Original article

Muscle "islands" in the tunica media of the goat thoracic aorta

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Abstract

Muscle islands in tunica media of the aorta may influence its mechanical properties and disease distribution. This study aimed to elucidate their, hitherto undescribed, characteristics in the goat thoracic aorta. Twenty four healthy male domestic goats (capra hircus) aged 6 – 24 months were used in the study. The animals were euthanized with sodium pentabarbitone. Samples were taken from the ascending, arch and descending thoracic aorta. 7 μ paraffin embedded sections stained with Mason's Trichrome and Weigert Resorcin Fuchsin/Van Gieson stains were examined by light microscopy for general organization of tunica media. Transmission electron microscopic examination of ultrathin sections stained with uranyl acetate and counterstained with lead citrate was done to study ultrastructure of the muscle islands. Fluorescent histochemical demonstration of adrenergic nerves was done by the sucrose-potassium phosphate-glyoxilic acid (SPG) method. Muscle "islands" were observed to interrupt elastic lamellae of the outer zone of tunica media in proximal aortic segments. These islands were preferentially vascularized and innervated. They comprised interconnected contractile smooth muscle cells linked to connective tissue fibres. These islands probably constitute an auxillary pump which supplements the windkessel function of the elastic lamellae, regulate blood flow and also strengthen the aortic wall.

Keywords: Muscle islands, aorta, goat, tunica media.

1 Introduction

Muscle islands in the aortic tunica media have been described in cows, sheep and goats (KNIERIEM, 1967; FUKUDA, PERRAUS and CRYSTAL, 1984; MALEK and OGENG'O, 2007). These islands may influence physicomechanical properties and pattern of disease in the aortic wall. Knowledge of their histomorphological organization may enhance understanding of aortic function, and serve as a baseline for studying alterations that occur in aging and disease processes such as atherosclerosis and aneurysms. In spite of this, the structural details of the muscle islands remain largely unreported. The goat is a suitable model for studying vascular disease, (ZHENG, QIU, ZHANG et al., 2000). This study therefore investigated the characteristics of muscle islands in its thoracic aorta.

2 Materials and methods

Aortae for this study were obtained from 24 male healthy domestic goats *(Capra hircus)* aged between 6 and 24 months and weighing 10 – 60 kg, purchased from private livestock farmers in the outskirts of Nairobi. Eight animals were used for light microscopic studies, eight for electron microscopy, and eight for histochemistry. For microscopic studies, the animals were weighed then euthanised with an overdose of sodium pentobarbitone 20 $mg.mL^{-1}$ injected intravenously. The abdomen and thorax were opened by midline incisions, and the pericardium slit to expose the heart. To clear off the blood, normal saline was introduced through the left ventricle and drained out through the right auricle. By the same means the animal was fixed by gravimetric perfusion using 3% phosphate buffered glutaraldehyde solution. In case of light microscopy, specimens taken from ascending, arch and thoracic segments were processed routinely for paraffin embedding and 7 μ sections stained with Weigert resorcin – fuchsin/Van Gieson stain for demonstration of elastic fibres; and with Mason's trichrome stain for collagen and cells. Specimens for transmission electron microscopy obtained from the same regions were post fixed with 1% phosphate buffered osmium tetroxide solution. The sections were then dehydrated in increasing grades of ethanol, cleared in propylene oxide, embedded in 100% durcupan with catalyst, and polymerized in an oven at 60 °C, for 48 hours. Ultrathin sections made with Reichert ultramicrotome©, were collected on 200 mesh copper grids, stained with uranyl acetate, counterstained with lead citrate and examined by EM 201 Phillips© electron microscope.

