Effects of aging on vasoconstrictor and mechanical properties of rat skeletal muscle arterioles

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Muller-Delp, Judy, Scott A. Spier, Michael W. Ramsey, Lisa A. Lesniewski, Anthony Papadopoulos, J. D. Humphrey, and Michael D. Delp. Effects of aging on vasoconstrictor and mechanical properties of rat skeletal muscle arterioles. Am J Physiol Heart Circ Physiol 282: H1843–H1854, 2002.—Exercise capacity and skeletal muscle blood flow during exercise are reduced with advancing age. This reduction in blood flow capacity may be related to increased reactivity of skeletal muscle resistance vessels to vasoconstrictor stimuli. The purpose of this study was to test the hypothesis that aging results in increased vasoconstrictor responses of skeletal muscle resistance arterioles. First-order (1A) arterioles (90–220 μm) from the gastrocnemius and soleus muscles of young (4 mo) and aged (24 mo) Fischer-344 rats were isolated, cannulated, and pressurized via hydrostatic reservoirs. Vasoconstriction in response to increases in norepinephrine (NE; 1 × 10−9–1 × 10−4 M) and KCl (20–100 mM) concentrations and increases in intraluminal pressure (10–130 cmH2O) were evaluated in the absence of flow. Responses to NE and KCl were similar in both soleus and gastrocnemius muscle arterioles from young and aged rats. In contrast, active myogenic responses to changes in intraluminal pressure were diminished in soleus and gastrocnemius arterioles from aged rats. To assess whether alterations in the mechanical properties of resistance arterioles underlie altered myogenic responsiveness, passive diameter responses to pressure and mechanical stiffness were evaluated. There was no effect of age on the structural behavior (passive pressure-diameter relationship) or stiffness of arterioles from either the soleus or gastrocnemius muscles. These results suggest that aging does not result in a nonspecific decrease in vasoconstrictor responsiveness of skeletal muscle arterioles. Rather, aging-induced adaptations of vasoreactivity of resistance arterioles appear to be limited to mechanisms that are uniquely involved in the signaling of the myogenic response.

norepinephrine; potassium chloride; orthostatic hypotension; stiffness; myogenic response

EXERCISE PERFORMANCE declines with advancing age. This decline in exercise capacity is due, in part, to an age-related decrease in the functional ability of the cardiovascular system to provide oxygen to working muscles. Although previous research demonstrates that part of the decreased functional capacity of senescent individuals is due to a diminished ability of the heart to elevate cardiac output during exercise (20, 22, 30, 34), age-related changes in the mechanisms of local vascular control also appear to contribute to reduced blood flow in skeletal muscle during exercise.

Reductions in skeletal muscle blood flow during exercise and muscle stimulation have been reported in aged humans and animals. Irion and co-workers (28) evaluated in situ hindlimb muscle blood flow during electrical stimulation and found that the flow capacity was reduced in old rats. Wahren and colleagues (29, 51) showed in humans that the rise in leg blood flow during exercise was less in older male subjects. Recently, Proctor et al. (39) reported that leg blood flow and vascular conductance during submaximal cycling exercise at a given level of whole body oxygen consumption are lower in older men compared with their young counterparts. The reduction in skeletal muscle blood flow capacity could be due to alterations in the intrinsic vasomotor responsiveness of resistance arteries or due to changes in the mechanical or structural properties of the vessels. More specifically, greater responsiveness to vasoconstrictor stimuli, increased vessel stiffness, or a reduced maximal diameter of resistance arteries could limit the exercise hyperemia in muscles of older individuals. Therefore, the purpose of the present study was to determine whether myogenic or agonist-induced vasoconstriction, stiffness, or maximal diameter of skeletal muscle arterioles is different between young and old animals. We hypothesized that the vasoconstrictor responses and stiffness of skeletal muscle resistance arterioles from both the soleus muscle, which is composed predominantly of slow-twitch fibers (18), and the gastrocnemius muscle, composed predominantly of fast-twitch fibers (18), would be greater in aged Fischer-344 rats.

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METHODS

Animals

All procedures performed in this study were approved by the Texas A&M University Laboratory Animal Care Committee. All methods conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC, Revised 1996).

Forty young (4 mo old) and thirty-eight aged (24 mo old) male Fischer-344 rats were obtained from Harlan (Indianapolis, IN). The animals were housed in a temperature-controlled (23 ± 2°C) room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum.

