Hindawi
International Journal of Zoology
Volume 2023, Article ID 8395360, 9 pages
https://doi.org/10.1155/2023/8395360

Research Article

A Preliminary Study on the Functionality of the Carotid-Vertebral Anastomotic Artery in the Regulation of Blood Flow in the Giraffe (*Giraffa camelopardalis*) by Duplex Ultrasound Examination

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Received 3 June 2022; Revised 7 February 2023; Accepted 10 February 2023; Published 27 February 2023

Academic Editor: Vipin Rana

Postural change intermittently between upright and head down in giraffes standing at a height of 4.5 meters is of physiological significance. The length of a giraffe's neck denotes the flow of blood against the force of gravity, to supply the brain over a 2 m distance. The force of gravity also affects the flow of blood toward the brain, with a posture change from erect to ground level. How do these changes in stance not result in fainting when the head is raised and brain damage when the head is lowered? Giraffe has an advanced interconnection of the common carotid artery and the vertebral artery. The connection is located at the midpoint of the atlas, as indicated by means of computerized tomography and dissection. Duplex ultrasound with Doppler waveform examination showed the unidirectional movement of blood with movement from the vertebral artery into the common carotid artery when the head is erect. The direction of flow allows the provision of blood to the maxillary artery that feeds the rostral epidural rete that supplies to the brain. The flow direction in the carotid-vertebral connection changes when blood moves in the direction of the head along with the force of gravity, when the head is lowered. The rerouting of blood to move from the common carotid into the vertebral artery prevents brain damage. We have confirmed, by utilizing a CT scan, Doppler sonar, and dissection of latex-filled arteries, the existence and blood flow direction within the anastomotic artery associated with variation in posture in the giraffe.

1. Introduction

The order Artiodactyla is recognized as very successful ungulates inhabiting a wide array of different and often characteristically challenging environments [1]. The giraffe (*Giraffa camelopardalis*) is not only challenged in its external environment but is also physiologically challenged. An extremely long neck, with the heart located 2 meters below the brain, high blood pressure of 200–400 mmHg [2–4], and long thin legs describe their unique build and highlight cardiovascular challenges faced by this species. Critical physiological issues result at this typical high blood pressure [2–4]. Continuous blood supply and subsequent oxygen supply to the brain, without any disruption in flow, are of utmost importance to prevent fainting and brain damage [1, 5–7]. Fainting is preceded by the dilation of blood vessels with the concomitant drop in blood pressure resulting in an inadequate flow of blood to the brain [8]. This is exacerbated by the fact that brain tissue is incapable of storing the fuel supply source, glucose [9]. The gravitational force exerted on the 2 m blood column from the heart to the head, when the animal reaches down to drink, renders it susceptible to protrusion of a wall of an artery, also known as a brain aneurysm or stroke [10]. High blood pressure in the body, neck, and head is overwhelmed by even higher blood pressures in the lower legs [11]. The combined influence of high blood pressure and the extreme gravitational effect on the extended blood column from the heart to the legs should, under normal circumstances, be physiologically ideal for the development of oedema in the lower extremities [11]. With blood pressures in the legs, recorded well above 400 mmHg, oedema is expected [12]. Several studies have explored possible adaptations to the physiological challenges giraffes face [11, 13–15]. One possible adaption to cerebral blood flow control is the unique carotid-vertebral arterial connection observed in giraffes [15]. The anastomosis between the two arterial pathways known to supply blood to the brain is significant when taken into consideration that the basilar artery in giraffe is reduced in size and not functional with regard to cerebral blood supply [13, 14]. The functionality of this connection in giraffes is unknown and not well researched. A study conducted on the effects of gravity on blood circulation in snakes indicated structural and functional countermeasures work in combination to adapt to body form [11]. We, therefore, aimed to confirm the location of the anastomotic arterial connection between the common carotid artery and vertebral artery in giraffes and the functionality thereof. We also investigated the flow of blood within the anastomosis, during head movements from upright, to ground level, and back to a fully erect position. Few studies describe the unified concept of how giraffes successfully circulate blood, avoid fainting and blackouts, avert brain aneurysms, and prevent oedema in the legs.

2. Materials and Methods

2.1. Experimental Model and Subject Details. Two mature giraffe heads were obtained. No animals were specifically sacrificed for use in this study. Unfortunately, these giraffes were culled by the reserve management due to overstocking densities and food scarcity. We only collected biological samples during the culling and were not involved in the actual process at all. Most reserves and game ranches either sell, translocate, or cull the excess number of animals every year. This is considered a common and frequent practice in Southern Africa Wildlife Industry to limit herbivore numbers to exceed the actual carrying capacity and to prevent death due to starvation.