Specimens for histochemical demonstration of adrenergic nerves were obtained as soon as possible after the animal was euthanised, without fixation, from the same regions and intervals as described for electron microscopy. They were wrapped in aluminium foil and stored in dry ice. Embedding of the material was done using OCT compound (Tissue – Tek II) in a cryostat chamber at –30 °C. Sections of 16 μm thickness were cut and picked by use of clean but non-treated glass slides at room temperature. Cut sections were prepared for demonstration of tissue monoamines by the sucrose-potassium phosphate – glyoxylic acid (SPG) method as described by De La Torre and Surgeon (1976). The sections were then given 3 dips (1dip/seconds) in the SPG solution. Excess solution was drained off and the slides dried at a temperature of 40 °C with a hair drier. The slides were then placed in an oven maintained at

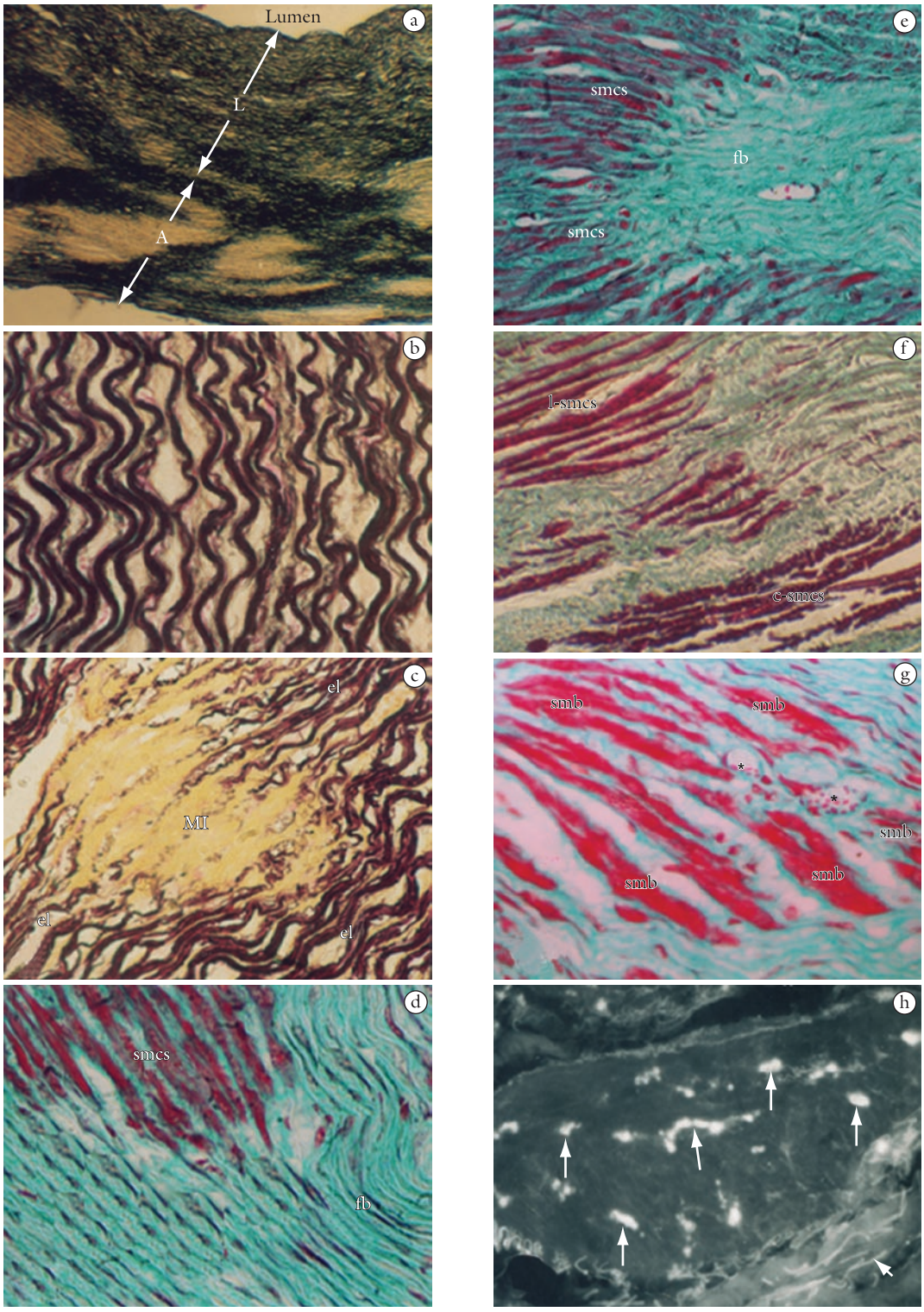

Figure 1 a-h. Photomicrographs of tunica media of the goat aorta: a)Full wall thickness showing transmedial zonation into luminal elastic (L) and adventitia musculoelastic (A) zones. Weigert elastic stain. 35×; b) Concentric elastic lamellae of the luminal zone. Weigert elastic stain. 250×; c)A muscle island (MI) interrupting elastic lamellae (el) in the adventitial zone. Weigert elastic stain. ×250; d) Smooth muscle cells (smcs) of the muscle islands terminating on a fibrous bundle (fb). Mason's Trichrome stain. 250×; e) Smooth muscle cells (smcs) in a muscle island converge from various directions on a fibrous bundle (fb). Mason's trichrome stain. 400×; f) Some smooth muscle cells run longitudinally, (l-smcs), while others run circumferentially (c-smcs). Mason's trichrome stain. 100×; g) Vasa vasora (stars) co-localised with muscle island in which they are surrounded by smooth muscle bundles (smb). Mason's trichrome stain. 250×; h) Adrenergic fluorophores (arrow) co-localised with a muscle island. The elastic fibres (arrowhead) are shown outside the island. 1,100×.

