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REVIEW ARTICLE

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Major gaps in human evidence for structure and function of the vasa vasora limit our understanding of the link with atherosclerosis

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Abstract

Atherosclerosis is the major pathology causing death in the developed world and, although risk factor modification has improved outcomes over the last decade, there is no cure. The role of the vasa vasora (VV) in the pathogenesis of atherosclerotic plaque is unclear but must relate to the predictability of diseased sites in the arterial tree. VV are small vessels found on major arteries and veins which supply nutrients and oxygen to the vessel wall itself while removing waste. Numerous studies have been carried out to investigate the anatomy and function of the VV as well as their significance in vascular disease. There is convincing evidence that VV are related to atherosclerotic plaque progression and vessel thrombosis, however, their link to the pathology of plaque initiation remains an interesting but neglected topic. We aim to present the evidence on the anatomy and functional behaviour of VV as well as their relationship to the initiation of atherosclerosis. At the same time, we wish to highlight inconsistencies in, and limitations of, the evidence available.

KEYWORDS

atherosclerosis, vasa vasora

1 | INTRODUCTION

From acute myocardial infarction to limb loss, atherosclerotic disease continues to cause overwhelming morbidity and mortality in the modern age. Over the years, many theories have been put forward to explain how the process is initiated and what the possible triggers are. Currently, endothelial dysfunction (Davignon & Ganz, 2004; Gimbrone & García-Cardeña, 2016) is the most accepted reason for the development of atherosclerotic plaque with other theories including inflammation and infection (Epstein et al., 1999; Tuttolomondo et al., 2012) less a focus of late.

The vasa vasora (VV) are small vessels supplying the vessel wall of larger arteries and veins (Figure 1). They supply oxygen and nutrients to the inner layers of the wall and remove waste products. Scholars and researchers over the last few decades have presented a significant body of evidence to suggest that the VV network could be a pivotal structure associated with disease processes of large arteries. Certainly, the links between the VV and plaque vulnerability to haemorrhage or rupture leading with consequent sudden thrombosis of large arteries are well established (Moreno et al., 2004; Sedding et al., 2018; Sun, 2014; Virmani et al., 2005), and thus not the subject of this review.

It has been suggested that VV could also be responsible for the initiation of atherosclerosis (Jarvilehto & Tuohimaa, 2009). To understand these links, we must first establish an understanding of basic structure and function, before we can consider how VV dysfunction can lead to atherogenesis.

2 | ANATOMY OF VASA VASORA

2.1 | Animal evidence

Kwon et al. (1998) studied eight porcine left anterior descending arteries and used micro-CT to reconstruct the orientation of the VV. It was found that normal VV originate from coronary branching arteries and run longitudinally along the target artery. These are named first-order VV. They further divide into second-order VV which encircle the coronary vessel with circumferential arches (Figure 2).

Similar findings were reported by Barker et al. (1992), who studied 25 rabbit carotid arteries using low-viscosity acrylic resin LR White. Barker describes the VV as visible to the naked eye with similar orientation to that described by Kwon in the porcine coronary arteries. Additionally, the cast of VV showed that they originate from both the arterial lumen as well as side branches.

Galili et al. (2004) and his team went a step further and studied different vascular beds in six pigs and compared the difference of VV count, density, mean diameter of first- and second-order VV as well as the ratio of first- and second-order VV between coronary, renal, carotid, femoral and internal thoracic arteries using micro-CT (Figure 3). Just like Kwon and Barker's team, they defined first- and second-order vasa in the same way.

Gossl et al. (2003b) also used micro-CT technology and studied porcine coronary arteries. However, they described VV very differently. Firstly, they also acknowledged the existence of VV interna defined as originating from the target artery lumen; externa as originating from a branching artery, and venous VV draining into a concomitant vein. Furthermore, they also described the branching structure of the VV system as tree-like with anastomoses in the distal parts between all three types of VV. Throughout their research, Gossl did not mention first-/second-order VV at all (Figure 4).

