Effect of Dietary Salt on the Skeletal Muscle Microvasculature in Dahl Rats

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The purpose of this study was to identify microvascular alterations that could contribute to increased peripheral vascular resistance in the Dahl salt-sensitive rat with salt-induced hypertension. Intravital microscopy was used to study the spinotrapezius muscle arteriolar network in anesthetized salt-sensitive rats fed either a high salt (7% sodium chloride) or low-normal salt (0.45% sodium chloride) diet for 4 weeks. Age-matched Dahl salt-resistant rats on high and low-normal salt diets served as controls. The high salt diet had no effect on arterial pressure in salt-resistant rats but increased arterial pressure in salt-sensitive rats. Mean resting diameter of arcade arterioles in salt-sensitive rats on high salt diet was reduced by 25% compared with salt-sensitive rats on low salt or salt-resistant rats on either diet. After abolition of vascular tone with 10⁻³ M adenosine, arcade diameters were comparable in all groups. No difference among groups was found in either resting or passive diameter of the more distal transverse arterioles. Measurement of vessel lengths and numbers in cleared muscle specimens revealed no differences among groups in the anatomic density of either arcade or transverse arterioles. These data suggest that a reduction in the resting diameter of arcade, but not transverse, arterioles may increase spinotrapezius muscle vascular resistance in hypertensive salt-sensitive rats. The similarity in vascular densities among groups indicates that structural rarefaction of arterioles does not contribute to any increase in spinotrapezius muscle resistance at this stage of salt-induced hypertension. (Hypertension 1990;15:420-426)

Between 30 and 50% of human patients with essential hypertension are salt-sensitive (i.e., increasing or decreasing dietary NaCl intake influences the severity of hypertension. Through selective breeding, Dahl and colleagues developed two rat strains that differ in their susceptibility to the hypertensinogenic effect of NaCl: a salt-sensitive strain (DS) that becomes hypertensive on a high NaCl diet, and a salt-resistant strain (DR) that does not. Since its introduction, the DS rat has been widely studied as a model of human salt-sensitive hypertension.

Similar to other forms of hypertension, the arterial pressure rise elicited by high salt intake in the DS rat is associated with an increase in total peripheral resistance. Consistent with this finding are reports that a progressive increase in hindquarters vascular resistance accompanies the development of hypertension in DS rats. However, little is known about this increase or the particular vessels involved. In other models of hypertension, observation with intravital microscopy has revealed microvascular alterations that contribute to elevated peripheral resistance. In the spontaneously hypertensive rat (SHR) with established hypertension, several studies indicate that there is a reduced number of perfused arterioles in resting skeletal muscle. This arteriolar rarefaction may occur either through a reversible closure of existing vessels (functional rarefaction) or a permanent loss of vessels from the network (structural rarefaction). In addition, some investigators have reported that resting arteriolar diameters are reduced in SHR skeletal muscle, although others have not found this to be the case.

Although less extensively studied, the skeletal muscle microvasculature in rats with deoxycorticosterone acetate (DOCA)–salt and one-kidney, one clip hypertension show important differences from SHR (and from each other) in the extent to which various structural and functional modifications arise during hypertension. Therefore, the specific microvascular abnormalities that develop in a given vascular bed...
may differ with the form of hypertension studied. In contrast to the above models of hypertension, there is little information available on microvascular characteristics in the DS rat.

The aim of this study was to identify specific functional and structural changes within the arteriolar network that could contribute to increased peripheral resistance in the hypertensive DS rat. Intravital microscopy was used to study the arteriolar network of the spinotrapezius muscle in DS and DR rats on high (7%) and low-normal (0.45%) NaCl diets. Microvascular alterations related to increased vascular tone and structural reorganization of the network were investigated.

