Protection against high intravascular pressure in giraffe legs

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1Department of Radiology, Aarhus University Hospital, Skejby, Denmark; 2Zoophysiology, Department of Bioscience, Aarhus University, Aarhus, Denmark; 3Department of Pathology, Vendsyssel Hospital and Center for Clinical Research, Aalborg University, Denmark; 4Department of Biomedicine, Aarhus University, Aarhus, Denmark; 5Department of Pharmacology and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada; 6Center for Zoo and Wild Animal Health, Copenhagen Zoo, Frederiksberg, Denmark; 7Department of CardioThoracic and Vascular Surgery and Institute of Clinical Medicine, Aarhus University Hospital, Skejby, Denmark; and 8Department of Anesthesiology, and The Copenhagen Muscle Research Center, Rigshospitalet, University of Copenhagen, Denmark

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Petersen KK, Hørløk A, Østergaard KH, Andresen J, Broegger T, Skovgaard N, Telinius N, Laher I, Bertelsen MF, Grøndahl C, Smerup M, Secher NH, Brøndum E, Hasenkam JM, Wang T, Baandrup U, Aalkjaer C. Protection against high intravascular pressure in giraffe legs. Am J Physiol Regul Integr Comp Physiol 305: R1021–R1030, 2013. First published September 4, 2013; doi:10.1152/ajpregu.00025.2013.—The high blood pressure in giraffe leg arteries renders giraffes vulnerable to edema. We investigated in 11 giraffes whether large and small arteries in the legs and the tight fascia protect leg capillaries. Ultrasound imaging of foreleg arteries in anesthetized giraffes and ex vivo examination revealed abrupt thickening of the arterial wall and a reduction of its internal diameter just below the elbow. At and distal to this narrowing, the artery constricted spontaneously and in response to norepinephrine and intravascular pressure recordings revealed a dynamic, viscous pressure drop along the artery. Histology of the isolated median artery confirmed dense sympathetic innervation at the narrowing. Structure and contractility of small arteries from muscular beds in the leg and neck were compared. The arteries from the legs demonstrated an increased media thickness-to-lumen diameter ratio, increased media volume, and increased numbers of smooth muscle cells per segment length and furthermore, they contracted more strongly than arteries from the neck (500 ± 49 vs. 318 ± 43 mmHg; n = 6 legs and neck, respectively). Finally, the transient increase in interstitial fluid pressure following injection of saline was 5.5 ± 1.7 times larger (n = 8) in the leg than in the neck. We conclude that 1) tissue compliance in the legs is low; 2) large arteries of the legs function as resistance arteries; and 3) structural adaptation of small muscle arteries allows them to develop an extraordinary tension. All three findings can contribute to protection of the capillaries in giraffe legs from a high arterial pressure.

THE GIRAFFE IS THE MAMMAL with the highest mean blood pressure of ~200 mmHg, and because of the height of the animal, its lower limbs are at least potentially exposed to a pressure of about 350 mmHg (8, 16). To ensure that this pressure is transmitted to the capillaries, it would likely provoke interstitial edema, and yet that does not manifest in the lower leg of the giraffe. Several mechanisms are suggested to prevent edema in the lower limb of the giraffe. The tight fascia in the limb of the giraffe may act as an

antigravity suit (8, 19), but tissue compliance in the lower limb has not been determined, and the interstitial fluid pressure in the lower limbs of a standing giraffe is only 44 mmHg (8). Secondly, thickened walls of resistance arteries in giraffe limbs could protect against the pressure load to which the distal capillaries in the giraffe might be exposed (7). However, when giraffe limb arteries become smaller than 400 μm, they are reported to be without thickened walls (7), and it appears that no structural differences are present between arteries supplying the skin of the leg and the neck (12). These findings are, however, based on histological sections of arteries not exposed to a well-defined mechanical stress during fixation. Therefore, it remains unknown whether in the giraffe, the structure of small arteries is different in the legs and the neck when evaluated under well-defined mechanical conditions. Finally, there is a sphincter-like structure in the isolated anterior tibial artery of the giraffe (9, 16), but whether it provides a relevant dynamic viscous resistance and, thus, regulates distal vascular pressure in the lower leg is not known.