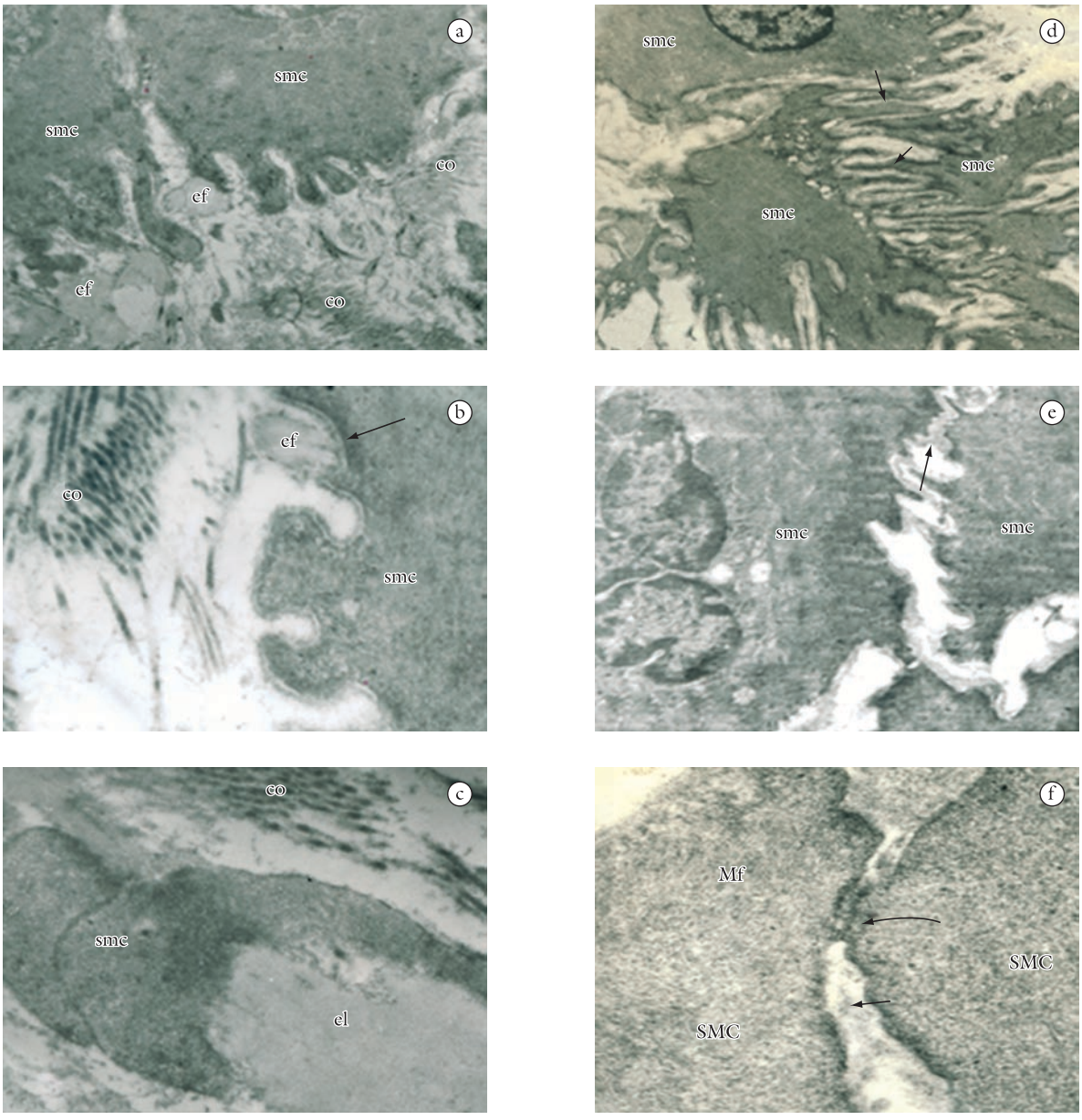

Figure 2 a-g. Electron micrographs showing muscle islands in the goat tunica media: a) Smooth muscle cells (smc) in the muscle islands. Note the irregular cell surface connected to elastic fibres (ef), and also interdigitating with collagen fibres (co). 27,800×; b) Irregular lateral surface of smooth muscle cell (smc) with the clefts displaying high electron density (arrow), and interdigitating with elastic fibre (ef) Note also the "weaved" collagen (co). 63,400×; c) An elastic lamella (el) connected to a smooth muscle cell (smc) process. Note collagen fibres (co) in the neighbourhood. 63,400×; d) Muscle cell (SMC) interdigitations, characterized by high electron density (arrow heads). 27,800×; e) The fusion of basal lamina (arrow) of the smooth muscles (smc). 27,800×; f) Adjacent cells in a muscle island showing gap (arrow) and tight (arrowhead) junction-like union between smooth muscle cells (smc) filled with myofilaments (mf). 63,400×.

100 °C under liquid paraffin for 5 minutes. They were removed and cover-slipped using fresh liquid paraffin. Examination of the slides was done under a Leitz ortholux© fluorescent microscope, using a 250/4 ultra high pressure mercury lamp with a Leitz Bp 546/filter block.

3 Results

The tunica media of the proximal segments down to T9 displays a transmural zonation in which there is a luminal elastic and adventitial musculoelastic zones (Figure 1a). The luminal zone comprises a uniform disposition of elastic lamellae (Figure 1b), while the adventitial one contains smooth muscle islands which interrupt some elastic lamellae (Figure 1c). In most of these muscle islands, the smooth muscle cells are disposed in series with thick elastic lamellae, with which they interdigitate. Some of the elastic lamellae and collagen fibres terminate into the muscle islands, while others transverse the island. Elastic lamellae and collagen

fibres between the islands are compact and form fibrous bundles onto which the muscle bundles insert (Figure 1d). The smooth muscle cells often converge onto the fibrous bundle from various directions (Figure 1e). Smooth muscle cells in the islands run circumferentially and longitudinally (Figure 1f). Further, the islands are colocalised with vasa vasora (Figure 1g) and adrenergic nerve terminals (Figure 1h).