Methods

Weanling male DR and DS rats (Brookhaven type) averaging 28 days of age were obtained from Harlan Sprague Dawley (Indianapolis, Indiana) and immediately placed on a natural grain diet containing 0.45% NaCl by weight (TD8831, Teklad, Madison, Wisconsin). After 1 week, one half of the rats of each strain were placed on a 7% NaCl diet with the other half remaining on the 0.45% NaCl diet. The 7% NaCl diet was made by supplementing the Teklad diet with additional NaCl.

Rats from all four experimental groups (DR-0.45% NaCl, DR-7% NaCl, DS-0.45% NaCl, and DS-7% NaCl) were studied between 4 and 5 weeks after initiation of the 7% NaCl diet. All rats were anesthetized with sodium thiopental (100 mg/kg, i.p.), and supplemental anesthetic (20% of initial dose) was administered intramuscularly if needed during the experimental period. The rat was placed on a heating mat to maintain a 37°C rectal temperature, and the trachea was intubated to ensure a patent airway. The right carotid artery was then cannulated for the direct measurement of systemic arterial pressure with a Gould P23ID pressure transducer (Cleveland, Ohio). The right spinotrapezius muscle was surgically prepared for microscopic observation as described by Gray21 and modified by Lash and Bohlen.22 Throughout the surgery and subsequent experimental period, the muscle was superfused with a bicarbonate-buffered physiological solution (119 mM NaCl, 25 mM NaHCO₃, 6 mM KCl, 3.6 mM CaCl₂) warmed to 35° C and equilibrated with a mixture of 95% N₂, 5% CO₂ (pH=7.35−7.40). The rat was positioned on its left side, and the muscle was extended ventral surface up over a transparent pedestal with Dow-Corning stopcock grease (Corning, New York). The superfusion solution was then directed through the reservoir at a flow rate of 4−6 ml/min to prevent equilibration with atmospheric oxygen. Measurements made with oxygen microelectrodes indicate that at this flow rate, sulfate PO₂, immediately above the muscle surface averages 14 mm Hg.14

The muscle was transilluminated with a 150 W halogen fiber-optic light source (American Volpi, Auburn, New York), and its microvasculature was observed with an Olympus BHMP intravital microscope (Hyde Park, New York) fitted with a Panasonic Newvicon video camera (Secaucus, New Jersey). Video images were displayed on a Panasonic high resolution television monitor and stored on videocassette tape for off-line analysis. All observations were made with a ×10 eyepiece and a Leitz ×25 water immersion objective (numerical aperture=0.60; Leitz-Wetzlar, Wetzlar, FRG), producing a final video magnification of ×1,940. The magnification of the videomicroscope was calibrated after each experiment by recording the image of a stage micrometer marked at 10 μm intervals. Arteriolar inner diameters were measured by ruler directly from the video screen during later tape playback.

Vessels chosen for study were classified according to location within the arteriolar network. In the rat spinotrapezius muscle, the largest arterioles and their immediate branches anastomose to form an arcading network that extends throughout the muscle. Arising directly from the arcade arterioles are the transverse arterioles, which do not anastomose with collateral vessels and whose branches perfuse discrete regions of tissue. In distinction to the arcade network, a transverse arteriole and all of its distal branches are collectively referred to as a transverse network. Vessels within the transverse network were assigned branch orders according to the method of Strahler.23 By this method, a terminal arteriole giving direct rise to capillaries is classified as a first-order arteriole. Two first-order arterioles meet to form a second-order arteriole, and this pattern continues proximally with two like-order vessels meeting to form a vessel of the next highest order. When two vessels of different branch order meet, the parent segment is assigned the higher order.

