as potential contributors to an elevated vascular resistance. Isolated skeletal muscle resistance arterioles exhibited a reduced passive diameter at all levels of intraluminal pressure and a left-shifted stress-strain curve in OZR versus LZR, indicative of structural remodeling of individual arterioles. Histological analyses using Griffonia simplicifolia I lectin-stained sections of skeletal muscle demonstrated reduced microvessel density (rarefaction) in OZR versus LZR, suggesting remodeling of entire microvascular networks. Finally, under maximally dilated conditions, constant flow-perfused skeletal muscle of OZR exhibited significant elevations in perfusion pressure versus LZR, indicative of an increased resistance to perfusion within the microcirculation. These data suggest that developing structural alterations to the skeletal muscle microcirculation in OZR result in elevated vascular resistance, which may, acting in concert with impaired arteriolar reactivity, contribute to blunted active hyperemic responses and compromised performance of in situ skeletal muscle with elevated metabolic demand.

MATERIALS AND METHODS

Animals. Fifteen-week-old male LZR (n = 16, Harlan) and OZR (n = 16, Harlan) maintained on standard rat chow and tap water, ad libitum, were used for all experiments. Rats were housed in an animal care facility at the Medical College of Wisconsin approved by the American Association for the Accreditation of Laboratory Animal Care, and all protocols received prior institutional animal care and use committee approval. Rats were anesthetized with an injection of pentobarbital sodium (60 mg/kg ip), and a carotid artery was cannulated for the determination of arterial pressure. In addition to being significantly heavier than LZR [mass = 361 ± 8 g, mean arterial pressure (MAP) = 111 ± 4 mmHg, oxygen tension (9) and elevated wall shear rate (12), and a “hypersensitivity” in response to elevated intraluminal pressure (10) and α-adrenergic stimulation (27)]. While the first three of these alterations in arteriolar reactivity in OZR appear to be a partial function of elevated vascular oxidant tone (9, 10, 12), the enhanced α-adrenergic reactivity appears to have its basis in both increased activity within the sympathetic nervous system per se (6) as well as in augmented sensitivity of skeletal muscle arterioles in response to norepinephrine (27).

Throughout the course of the previous studies, it has been consistently determined that the diameter of passive skeletal muscle arterioles of OZR is consistently reduced below that identified for comparable vessels in its control animal strain, the lean Zucker rat (LZR) (9, 10, 12, 27). With specific regard to the structure of skeletal muscle microvessels and microvascular networks, a previous study by Lash et al. (20) determined that the skeletal muscle capillary basement membrane thickness in adult OZR versus LZR, with a reduction capillary density within plantar muscles. These previous observations may have significant implications for the patterns of blood flow within the skeletal muscle microcirculation, due to elevated peripheral vascular resistance (14, 15, 23). The purpose of the present study was to determine whether structural modifications to individual skeletal muscle resistance arterioles and the skeletal muscle microvascular network develop in OZR and to evaluate the impact of these alterations on the resistance to perfusion within the skeletal muscle microcirculation.

Address for reprint requests and other correspondence: J. C. Frisbee, Dept. of Physiology, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (E-mail: jfrisbee@mcw.edu).
blood [glucose] = 135 ± 11 mg/dl, OZR (mass = 604 ± 13 g, MAP = 138 ± 4 mmHg, blood [glucose] = 402 ± 28 mg/dl) demonstrated significant hypertension and hyperglycemia.