Microvessel Preparation

The rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and euthanized by decapitation. The gastrocnemius-plantaris-soleus muscle group from each hindlimb was carefully dissected free and placed in cold (4°C) physiological saline solution (PSS) with 1 g/100 ml bovine serum albumin as previously described (13). First-order (1A) arterioles from the soleus muscle and the superficial white portion of the gastrocnemius muscle were isolated and removed from the surrounding muscle tissue. In soleus muscle, 1A arterioles were defined as the first branch that occurred after the feed artery had entered the muscle tissue. In gastrocnemius muscle, 1A arterioles were defined as the first branch off the feed artery that runs over the superficial portion of the muscle. The arterioles (0.5–1.0 mm in length, 90–220 μm in inner diameter) were transferred to a Lucite chamber containing PSS equilibrated with room air. Each end of the arteriole was cannulated with a micropipette (60- to 80-μm diameter tip) and secured with sutures (Alcon 11-0 nylon monofilament). After cannulation, the microvessel chamber was transferred to the stage of an inverted microscope equipped to measure and record arteriolar intraluminal diameter (13). Arterioles were initially pressurized to 60 cmH2O with two independent hydrostatic pressure reservoirs.Leaks were detected by pressurizing the vessel, closing the valves to the reservoirs, and then verifying that intraluminal pressure remained constant. Arterioles that exhibited leaks were discarded. Arterioles that were free from leaks were warmed to 37°C.

Experimental Design

To determine whether vasoconstritor responsiveness, stiffness, or maximal diameter of skeletal muscle arterioles is altered by old age, three series of experiments were performed. Each of the series involved isolation and cannulation of 1A arterioles from the soleus and gastrocnemius muscles. One arteriole from the soleus muscle and one arteriole from the gastrocnemius muscle were studied from each animal. In the first series of experiments, spontaneous tone development, active myogenic response, and maximal inner arteriolar diameter were determined. In the second series of experiments, KCl and norepinephrine (NE) responses were sequentially determined, and intraluminal maximal diameter was measured. Finally, in the third series of experiments, the passive pressure-response relationship and maximal diameter were determined. The vessels were then fixed and sectioned for measurement of wall thickness (WT) and vessel cross-sectional area (CSA) to determine whether the stress/strain relationship (stiffness) is altered with old age.

Series 1: evaluation of myogenic response. Vessels were equilibrated at 37°C and 60 cmH2O for 60 min, which allowed for the development of spontaneous tone. After equilibration, intraluminal pressure was increased in increments of 10 cmH2O up to 130 cmH2O, decreased from 130 to 10 cmH2O, and finally raised back up to 60 cmH2O. After each step change in intraluminal pressure, diameter was recorded continuously for 5 min. All pressure changes occurred in the absence of intraluminal flow.

To determine maximal diameter at 60 cmH2O, the vessel chamber and pressure lines were filled with calcium-free PSS containing 2.0 mM EDTA. Arterioles were rinsed every 15 min during a 60-min period to facilitate complete relaxation of the arteriolar smooth muscle. In a subset of arterioles, 100 μM sodium nitroprusside was also added to the bathing solution. The diameter of vessels in calcium-free PSS did not increase further when supplemented with sodium nitroprusside.

Series 2: evaluation of vasoconstrictor responses to pharmacological agents. A concentration-response relationship to the non-receptor-mediated vasoconstrictor agent KCl was established by measuring changes in vessel diameter that occurred in response to cumulative additions of KCl (20–100 mM) to the vessel bath. Results from the first series of experiments demonstrated that soleus muscle arterioles from aged animals developed less spontaneous tone than those from young rats. To avoid the potential problem of comparing agonist-induced vasoconstrictor responses between groups having different levels of baseline tone, KCl (and NE) responses were initiated before the development of significant spontaneous tone, i.e., the level of spontaneous tone was minimal and similar between arterioles from all groups. After each addition of the vasoconstrctor agonist, diameter was monitored continuously until a steady-state constriction was recorded.

To determine the vascular reactivity to a receptor-mediated vasoconstrctor agent, the adrenergic agonist NE was used. Diameter changes that occurred in response to cumulative additions of NE (1 × 10⁻³–1 × 10⁻⁴ M) to the bathing solution were recorded continuously after each dose. At the end of the experiment, maximal diameter was determined in calcium-free PSS as described above.

