Ascending aorta of hooded seals with particular emphasis on its vasa vasorum

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Blix AS, Kuttner S, Messelt EB. Ascending aorta of hooded seals with particular emphasis on its vasa vasorum. Am J Physiol Regul Integr Comp Physiol 311: R144–R149, 2016. First published April 27, 2016; doi:10.1152/ajpregu.00070.2016.—The pressure-volume relationship in the ascending aorta (“windkessel”) of the hooded seal was determined and the morphology of its vasa vasorum described in some detail. We found that the ascending aorta has a high compliance and can easily accommodate the entire stroke volume when the peripheral vascular resistance becomes much increased and maintain perfusion pressure during the much extended diastole and thereby reduce cardiac stroke work during diving. We also found that the 3- to 5-mm thick wall of the ascending aorta had a very elaborate vasa vasorum interna with a hitherto undescribed vascular structure that penetrates the entire vascular wall. If similar structures with similar importance for the nutrition of the wall of the vessel are found in humans, important implications for the understanding of pathological conditions, such as aneurisms, may be indicated.

hooded seal; vasa vasorum; aortic aneurysm

Scholander (29) demonstrated, indirectly, that seals may respond to diving with a widespread peripheral arterial vasoconstriction, which secures delivery of oxygenated blood to the brain at the expense of muscles and visceral organs. This was subsequently confirmed by muscle blood flow measurements (15), and Irving et al. (19) demonstrated that the resultant enormous increase in peripheral vascular resistance was compensated with a dramatic bradycardia, which had been observed, for the first time, in the duck, as early as 1870 (1). More important, Irving et al. (19) also demonstrated that central arterial blood pressure was by and large maintained during diastole, despite heart rates as low as 10 beats/min during diving. However, it was not until Elsner et al. (10), who also measured arterial blood pressure in seals, that the importance of the bulbous ascending aorta was recognized as a necessary “windkessel” for maintenance of blood pressure during the extended diastole during diving. The presence of a huge bulbous ascending aorta in seals (Fig. 1) was known already to early anatomists (4, 22) but did not receive much attention until Drabek (6) examined it in some detail in several Antarctic seals. He found that the tunica media consisted mainly of anastomosing elastic fibers, some collagen and a few smooth muscle cells. He also measured the diameter of previously Formalin-fixed bulbs and concluded that the size of the bulbs, expressed as a percent increase in the aorta’s greatest diameter, relative to the diameter of the base of the aorta, was bigger in deep diving species than in the more shallow divers. He also found that bulb (and heart) was smaller in newborn and juvenile Weddell seals than in the adult. Drabek and Burns (7) subsequently measured the diameter of fresh bulbs from the hooded seal and found that the size of those bulbs, proximal to the brachiocephalic artery, was greater than in Ross and leop-ard seals, but smaller than in Weddell and crabeater seals. They also found that the size of the aortic bulb (and the heart) of juvenile hooded seals, relative to lean body mass, was similar to that of adults, unlike in Weddell seals, but as in the common seal (32). In all three studies (6, 7, 32) vasa vasorum (VV) is easily recognized in their histological sections, but never mentioned in their text. To our knowledge the only mention of a VV in arteries of seals of any kind is in Rhode et al. (26) who studied static mechanical properties of the thoracic aortas of harbour and Weddell seals and mention the presence of VV in the aortas, in passing, without further comment. It is also conspicuous that most major medical textbooks do not mention VV and that the few that do seem to be under the impression that it consists only of vessels that penetrate the adventitia and, in some cases, parts of the media of major arteries, from the outside. That this VV externa provides nutrition to the outer part while the inner part of the vascular wall depends on diffusion from the lumen, is also the prevailing notion in many much cited papers (e.g., 16, 17, 30, 34). This is puzzling for at least two reasons. First, Schönenberger and Müller (28) already in 1960, in a little known paper, reported on an elaborate VV interna in the aorta of the cow and Gössl et al. (14) found evidence also for a VV interna in the coronary artery of the swine. Ritman and Lerman (27) have even elaborated on its importance and medical implications in health and disease. Second, it is hard to envisage that the capillary pressure in a VV originating from the outside of the aorta will not be exceeded by the transmural pressure and thereby occlude the capillaries and prevent perfusion.