Preparation of isolated vessels. The intramuscular continuation (first-order arteriole) of the right gracilis artery (the small muscular branch of the right femoral artery supplying the gracilis muscle) was surgically removed from the anesthetized rat, with care taken to minimize stretching and handling arterioles by their surrounding connective tissue only. 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) from separate reservoirs. The PSS used in these experiments was equilibrated with a 21% O2-5% CO2-74% N2 gas mixture and had the following composition (in mM): 119 NaCl, 4.7 KCl, 1.17 MgSO4, 1.6 CaCl2, 1.18 NaH2PO4, 24 NaHCO3, 0.026 EDTA, and 5.5 glucose. Vessels were cannulated at both ends with glass micropipettes and secured to inflow and outflow pipettes using 10-0 nylon sutures. Any side branches were ligated with a single strand from a 6-0 silk suture. The inflow pipette was connected to a reservoir and superfusate PSS were replaced with Ca2+-free PSS. Vessels were challenged with 10−6 M norepinephrine until all vascular reactivity was eliminated and active tone at rest was discarded. Active tone at the equilibration pressure in Ca2+-free PSS. Vessels of any vessel that did not demonstrate active tone at rest was discarded. Active tone at the equilibration pressure was calculated as (ΔID/IDmax) × 100, where ΔD is the diameter increase from rest in response to Ca2+-free PSS and Dmax is the maximum diameter measured at the equilibration pressure in Ca2+-free PSS. Active tone for vessels in the present study averaged 13 ± 3% in LZR and 30 ± 4% in OZR.

Determination of passive mechanics of the arteriolar wall. Before the experimental procedures began, the perfusate and superfusate PSS were replaced with Ca2+-free PSS. Vessels were challenged with 10−7 M norepinephrine until all vascular reactivity was eliminated and active tone was lost. At this time, intraluminal pressure within the isolated vessel was altered, in 20-mmHg increments, to between 0 and 160 mmHg. Circumferential stress from the derivative of the exponential least squares, (ordinary least squares, (ε)) was then estimated at different values of intraluminal pressure from 5 to 160 mmHg. *P < 0.05 vs. LZR at that intraluminal pressure.

All calculations of passive arteriolar wall mechanics (used as indicators of structural alterations to the individual microvessel) are based on those used previously by Baumbach and Hadju (2), with minor modifications. Vessel wall thickness (WT; in μm) was calculated as

$$\text{WT} = \frac{(OD - ID)}{2}$$

where OD and ID represent the outer and inner diameter, respectively (in μm).

The arteriolar cross-sectional area (CSWA; in μm2), assuming the arteriole is round, was calculated as

$$\text{CSWA} = \pi \left(\frac{OD}{2}\right)^2 - \frac{1}{2}$$

where OD and ID represent the arteriolar outer and inner diameter, respectively (in μm).

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

$$\text{DISTINC} = \text{ΔID}/\text{ΔP}_{\text{IL}} \times 100$$

where ΔID represents the change in internal arteriolar diameter for each incremental change in intraluminal pressure (ΔPIL).

Circumferential strain (ε) was calculated as

$$\epsilon = \frac{(ID - ID_0)/ID_0}{(ID - ID_{0})/ID_0}$$

where ID5 represents the internal arteriolar diameter at the lowest intraluminal pressure (i.e., 5 mmHg).

To determine the tangential elastic modulus (ET), the stress-versus-strain curves from each vessel were fit (ordinary least squares, r2 > 0.85) with the following exponential equation

$$\sigma = \sigma_0 e^{\beta \epsilon}$$

where σT represents the circumferential stress at ID5 and β is the ratio coefficient, which is a function of the independent variable ε. ET was then estimated at different values of circumferential stress from the derivative of the exponential curve