Series 3: evaluation of passive pressure-diameter relationship and stiffness. Because alterations in the mechanical or structural properties of arteries can affect active myogenic vasoconstriction, passive pressure-diameter relationships and stiffness were determined in arterioles from young and old animals. Previous investigation of arteries from normotensive and hypertensive animals indicates that the structural behavior of a vessel segment may remain unchanged despite an alteration in the material properties of the vascular wall (40). Therefore, the passive pressure-diameter relationship and measures of stiffness were used to assess the structural behavior and material properties of the arterioles, respectively. Arterioles equilibrated at 37°C for 60 min with the vessel chamber and pressure lines filled with calcium-free PSS containing 2.0 mM EDTA. The arterioles were rinsed every 15 min during the 60-min period to induce complete vasorelaxation. The intraluminal pressure was then lowered to 0 cmH2O, and a pressure-diameter relationship was established by increasing pressure in increments of 10 cmH2O up to 130 cmH2O. The inner diameter was recorded continuously for 3 min after each step increase in pressure. After the last pressure step, intraluminal pressure was returned to 60 cmH2O for 5 min. The vessel was then fixed with Bouin’s solution, stained with eosin, and embedded in paraffin. Paraffin-embedded vessels were cut into 5-μm-thick cross sections, mounted on glass microscope slides, and stained with eosin and hematoxylin. The medial CSA of arterioles was measured as previously described (17).
Medial wall thickness at each pressure step was then calculated according to the following equation

\[ V = \pi ((ID + 2WT)^2 - ID^2)L = \pi (ID_o + 2WT)^2 - (ID_o)^2L \]  

where \( V \) is wall volume (a constant), \( ID \) is inner diameter at any given pressure other than zero, \( WT \) is wall thickness, \( L \) is vessel segment length, \( ID_o \) is original diameter measured at 0 cmH2O, and \( WT_o \) is original wall thickness measured at 0 cmH2O. The validity of estimating WT at intraluminal pressures other than 60 cmH2O is based on the assumption that wall volume remains constant with changes in diameter. This assumption has been verified for isolated cheek pouch muscle arterioles (11, 52).

Both circumferential Cauchy (\( \sigma \)) and circumferential second Piola-Kirchhoff stresses (\( S_\text{c} \)) (6, 26) were calculated from intraluminal pressure (\( IP \)), inner diameter, and wall thickness in the following manner

\[ \sigma = (IP \times ID)/(2WT) \]  

and

\[ S_\text{c} = \sigma_s \times \left[1/(\lambda_o)^2 \right] \]

where \( \lambda_o \) is the principal stretch (extension) ratio in the circumferential direction, which equals current inner radius (\( R \)) divided by initial inner radius (\( R_o \)). In these arterioles, \( \lambda_o \) can be accurately represented by measurements of the inner radius because the wall thickness is minimal, i.e., on average, wall thickness is \( \approx 4\% \) of outer diameter. Because the deformations of the arterioles are large, principal stretch ratios were appropriate measures for this study (6). The Cauchy stress, or true stress, is defined as the actual force acting on an oriented differential area in the current (deformed) configuration and is related to the principal stretch ratio (\( \lambda \)). The second Piola-Kirchhoff stress, on the other hand, is defined as a theoretical force acting on an oriented differential area in the reference (undeformed) configuration and is conjugate to the Green’s strain (\( E \)), where conjugate indicates that the stress can be determined directly from an energy function by differentiating with respect to the conjugate measure of deformation (26). The circumferential Green’s strain is calculated in the following manner

\[ E = \frac{1}{2}(\lambda^2 - 1) \]

Stiffness can be determined from either the stress/strain relationship or the stress/stretch relationship. In the present study, incremental stiffness was calculated as the change in stress/change in strain for each datum point. Incremental stiffness points were plotted as a function of their corresponding stress points, and linear regression analysis was used to calculate the slope of that relation (see Fig. 6, A and B). The slope was then used as a measure of the overall relative stiffness for each arteriole.