This study aims to provide quantitative assessments of three mechanisms proposed that are important for prevention of edema in the lower leg of the giraffe. Thus, the study evaluated whether 1) there is a sphincter-like structure in the median artery of the foreleg, as has been reported for the hindleg (6, 13), and whether it affects pressure in the lower leg; 2) whether the structure of small arteries in the leg of giraffes is similar to that of the small arteries of the neck when examined under mechanistically well-defined conditions; and 3) whether tissue compliance is smaller in the lower leg than in the neck of the giraffe.

METHODS

Eleven male giraffes belonging to a subspecies (Giraffa camelopardalis giraffa), encompassing at least 12,000 animals were studied at an age between 3 and 5 yr after being bred in privately owned parks. The lengths of the forelegs (hoof to shoulder joint) and hindlegs (hoof to hip joint) were measured in 10 giraffes. The distance from ground to the sphincter was measured in four hindlegs and six forelegs. Approval for the experiments was obtained from the Animal Ethics Screening Committee at the University of Witwatersrand (Johannesburg) and the Animal Use and Care Committee at University of Pretoria, South Africa. Local animal ethical committee members supervised the experiments and the Gauteng Province of South Africa granted permission for euthanasia.

Anesthesia. Following overnight deprivation of food and water, giraffes were premedicated with medetomidine (5.5 μg/kg im) and ~10 min later anesthesia was induced with etorphine (6.5 μg/kg im)
and ketamine (0.65 mg/kg im). A rope connected to a halter and a pulley allowed control of the head when the giraffes became recumbent after 3–7 min. The giraffes were intubated immediately (ID 20 mm) for assisted ventilation with oxygen using a demand valve (Hudson RCI, Limerick, Pennsylvania). Following induction of anesthesia, animals were moved to an adjacent room and placed in right lateral recumbence with the head and neck elevated. Anesthesia was maintained by intravenous α-chloralose (15 mg/ml; KVL-Pharmacy, Copenhagen, Denmark; 30 mg/kg/h)—a dose that was gradually reduced depending on reflexes and breathing pattern. Anesthesia was monitored using ECG, invasive and noninvasive arterial blood pressure, and recording of the end-tidal CO2 tension (PM9000Vet; E-Vet, Haderslev, Denmark). Additionally, arterial and venous blood gas variables were measured at regular intervals (GEM Premier 3500; Instrumentation Laboratory, Bedford, MA). Unless otherwise stated, the anaesthetized giraffes were maintained in the upright position by placing the limbs into straps suspended from the ceiling, leaving the thoracic and abdominal regions free of external pressure, as described previously (4).

Ultrasound of the foreleg. The luminal diameter of the median artery and the thickness of the intima-media complex were determined on both forelegs by ultrasound (GE Healthcare, Vivid I, Israel) using a 12-MHz linear transducer after shaving the medial aspect of the forelegs immediately distal to the elbow by experienced radiologists. Flow velocity was determined by pulsed Doppler.

Foreleg arterial and interstitial fluid pressures. Using ultrasound guidance and Seldinger technique, we placed two 20-gauge catheters in the median artery; one proximal to the sphincter and one about 20 cm proximal to the hoof. Pressures were recorded with Edwards, Baxter disposable pressure transducers (Baxter Healthcare, Irving, CA) and a monitor (Dialogue 2000, IBC-Danica, Copenhagen, Denmark), and they were sampled using a Biopac 100 data acquisition system (Goleta, CA). The contribution from gravity to the pressure difference between the two catheters was eliminated. This was done by subtracting the hydrostatic pressure calculated from the distance from the heart to the catheters from the readings.