$$E_T = \frac{d\sigma}{d\epsilon} = \beta \sigma e^{\beta \epsilon}$$

Determination of microvessel density. While the rat was under anesthesia, the right gastrocnemius muscle was removed, rinsed in PSS, and lightly fixed in 1% formalin. Muscles were embedded in paraffin and cut into 5-μm cross sections.
sections, which were stained with *Griffonia simplicifolia I* lectin (Sigma), as described previously (13, 17). This procedure selectively stains all microvessels with a diameter ≤ 20 μm, preferentially arterioles and capillaries versus venules, regardless of perfusion status (13). After the exposure to lectin, sections were rinsed three times in PSS and mounted on microscope slides with a water-soluble mounting medium (SP, ACCU-MOUNT 280, Baxter). With the use of epi-fluorescence microscopy, localization of labeled microvessels was performed with a Nikon E600 upright microscope with a 10× objective lens (Plan Fluo phase, numerical aperture 0.5). Excitation was provided by a 75-W xenon arc lamp through a Lambda 10-2 optical filter changer (Sutter Instruments; Novato, CA) controlling a 595-nm excitation filter and 615-nm emission filter. The microscope was coupled to a cooled charge-coupled device camera (Micromax, Princeton Instruments; Trenton, NJ). All acquired images from individual sections were analyzed for the number of microvessels and number of skeletal muscle fibers using MetaMorph Imaging software (Universal Imaging; Downingtown, PA).

**Determination of skeletal muscle vascular resistance.** After completion of the procedures described above, the contralateral (left) leg of the anesthetized rat received a medial incision from the distal calcaneus to the femoral triangle. The saphenous artery and vein were tied and removed, after which the semitendinosus, semimembranosus, gracilis, and caudofemoralis muscles were removed from the leg, exposing the gastrocnemius muscle and the vascular supply to the lower leg. The sciatic nerve was sectioned to eliminate neural input to the gastrocnemius muscle. Subsequently, all branches from the femoral/popliteal artery that did not directly perfuse the gastrocnemius muscle were ligated or cauterized. These procedures included vessels serving biceps femoris, vastus lateralis, and adductor magnus muscles, the superior genicular vessels, the posterior saphenous artery, the anterior and posterior tibial arteries, and the peroneal arteries. This procedure was repeated proximally along the length of the femoral artery leading to the iliac artery, thus creating a length of vessel, devoid of branches, extending from the iliac artery to the gastrocnemius muscle. The entire preparation was covered in PSS-soaked gauze and plastic film to minimize evaporative water loss and placed under a lamp that maintained the temperature at ~37°C.

After the completion of these procedures, an external jugular vein was cannulated, and heparin was injected into the animal (1,500 IU/kg) to prevent blood coagulation. Subsequently, the left femoral artery was cannulated at its origin from the iliac artery. This cannula was connected to a syringe infusion pump and contained a side branch for monitoring perfusion pressure. After the insertion of the femoral artery cannula, Ca²⁺-free PSS containing 10⁻³ M sodium nitroprusside and 10⁻⁴ M papaverine was infused at 1.0 ml/min via the cannula to flush and maximally dilate the microcirculation of the gastrocnemius. During this infusion period, the
carotid artery and jugular vein cannulae were opened, allowing for the removal of excess blood volume. These procedures resulted in 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, or 2.0 ml/min for 5 min each, and the perfusion pressure was continuously monitored. Because the perfusate flow = perfusion pressure × vascular resistance, at a constant perfusate flow, resistance within the microvascular bed of the gastrocnemius muscle can be calculated from changes in perfusion pressure. Gastrocnemius muscle mass did not differ between LZR (2.35 ± 0.13 g) and OZR (2.29 ± 0.15 g) in the present study.

Statistical analyses. All data are presented as means ± SE. Differences in passive mechanical characteristics between LZR and OZR and in resistance or perfusion pressure through the microvascular network were assessed using ANOVA or regression analyses, with Student-Newman-Keuls test post hoc, as appropriate. Differences in microvessel density within the gastrocnemius muscle between LZR and OZR were assessed using Student’s t-test. In all cases, P < 0.05 was taken to reflect statistical significance.

RESULTS

Arteriolar wall mechanics. Data describing the change in inner (A) and outer diameter (B) of isolated gracilis muscle arterioles from LZR and OZR in response to altered intraluminal pressure are presented in Fig. 1. Under Ca\(^{2+}\)-free conditions, throughout the imposed range of intraluminal pressure, both inner and outer diameters of isolated vessels from OZR were significantly smaller than those determined in arterioles from LZR.