A third, living adult giraffe male was used for the second experiment.

Experiment part 1: Two adult giraffe (*G. camelopardalis*) heads $n = 1$ male and $n = 1$ female were obtained.

Experiment part 2: One male giraffe (*G. camelopardalis*) was used for this experiment. The experiment was conducted on only one giraffe due to the following factors:

(1) High incidence of fatality of a giraffe during chemical immobilization

(2) Availability of a highly specialized ultrasound device suitable for use in the field

(3) Availability of human cardiac radiologists to conduct a field operation

Experiment part 3: Two adult giraffes (*G. camelopardalis*) $n = 1$ male and $n = 1$ female were obtained. These are the same animals used in experiment 1.

3. Method Details

Experiment part 1: The two giraffe heads were obtained. The specimens included the head and neck, which were severed at the position of the fourth cervical vertebra. The vascular system was manually rinsed out with warm water. The specimens were elevated with the nasal region pointing upwards and rinsed until water drainage ceased.

Two different mixtures were prepared for the arterial and venous systems, respectively. Barium sulphate ($BaSO_4$) (X-ray grade, Kyron Powder, Kyron, SA), Latex moulding rubber (A. Shak (Pty) Ltd, SA), and red and blue pigments were used. Arteries were colored red (Stamp ink, Office mate, SA) and veins were colored blue (Print ink, Treeline, SA) for differential purposes. The mixture used for arteries included $BaSO_4$ powder of 40 percent volume, 58 percent latex volume, and 2 percent volume of red ink. The mixture used for veins included $BaSO_4$ powder of 20 percent volume, 78 percent latex volume, and 2 percent volume blue ink.

After flushing with water, the vascular system was infused with the variable latex mixtures by means of a pipe (10 mm PVC tubing), held 2 meters above the giraffe head, thus creating a pressure gradient. Tubing was fixed with adhesives and ligatures. The red latex mixture was infused into the common carotid artery. The blue latex mixture was infused into the jugular vein. Following latex infusion, all arteries and veins were ligated with catgut (Catgut 3, Kyron, SA). The specimen heads were placed at a 30° angle for 24 hours, allowing the latex to set. Specimens were stored frozen until computerized tomography (CT) scanning could be performed. The heads were CT scanned at Netcare Kroon Hospital, Radiology Department, South Africa. The CT scan was performed by a qualified radiologist using a Siemens SOMATOM Emotion 6, CT scanner. Specimens were scanned with a resolution of 1.25/6x100, p0.7 slice thickness, 130 kV, and 79 mA. In both the heads, the arterial and venous systems were injected. The General Electronic Computerised Tomography with associated software was used to take the CT scan illustrating the latex-filled arterial and venous system. A medically associated photoshop program (Photoshop version CS.6) allows the radiologist to remove, for instance, bone showing only soft tissue. In this case, the photo was manipulated to remove the venous system and enhance the arterial system. We used two different colorants in the latex, a typical red colorant was used for the arterial system and a blue colorant was used for the venous system. That made it possible afterward to only select one set of systems at a time using the photoshop program. With the help of Dr. Christopher Basu at the Royal Veterinary College of London, we used the Sketchfab software to digitally process the scans. The Sketchfab software is a 3D program typically used in the medical world for explaining complex anatomical structures to teach people about physiology and anatomy. Following the CT scanning of specimens, giraffe heads were manually dissected to expose the area where the anastomosis was located according to the CT scan results. The anastomosis, a prominent artery, between the common carotid artery and the vertebral artery, was exposed and photographed with a Canon D5 camera, with 24-105 Canon lens.

Experiment part 2: One male giraffe (*G. camelopardalis*) was used for this experiment.

A young giraffe bull aged 7-8 years was located and was immobilised with a trained and experienced giraffe capture team for the cardiology radiologists to perform a sonar. The giraffe was darted in the rump, with a dart gun (Pneu-Dart, 389). The dart contained a mixture of A3080 (Thiafentanil) and Stresnil (Azaperone). After 10 minutes, the typical immobilizing effect of the drugs was seen in the giraffe, whereafter it became recumbent. The ears and eyes were closed immediately to prevent stimulation by sight and sound from subsequently causing stress. A partial antagonist drug, M5050 (Diprenorphine), was used as the antidote against the immobilizing drug (A3080). This allows for the calm regaining of consciousness. The blood pressure of the giraffe is highly sensitive to the drugs used to immobilize the animal, and the drugs must, therefore, be reversed immediately after the animal becomes recumbent in order to prevent death [16]. The giraffe was held in a recumbent position, with the head and neck moved to simulate a head upright (Figure 1(a)) and head-down position (Figure 1(b)).