Leg and neck interstitial fluid pressures were measured with an Arrow 14-gauge catheter (Teflex, Reading, PA) that was advanced below the fascia for 15 cm or by a 18-gauge catheter (Periflex, Braun, Melsungen, Germany) that was advanced 8 cm by a Tuohy needle. The Tuohy needle was retracted and the catheters, which have side holes, were left in place for pressure measurements. The transducer reading was set to zero; thereafter, tissue compliance was assessed from the pressure rise following injection of 3 ml of saline through the catheter. We verified that the skin surrounding the catheter remained tight when subjected to a pressure of up to 350 mmHg following injection of an additional 3 ml of saline.

Isolated small arteries. Following euthanasia by pentobarbital sodium (50 mg/kg iv), muscle samples from the hind limbs (about 90 cm below the heart) and the neck (about 30 cm [low neck] and about 130 cm [high neck] above the heart) were obtained together with skin biopsies from the hind limbs (about 140 cm below the heart), and small arteries were dissected under a stereomicroscope. Two arteries (in a few cases only one artery) that were closely size-matched, according to lumen diameter, were dissected from each muscle biopsy and mounted in a myograph for isometric force recording: arteries according to lumen diameter, were dissected from each muscle biopsy and mounted in a myograph for isometric force recording. The diameters were increased to obtain a passive force of about 50–100 mN, after which the arteries were stimulated with NEK.

The responses were expressed as 1) active wall tension, which is the force measured divided by 2× segment length; 2) active media stress, the active wall tension divided by the media thickness; and 3) as equivalent active pressure, the active wall tension divided by the arterial radius as a measure of the transmural pressure the artery would be able to constrict against.

The physiological salt solution (PSS) used for the in vitro experiments contained (in mM): 119 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4, 25 NaHCO3, 1.6 CaCl2, 0.026 EDTA, and 5.5 glucose. The solution was gassed with 5% CO2 in air and adjusted to a pH of 7.4. K-PSS was PSS, in which NaCl was substituted with KCl on an equimolar basis.

Histology. Arteries (7 from the median artery, 10 from tibial artery) from nine giraffes were obtained. The sphincter area was located, and eight transverse sections of the arteries were cut from 5 cm proximal to 5 cm distal to this area, with ~1 cm between each block. The blocks were fixed in 4% buffered formaldehyde, embedded in paraffin, and two 3-μm sections were cut from each block. For immunohistochemical detection of nerves, one section was stained with S100 (1:2,000 polyclonal Z 0311, protein kinase K S3020, DAKO, autostainer LAB VISION) and one with anti-tyrosine-hydroxylase (TH) (1:2,500, Abcam, ab112). The sections were examined using an Olympus MVX10 macroscope equipped with a camera connected to a PC. For the number and area of nerves in the arteries, the 2D nucleator software from newCAST (Visiopharm, Copenhagen, Denmark) was used.

The small muscular arteries from the leg and upper part of the neck were embedded in plastic and two longitudinal sections were produced. The stereological technique “disector” (13) provided an unbiased estimate of smooth muscle cell (SMC) volume, number per segment length, length, cross-sectional area, and cell layers. The estimates of the variables listed are based on the assumption that each SMC contains only one nucleus.

Statistics. When several arteries from the same giraffe biopsy were investigated, the mean value was taken to represent data from that giraffe. The small arteries from the muscle biopsies (leg, proximal neck, and distal neck) were compared with ANOVA. When the ANOVA was significant, a test for a linear trend between the values at the three sites was performed (GraphPad Prism 5). The effect of NE on median/tibial artery structure and pressure differences between the catheters, as well as the indices of innervation, were tested with a paired t-test. For tissue pressures, the values at the lowest and highest positions were compared with a paired t-test. Data are presented as means ± SE, with n indicating the number of giraffes and P value <0.05 was considered statistically significant.