The reduced diameters of arterioles from OZR in response to elevated intraluminal translated into significant reductions in vessel wall thickness (Fig. 2A) and cross-sectional wall area (Fig. 2B) versus LZR. Furthermore, the arteriolar wall-to-lumen ratio in OZR was significantly reduced up to an intraluminal pressure of 40 mmHg versus values determined for LZR (Fig. 2C), whereas incremental distensibility was significantly reduced in OZR up to an intraluminal pressure of 60 mmHg (Fig. 2D). At pressure levels higher than 40 mmHg (for the wall-to-lumen ratio) and 60 mmHg (for distensibility), there were no differences in the response of vessels to elevated intraluminal pressure between OZR and LZR.

Figure 3 presents data describing the circumferential stress-versus-strain relationship in passive arterioles of LZR and OZR. In OZR, a significant leftward shift of the stress-strain curve was identified versus LZR. As such, the slope coefficient (β) from the exponential regression equation describing these relationships was significantly increased in OZR compared with LZR.

Data describing the relationship between the tangential elastic modulus (E\(_T\)) and circumferential stress in arterioles of LZR and OZR are presented in Fig. 4. In response to elevated wall stress, E\(_T\) in OZR was consistently increased versus that from LZR, such that the slope of this relationship was significantly greater in OZR than LZR.

Skeletal muscle microvessel density. Figure 5 presents representative cross-sectional images taken using epifluorescence microscopy of the lectin-stained gastrocnemius muscles from LZR and OZR. Figure 6 presents summary data from the experimental procedures determining microvessel density. In OZR, the microvessel number per millimeter squared (Fig. 6A) was decreased, whereas the number of skeletal muscle fibers per millimeter squared (Fig. 6B) was increased compared with values determined in muscles of LZR.
As such, when expressed as microvessels per muscle fiber, the microvessel density in the gastrocnemius muscle of OZR was significantly reduced versus the value determined in LZR (Fig. 6C). During the course of the present experiments, no significant differences between the “red” and “white” heads of the gastrocnemius muscle were evident within LZR and OZR. As such, all data describing microvessel density were pooled.

**In situ skeletal muscle vascular resistance.** Gastrocnemius muscle mass was not different between LZR (2.35 ± 0.13 g) and OZR (2.29 ± 0.15 g). Data describing changes in the perfusion pressure and vascular resistance during perfusion of the in situ gastrocnemius muscle of LZR and OZR at flow rates of 0.5, 1.0, 1.5, or 2.0 ml/min are presented in Fig. 7. In response to an increased volume flow rate to the muscle, perfusion pressure in OZR, while not different from that in LZR at 0.5 and 1.0 ml/min, was significantly elevated at the two highest flow rates (Fig. 7A). With the use of these data, the calculated vascular resistance of the in situ skeletal muscle in OZR was also significantly increased at flow rates of 1.5 and 2.0 ml/min compared with values calculated in LZR (Fig. 7B).

**DISCUSSION**

With the use of the OZR model of syndrome X, numerous alterations to the reactivity of skeletal muscle arterioles that develop with the progression of obesity, hypertension, and Type II diabetes mellitus have been identified. Among these alterations is an impaired arteriolar reactivity in response to numerous physiological stimuli (9, 10, 12, 27). Associated with these alterations in reactivity, a reduction in the passive diameter of skeletal muscle arteries in OZR versus LZR was consistently observed throughout the course of these previous studies. When compared with previous observations suggesting a reduction in capillary density in plantar muscles of OZR versus LZR (20), determining the impact of potential structural alterations to the skeletal muscle microcirculation on the resistance to perfusion is warranted.