The hooded seal has a very prominent ascending aorta (7; Fig. 1) with a wall thickness of 3–5 mm. For that reason a VV interna is obviously needed and in the bulb of their aortas the entrances to the VV interna from the lumen of the vessel are easily seen with the naked eye. In the present study we have determined the pressure-volume relationship in freshly isolated ascending aortas and examined the morphology of their VV interna in detail in samples that were perfusion fixed under arterial pressure. This revealed novel features that may have important implications also in humans.

MATERIALS AND METHODS

Animals

A total of six female hooded seals (Cystophora cristata), 154–220 kg body mass, were used. The animals were killed for other purposes, by a shot to the head, during a research expedition to the pack ice off East Greenland, under permits from the Royal Norwegian Ministry of Fisheries and the Royal Danish Ministry of Foreign Affairs. The

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The ascending aorta, from the exit of the coronary arteries to immediately after the exit of the brachiocephalic artery, was removed, and the openings at each end of the excised aorta were closed with plastic discs with rim groves that were secured in position by use of 0010A umbilical tape (Ethicon, Sommerville, NJ)(Fig. 2). The brachiocephalic artery was then cannulated with a silicone tube (inner diameter $\frac{10}{1000}$ mm) that ended in a graded fluid reservoir. The reservoir was filled with saline and lifted along a graded ruler for reading of pressure (in cmH$_2$O, later to be converted to kPa). The volume changes of the ascending aorta were recorded as changes of volume in the graded reservoir, with the ascending aorta immersed in saline.

Morphology

Pretreatment. The isolated ascending aortas were mounted as described above (Fig. 2) and first perfused under arterial pressure (14 kPa) with saline and thereafter with 4% glutaraldehyde in 0.1 mol/l phosphate buffer adjusted to pH 7.2. After 2 h, under arterial pressure, the walls of the aortas were cut into pieces ($2 \times 2 \text{ cm}^2$) and kept in 2% glutaraldehyde until processing.

Light microscopy. Samples intended for light microscopic examination were subsequently dehydrated in graded series of ethanol up to 100% and embedded in Epon (Electron Microscopy Sciences, Hatfield, PA), then serial sectioned on an Ultracut UCT microtome (Leica, Wetzlar, Germany), stained with an aqueous solution of 0.1% toluidine blue adjusted to pH 8.9 with 0.067 M Na$_2$HPO$_4$, and examined using a color View Camera with accompanying software (Soft Imaging System, Münster, Germany).

Transmission electron microscopy. Samples intended for transmission electron microscopy (TEM) were pretreated as for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a CM120 transmission electron microscope (Phillips, Amsterdam, The Netherlands). Images were captured using a Morada camera with accompanying software (Soft Imaging System).

Computed tomography. A fresh aorta mounted as described above was perfused with barium sulfate solution (Mixobar Colon 1 g/ml; Astra Tech AB, Mölndal, Sweden) under arterial pressure (14 kPa) and the convex wall subsequently cut in two $3 \times 5 \text{ cm}^2$ samples that were fastened to pieces of cardboard with pins and frozen at $-20^\circ$C with the inner surface facing up. The samples were later positioned in the center of the field of view inside a Triumph II XO CT small animal PET/SPECT/CT scanner (Trifoil Imaging, Chatsworth, CA), while still frozen. Computed tomography (CT) was performed using 80 kVp, $1 \times 1$ binning, 1,024 projections, and a magnification factor varying between 1.3 and 3.5. The corresponding pixel size varied between 65 and 177 $\mu$m. The projection data were reconstructed into three-dimensional images using filtered back projection with the vendor’s software. Image analysis and postprocessing were performed with PMOD (PMOD Technologies, Zürich, Switzerland) by masking out the sample, forming maximum intensity projections, and applying a color table.