A SonoScape S8 Exp Ultrasound device, equipped with a 3.6–5 MHz convex probe, was used to determine the flow direction in the anastomotic artery between the common carotid artery and the vertebral artery. The hair was shaved in all the areas where measurements were taken. Coupling gel was applied, and the probe was placed posterior to the sternocleidomastoid muscle. After locating the carotid-vertebral anastomosis, the Doppler sample volume was placed in the anastomotic artery approximately 2 cm from its confluence with the carotid artery (Figure 2). A Doppler waveform was obtained to determine the direction of flow in the anastomotic artery (away from or towards the carotid artery), whilst in a head upright as well as a head-down position. The ultrasound device was also used to measure the diameter of the proximal and distal common carotid artery and the internal jugular vein in a head upright and head-down position.

After completion of the measurements, the giraffe was given the full dose of antidote intravenously. The ear and head covers were removed, and the animal regained complete consciousness.

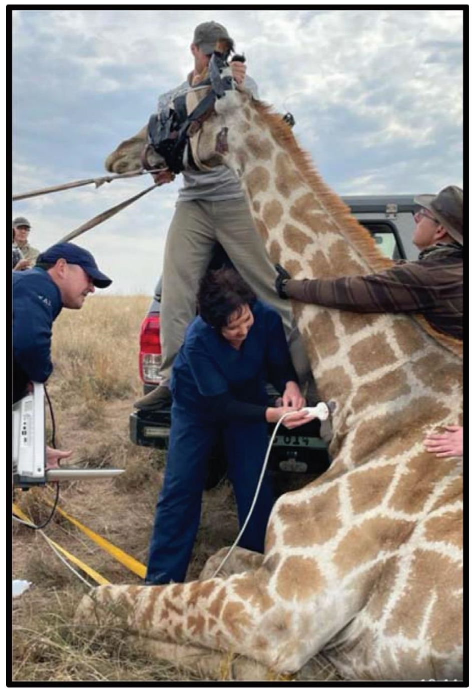
Experiment part 3: Samples were retrieved from the two giraffes. The neck of the specimens was severed at the position of the fourth cervical vertebra. The neck was opened from this point along the length of the neck up to the connection between the seventh cervical and the first thoracic vertebra, exposing the jugular vein and carotid artery. The jugular vein and carotid artery were removed and manually rinsed out with warm water. Both were cut open to expose the lumen, to confirm the presence of valves along the entire length of the jugular vein and the absence of valves along the length of the carotid artery. Experiment three confirmed that speculations on possible arterial valves are not present [17].

4. Results

Experiment part 1: The CT scans performed on both male and female giraffe illustrated the position of the anastomotic artery, which provides a direct connection between the common carotid artery and the vertebral artery (Figure 3(a)). The anastomotic artery is situated at the center of the atlas, where it is observed as a prominent vessel that branches off the common carotid artery. At the location where the anastomotic artery branches from the common carotid artery, it transits through a foramen, known as the alar fossa [11, 15]. Two canals are evident within the alar fossa, with the medially situated, larger canal allowing a notable link from the anastomotic artery straight into the vertebral arteries (Figure 3(a)). In the giraffe, it is evident that blood supply from the vertebral arteries does not contribute to the cerebral arterial circle, due to an underdeveloped basilar artery (Figure 3(b)) and that the vertebral arteries course within the vertebral column through the axis and atlas bones where they connect to the anastomotic artery.

Experiment part 2: The duplex ultrasound examination showed blood flow direction during head upright and head-down positions. The Doppler waveform with color flow images demonstrated a preferential blood flow in the anastomotic artery in the direction away from the vertebral artery towards the common carotid artery when the head is in an upright position (Figure 4(a)). The blue color in the anastomotic artery and the Doppler waveform below the basis line indicate flow within the anastomotic artery, which is away from the sample volume. Thus, the flow direction in the anastomotic artery is away from the vertebral artery towards the common carotid artery. The blood flow direction as observed on the ultrasound device is demonstrated in the illustrations of the dissected specimens where the common carotid artery, the anastomotic artery, and the vertebral artery are present (Figure 4(b)). Supplementary Figures 1(a) and 1(b) show additional illustrations of raw dissected specimens and specimens with red and blue colors added, respectively, to distinguish arteries and veins.