RESULTS

Ultrasound and pressure recordings of the median artery. The length of giraffe forelegs (hoof to shoulder joint) was 206 ± 19 cm and of the hind legs (hoof to hip joint) 191 ± 14 cm; n = 10. The median artery (the extension of brachial artery) was visualized by ultrasonography (Fig. 1). The lumen diameter...
was reduced, and a thick intima-media complex became apparent about 5–10 cm below the elbow (Figs. 1 and 2). This structural change took place over less than 1 cm and was maintained in the distal part of the artery. Figure 1C shows the isolated median artery, where the structural change can be seen. The mean distance from ground to sphincter was 134 ± 8 cm (n = 6, forelegs) and 123 ± 13 cm (n = 4, hindlegs).

With ultrasound, we observed what appeared to be a fully dilated artery (Fig. 1A), but on one occasion, the artery was constricted (Fig. 1B), and the media-intima complex thickness was increased, indicating a spontaneous constriction. Pressure recordings revealed a pressure difference between the distal and proximal catheters of about 10 mmHg when the artery was dilated (Fig. 3). Sometimes, a spontaneous, transient (lasting 5–15 min) increase in the pressure difference was seen (Fig. 3A). Such a transient was seen in the artery, which was constricted on the basis of the ultrasound recording (Fig. 3A), supporting the conclusion that the artery can constrict spontaneously. During an orthostatic challenge, no consistent changes in the pressure difference or artery diameter were apparent.

Intra-arterial injection of 1 μg NE proximal to the elbow had no effect on the sphincter, but in two out of seven cases, 10 μg NE caused a constriction. Intra-arterial injection of 100 μg NE, however, caused constriction in all cases (Figs. 2 and 4). Constriction was, on occasion, restricted to the sphincter area (Fig. 2B), while in most cases, the area distal to the sphincter

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Fig. 1. A and B: ultrasonography imaging of the sphincter region of the median artery of the giraffe. A: no apparent contraction. B: spontaneous contraction that is most pronounced distal to the sphincter region. The composite images (indicated by broken lines in A and B) are composed of scans obtained at adjacent positions along the leg with the ultrasound transducer (which has a limited field of view) (C) the isolated median artery.

Fig. 2. Ultrasonography imaging of the sphincter region of the median artery of the giraffe: control (A) after injection of 100 μg norepinephrine (B and C).
also constricted (Fig. 2C and Supplemental Video S1). Within 10 s after injection of NE, the mean flow velocity in the artery decreased to almost zero (Supplemental Video S1) and then slowly returned to re-establish preinjection values over 10–15 min as the artery constriction waned. On the other hand, there was no constriction in response to administration of NE proximal to the sphincter, no constriction in the contralateral foreleg, and no effect on the blood pressure, indicating no systemic effect.

Intra-arterial NE injection was associated with a ~2-min decrease in the pressure difference between the two sites of recording in six of seven giraffes (Fig. 3), followed by an increase to an average of about 30 mmHg (Figs. 3 and 4). The increase of the blood pressure difference developed slowly (over 5–10 min) and then slowly waned but closely followed the reduction of the lumen diameter and thickening of the media-intimal complex (Fig. 3B).

**Histology of median/tibial arteries.** Staining of the arteries revealed TH-positive (Fig. 5, A and C) and S100-positive cells at the sphincter, with very little staining proximal to the sphincter (Fig. 5B). The number of TH-positive structures in the most proximal slice, i.e., proximal to the sphincter, was 0.8 ± 0.2, while in a slice at the sphincter, it was 10.2 ± 1.4 (Fig. 6A). The area of TH-positive structures at the proximal slice was 2.0 ± 10^{-3} ± 7 × 10^{-3} mm², while at the sphincter, it was 32 × 10^{-3} ± 7 × 10^{-3} mm² (Fig. 6B).