**Statistical Analysis**

Linear regression analysis was used for the data presented in Fig. 2.

**RESULTS**

**Gross Anatomy**

The ascending aorta is very prominent in the hooded seal (Fig. 1), where in adult animals it has a wall thickness of 3–5 mm.

![Fig. 1. The bulbous ascending aorta (A) of a juvenile hooded seal. (Photo: A. S. Blix).](image)

![Fig. 2. Pressure (kPa)-volume (ml) relationships of the ascending aorta of 2 female adult hooded seals. The relationship is described by the equation: volume (ml) = $69.24 \times \text{pressure (kPa)} - 35.98; \ N = 2 \text{ seals; } n = 14 \text{ experiments; } r^2 = 0.91$. Inset: arrangement for the pressure-volume testing and the perfusion of the vasa vasorum interna of the ascending aorta. The excised aorta was closed by plastic discs at the level of the coronaries and just distal to the brachiocephalic artery, which was cannulated and connected to a reservoir.](image)
Pressure-Volume Relationships

The pressure-volume relationships of the ascending aorta of two hooded seals is shown in Fig. 2. A linear model was assumed, and the following regression line was obtained:

\[ \text{volume (ml)} = 69.24 \times \text{pressure (kPa)} - 35.98; N = 2; n = 14; r^2 = 0.91, \]

the volume of the neutral aortas (transmural pressure = zero) being \( \approx 330 \text{ ml} \). The response to increased pressure was linear within normal variations in central arterial pressures, and the expansion was immediate, as was the recoil when pressure was released. Most of the expansion occurred in the convex part of the vessel.

Morphology

The tunica media of the wall of the ascending aorta consisted almost entirely of elastic fibers interspersed with an abundance of VV (Fig. 3, A and B). The many entrances to the VV from the lumen of the aorta could easily be seen by the naked eye (Fig. 4A), while microscopic examination revealed a complex network of vessels inside the wall (Fig. 4, B and C; Fig. 5, A and B) that seems to terminate in a profusion of veins (wall thickness/internal radius = 1/15; 5) near the outer (adventitial) surface (Fig. 6, A and B). The walls of the VV interna, unlike other known vasculature, regardless of great differences in diameter, consisted only of a monolayer of cells (Fig. 7, A and B), and the normal order of steadily decreasing diameter as an artery branches up into the vascular tree was not apparent. Instead, vessels with any diameter may branch off at any stage in the tree (Fig. 4, B and C; Fig. 5, A and B) and anastomoses occurred between trees that had started from different entrances in the lumen of the aorta. Injection of the barium sulfate solution into the VV interna, under arterial pressure, revealed that some branches of the system that started from the lumen of the aorta penetrate the wall and spread out in a normal arterial fashion on the outer (adventitial) surface of the vessel (Fig. 8).

DISCUSSION

Several investigators (6, 10, 26, 32) have pointed out the importance of the aortic bulb for maintenance of central blood pressure during the sometimes very long diastole in the diving seal. Moreover, Drabek (5) and Drabek and Burns (7) have argued that the size of the aortic bulb is related to the diving capability of the species, but this argument is difficult to follow and is hardly borne out by their data: In Drabek’s Table 2 (6), the crab-eater seal (Lobodon carcinophagus), which is known to be a shallow diver (24), has a bigger aortic bulb than the Weddell seal (Leptonychotes weddelli), which is an accomplished diver (21). In Drabek’s Plate 7 (6), on the other hand, the crab-eater seal has the second smallest bulb; only another shallow diver, the leopard seal (Hydrurga leptonyx) (23), has, as would be expected from their argument, a smaller one. One would assume that the data in the table are wrong, but the same value is repeated without comment in Drabek and Burns (7), in which the hooded seal, which is also a very accomplished diver (11), has a bulb size that is comparable to that of the Weddell seal. Drabek (6) also found that the size of the aortic bulb was much smaller in the pups than in juvenile and adult Weddell

![Fig. 3. A and B: thin sections through the tunica media of the ascending aorta of female adult hooded seals showing a great density of elastin strands and several vasa (VV), one, in A, with a long branch (arrow).](image)