**Data Analysis**

The development of spontaneous tone was expressed as the percent constriction relative to maximal diameter and was calculated as follows

\[ \text{Spontaneous tone (in %)} = (ID_{\text{max}} - ID_\text{b})/ID_{\text{max}} \times 100 \]

where \( ID_{\text{max}} \) is the maximal inner diameter recorded at a pressure of 60 cmH2O and \( ID_b \) is the steady-state baseline diameter. Active myogenic responses and passive diameter measurements recorded in response to pressure changes were normalized according to the following formula

\[ \text{Normalized diameter} = (ID_b / ID_{\text{max}}) \]

where \( ID_b \) is the steady-state diameter measured after each incremental pressure change. The data are expressed as normalized diameter to account for differences in vessel size between young and old animals. Vasoconstrictor responses to KCl and NE were expressed as the percent change from baseline diameter according to the following formula

\[ \text{Constriction (in %)} = (ID_b - ID_s)/ID_b \times 100 \]

where \( ID_s \) is the initial baseline diameter recorded immediately before the addition of the vasoconstrictor agonist and \( ID_b \) is the steady-state diameter measured after each dose of the drug. Two-way repeated-measures ANOVA was used to detect differences between (young vs. old or soleus vs. gastrocnemius) and within (drug concentration or pressure level) factors. Post hoc analyses were performed using Schef-fee's test for pairwise comparisons. Differences in animal weight between old and young groups were compared with t-tests. All data are presented as means ± SE. In all statistical analyses, \( n \) is the number of animals from which vessel responses were measured. Significance was defined as \( P \leq 0.05 \).

**RESULTS**

**Animals**

Body weight was significantly greater with old age. Young rats weighed 348 ± 6 g and aged rats weighed 420 ± 7 g.

**Characteristics of Isolated Vessels**

Vessel characteristics are reported in Table 1. Maximal inner diameters of arterioles from the soleus muscle ranged from 59 to 214 μm in young animals and from 80 to 213 μm in aged animals, with no differences between age groups. Maximal inner diameters of arterioles from

<table>
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<th>Table 1. Characteristics of first-order arterioles from the soleus muscle and the superficial portion of the gastrocnemius muscle</th>
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<tr>
<td><strong>Soleus Muscle</strong></td>
</tr>
<tr>
<td>Maximal diameter, μm</td>
</tr>
<tr>
<td>Young</td>
</tr>
<tr>
<td>128 ± 7 (n = 29)</td>
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<tr>
<td>Wall thickness, μm</td>
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<tr>
<td>4.6 ± 0.3 (n = 12)</td>
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<tr>
<td>Medial cross-sectional area, μm²</td>
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<td>1,318 ± 227 (n = 11)</td>
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<tr>
<td>Spontaneous tone, %</td>
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<tr>
<td>52 ± 3 (n = 8)</td>
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Values are means ± SE; \( n \) = no. of animals. †Significant difference between young and old groups (\( P < 0.05 \)); ‡significant difference between soleus and gastrocnemius muscle arterioles (\( P < 0.05 \)).
the superficial gastrocnemius muscle of young rats ranged from 108 to 196 μm and in aged rats from 122 to 214 μm; maximal diameter of the old animals tended to be larger (P = 0.06) than that of the young group. The average diameter of soleus muscle 1A arterioles was significantly less than the diameter of gastrocnemius muscle 1A arterioles in both age groups.

The level of development of spontaneous tone at 60 cmH₂O varied with age and muscles (Table 1). Arterioles from the soleus muscle of young animals developed more tone than those from aged animals. Average tone development in arterioles from the gastrocnemius muscle did not differ between young and old animals (P = 0.12). Finally, arterioles from the soleus muscle displayed greater spontaneous tone than arterioles from gastrocnemius muscle (P < 0.05).

**Myogenic Responses**

Figure 1 illustrates active and passive pressure-diameter relationships as intraluminal pressure was increased stepwise from 10 to 130 cmH₂O. Arterioles from both the soleus and gastrocnemius muscles of young and old rats displayed active myogenic constriction. In addition, active responses of arterioles from both the soleus and gastrocnemius muscles from aged rats were significantly less than those of arterioles from young rats and more closely resembled passive responses to increasing pressure. The differences between the active responses of arterioles from young and old animals were present either when pressure was increased or decreased from 10 to 130 cmH₂O. No significant hysteresis was detected in the active responses to pressure in any group.