### Table 1

<table>
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<tr>
<th>Time (minutes)</th>
<th>Proximal pressure (mmHg)</th>
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### Figure 3

**A**: hydrostatic pressure immediately proximal to the sphincter region (top, proximal) of the median artery of a giraffe and at a location about 20 cm above the hoof (middle, distal). The contribution to hydrostatic pressure from the effect of gravity from the heart to the catheter tip was subtracted from the recorded pressures, so that differences between the recordings from the two catheters represent a viscous pressure drop between the catheters. The lower curve (pressure difference) shows the distal minus the proximal pressures (a measure of the viscous pressure drop between the two catheters). A: values indicate the internal diameter of the median artery measured with ultrasonography distal to the sphincter region at the two time points indicated by the arrows. B: arrows indicate intra-arterial injections of norepinephrine just proximal to the sphincter region. C: records (typical of 7 experiments) of the distal minus the proximal pressure of the median artery (pressures recorded as described in 3B), and proximal median artery lumen diameter and intima-media complex thickness. At time 0, 100 μg norepinephrine was injected intra-arterially.
In the S100-stained arteries, the number of nerves proximal to the sphincter was 0.9 ± 0.2, while at the sphincter, it was 17.2 ± 3.2 (Fig. 6C). The corresponding nerve areas were $2 \times 10^{-3} \pm 0.4 \times 10^{-3} \text{mm}^2$ and $40 \times 10^{-3} \pm 8 \times 10^{-3} \text{mm}^2$ (Fig. 6D). All indices of innervation were significantly greater at the sphincter compared with proximal to the sphincter.

Interstitial fluid pressure in the foreleg. The interstitial fluid pressure in the hindleg (20–40 cm above the hoof) in four giraffes suspended in an upright position was 47 ± 10 mmHg. In eight additional giraffes, the interstitial fluid pressure was measured (Fig. 7A) at four positions along the foreleg (13–15, 30–40, 60–80, and 120–150 cm from the hoof) and in two positions in the neck (−200 and 350 cm from the hoof) with the head placed on a 70-cm-high support, such that the head was 150 cm above the hoofs. The interstitial fluid pressure varied from 20 to 25 mmHg at the two lowest positions and was close to zero at the other positions (Fig. 7B). Thus, the pressures at the lowest and highest positions were significantly different. At the same sites, injection of 3 ml saline caused pressure to increase for 5–20 s. After the transient increase, the pressure returned to a value close to the preinjection value, probably as a consequence to saline spreading into the tissue, which supports that interstitial fluid pressure was recorded by our approach. The peak pressure increase was larger at the lowest position compared with the highest position (Fig. 7C).

Function of isolated arteries. Arteries with a normalized internal diameter of about 200 µm were dissected from muscle biopsies. Arteries were easy to distinguish from the accompanying veins in the biopsies from all three sites. The small arteries responded to NEK with rapid force development reaching a steady-state force within 10–15 s (Fig. 8A). In contrast, the median artery constricted much slower and the time from application of NEK to reaching steady-state force varied from 2 to 3 to 10 min or more (Fig. 8B).

The length tension curves for small muscular arteries are shown in Fig. 8, C and D. These curves demonstrate that with the circumference set to 0.9xL100, all arteries developed near-maximal active tension.

The morphological characteristics of the small arteries from the muscles are shown in Fig. 9. Although the arteries dissected had the same normalized diameter (Fig. 9A), the media thickness increased inversely with the distance from the ground. The media was about twice as thick (and with a significantly increased media area; not shown) in arteries from the leg compared with those from near the head (Fig. 9B), resulting in
a significantly increased media thickness to lumen diameter ratio in the arteries near the ground (Fig. 9C). Morphometric analysis indicated that the large media area in the arteries from the legs resulted from an increased number of smooth muscle cells per segment length. Smooth muscle cells were organized in an increased number of layers, with no increase in size (length and cross-sectional area) of each cell (Table 1).