The Doppler waveform with color flow images demonstrated a preferential flow in the anastomotic artery directed away from the common carotid artery towards the vertebral artery, in the head-down position (Figure 5(a)). The red color in the anastomotic artery and the Doppler waveform above the basis line indicate that flow is towards the sample volume. Thus, the flow direction within the anastomotic artery is away from the common carotid artery towards the vertebral artery. The blood flow direction as observed on the ultrasound device is demonstrated in the illustrations of the dissected specimens where the common carotid artery, the anastomotic artery, and the vertebral artery are present (Figure 5(b)). This equates to a summary confirmation of blood flow within the anastomotic artery by means of a CT scan, physical dissection of the anastomotic and its associated arteries, and the Doppler flow experiment. The CT scan illustrates the potential for blood flow direction. The physical dissection of latex-filled arteries proved the existence and possible blood flow direction within the



(a)

(b)

Figure 1: (a): Doppler waveform measurement in the head upright position. (b) Doppler waveform measurement in the head-down position.

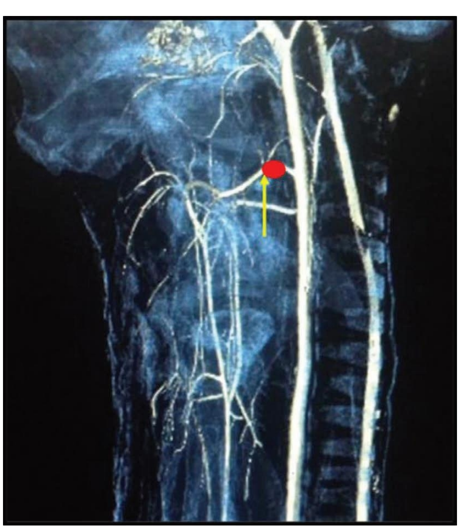
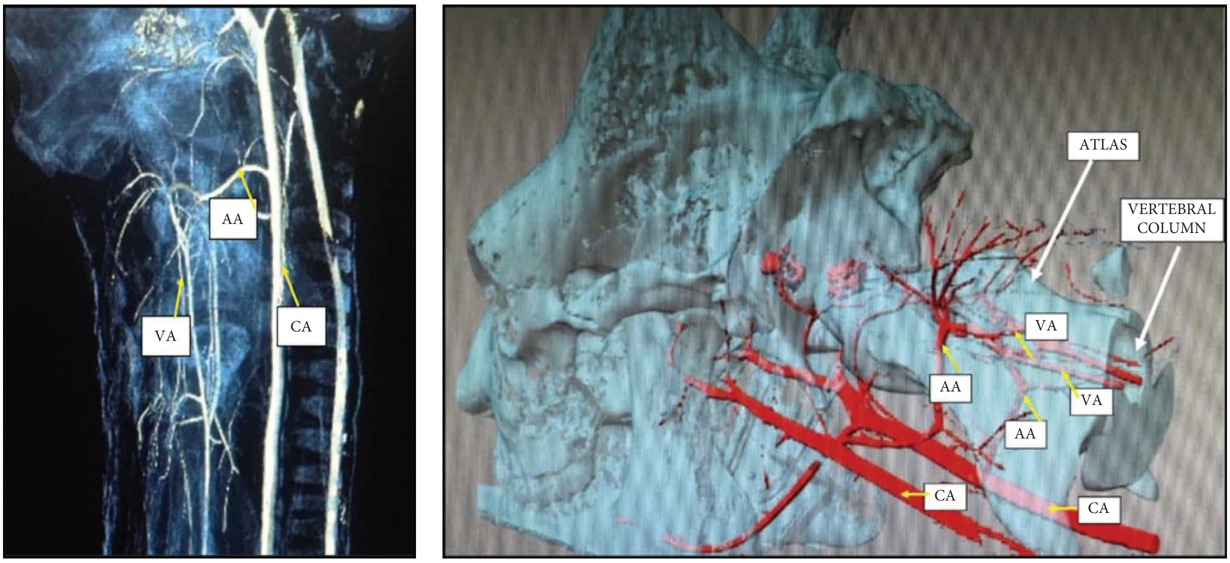


Figure 2: Position of Doppler sample volume in the anastomotic artery on the CT scan.

anastomotic artery in different head positions. Most importantly, the Doppler experiment provides evidence on the blood flow direction within the anastomotic artery in different head positions.