![Fig. 4. Scanning electron micrograph of the ascending aorta of a female adult hooded seal. A: showing the inner (lumen) surface of the vessel with an entrance to the vasa vasorum interna (top), with several branches of very different diameter in the media, in cross section after freeze-cracking of the sample (bottom). B and C: scanning electron micrographs of cross sections through the media of the ascending aorta of a female adult hooded seal showing the complexity of the vasa vasorum interna.](image)
seals, and he attributed that to the fact that the young pups of this species do not dive. This notion was later supported by van Nie (32) who found that the size of the aortic bulb relative to heart weight (and lean body mass) did not change with age in the common seal (Phoca vitulina vitulina), which starts to dive shortly after birth. The hooded seal also starts diving and develops a remarkable diving capacity shortly after birth (12), and while the heart is a much larger fraction of the total muscle mass in pups than in adults (3), the size of the aortic bulb is smaller in pups than in adults, but still fairly large (7). In any case, it is difficult to accept that the size of the aortic bulb should in any way be directly related to the animal’s ability to dive deep for long periods of time, since the importance of this structure is in its ability to act as a “windkessel” and store the stroke work in each individual systole. Thereby a disastrous increase in blood pressure during systole is avoided, and blood pressure during the extended diastole that occurs in prolonged dives (25) is maintained. It follows, that the size of the bulb should rather be related to the lean body mass of the seal. Unfortunately, the body mass of the individuals used by Drabek (6) is not available and that relationship can therefore not be tested at present.

The stress-length relationship of the hooded seal ascending aorta was found by us to be linear over the normal range of blood pressures (Fig. 2B). The stroke volume has not been measured in hooded seals, but assuming that it scales linearly with body mass, it can be extrapolated from data from other seal species (20, 31, 35) that our hooded seals would have a stroke volume in the order of 300 ml. It follows from our data (Fig. 2) that the ascending aorta of the hooded seal has the capacity to store the entire stroke volume without excessive increase in systolic pressure, which, in turn, is a prerequisite for the development of the cardiovascular responses to prolonged diving (25).

Heistad et al. (18) found that the blood flow through “the outer two-thirds of the media of the thoracic aorta” of dogs receive 10 ml·min⁻¹·100·g⁻¹ through the VV, whereas flow to “the inner third of the aorta” was 1 ml·min⁻¹·100·g⁻¹. Later they measured blood flow through the VV in the wall of the arch of the aorta “at the origin of the great vessels” in the dog and found it to be 0.5, 5, and 3 ml·min⁻¹·100·g⁻¹ in the inner, middle, and outer part, respectively (17). Finally, they reported flow values from the media of the aortic arch of other dogs in three different experiments and found 9, 6, and 8 ml·min⁻¹·100·g⁻¹, with the astonishingly high values of 29, 22, and 26 ml·min⁻¹·100·g⁻¹ in the adventitia, respectively (16). Heistad and co-workers (16) assumed that these flow values represent flow in the VV externa, but their use of radioactive microspheres does not allow discrimination between the flow in VV interna and externa. In a previous study we (2) also measured the blood flow in the wall of the aortic bulb of spotted seals (Phoca vitulina largha) and gray seals (Halichoerus grypus) with radioactive microspheres. We found that the flow in the inner (media and interna) part was fairly stable at 1.4 ml·min⁻¹·100·g⁻¹ before, during, and after 10-min dives, whereas the flow in the outer (adventitia) part...
Fig. 7. A and B: transmission electron micrographs from the tunica media of the ascending aorta of a female adult hooded seal showing the thin endothelial walls of the vasa (arrow).