The high active tension developed in response to NEK in arteries from the lower leg was related to increased media thickness, as there was no increase in media stress development to NEK in these arteries compared with the arteries from the neck (Fig. 10A). In contrast, the equivalent active pressure was larger in arteries closer to the ground, indicating that the arteries from the limbs could constrict against transmural pressures of more than 500 mmHg and significantly more than...
the arteries from the upper part of the giraffe (transmural pressures about 300 mmHg) (Fig. 10B).

All arteries from the leg muscles responded to NE with an EC\textsubscript{50} between 1 and 5 \textmu M. In contrast, only 50% of the arteries from the neck developed tension to cumulative NE concentrations. For those arteries that developed tension in response to administration of NE, the EC\textsubscript{50} also varied between 1 and 5 \textmu M. All of the arteries relaxed to increasing concentrations of ACh (most of the arteries relaxed fully) with EC\textsubscript{50} between 30 and 100 nM.

Skin arteries with internal diameters larger than 500 \textmu m ran parallel to and just outside the dense connective tissue of the skin. Branches of these arteries running obliquely into the skin were dissected and mounted in myographs. The arteries had a mean diameter of 363 ± 43 \textmu m (n = 8) and produced a maximal tension of 4.70 ± 0.83 N/m, which is equivalent to an effective pressure of 190 ± 28 mmHg. It was not possible to visualize the media in the myographs, as was the case for arteries from the muscular bed.

**DISCUSSION**

Of all mammals, giraffes have the highest blood pressure, which combined with their extreme height, challenges the cardiovascular systems and especially so in their lower extremities. In anesthetized giraffes, similar to the giraffes used in the present study, we have reported a mean blood pressure of 193 mmHg in the upright position (2) and a mean arterial pressure of up to 350 mmHg in the lower limbs (13), which are values similar to values reported in awake giraffes (5). Such a pressure might lead to edema, but healthy giraffes do not experience edema in their lower limbs.

A large viscous resistance in the arteries would reduce the capillary pressure and the likelihood of edema development in...
the lower legs of giraffes. A sphincter-like structure in the (anterior) tibial artery, immediately beneath the knee, has been reported in isolated arteries (9, 16). Whether a similar sphinc-
ter-like structure also exists in the forelegs and whether it
provides a significant visco-resistant resistance was not known pre-
vious to this study. In addition, a substantial precapillary
viscous resistance could be developed by a large wall thick-
ness-to-lumen diameter ratio in small limb arteries. A large
wall thickness-to-lumen diameter ratio is established in limb
small arteries of the giraffe, but apparently not in arteries with
diameters less than 400 μm and not in those at the level of the
ankle (7). These measurements were obtained without defini-
tion of the mechanical strain to which the arteries were
exposed during measurements. Here, we determined wall
thickness of arteries <400 μm outer diameter in giraffe legs
exposed to a well-defined strain. We tested the hypothesis that
the structural and/or functional characteristics of small arteries
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small arteries of the hind legs of giraffes, but apparently not in arteries with
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small arteries of the hind legs of giraffes, but apparently not in arteries with

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<th></th>
<th>Cell Volume, μm³</th>
<th>Cells per Unit Length, μm⁻¹</th>
<th>Cell Length, μm</th>
<th>Cell Cross-Sectional Area, μm²</th>
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<td>Leg arteries</td>
<td>2462 ± 178</td>
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<td>95.7 ± 13.4</td>
<td>29.2 ± 4.0</td>
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<td>Upper part of neck</td>
<td>2425 ± 353</td>
<td>2.98 ± 1.03</td>
<td>85.5 ± 6.9</td>
<td>27.6 ± 2.3</td>
<td>2.24 ± 0.12</td>
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*P* value

- *ns*
- *P < 0.05*

Values are expressed as means ± SE. *ns*, not significant.