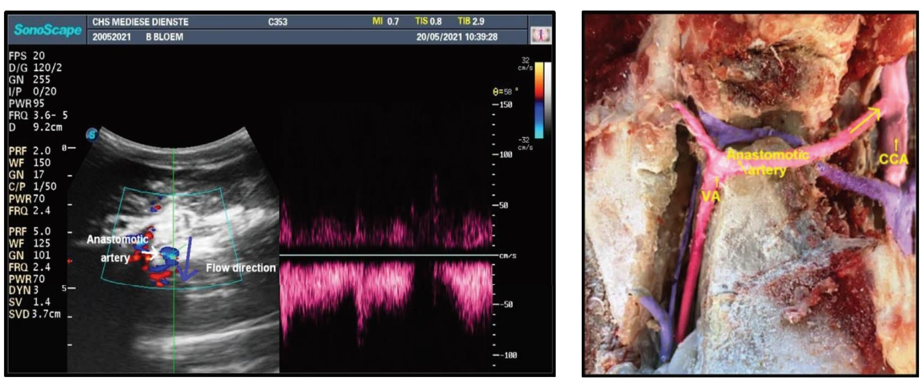
Experiment part 3: The jugular vein in the giraffe contains several valves throughout the length of the neck. However, no valves were observed anywhere along the length of the carotid artery throughout the length of the neck (Figure 6). Blood flow can, therefore, occur bidirectionally in arteries, but in veins, it can only occur in a single direction due to the presence of valves.

Blood movement within the anastomotic artery is thus posture dependent, as illustrated diagrammatically in Figure 7. With posture changing to fully erect, the blood supply to the brain route entails blood flow from the vertebral artery through the anastomoses into the common carotid artery. Blood then follows the normal route via the maxillary artery supplying the rostral epidural rete mirabile with subsequent brain perfusion.



(a) (b)

Figure 3: (a) Computerised tomography image illustrating the common carotid artery (CA), anastomotic artery (AA), and the vertebral artery (VA). (b) An illustration of the common carotid artery (CA), anastomotic artery (AA), and vertebral artery and the course of the vertebral arteries within the vertebral column through the atlas where they connect to the anastomotic artery.



(a) (b)

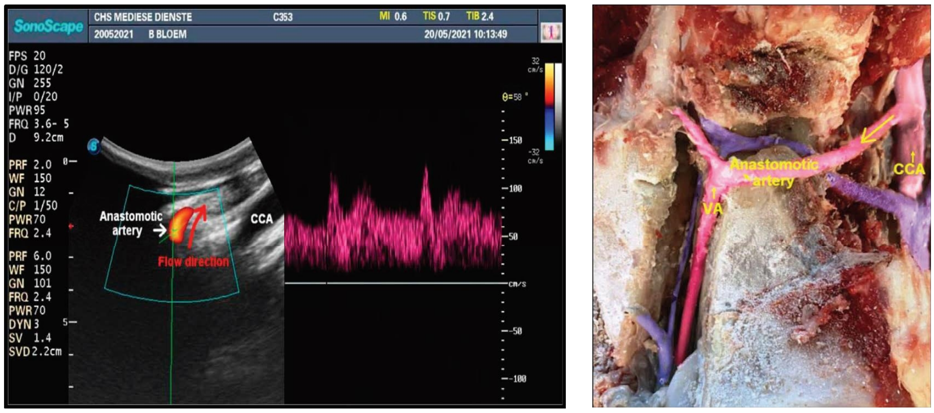
Figure 4: (a) Doppler waveform with color flow images demonstrated preferential flow in the anastomotic artery directed away from the vertebral artery towards the common carotid artery in the head upright position. (b) Visual illustration of the latex model during detailed dissection of the carotid-vertebral anastomosis with the direction of flow (yellow arrow) away from the vertebral artery (VA) towards the common carotid artery (CCA), in the head upright position.

The blood flow direction associated with posture change where the head is at ground level is altered flowing from the common carotid artery into the vertebral artery. Possible brain damage associated with blood moving towards the head as a result of the force of gravity is prevented by the diversion in flow out of the common carotid artery via the anastomoses, into the vertebral artery.