averaged 3.4, 1.2, and 4.2 ml·min⁻¹·100·g⁻¹ before, during, and after 10-min dives (Blix AS, Elsner R, Kjekshus JK; unpublished data). Our values are considerably lower than those of Heistad and associates (16–18). The reason for this may be that our samples were limited to the ascending aorta, where smooth muscle is all but absent, while that may not have been the case in “the aortic arch” of dogs and therefore the need for oxygen and nutrition may be less in the former. It is however intriguing that the flow in the media of the seal aorta did not change in response to diving, which may indicate an additional source of blood supply to the outer parts (Fig. 8). Heistad and associates (16, 33, 34) argue that the source of the VV in the thoracic aorta of dogs arise from the intercostal arteries, the great vessels, and coronary arteries and that nourishment of the wall of the aorta is accomplished by diffusion from the lumen of the vessel, diffusion from adventitial VV, and in thick-walled vessels by blood flow through medial VV. They (16) also reported that the circumflex coronary artery in dogs has little or no blood flow through VV in the media. It is difficult to comprehend that it may be possible to perfuse the deeper parts of the media of walls of substantial thickness, from the outside, against a very high transmural pressure. Accordingly, already Schöenenberger and Müller (28) described the presence of VV interna, originating from the lumen of the aorta in cows, and they found anastomoses among VV externa and interna. Gössl et al. (14) also found evidence for a VV interna in the walls of 5 of 10 coronary arteries of the swine and reported that the branching architecture of the VV trees was surprisingly similar to that of vasculature in general. Moreover, Gössl et al. (13) found that the VV of the LAD coronary artery of dogs are functional end arteries that are not connected via a plexus, but their CT technique did not allow detection of small vessels. In the hooded seal we found anatomical evidence for anastomoses between the VVs of different origin in the lumen of the aortic bulb, but such connections were not abundant.

In the present study of the VV interna of the hooded seal we used samples of the aortic bulb that was perfusion fixed under arterial pressure. This allowed, for the first time, exposure of very small vessels and delicate structures that might otherwise have collapsed and been obscured. We found, unlike the tree-like structure described by Gössl et al. (14), that the branching of the VV of the hooded seal, from its start as a big hole in the inner wall of the aorta, seemed rather haphazard, with branches of any diameter taking off at any time throughout the media (Fig. 4, A–C). Moreover, the walls of the VV were very delicate, consisting in most cases only of an epithelial lining (Fig. 7, A and B), regardless of the diameter of the vessel. We did not detect any differentiation into arteries and veins in the hooded seal VV, instead it appears that vessels of different diameters terminate in an abundance of veins in the outer adventitia of the aorta (Fig. 6, A and B).

In addition, our injection of the barium sulfate solution into the lumen of the aortic bulb, under arterial pressure, revealed that some vessels originating in the lumen of the aorta traverse the wall and emerge at the outer surface of the bulb, from where they differentiate into an ordinary arterial tree in the adventitia (Fig. 8). In the present study we did not investigate any presence of a VV externa, but our data on blood flow within the wall (2) suggest that there is a VV externa also in seals.

The possible presence of adventitial lymphatics, as identified by use of immunohistochemical staining by Drozdz and associates (8, 9) in the human carotid artery, was not investigated in this study. We have noticed, however, that the histological cross sections of their lymphatic vessels have a visual appearance very much like those of the vasa vasorum we have described in the media of the hooded seal aorta. This, as well as the differentiation between lymphatic vessels and those of the vasa vasorum externa, obviously warrants further study.

Perspectives and Significance

This paper documents for the first time the presence of an elaborate vasa vasorum interna in the wall of the ascending aorta of the hooded seal. The blood flow through this vascu-
lature may be rather small, but may nevertheless be decisive in providing the necessary nutrition to maintain the integrity of wall. If a similar kind of VV interna is to be found also in humans, obliterating its entrances from the aortic lumen by, for example, atherosclerotic plaques (27), may lead to ischemia and aneurysms.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.S.B. conception and design of research; A.S.B. and E.B.M. performed experiments; A.S.B. and S.K. analyzed data; A.S.B., S.K., and E.B.M. interpreted results of experiments; A.S.B., S.K., and E.B.M. prepared figures; A.S.B. drafted manuscript; A.S.B. edited and revised manuscript; A.S.B., S.K., and E.B.M. approved final version of manuscript.

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