A major function of the leg arteries in giraffes is to provide pressure resistance against gravity. The high pressures generated by the heart are insufficient alone to prevent the arterial pressure in the legs, consistent with previous pressure recordings (8, 16).

The initial decrease of the pressure difference between the proximal and distal site after injection of NE was associated with an almost complete cessation of blood flow, suggesting that the resistance arteries distal to the distal catheter con-
stricted more rapidly than the median artery, and so indicating an extraordinarily powerful regulatory capacity of the leg arteries. This suggestion, derived from in vivo measurements showing that the distal arteries constricted faster than the median arteries, was supported by the much faster force de-
velopment of small muscular arteries compared with the med-
ian artery observed in vitro. However, we did not visualize the
dynamics of a spontaneous constriction in vivo to see whether this was equally slow in the median arteries. Arteries from
giraffes have a rich sympathetic innervation (10, 15), as con-
firmed here by staining for the sympathetic nerve marker
tyrosine hydroxylase in the adventitia, and the staining was
most pronounced near the sphincter, suggesting that the sphincter
is richly innervated by sympathetic nerve fibers. In support,
staining of Schwann cells with S100, followed a similar pattern
and confirms previous observations on the hind leg of giraffes (9, 16). Our findings, thus, suggest that giraffes can increase viscous
resistance in the main arteries of the legs by sympathetic activa-
tion and, hence, reduce capillary pressure in the lower legs.

**Structural adaptation of resistance arteries in the leg has functional consequences.** Our functional data on small muscular
arteries in the legs of the giraffe provide further evidence for their role in protection against interstitial edema in the
lower leg. We compared the structure and function of small
arteries from muscular tissue at three levels above the hoof of the giraffe. Media thickness correlated with the distance of the artery from the ground, as did the ratio of media thickness-to-lumen diameter, lending additional support to the hypothesis that vascular structure varies with transmural pressure. The increased media thickness (and media volume per segment length) was due to an increased cell number organized in a greater number of layers in the arteries from the lower leg of the giraffe, while cell length and cross-sectional area were similar at all three positions from which the arteries were obtained. This hyperplastic response in arteries exposed to a high transmural pressure is different from that seen in human essential hypertension, for which a eutrophic increase in media thickness-to-lumen diameter ratio is associated with unchanged smooth muscle numbers and size (11). A similar wall-to-lumen ratio of skin arteries from the leg and neck of giraffes was reported by Mitchell and Skinner (12), suggesting that small arteries of the skin do not adapt structurally when exposed to the high pressures in the legs. In agreement with this notion is our observation that small arteries of the leg skin had low contractility compared with those from the skeletal muscles of the leg. The skin arteries may be protected from the high blood pressure in the lower leg of giraffes by the stiff collagen content of the skin.

Arteries from giraffes studied in vitro constricted to NE and relaxed to ACh, demonstrating that these regulatory functions are maintained in small arteries from giraffes. We observed that only some arteries from the neck responded to NE (although they all constricted to K-PSS), suggesting that sympathetic innervation is more pronounced in the legs of giraffes than in the neck. The ability of the arteries to constrict against a transmural pressure was related to their distance from the ground, i.e., arteries from the legs were able to constrict against a higher transmural pressure than those from the neck. This increased contractility of the arteries from the legs was not caused by stronger constriction per smooth muscle cross section per se, since there was no change in media stress and distance from the ground and is explained by the altered vascular morphology. Furthermore, length-tension curves confirmed that arteries from the three sites were investigated under conditions where maximal active force production was obtained. Therefore, we conclude that small arteries with diameter <400 µm are structurally changed and that this has functional consequences, which are likely to contribute to the high precapillary resistance, which explains a cardiac output that is not larger than that of animals of similar weight and a high blood pressure (5). We also suggest that these structural characteristics make it possible for NE to almost completely stop blood flow in the legs as reported here.