5. Discussion

The distinctive build of the giraffe results in unique cardiovascular and circulatory challenges. Several aspects of individual challenges have evoked the interest of scientists worldwide. Most articles focus on a specific structure as a single solution. The information shared in this paper also specifically discusses a single structure, but the authors believe that a group of mechanisms work in unison to allow the giraffe to successfully overcome these physiological challenges.

An exceptional mechanism for cerebral blood flow control lay within the diversion of large blood volumes via the anastomosis characteristic of artiodactyls, between the common carotid artery and the vertebral artery [5, 15, 18–20]. This anastomotic artery is also known as the alar artery. The occurrence of blood volumes shunted away from the cranium provides the support of the carotid-vertebral anastomosis that exerts a meaningful influence during the movement of the giraffe when in the upright



(a) (b)

Figure 5: (a) Doppler waveform with color flow images demonstrated preferential flow in the anastomotic artery directed away from the common carotid artery towards the vertebral artery, in the head-down position. (b) Visual illustration of the latex model during detailed dissection of the carotid-vertebral anastomosis with the direction of flow (yellow arrow) away from the common carotid artery (CCA) towards the vertebral artery (VA), in the head-down position.

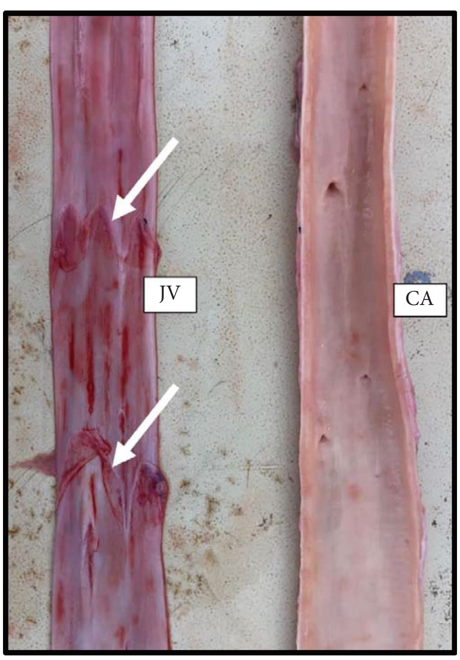


Figure 6: Photograph of the giraffe jugular vein (JV) with valves indicated by white arrows and the common carotid artery (CA) without valves.

position and at ground level, in altering blood pressure [15, 20]. In a giraffe, blood flow to the head is described as uncontrolled, during the head-down position [20]. Is the flow uncontrolled or specifically controlled by means of shunting blood rushing to the brain from the common carotid artery through the anastomosis into the vertebral artery? Furthermore, blood flow occurs bidirectionally in arteries and is not, as in veins, inhibited or directed towards a specific direction, by valves [15]. When the giraffe lifts its head, extracranial vasoconstriction is the mechanism, together with the movement of common carotid blood within the carotid-vertebral anastomosis towards the brain, that

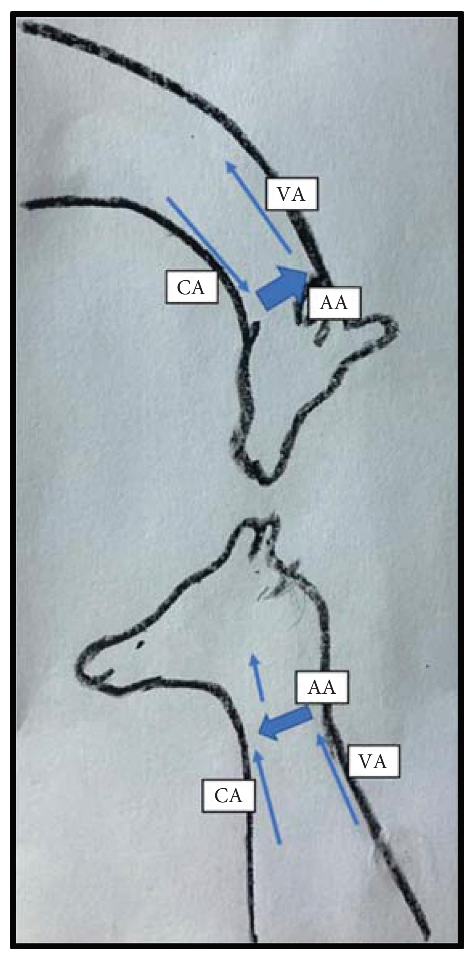


Figure 7: Diagrammatic presentation of the blood flow pattern through the anastomotic artery illustrating the shunting of blood towards or away from the brain depending on the position of the head.