Experimental Protocol

After a postsurgical equilibration period of 45−60 minutes, a portion of the arteriolar network in the central region of the muscle was scanned, and a number of arcade and transverse arterioles were randomly selected for study. A 1-minute video recording of each vessel was made for off-line measurement of internal diameter. After completion of the scan sequence, vascular tone was abolished by adding adenosine to the superfusion solution to achieve a final concentration of 10⁻³ M, and the scan sequence was repeated. The maximally dilated vascular bed was then filled with Microfil (Canton Bio-Medical Products, Boulder, Colorado) to permit the identification and mapping of all vascular segments for analysis of network architecture. During continued exposure of the muscle to adenosine, the
abdominal aorta was cannulated and the animal's blood isovolumetrically replaced with a heparinized electrolyte solution containing 0.04 mg/ml papaverine HCl to maximally dilate the peripheral vasculature. The displaced blood was drained through a cut in the abdominal vena cava. A 10% buffered formaldehyde solution (formalin) was then infused to fix the vasculature in situ, followed by Microfil infusion. The formalin and Microfil infusions were made at a pressure equivalent to the animal's mean arterial pressure. The muscle was then removed and secured at its in situ length in a Petri dish by ligatures embedded in a perimeter of modeling clay. After dehydration in progressively increasing concentrations of ethyl alcohol, the muscle was cleared with methyl salicylate.

Data Analysis
The cleared muscle specimen was photographed with a 35 mm camera at ×40 magnification, and the individual prints were assembled into a montage of the vascular bed. The exact magnification of the photomontage was established by photography of a stage micrometer under the same conditions as the muscle specimen. The entire arcade arteriole network was then traced onto an acetate sheet overlying the montage. Muscle specimens with incomplete network filling were excluded from analysis. The identification of all arcade segments in the montage was verified through repeated comparison with the cleared specimen under the microscope. For the purposes of this analysis, a branch point formed by the junction of three arcade segments was defined as a node. A central rectangular region representing 50% of the total muscle surface area was outlined on the acetate overlay, and the node-to-node length of each individual arcade segment within the region was measured. The number of arcade “loops” lying completely within the region was also counted. Ten to twenty arcade segments were then randomly selected and examined in the cleared specimen at higher (×200) magnification to count the transverse arterioles branching from each segment. Randomly selected transverse networks were then mapped in their entirety, and the total number of vessels of each branch order was determined for each network. Because the Strahler method of assigning branch orders requires the identification of all terminal vessels, only those transverse networks whose distal branches were filled down to the capillaries were included in this analysis. Otherwise, no special selection criteria were used.

For each muscle, the cumulative arcade vessel length, number of individual arcade segments and number of arcade loops within the counting region were normalized to regional surface area and expressed as per millimeter squared tissue surface area. The average number of transverse arterioles per millimeter of arcade segment was calculated by dividing the total number of transverse arterioles counted by the cumulative length of their parent arcade segments. The total number of transverse arterioles within the counting region was estimated by multiplying the number per millimeter arcade by the cumulative arcade vessel length, and this value was expressed as the number of transverse arterioles per millimeter squared tissue surface area.

Statistics
All data are expressed as mean±SEM. For each variable, simultaneous multiple comparison of group means was made by using analysis of variance in a 2x2 factorial design to evaluate treatment effects (strain, diet) and potential strain–diet interaction. Post hoc analysis of any differences was made by using the Newman-Keuls multiple range procedure. In all tests, significance was assessed at the 95% confidence level (p<0.05).

Results
Arteriolar diameter measurements were made in a total of 14 DR (seven on 0.45% NaCl, seven on 7% NaCl) and 14 DS (seven on 0.45% NaCl, seven on 7% NaCl) rats. In both strains, mean body weight of rats on the 7% NaCl diet tended to be lower than that of rats on the 0.45% NaCl diet, but this difference was only significant in DR rats (Table 1). In DR rats, part of this difference may be attributable to the fact that rats of this strain on 7% NaCl tended to be slightly younger than those on 0.45% NaCl at the time of study. The 7% NaCl diet had no effect on mean arterial pressure in DR rats but produced marked hypertension (mean arterial pressure=155 ±4 mm Hg) in DS rats. In addition, mean arterial pressure in DS rats on 0.45% NaCl (128±6 mm Hg) was significantly greater than that of DR rats on either 0.45% or 7% NaCl (104±7 and 101±5 mm Hg, respectively).