Within these islands, smooth muscle cells pocess projections which interdigitate with the matrix (Figure 2 a,b). The parts of the cell adjacent to the matrix show areas of high electron density. The collagen and elastic fibres are haphazardly arranged (Figure 2 a,b), and some of the elastic fibres terminate in series with the smooth muscle cell processes (Figure 2c). Smooth muscle cells also show lateral interdigitations where they come in contact with each other. At these sites, the cell processes interlock with each other (Figure 2d), and in some of them, the basal laminae of the cells fuse (Figure 2e). The smooth muscle cells are rich in myofilaments and their processes establish cytoplasmic continuity in a manner akin to gap junctions (Figure 2f).

4 Discussion

Observations of the present study reveal that in the goat ascending aorta, aortic arch and proximal thoracic aorta down to T9, tunica media comprises an adventitial zone in which muscle islands interrupt elastic lamellae. These findings are concordant with those reported in the goat, cow and sheep (KNIERIEM, 1967; FUKUDA, PERRAUS and CRYSTAL, 1984; MALEK and OGENG'O, 2007). The present study further reveals that smooth muscle cells in these islands are characterized by abundance of myofilaments, cell-cell, cell-matrix interlinkages, colocalization with vasa vasora and adrenergic nerves. Abundance of myofilaments, nexuses and other intercellular junctions are considered to be features of contractile cells (DINGEMANS, JANSEN and BECKER, 1981; SOSA-MELGAREJO, BERRY and ROBINSON, 1991). In addition, areas of high electron density in the smooth muscle cells are known to represent points of anchorage of contractile filaments (BEZIE, LACOLLEY, LAURENT et al., 1998). On the other hand, adrenergic nerves modulate contraction while vasa vasora deliver required oxygen and nutrients. This suggests that the function of the islands involves active regulated contraction, which may serve several functions.