2.2 | Human evidence

A much older piece of literature from Japan by Okuyama et al. (1988) is important to discuss as it is one of the rare studies carried out on human tissue; in this case, the aorta was studied specifically. Without modern technology, Okuyama and his team still managed to

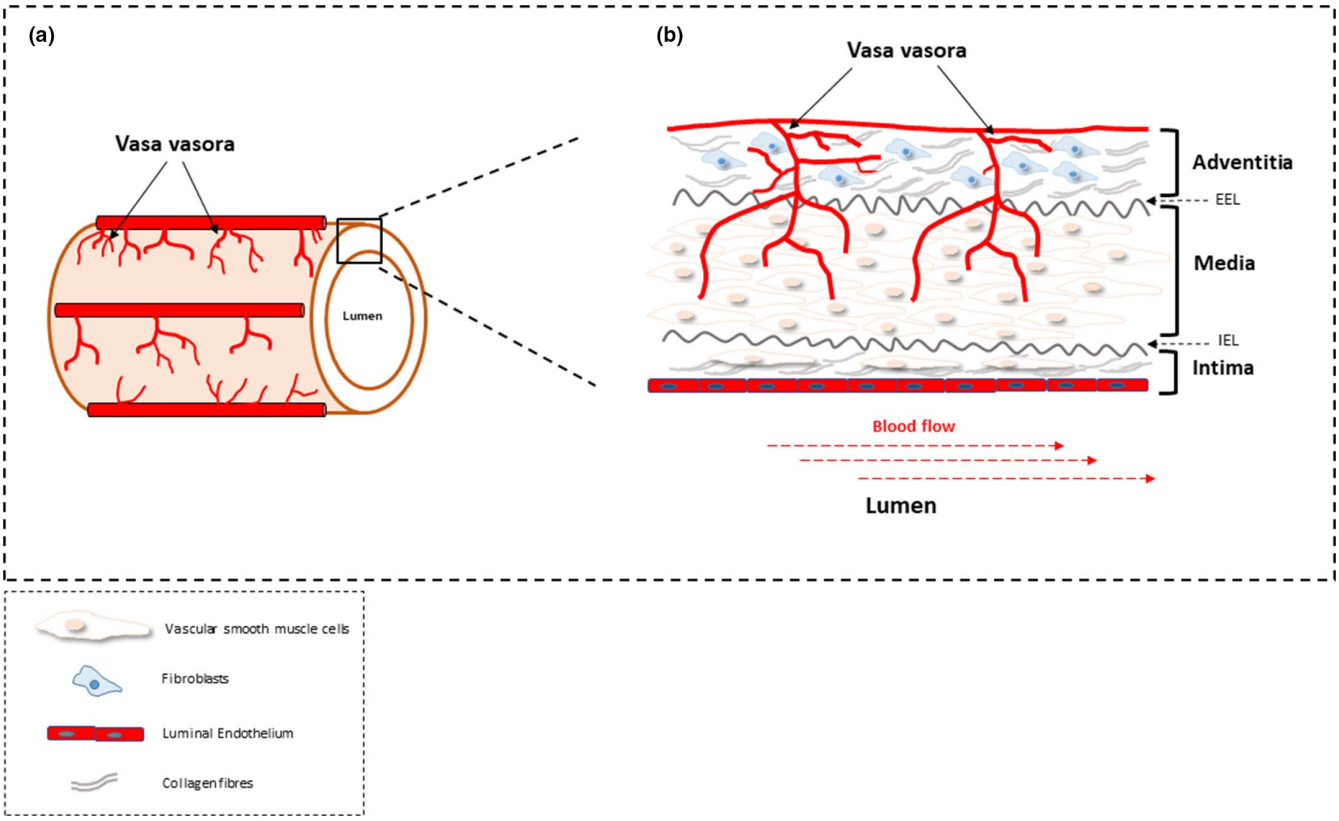

FIGURE 1 Vasa vasora (VV) structure and localisation in the arterial vessel. (a) VV surrounding the outer layer of the artery (Adventitia). (b) A cross section of the arterial vessel showing three layers (adventitia, media and intima) and VV branches penetrating adventitial and medial layers in large blood vessels. EEL, External Elastic Lamina; IEL, Internal Elastic Lamina