Arterioles from the soleus muscle displayed significantly greater myogenic responses than arterioles from the gastrocnemius muscle in both young and old animals.

**Vasoconstrictor Responses to Pharmacological Agents**

There were no age-related or muscle type-related differences in the vasoconstrictor responses to pharmacological agents. Both sensitivity (EC₅₀) and maximal...
constriction to KCl and NE were similar in arterioles from young and old animals and from the soleus and gastrocnemius muscles (Figs. 2 and 3).

Pressure-Diameter Relationship and Stiffness

Passive changes in diameter that occurred as pressure was increased from 0 to 130 cmH2O were not altered by age in arterioles from either the soleus or gastrocnemius muscle (Fig. 1). Stress/stretch analysis indicated that the mechanical behavior of arterioles from young and old animals did not differ once a minimal load (10 cmH2O intraluminal pressure) was placed on the vessels. If the initial, undeformed radius used to calculate stretch was defined as the radius measured when intraluminal pressure was zero, a separation was present in the stress/stretch relationships for arterioles from young and old animals (Figs. 4A and 5A). If the stress/stretch relationship was established using the radius measured when intraluminal pressure was set at 10 cmH2O, this difference in the curves was eliminated (Figs. 4B and 5B). Linear regression analysis and calculation of the slope of the incremental stiffness-stretch relation in each vessel indicated that this measure of nongeometric stiffness of arterioles (for calculations, see Fig. 6, A and B) tended to be higher in soleus muscle arterioles from old rats (young: 2.45 ± 0.60 dyn/cm²; old: 3.78 ± 0.54 dyn/cm², P = 0.10) but was not different in arterioles from the gastrocnemius muscle (young: 3.69 ± 0.67 dyn/cm²; old: 3.57 ± 0.57 dyn/cm², P = 0.38). Additionally, this measure of stiffness was not different between arterioles from the soleus and gastrocnemius muscles in either young or old rats.

DISCUSSION

Previous work has demonstrated that skeletal muscle hyperemia during exercise is reduced with old age.
The purpose of the present study was to test the hypotheses that with old age, skeletal muscle arterioles are more reactive to vasoconstrictor agents or transmural pressure changes; are passively more resistant to distension, i.e., they are stiffer; and have structurally remodeled so that maximal diameter is smaller. Six main findings emerge from this investigation. First, aging reduces rather than increases myogenic responses of 1A arterioles from both the gastrocnemius and soleus muscles. Second, vasoconstrictor responses to the pharmacological agents KCl and NE are not altered by age in 1A arterioles from either muscle type. Third, wall thickness increases with age in soleus muscle arterioles, whereas maximal diameter of 1A arterioles from the soleus and gastrocnemius muscles is not altered by old age. Fourth, the functional mechanical properties and material properties (i.e., the passive pressure-diameter relationship and stiffness) of resistance arterioles from the soleus and gastrocnemius muscles are similar in young and old rats. Fifth, spontaneous tone development and myogenic activity differ between arterioles from the soleus muscle, a highly oxidative postural muscle, and the highly glycolytic portion of the gastrocnemius muscle (18). Finally, the mechanical distensibility (stretch) of skeletal muscle arterioles exceeds that of other arterial vessels reported in the literature.

The effects of aging on the intrinsic responsiveness of arteries to vasoconstrictor stimuli appear to vary along the arterial vascular network and between tissues. For example, in the rat aorta, maximal tension produced by NE, vasopressin, and KCl decreases with aging (16). In human subcutaneous resistance arteries, it has been reported that maximal constrictor responses to α-adrenergic agents are also decreased by aging (38). In contrast, Cook et al. (7) reported that NE-induced vasoconstriction was not altered in resistance arterioles from the cremaster muscle (a striated muscle that does not have skeletal connections) of aged rats. In the present study, we were specifically interested in