Smooth muscle contraction may synergise the windkessel function of the aorta in these animals; a function hitherto attributed to elastic lamellae alone (ROBERT, JACOB and FULOP, 1995). This role may derive from the myogenic response of vascular smooth muscle (JOHANSSON, 1989). Accordingly, during ventricular contraction, and rapid ejection of blood into the aorta, because of the inter-linkages between smooth muscle cells and matrix fibres, stretching of elastic fibres pulls the muscle islands, thus causing them to stretch as well. The smooth muscle cells then respond to this stretch by contracting, which in consonance with the elastic recoil, at the onset of diastole, facilitates the onward blood flow. In this way, the outer zone of the tunica media of the proximal parts of the aorta probably constitutes an "auxillary pump".

The myogenic response of smooth muscle cells in the islands could increase the mechanical strength of the aortic wall. Pertinent to this suggestion are observations of previous studies that active vascular smooth muscle resists distention upto 150 – 250 mmHg (DOBRIN, 1984). In addition, aortic smooth muscle may change its contractile state to keep intramural strain distribution uniform against temporary changes in blood pressure (MATSUMOTO, TSUCHIDA and SATO, 1996). Coincidentally, some of the smooth muscle cells of the goat aortic tunica media are in-series with the matrix, a feature which may be designed to enable the aorta achieve greater mechanical strength, especially at higher strains. In these circumstances, behaviour of the vessel wall has been shown to be consistent with an in-series arrangement of collagen and smooth muscles (SILVER, SNOWHILL and FORAN, 2003).

The muscle islands are probably involved in regulation of blood flow to the cranial parts of the animal. This suggestion is supported by the proposal that in birds, transmural zonation into a luminal elastic and adventitial muscular zones permits changes in vessel radius to modify the effects of drastic circulatory changes in the posterior part of the body during flight (BERRY, GERMAIN and LOVELL, 1974). A similar blood flow regulation role has been suggested for the muscular zone of the giraffe brachiocephalic and bicarotid trunks (KIMANI and OPOLE, 1991).

The mode of adrenergic innervation demonstrated by the present study in which the nerve terminals co-localise with the muscle islands probably regulates muscle contraction. Further, sympathetic nerves in the smooth muscle islands may serve to suppress the proliferation, differentiation (CHAMLEY, CAMPBELL and BURNSTOCK, 1974) and retro-differentiation of the smooth muscle cells into morphogenic cells, (KACEM, SEYLAZ, ISSERTIAL et al., 1995) thus maintaining the contractile phenotype. The importance of neurohumoral influences on the mechanical properties of the aorta has been suggested (GEROVA, GERO, DOLEZEL et al., 1973). Increased sympathetic activity alters the aortic diastolic pressure diameter relationship, reducing the diameter for any given pressure (PANGANI, SCHWARTZ, BISHOP et al., 1975). It is probable, therefore, that neurogenic contraction of smooth muscle cells in the islands contributes to aortic strength, thus constituting part of the mechanism for preventing aortic rupture in the wake of systolic pressure.

5 Conclusion

Smooth muscle islands showing features of predominantly contractile smooth muscle cells interlocked with matrix and co-localised with adrenergic nerves, and vasa vasora in the proximal segments of the aorta are probably designed to constitute an auxillary pump, for augumenting elastic recoil during diastole, a mechanism for strengthening the aortic wall, and to regulate blood flow.

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References

BERRY, CL., GERMAIN, J. and LOVELL, P. Comparison of aortic lamellar unit structure in birds and mammals. *Atherosclerosis*, 1974, vol. 19, no. 1, p. 47-59.