The results from the present study indicate that structural alterations to the skeletal muscle microcirculation occur at two levels of spatial resolution: 1) the individual microvessel and 2) the entire microvascular network. Compared with LZR, a remodeling of individual arterioles of OZR occurs, which results in the thinning of the microvessel wall, a reduced cross-sectional wall area, and a significant leftward shift in the stress-versus-strain relationship. Given that arterioles, a non-homogeneous structure, exhibit a curvilinear stress-strain relationship, the elastic modulus will vary with the degree of extension. As such, the slope of the $E_T$, the tangent to the stress-strain relationship at a given point, versus wall stress can provide additional information (23). In the present experiments, the slope of the $E_T$-versus-wall stress relationship was significantly greater in arterioles of OZR compared with vessels from LZR. These results suggest that not only are passive arterioles of OZR stiffer and less deformable than those of LZR, but that this relationship is present at all levels of intraluminal pressure or wall stress. Additional studies will be necessary to determine whether this arteriolar remodeling occurs in the
microcirculation of other vascular beds in OZR as well or if arterioles of other tissues exhibiting chronic elevations in metabolic demand or a stronger degree of autoregulation are protected.

The development of an increased stiffness of the microvessel wall in many models of hypertension (3, 8, 18, 21, 22, 28), diabetes mellitus (3, 7, 21, 22, 26), and to some extent dyslipidemia (21, 22, 26, 30) has been demonstrated previously. However, that this increased stiffness was associated with a thinning of the vessel wall (i.e., a lack of a net hypertrophic response) and a reduced cross-sectional wall area is novel and suggests that remodeling of individual microvessels in OZR may be far more complicated than that which develops in the face of chronic elevations in perfusion pressure alone. The development of thinner, less distensible microvessels suggests that fundamental alterations in the nature of connective tissue deposition in skeletal muscle arteriolar walls evolve in OZR versus LZR. Previous studies have suggested that development of diabetes is associated with decreased matrix metalloproteinase (MMP)-1 and MMP-2 activity, with a consequent increase in collagen deposition in the vascular wall (24, 25, 29). Additionally, Kuzuya et al. (19) examined the effects of extracellular matrix glycation on arterial stiffness in diabetics and determined that glycation-induced cross-linking of collagen fibrils contributes to arterial stiffness in diabetes and that this may be due to an inhibition of MMP-2 activity along with a reduction of collagen fibril susceptibility to proteolytic enzymes. While these pathways may be highly relevant to altered arteriolar structure in OZR, additional studies examining the extent to which the individual systemic pathologies that develop contribute to the structural remodeling in OZR and the mechanisms through which these alterations manifest themselves are needed.

The second major observation of the present study was that of a significant reduction in the density of skeletal muscle distal arterioles and capillaries in the gastrocnemius muscle of OZR compared with levels determined in LZR (Figs. 5 and 6). With the use of G. simplicifolia I lectin staining (13, 17), this rarefaction of the skeletal muscle microvascular network was evident regardless of whether microvessel density was normalized as per unit area or per muscle fiber. Previous studies have indicated that, as a means of long-term autoregulation of skeletal muscle blood flow, the density of microvessels within muscle is reduced, thus decreasing the number of parallel pathways within a network and increasing the resistance to blood flow in the face of chronic elevations in perfusion pressure (8, 28). Clearly, this decrease in skeletal muscle microvessel number can contribute to an increase in the resistance to blood flow in the microvascular network of the gastrocnemius muscle. Additionally, this reduction in the density of skeletal muscle microvessels is comparable to existing observations of microvessel rarefaction in experimental models of diabetes mellitus (7, 21) and may have significant implications for tissue oxygenation (15), especially during periods of elevated metabolic demand.