![Fig. 3. A: concentration-response relationship of soleus muscle arterioles from young (n = 7) and old rats (n = 7) to norepinephrine (NE). B: concentration-response relationship of gastrocnemius muscle arterioles from young (n = 8) and old rats (n = 11) to NE. Vasoconstrictor responses to NE were not different between young and old animals, and no differences in responsiveness to NE were detected between arterioles from the soleus and gastrocnemius muscles.]
evaluating the effects of age on vasoconstrictor responses of resistance arterioles from skeletal muscle. We found that the responses to both NE, which causes contraction through receptor binding and activation of an intracellular second messenger system to increase intracellular Ca\(^{2+}\) in smooth muscle, and KCl, which acts through a receptor-independent mechanism to increase intracellular Ca\(^{2+}\), were similar in arterioles from young and old rats. However, responses to transmural pressure changes (the myogenic response) were blunted by old age. This differential effect of aging on vasoconstrictor responses to pharmacological agents and the myogenic response suggests that, unlike the general blunting of vasoconstrictor responses reported in the aorta of senescent rats (16), aging-induced adaptations in skeletal muscle arterioles stem from a change in mechanisms uniquely involved in the myogenic response. Therefore, changes in components of the signaling pathways that are common to KCl-, NE-, and pressure-induced constriction, such as Ca\(^{2+}\) entry through voltage-gated channels, Ca\(^{2+}\) release from the sarcoplasmic reticulum, and contractile protein activity (9, 10, 33, 35, 36), are unlikely to have been altered by aging in these vessels.

One means through which myogenic responsiveness can be altered is via a change in vascular structure. For example, in rats where the hindlimb has been unloaded via tail suspension, there is a diminished myogenic responsiveness of gastrocnemius muscle arterioles to incremental changes in transmural pressure (13); this myogenic alteration is paralleled by a structural remodeling of the arteriole, i.e., smooth muscle atrophy and thinning of the medial layer (17). In the hindlimb unloading model, the thinning of the arteriolar media blunted both the myogenic response and vasoconstriction induced by pharmacological agents.

Fig. 4. Relationship between circumferential stress and circumferential stretch in isolated first-order (1A) arterioles from the soleus muscle of young \(n = 11\) and old rats \(n = 11\). A: circumferential stretch \((r/R)\), where \(r\) is the current inner radius and \(R\) is the initial inner radius) was calculated using the radius measured when intraluminal pressure was set at 0 cmH\(_2\)O as the undeformed radius \((R)\). B: circumferential stretch was recalculated using the radius measured when intraluminal pressure was set at 10 cmH\(_2\)O as the undeformed radius \((R)\).
Therefore, if gross structural changes were responsible for the blunted myogenic responsiveness that occurs with old age, a reduction of all vasoconstrictor responses might be expected. Instead, aging selectively impaired the myogenic response, and this reduction in the myogenic responsiveness was accompanied by an increase in arteriolar wall thickness. These results suggest that the impaired myogenicity of skeletal muscle resistance arterioles from aged rats does not result from gross structural changes in the vascular smooth muscle but may be due to an alteration of the relative composition of the vascular wall, i.e., changes in collagen isoforms or extracellular matrix proteins.

To determine whether the change in the myogenic response was related to a change in the structural behavior or the intrinsic material properties of arterioles from aged animals, we recorded passive diameter in response to pressure changes and calculated vascular stiffness. The data indicate that the structural behavior of these vessels remains constant with age (Fig. 1). Furthermore, the data indicate that aging did not alter the intrinsic material properties of either soleus or gastrocnemius muscle arterioles (Figs. 4B and 5B). The shift in the stress/stretch relationship that occurred (Figs. 4A and 5A) may indicate that the vessels do change with age, resulting in a resetting of this relationship. The wall thickness of soleus muscle arterioles was significantly increased in aged animals. As a result of this increase, the maximal stress recorded in soleus muscle arterioles was reduced compared with arterioles from young animals. Similarly, the maximal stress recorded in gastrocnemius muscle arterioles was lower than that recorded in soleus muscle arterioles because of the difference in wall thick-
A measure of nongeometric material stiffness. If nonlinear regression analysis revealed an $R^2$ value $>0.9$, incremental stiffness ($\Delta s_0/\Delta \lambda_0$) was calculated from the data points. B: incremental stiffness plotted versus Cauchy stress for the same vessel. Linear regression analysis was used to calculate the slope of the incremental stiffness versus stress relation, a measure of nongeometric material stiffness.

Data from the present study suggest that arterioles from soleus muscle do undergo some remodeling with age, but this does not impact the mechanical response of the vessels as pressure (stress) increases. Therefore, the decrease in the myogenic tone in skeletal muscle arterioles is likely due to an impairment of a pressure-sensitive signaling mechanism (possibly linked to a change in the composition of the vascular wall) but not directly related to a structural impairment of the vessel wall.