The mean internal diameter of the arcade arterioles in each group before and after adenosine superfusion is illustrated in Figure 1. Mean resting diameter in DR rats on 7% NaCl (33±2 μm) was not significantly different from that in DR rats on 0.45% NaCl (32±3 μm). In contrast, mean resting diameter in DS rats on 7% NaCl was significantly reduced compared with DS rats on 0.45% NaCl (25±2 vs. 32±2 μm, p<0.05) and DR rats on either diet. However, after abolition of vascular tone with aden-
osine, no significant differences in arteriolar diameter were found among groups. In contrast to the arcade arterioles, no intergroup differences related to either diet or strain were found in resting transverse arteriole diameter (Figure 2). As with the arcade arterioles, no significant differences in passive transverse diameter were found among groups.

Morphometric analysis of the filled, maximally dilated arteriolar network was made in cleared muscle specimens from six rats in each experimental group. Characteristics of the arcading network in each group are shown in Table 2. On the 0.45% NaCl diet, there was no significant difference between the DR and DS rat strains in total arcade segment length, number of individual arcade segments or number of arcade "loops" per millimeter squared tissue surface area. In addition, in both DR and DS rats, the 7% NaCl diet had no significant effect on any of the these variables.

The number of transverse networks branching from the arcade arterioles was also similar in all experimental groups. Muscles from DR and DS rats maintained on 0.45% NaCl did not differ in either the average number of transverse arterioles per millimeter arcade segment length or the estimated number of transverse arterioles per millimeter squared tissue surface area (Table 2). As with the more proximal arcade arterioles, the high salt diet did not have a significant effect on either of these variables in DR or DS rats.

Twenty transverse networks were randomly selected from each group and all of the vessels within these networks were mapped in their entirety. The size of these networks was highly variable in each group, with anywhere from five to 32 capillary bundles supplied by the branches of a single transverse arteriole. Despite this size range, each network examined was found to contain either three or four branching generations from its point of origin at the arcade arteriole to the origin of the capillaries. The average number of vessels of each branch order per network is shown for each experimental group in Table 3. The number of vessels of each branch order was similar in all groups, with no significant strain- or diet-related differences observed.

**Discussion**

In the DS rat, cardiac output is elevated only for the first few days after initiation of a high NaCl diet, whereas total peripheral resistance is elevated throughout the development of NaCl-induced hypertension. Whole-organ studies suggest that alterations in the skeletal muscle vascular bed contribute to this resistance increase. The results of this study indicate that in the DS rat made hypertensive by a 4-week period of high salt intake, spinotrapezius muscle vascular resistance may be increased at least in part because of a reduction in the resting diameter of proximal, but not distal, arterioles. On the other hand, we found no evidence of structural rarefaction at any level of the arteriolar network in hypertensive DS rats, arguing against the possibility that a permanent change in network architecture contributes to increased resistance at this stage of the hypertensive process.

The mean resting diameter of arcade arterioles in DS rats on the 7% NaCl diet was found to be 20–25% smaller than that of age-matched DS rats on 0.45% NaCl or DR rats on either diet (Figure 1). A reduction in resting arteriolar diameters has also been reported in skeletal muscle of Sprague-Dawley rats with experimentally induced DOCA-salt hypertension. In contrast, most investigators have not found evidence of reduced arteriolar diameters in skeletal muscle of SHR. These different earlier observations emphasize that the specific microvascular alterations associated with hypertension vary depending on the model of hypertension studied.