prohibits fainting [20]. The anatomical structure of this anastomosis that is exceptionally well developed in giraffes [19, 21] in comparison to its presence in other artiodactyls can be a part of the list of structures that in conjunction aid in effective blood circulatory control in long-necked giraffes. Similarly, a study showed that in Alpaca (*Vicugna pacos*), this anastomosis known as the alar artery is present [22]. Therefore, the anastomosis exists in giraffes, camels, and alpacas, all animals with characteristically long necks. In a descriptive analysis of the cerebral arterial vascular system of the dromedary camel (*Camelus dromedarius*), the anastomotic artery called the occipital artery was also described as a notable vessel [18]. The alar canal through which the anastomotic artery connects the two cerebral arterial pathways is a relatively short canal diagonally through the atlas wing acting as a link between the ventral and dorsal openings. The anastomotic artery exits the common carotid artery passing into the ventral opening of the alar canal. The course of the vertebral artery runs through the transverse foramen situated at the wing of the atlas following through the transverse canal. Within the alar canal, the connection with the vertebral artery occurs. This course is similar to dromedary camels [18] and is also evident in our study, on giraffes. In camels, however, the vertebral arteries branch several times to meet the basilar artery, which contributes significantly to the cerebral arterial circle. In a giraffe, the basilar artery is rudimentary and does not contribute to the supply of blood towards the cerebral arterial circle [13, 15, 23–26]. The functionality of the anastomotic artery between both long-necked dromedary camels and giraffes shares similarities. However, the degree of importance of this anastomosis in the longer-necked giraffe, lacking basilar contribution to cerebral supply, might be extensive in exerting control on blood movement to and from the brain during alteration of head position at head upright and head down. A study on dromedary camels described the internal carotid—rostral epidural rete mirabile joining, the anastomotic connection of the common carotid, and vertebral artery, as well as a third connection between the vertebral and basilar arteries—as diversions employing an essential part in both blood pressure and blood flow control mechanisms during head movements of long-necked dromedaries [18, 27]. Removing the vertebral-basilar connection, our study also supports this idea in giraffes to an even greater extent.

The connection of the common carotid artery and the vertebral artery in the giraffe plays an essential role in blood pressure and blood flow control during the movement of the head from the head down, to the upright position and again to the head-down position. In combination with other structures, such as the rostral epidural rete mirabile, large blood volumes rushing to the brain, when the head moves down, are diverted from the common carotid artery towards the vertebral arteries, away from the cerebral supply. It has also been hypothesized although not yet proven that the rostral epidural rete mirabile meshwork offers resistance to flow, due to its structure [5, 27–29] adding to the slowing down of the blood column rushing down with gravitational force, towards the brain. Furthermore, blood flow is directed from the vertebral arteries through the anastomotic artery, into the common carotid artery, supplying essential blood that can be moved quickly to the rostral epidural rete mirabile directly to the circle of Willis. This occurs at a time when a centripetal force causes the blood to flow downwards, away from the brain during head movement to an upright position. As previously mentioned, the vertebral veins run through the transverse foramina, closely associated with connective tissue and the back muscles, and run through the axis and atlas to connect to the anastomotic artery. Unlike the common carotid artery that expands when the head moves to a fully erect position, the tight connective tissue and bony structures enclosing the vertebral veins prevent expansion. Less effort is thus required from the heart to pump blood towards the brain via the nonexpandable vertebral artery route, through the anastomotic artery, into the common carotid artery and the brain. Fainting is thus prevented by the supply of blood instantly to the brain from the vertebral artery via the anastomotic artery into the common carotid artery, as well as a small amount of blood that is already within the meshwork of rostral epidural rete mirabile arteries.

In summary, we conclude that the well-developed anastomotic artery greatly assists in blood flow and blood supply to the brain of the giraffe during the movement of the head from fully erect to ground level and back to fully erect. The anastomotic artery allows for adequate cerebral perfusion at the stage where blood flow, against gravity, away from the brain. This is by means of an alternative route for blood flow from the heart via the vertebral arterial pathway, which is protected from both collapse and expansion by surrounding structures. Blood flow from this point follows the usual route from hereon, via flowing from the common carotid into the maxillary and subsequently through the rostral epidural rete mirabile network, to reach the brain. In addition, the anastomotic artery also assists in the prevention of brain damage by providing the route change as head posture is altered moving towards the ground. The large volume of blood flowing with gravity and centripetal force from the heart to the brain is diverted from the common carotid artery through the anastomotic artery into the vertebral artery, preventing the large volume from entering the rostral epidural rete mirabile and circle of Willis. In other species, the blood shunted toward the vertebral arteries would have still entered the brain via the basilar artery, but uniquely in the giraffe, the basilar artery is rudimentary and does not contribute blood to the brain.