The third major observation of the present study was that, in a maximally relaxed microvascular bed perfused at increasing levels of volume flow, the resistance to perfusion, while not different at the two lowest perfusion rates, was elevated in OZR compared with calculated values in LZR. When integrated with the other observations presented in this study, these data support the hypothesis that the increased stiffness of the microvessel wall (Figs. 3 and 4), the reduced diameter of passive arterioles (Fig. 1), and the rarefaction of the skeletal muscle microvascular network can all contribute to an increased vascular resistance to perfusion in OZR versus LZR. It may be that the comparable levels of resistance calculated for the two lowest volume perfusion rates are the results of the rarefaction of the microvessel bed and/or the increased stiffness of individual resistance arterioles not being sufficiently severe to result in an increased resistance. In contrast, as perfusion levels increase to 1.5 and 2.0 ml/min, these structural alterations to the skeletal muscle microcirculation are of sufficient magnitude to significantly impede the flow of perfusate (or blood) to the skeletal muscle. One issue arising from the results of the present study was the lack of increased vascular resistance at low perfusion rates. At the two low flow rates, perfusion pressure was between 50 and 80 mmHg (Fig. 7A). However, these levels of pressure were associated with decreased arteriolar diameters in OZR versus LZR (Fig. 1). Because of narrowing of resistance arterioles, vascular resistance should have been elevated; a result that was not evident in the present study. Potential explanations for this similar-
Microvascular Remodeling in Zucker Rats

The author thanks C. A. Bobrowitz and G. R. Slocum for expert technical assistance.

This work was supported by American Heart Association Scientist Development Grant 0330194N, a Medical College of Wisconsin Faculty Development grant, and National Heart, Lung, and Blood Institute Grants HL-65289 and HL-29587.

REFERENCES


ity in vascular resistance between LZR and OZR at lower flow rates may due to the density of larger arterioles in the gastrocnemius muscles of OZR (not determined in the present study) or to an imperfect comparison between isolated pressurized arterioles under no-flow conditions and in situ perfused microvascular networks. A better understanding of similarities in vascular resistance between the gastrocnemius muscles of LZR and OZR perfused at a low flow rate will require additional study. For the present study, the gastrocnemius muscle mass was nearly identical between LZR and OZR. As such, it is extremely unlikely that differences in calculated vascular resistance stem from a relative over- or underperfusion of the gastrocnemius muscle from either strain rather than due to alterations in the structure of the microcirculation itself.

A previous study by Greene et al. (14) modeled the contribution of structural narrowing of microvessels and a reduction in microvessel density within a network on tissue vascular resistance during hypertension. The authors constructed a mathematical network model of the hamster cheek pouch microcirculation, based on Poiseuille flow and published data describing microvessel lengths, diameters, and connections of the feeding arterioles of the cheek pouch. By altering the density of third- and fourth-order arterioles (0%, 17%, and 42% rarefaction; randomly distributed) and/or the diameter of arterioles (0%, 10%, and 30% constriction; all vessels of a particular order), the authors demonstrated that a narrowing of the arterioles in question was an extremely potent contributor to the increased vascular resistance that develops with chronic hypertension. In addition, the authors also demonstrated that the reduction in microvessel density (rarefaction) that evolves with the chronic increases in perfusion pressure also contributes significantly to the increased tissue vascular resistance in hypertension, although not to the extent of structural narrowing. Thus it appears to be highly likely that the combination of both reduced microvessel density (20–25%) and the remodeling of individual arterioles reducing passive diameter (~30%) contributes to the increased vascular resistance to blood flow that develops in OZR. However, this effect does not appear to exert a significant influence under conditions of lower flow (0.5 and 1.0 ml/min) as calculated vascular resistance under these conditions was not different between LZR and OZR.

Functional implications. The key observation of the present study was that, with the development of syndrome X, the skeletal muscle microcirculation experiences a structural remodeling that leads to 1) a narrowing of individual resistance arterioles and an increased stiffness of the vessel wall, 2) a rarefaction of the microvascular network, and 3) an increased vascular resistance to perfusion with increased volume flow under conditions of a relaxed microcirculation. With regard to perfusion of the skeletal muscle microcirculation, these demonstrated alterations to the structure of individual microvessels and microvascular networks would have their most significant implications under conditions of microvessel dilation (e.g., during elevated metabolic demand). Increased vascular resistance during periods of elevated metabolic demand can impair hyperemic responses within the skeletal muscle and may have profound effects on the ability of the microcirculation to transport and exchange metabolic substrate and waste products with the surrounding skeletal muscle cells, thus compromising the ability of skeletal muscle to maintain performance.