Another means through which myogenic responsiveness could be blunted with old age is through alterations in the vascular endothelium. For example, an increased release of endothelium-derived vasodilator substances in arterioles from old animals could function to attenuate active constrictor responses to increases in transmural pressure. Preliminary evidence from arterioles of the soleus and gastrocnemius muscles, however, does not support such a contention (45, 46). We have found that neither acetylcholine nor flow-induced vasodilation is increased in arterioles from old rats; on the contrary, these endothelium-dependent vasodilatory responses are diminished with aging. Although these preliminary results do not definitively exclude the involvement of endothelium in the diminished myogenic responsiveness, they do suggest it is not likely the result of an enhanced release of an endothelium-derived vasodilator substance.

Finally, it is possible that aging-induced alterations in skeletal muscle fibers could modify the chemical milieu surrounding the resistance vasculature to provide a stimulus for vascular adaptation. For example, there is evidence in human skeletal muscle that aging alters both the fiber composition and oxidative capacity of muscle (25). In the aging rat, there does not appear to be significant alterations in muscle fiber composition (1, 5), particularly in the soleus muscle, but there are age-related reductions in the oxidative capacity of slow-twitch and fast-twitch muscles (47). Thus one could speculate that age-induced alterations in skeletal muscle metabolism could alter metabolite release and correspondingly influence the intrinsic myogenic responsiveness of vascular smooth muscle.

Evidence from rats and human subjects suggests that skeletal muscle blood flow capacity is reduced by old age (28, 39, 51). In contrast to our hypothesis that increased reactivity of skeletal muscle arterioles to vasoconstrictor agents could contribute to the phenomenon of reduced skeletal muscle blood flow, we found that vasoconstrictor responses to KCl and NE were unchanged and that the myogenic response was reduced in aged rats. Although these results do not appear to explain the reduction in blood flow capacity that occurs with advancing age, impaired myogenic responses of skeletal muscle resistance arterioles may be important in determining overall control of skeletal muscle blood flow in the elderly. The myogenic response has been postulated to contribute to the maintenance of constant blood flow and capillary hydrostatic pressure during changes in arterial pressure (11, 21). For example, during the assumption of an upright posture in humans, myogenic constriction of resistance arterioles buffers against an increase in capillary hydrostatic pressure in the lower extremities and contributes to an increase in total peripheral resistance (21, 27). Approximately 20% of the increase in total peripheral resistance during orthostasis occurs as a result of skeletal muscle vasoconstriction (41). Thus an age-induced blunting of the myogenic responsiveness of skeletal muscle arterioles could impact the ability of skeletal muscle to increase vascular resistance and, therefore, overall peripheral vascular resistance. This is consistent with observations that the mechanisms that mediate increases in peripheral vascular resistance in response to head-up tilt and orthostatic challenges are altered in the elderly (12, 37, 43, 44, 48).
Furthermore, it has been reported that older subjects increase splanchnic resistance to a greater extent but demonstrate less of an increase in skeletal muscle vascular resistance in response to head-up tilt (37, 43, 48). Thus a diminished myogenic vasoconstrictor responsiveness of the rodent skeletal muscle resistance vasculature with aging is consistent with these observations in humans.

The results of this study also indicate that the intrinsic ability of blood vessels to respond to changes in transmural pressure is differentially regulated in resistance arterioles from muscles composed of different fiber types, e.g., the highly oxidative soleus muscle and the glycolytic superficial portion of the gastrocnemius muscle. In contrast, constrictor responses to KCl and NE were similar in arterioles from these two muscle types. In addition to the oxidative capacity and fiber composition (18), the recruitment order (2, 3, 18) and blood flow patterns at rest and during exercise (2, 14, 32) vary between these two muscle types. Any of these differential characteristics could influence the intrinsic responses of the resistance arteries within the muscle. Laughlin and Armstrong (31) showed that in conscious rats, α-adrenergic blockade increased blood flow to fast-twitch muscle but did not change blood flow to slow-twitch muscle. Delp and Armstrong (14) have shown that under resting conditions, blood flow is much greater in the soleus muscle compared with the white portion of the gastrocnemius muscle; however, upon denervation, blood flow to the white gastrocnemius muscle increases, whereas blood flow to the soleus muscle decreases. These findings suggest that under normal resting conditions, higher adrenergic tone is present in the white portion of the gastrocnemius muscle, composed of fast-twitch fibers, whereas blood flow to highly oxidative soleus muscle is predominantly under metabolic control. The difference in tonic adrenergic input between these two muscles may influence the myogenic responsiveness of the resistance vasculature, resulting in a greater development of a myogenic mechanism in the soleus muscle, where adrenergic tone is less dominant.