What is the possible reason and advantages for this evolutionary development in a giraffe? The effectiveness of the anastomotic artery in giraffes in aiding in the control of blood pressure is critical in the following naturally occurring situations: (a) when the head is moved in a relatively fast motion to ground level for the animal to drink, (b) when the head is moved in a relatively fast motion from ground level to fully erect, and (c) during the fighting when the males swing their heads and necks rapidly, hitting the rival with the full force of the head. Furthermore, the anastomotic artery assists in blood pressure control during darting, when the animal not only experiences a sudden drop to the ground when it loses consciousness due to the effect of the morphine drug on the neuroreceptors in the brain but also the additive effect of the alpha-2 agonist that is used in combination with the morphine drug during darting, on blood pressure, causing a primary rise and then a quick fall in blood pressure due to the drop in cardiac output [16]. In this emergency circumstance, the mechanism of the anastomotic artery prevents death.

Another question arises as to when this anastomotic artery evolved. Was the cerebral circulation of extant giraffe similar to that of extinct giraffe of 5 million years ago? Preliminary work in our study showed that, unlike extant giraffes, extinct giraffids do have a fully functional basilar artery, contributing to the supply of blood to the brain. Whether a connection existed between the common carotid and vertebral systems may only be speculated. The mechanism would have been less effective in blood pressure control because the diversion of large blood volumes in the head-down position would not be possible due to blood that could still move to the brain through the basilar artery. Why would a giraffe living 5 million years ago, not have to regulate its blood pressure by means of the anastomotic artery mechanism? Did these giraffes not fight by means of head butting or was it because of the shorter heart-to-head distance in comparison to extant giraffes? The results of this preliminary study are of major significance, contributing to the understanding of the concurrent evolution of circulatory control in parallel to neck elongation in giraffes and stimulating further research on larger groups to confirm the findings.

6. Conclusions

This is the first study that indicates blood flow direction within the anastomotic artery in the giraffe, by means of Doppler flow waveforms by duplex ultrasound examination, CT scans of the anastomotic arterial connection, and physical dissections to prove our findings. We conclude that the anastomosis, connecting the common carotid artery and the vertebral artery, in combination with other significant structures, is of great importance in helping to overcome the specific physiological challenges faced by giraffes.

Data Availability

All data are available from the main author upon request.

Additional Points

Direct communication between the common carotid artery and the vertebral artery at the midpoint of the atlas called anastomotic artery. Blood flow in anastomotic artery influenced by posture: head-upright and head-down positions. Head upright: flow direction away from vertebral artery towards the common carotid artery. Head down: flow direction away from the common carotid artery towards the vertebral artery

Ethical Approval

This study obtained the approval from the Animal Ethics Research Committee at the UFS, SPCA, and DESTEA, Ethics approval no. UFS-AED2020/0083.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors would like to thank Gerhard Neethling for giving them permission to chemically immobilize a giraffe and do the measurements with the Doppler Sonar on his farm and Corna Serfontein (Vascular Technologist) and Hennie Cloete (Cardiac clinical Technologist) at Bloemfontein Mediclinic for their willingness to bring their expensive fragile equipment into the field and take the measurements of the giraffe. The authors would like to thank Corna Serfontein for giving them an extensive report on her findings, Dr. Willem Daffue and Dr. Chris van der Walt for chemical immobilization of the giraffe and Kroonstad Dierehospitaal for the use of the animal hospital premises and their staff, especially Ben Koloti and Daniel Mthembu, for experimental work, and Netcare Kroon Hospital, in particular, Mr. Tommie Hugo, for allowing them to use the CT-Scanner of the hospital and the expert service and help of Mrs. Manje Brits for conducting the scans and converting the images. This work was supported by Midlands Veterinary Wholesalers Pty Ltd., South Africa.

Supplementary Materials

Supplementary Figure 1a and Figure 1b illustrate the physical detailed dissection exposing the carotid-vertebral anastomoses, the vertebral artery, and the common carotid artery located in the neck, at the center of the atlas. Figure 1a shows the raw dissection, and Figure 1b illustrates the dissection with the arteries highlighted in red and the veins highlighted in blue. (*Supplementary Materials*)

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