BEZIE, Y., LACOLLEY, P., LAURENT, S. et al. Connection of smooth muscle cells to elastic lamellae in aorta of spontaneously hypertensive rats. *Hypertension*, 1998, vol. 32, no. 1, p. 166-169.

CHAMLEY, JH., CAMPBELL, GR. and BURNSTOCK, G. Dedifferentiation, redifferentiation and bundle formation of smooth muscle cells in tissue culture: the influence of cell number and nerve fibres. *Journal of Embryology and Experimental Morphology*, 1974, vol. 32, no. 2, p. 297-323.

DE LA TORRE, JC. and SURGEON, JW. A methodology approach to rapid and sensitive monoamine histo-fluorescence using a modified glyoxylic technique – The SPG method. *Histochemistry*, 1976, vol. 49, no. 2, p. 81-93.

DINGEMANS, KP., JANSEN, N. and BECKER, AE. Ultrastructure of the normal human aortic media. *Virch Arch (Path and Anat)*, 1981, vol. 392, no. 2, p. 199-216.

DOBRIN, PB. Mechanical behaviour of vascular smooth muscle in cylindrical segments of arteries in vitro. *Annals of Biomedical Engineering*, 1984, vol. 12, no. 5, p. 497-510.

FUKUDA, Y., PERRAUS, VJ. and CRYSTAL, RG. Development of elastic fibres of nuchal ligament, aorta and lung of fetal and postnatal sheep. An ultrastructural and electron microscopic immunohistochemical study. *American Journal of Anatomy*, 1984, vol. 170, no. 4, p. 597-629.

GEROVA, M., GERO, G., DOLEZEL, S. et al. Sympathetic control of canine Abdominal Aorta. *Circulation Research*, 1973, vol. 33, no. 2, p. 149-159.

JOHANSSON, B. Myogenic tone and reactivity: definitions based on muscle physiology. *Journal of Hypertension*, 1989, vol. 7, no. 4, p. S5-8.

KACEM, K., SEYLAZ, J., ISSERTIAL, O. et al. Chemical sympathectomy favours vemetin expression in arterial smooth muscle cell of young rats. *Journal of the Autonomic Nervous System*, 1995, vol. 53, no. 1, p. 57-68.

KIMANI, JK. and OPOLE, IO. The structural organization and adrenergic innervation of the carotid arterial system of the Giraffe (Giraffa Camelopardalis). *The Anatomical Record*, 1991, vol. 230, no. 3, p. 369-377.

KNIERIEM, HJ. Electron microscopic study of bovine atherosclerotic lesions. *The American Journal of Pathology*, 1967, vol. 50, no. 6, p. 1053-1065.

MALEK, AKA. and OGENG'O, JA. Regional differences in the tunica media of the aorta of the goat *(cabra ibex)*. *Journal of Anatomy*, 2007, vol. 210, no. 6, p. 767.

MATSUMOTO, T., TSUCHIDA, M. and SATO, M. Change in intramural strain distribution in rat aorta due to smooth muscle contraction and relaxation. *American Journal of Physiology*, 1996, vol. 271, no. 4, pt. 2, p. H1711-6.

PANGANI, M., SCHWARTZ, PJ., BISHOP, VS. et al. Reflex sympathetic changes in aortic diastolic pressure-diameter relationship. *American Journal of Physiology*, 1975, vol. 229, no. 2, p. 286-290.

ROBERT, L., JACOB, MP. and FULOP, T. Elastin in blood vessels. *Ciba Foundation Symposium*, 1995, vol. 192, p. 286-299.

SILVER, FH., SNOWHILL, PB. and FORAN, DJ. Mechanical behaviour of vessel wall: a comparative study of aorta, venacava, and carotid artery. *Annals of Biomedical Engineering*, 2003, vol. 31, no. 7, p. 793-803.

SOSA-MELGAREJO, JA., BERRY, CL. and ROBINSON, NA. Effects of hypertension on the intercellular contacts between smooth muscle cells in the rat thoracic aorta. *Journal of Hypertension*, 1991, vol. 9, no. 5, p. 475-480.

ZHENG, JW., QIU, WL., ZHANG, ZY. et al. Anatomical and Histologic study of the cervical vessels in goats. *Shangai Kou Qiang Yi Xue*, 2000, vol. 9, no. 1, p. 39-41.

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