Results of the present investigation indicate the intrinsic material properties of skeletal muscle arterioles are unique compared with other arterial vessels. This is based on the findings that the stretch ratios measured in the skeletal muscle arterioles (Figs. 4 and 5) are greater than those reported for conduit arteries (8, 40) and cerebral arterioles (24). We presume that the variation in arteriolar distensibility between vascular beds is functionally important and reflects differences in the perfusion demand among organs. For example, exercise represents a significant metabolic stress for both the brain (15, 49) and skeletal muscle (2, 4, 42). In going from rest to high intensity exercise, brain blood flow increases ~20–40 ml·min⁻¹·100 g⁻¹, which represents approximately a 25–50% increase in perfusion (15, 49). In contrast, skeletal muscle perfusion is elevated up to 200–500 ml·min⁻¹·100 g⁻¹ above that at rest, a 10 to 20-fold increase (2, 4, 42). Although there are a number of mechanisms through which the skeletal muscle vasculature can accommodate such high flow rates during exercise (19), a high stretch ratio of skeletal muscle arterioles appears to be a necessary feature.

In addition to higher stretch ratios relative to other arteries, the stretch ratios for skeletal muscle arterioles in the present study are greater than what can be estimated from pressure-diameter relationships previously published for isolated cheek pouch (11) and isolated gracilis muscle arterioles (50). These differences may be related to differences in the resting vessel length established by the investigator. In the present study, arterioles were pressurized at 60 cmH₂O and then stretched longitudinally to a length at which no bending of the vessel was evident. Images published by Davis and Gore (11) indicate that similar vessel lengths were used in the study of isolated cheek pouch arterioles; however, the resting length of gracilis muscle arterioles was not published (50). Differences in the stretch ratios of skeletal muscle arterioles among the present and previously published studies may also be attributable to our use of an undeformed vessel radius measured at an intraluminal pressure of 0 cmH₂O (Figs. 4A and 5A). If arteriolar diameter at an intraluminal pressure of 10 cmH₂O is used as the undeformed radius in calculating arteriolar distensibility (the lowest pressure previously used to establish a pressure-diameter relation for skeletal muscle arterioles (11, 50)), then the stretch ratios of skeletal muscle arterioles from the present study (Figs. 4B and 5B) are virtually identical to those that can be estimated from these previous reports. Therefore, these data suggest that the stretch ratio of skeletal muscle arterioles may be fairly uniform across muscles when a true undeformed vessel radius is determined and that skeletal muscle arteriolar distensibility is greater than that of conduit arteries and arterioles in other tissue.

In summary, the results of the present study demonstrate that neither myogenic, KCl, nor NE vasoconstrictor responses are enhanced in skeletal muscle 1A arterioles with aging. Rather, these data demonstrate that myogenic responses of isolated resistance arterioles from both the soleus and the superficial portion of the gastrocnemius muscles are impaired in aged Fischer rats. This impairment of the myogenic response is not a result of a generalized reduction of all vasoconstrictor responses in these resistance arterioles, because responses to KCl and NE were similar between young and aged rats. In addition, the reduction of the myogenic response is not a consequence of altered stiffness in arterioles from either soleus or gastrocnemius muscles. These findings suggest that aging specifically alters mechanisms that are critical to transduction of the myogenic response. Such an alteration in the myogenic responsiveness of the resistance vasculature of skeletal muscle may impact the ability of the aged skeletal muscle vasculature to elevate resistance during varying physiological challenges, such as during the assumption of an upright posture. Results from the present study also demonstrate that spontaneous tone development and myogenic activity
are greater in arterioles from the soleus muscle, a highly oxidative postural muscle, than in resistance vessels from the highly glycolytic, low-oxidative portion of the gastrocnemius muscle (18). Finally, the mechanical distensibility of skeletal muscle arterioles exceeds that of other arterial vessels previously reported in the literature.

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