After abolition of vascular smooth muscle tone with adenosine, the mean arcade diameter in DS rats...
Tissue mass are most accurately assessed by vascular wall. An increase in smooth muscle tone hypertensive animals than in the normotensive ani-
would suggest that passive wall stiffness is increased under these conditions may be attributable to several factors, including increased sympathetic neural activity,5-9-25 increased vascular responsiveness to circu-
lating or neurally released vasoconstrictor sub-
stances1113 and autoregulatory (metabolic or myo-
.genic) responses.1326 However, the possibility of

diameter measurements alone. If pressure in the passive arcade or transverse arterioles is greater in the hypertensive group. Because microvascular
pressures were not measured in this study and have not been previously reported for the Dahl rat, this possibility cannot be discounted. In addition, lumenal diameter measurements would not reveal a structural thickening of the arteriolar wall if it had occurred toward the outside of the vessel wall. Changes in vascular wall mass are most accurately assessed by direct morphological measurement of wall cross-sectional area. Such measurements have demonstrated that wall hypertrophy does occur in large and small arteries of hypertensive DS rats fed 8% NaCl for 6-7 weeks.27 Unfortunately, comparable data for microvessels in the hypertensive DS rats are lacking, and microvascular wall hypertrophy has not been found in all forms of hypertension. Structural thickening of the arteriolar wall occurs in skeletal muscle of rats with one-kidney, one clip renal hypertension28 but not in the SHR.10,29 and data from rats with coarctation hypertension are contradictory.26,30

In contrast to the arcade arterioles, the resting diameter of transverse arterioles in DS rats on 7% NaCl was not reduced (Figure 2). However, the lack of a detectable decrease in resting diameter does not necessarily mean that these vessels have normal vascular tone. If transverse arteriolar pressure is elevated in hypertensive DS rats, then a greater force development by the vascular smooth muscle would be required to maintain a normal resting diameter in the face of the increased distending pressure.

In the SHR, structural rarefaction of arterioles has been reported in a variety of organs, including the cremaster muscle and gracilis muscle vascular beds.10,12,31-33 In contrast, Engelson and coworkers34 found a "denser" arteriolar network in the spinotrapezius muscle of mature SHR, suggesting that at least in this model of hypertension, structural rar-
faction may not occur in all skeletal muscles. In the present study, we found no evidence of structural rarefaction in the spinotrapezius muscle of DS rats with NaCl-induced hypertension (Tables 2 and 3). The arteriolar network in DS rats on 7% NaCl was similar to that of all other groups in terms of total arcade length and number of arcade loops per millimeter squared tissue surface area. In addition, the number of transverse arterioles and number of ves-
sels within each transverse network were not reduced. The mass of the counting region was not
determined in this study, but we found no significant
difference among experimental groups in tissue mass

### Table 2. Arcade Network Characteristics and Transverse Arteriole Density in Each Experimental Group

<table>
<thead>
<tr>
<th>Measurement</th>
<th>DR, 0.45% NaCl</th>
<th>DR, 7% NaCl</th>
<th>DS, 0.45% NaCl</th>
<th>DS, 7% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcade arterioles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length/surface area</td>
<td>1.11±0.10</td>
<td>1.26±0.05</td>
<td>1.20±0.07</td>
<td>1.12±0.05</td>
</tr>
<tr>
<td>(mm/mm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segments/mm² surface area</td>
<td>1.0±0.12</td>
<td>1.27±0.08</td>
<td>1.10±0.16</td>
<td>1.08±0.12</td>
</tr>
<tr>
<td>Arcade loops/mm² surface area</td>
<td>0.17±0.04</td>
<td>0.24±0.04</td>
<td>0.22±0.05</td>
<td>0.21±0.03</td>
</tr>
<tr>
<td>Transverse arterioles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessels/mm arcade length</td>
<td>2.48±0.05</td>
<td>2.34±0.13</td>
<td>2.31±0.13</td>
<td>2.60±0.15</td>
</tr>
<tr>
<td>Vessels/mm² surface area</td>
<td>2.75±0.22</td>
<td>2.96±0.20</td>
<td>2.78±0.23</td>
<td>2.92±0.19</td>
</tr>
</tbody>
</table>

Values are mean±SEM. DR, Dahl salt-resistant rats; DS, Dahl salt-sensitive rats.