Low tissue compliance in the leg. In addition to providing a means of limiting edema formation due to high arterial pressure, it is important to note that blood vessels in giraffe legs also possess a thick basement membrane (21), appear impermeable to protein (8), and are enclosed within relatively thin (compared to the trunk) but inflexible skin (12), due to a high-collagen density (18). These features are likely to all contribute to preventing edema formation in the lower legs (8). However, the functional consequence of an inflexible skin, i.e., subcutaneous compliance has not been assessed previously. The antigravity suit-like function of the tight skin in the leg (8) is strongly supported by the finding that injection of a small volume of saline into the interstitial space caused larger pressure increases in the leg than in the neck of giraffes. The low compliance that we demonstrate is likely to contribute to the reported large fluctuations in interstitial fluid pressure in the leg of walking giraffes (8).

Limitations. The evaluation of the sphincter function and its response to NE were made in anesthetized giraffes, and it is important to confirm the pressure recordings in the legs of free-ranging animals.

It may be controversial to measure interstitial pressure by a perforated catheter to assess vascular transmural pressure and tissue compliance. Guyton et al. (6) suggested that interstitial pressure represents the interstitial fluid pressure and the pressure exerted by solid tissue elements that together constitute the total tissue pressure exerting the outside pressure on the vessel walls. Later, Aukland and Reed (2), however, concluded that a distinction between the interstitial fluid pressure and the pressure in solid tissue elements is irrelevant since solid tissue pressure is likely to be zero. The interstitial fluid pressure of importance is the pressure measured with “fluid equilibration techniques,” i.e., in a saline-filled column brought in contact with the interstitium that represents the filling pressure of the lymph vessels. In their giraffe study, Hargens et al. (8) used wick catheters to measure interstitial fluid pressure. With this technique, the wick secures contact with the interstitium, but this group also demonstrated that a solid-state microtransducer inserted into the tissue (17) provides, over a range of pressures, similar results as techniques involving wicks (20). Thus, a wick is not needed to increase the contact area with interstitial fluid. Leakage in the recording system might have resulted in loss of continuity in the saline-filled column between the interstitium and the transducer, but for all experiments, it was secured that there was no leakage. Taken together, these considerations support that the catheters used in this study recorded the interstitial fluid pressure.

We report tissue compliance as ∆V/∆P, where ∆V was the injected volume of isotonic saline (3 ml) and ∆P is the resulting immediate increase in interstitial fluid pressure. Because the local interstitial fluid volume was not determined, ∆V/∆P is not the true compliance of the interstitium [for discussion, see Ref. 20 since ∆V/∆P is influenced by the ability of the tissue to distribute volume vs. structures restricting tissue expansion, including the tight skin ensheathing the limb of the giraffe as, e.g., in the rat tail (3)]. True tissue compliance could be provided by inflating a balloon within the interstitium but ∆V/∆P provides at least some information on the counter-pressure exerted by the interstitium to volume expansion at different levels of the giraffe and, thereby, an indication for how edema is prevented in their lower leg.

It should be further stressed that our measurements are made in anesthetized giraffes, and although the interstitial fluid pressure that we measure is similar to that measured in awake giraffes (8), it would be important to obtain compliance measurements also in awake giraffes.

Conclusions. Vascular adaptations in the giraffe limbs appear to protect distal capillaries against high hydrostatic pressures. The large leg arteries of the giraffe have a pronounced and richly, sympathetically innervated narrowing that can regulate viscous resistance to the extent that it eliminates 20% or more of the pressure facing the distal arteries. In addition, isolated small-resistance arteries demonstrate structural adap-
tations that enable them to contract against transmural pressures reaching 500 mmHg, or more than reported for any isolated resistance artery in the animal kingdom. The steady-state interstitial fluid pressure of 40–50 mmHg in combination with low tissue compliance in the legs confers vascular protection. However, the role of these adaptations for hemodynamic control in free-ranging giraffes has yet to be examined.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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