### Table 3. Number of Vessels of Each Branch Order per Transverse Network in Each Experimental Group

<table>
<thead>
<tr>
<th>Group</th>
<th>First-order</th>
<th>Second-order</th>
<th>Third-order</th>
<th>Fourth-order</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR, 0.45% NaCl</td>
<td>15.6±1.7</td>
<td>5.6±2.8</td>
<td>2.0±0.2</td>
<td>0.60±0.12</td>
</tr>
<tr>
<td>DR, 7% NaCl</td>
<td>18.0±1.7</td>
<td>6.2±3.1</td>
<td>2.0±0.3</td>
<td>0.55±0.14</td>
</tr>
<tr>
<td>DS, 0.45% NaCl</td>
<td>17.3±1.9</td>
<td>6.7±3.5</td>
<td>2.0±0.2</td>
<td>0.60±0.12</td>
</tr>
<tr>
<td>DS, 7% NaCl</td>
<td>16.6±1.3</td>
<td>5.8±1.7</td>
<td>2.0±0.2</td>
<td>0.60±0.12</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Branch orders are assigned according to Strahler23; first-order equals most distal vessels and fourth-order equals most proximal vessels. DR, Dahl salt-resistant rats; DS, Dahl salt-sensitive rats.
per millimeter squared surface area as calculated from whole muscle measurements. This value averaged 0.63±0.07 g/mm² for all DR rats and 0.68±0.04 g/mm² for all DS rats, with no significant effect of diet found in either strain. This indicates that our data accurately reflect an equivalent vascular density per unit tissue mass in all groups. These findings are consistent with whole-organ data suggesting that structural changes in the peripheral vasculature do not contribute to the elevation of total peripheral resistance at this stage of NaCl-induced hypertension in DS rats. After 4 weeks on an 8% NaCl diet, hypertensive DS rats have increased resting but not minimum hindquarters vascular resistance.9 However, Mueller8 reported that minimum hindquarters resistance in DS rats is significantly increased by the eighth week of high NaCl intake. Therefore, the possibility of a later-developing structural rarefaction in the spinotrapezius muscle of hypertensive DS rats cannot be ruled out.

The analysis of network architecture in each group indicates that regardless of NaCl intake or arterial pressure, the topology of the spinotrapezius muscle arteriolar network in 9–10-week-old DS rats is similar to that of the age-matched DR rat. In light of this finding, it seems reasonable to conclude that there are no inherent differences in network architecture between the DR and DS rat strains. This information is important because, although DR and DS rats are descended from the same parent population,3,4 the genetic isolation of the strains brought about by years of selective breeding raises the possibility that microvascular differences unrelated to salt-sensitivity or blood pressure could have arisen between the strains as a consequence of genetic drift.

The findings of this study do not preclude the possibility of functional arteriolar rarefaction in the spinotrapezius muscle of hypertensive DS rats. In resting skeletal muscle of both SHR and rats with one-kidney, one clip hypertension, a significant fraction of the distal arterioles are constricted to the point of closure,10,11,35,36 possibly in part due to a hyperresponsiveness to norepinephrine.11 Our experimental protocol required a detailed in vivo mapping of the arcade network before the initial control video scan, and during the subsequent experimental period, there were no instances in which additional arcade segments became perfused on abolition of vascular tone with adenosine. Thus, we found no evidence for a functional rarefaction of the arcade arterioles in any of the four experimental groups. However, a quantitative assessment of functional rarefaction of the more distal arterioles was beyond the scope of this study. Because of the thickness of the spinotrapezius muscle, accurate in vivo counts of these smaller arterioles (<10 μm i.d.) would not have been possible with the conventional bright-field microscopy used in this study.

In summary, in the spinotrapezius muscle of hypertensive DS rats on a high salt diet, the luminal diameter of arcade arterioles is significantly reduced at rest but not in the passive state. This observation suggests that in these animals, spinotrapezius muscle vascular resistance is increased by active constriction of the proximal arterioles. The anatomic density of arterioles in hypertensive DS rats was similar to that in normotensive controls, indicating that structural rarefaction does not contribute to increased spinotrapezius muscle resistance at this stage of NaCl-induced hypertension.

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