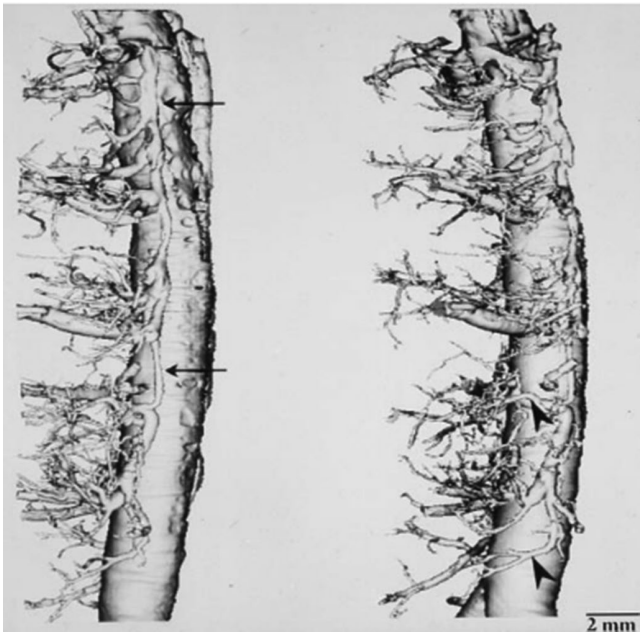

FIGURE 2 Kwon et al. (1998) shows a normal coronary artery with associated VV. Black arrows show the first-order VV originating from side branches and arrowheads show the second-order VV originating from the first-degree VV. The first-order VV can be seen running longitudinally down the main coroanry artery and the second-order VV running circumferentially. From "Enhanced Coronary Vasa Vasorum Neovascularization in Experimental Hypercholesterolemia" by H. M. Kwon, G. Sangiorgi, E. L. Ritman, C. McKenna, D. R. Holmes Jr, R. S. Schwart, A. Lerman, 1998, Journal of Clinical Investigation, volume 101, p. 1551. Copyright 2020 by the American Society for Clinical Investigation provided by the copyright clearance centre. Reprinted with permission

reconstruct the VV in 3D. They prepared serial sections of the aortic wall that were 4 micrometres thick, projecting an area of 3 × 3 mm using a profile projector under 200 times magnification. The team then painstakingly delineated the VV as well as important landmarks such as the medial-adventitial border. This was repeated every 2 sections (with 8 micrometre intervals between sections) and 40–60 drawings were done for each serial section. All images were then used to reconstruct a 3D image using computer software (Yaegashi et al., 1987).

It is also important to note that during dissection, the aorta was removed en bloc with the inferior vena cava. The team then proceeded to inject barium sulphate solution into the aorta and india ink solution into the vena cava, allowing, once again, for venous VV to be identified. Okuyama and his team made an interesting discovery; First and foremost, in the innermost adventitia, they found finely anastomosing venous plexuses with small amounts of arterials interlacing among the venues. No other author has mentioned this finding prior or since. Secondly, Okuyama describes 'vascular cords' consisting of an arterial vasa and a venous vasa branching off, then penetrating the media in a sheath of connective tissue. The author goes on to describe that these vascular cords were only sparse in the media and divided to form a tree-like system.

While there is some consistency in the human evidence presented above, it is clear there is also discrepancy in the findings, perhaps as a result of the historical limitations in imaging technology or arterial specimens from different regions of the vascular tree being studied.

Another concern regarding the current evidence is to what degree can animal studies be applied to humans. An early ground-breaking study done in 1967 by Wolinsky and Glagov (1967) showed that human and animal vessels were fundamentally different. In the paper, the authors clearly demonstrated that 29 lamellar units was the minimum number for medial VV to be required to nourish the vessel wall. This was done by studying 12 different sized mammalian species, from the mouse to the horse.

In summary, animal vessels provide limited evidence to extrapolate to the human. Furthermore, evidence conflicts as to whether VV are arranged in a branching form with different orders surrounding the target vessel circumferentially, or in a tree-like arrangement with limited anastomoses like end arteries. This distinction is important, as it fundamentally affects the arterial wall and its vulnerability to disruption of vasal blood supply. The relationship between anatomy and functional behaviour of VV can further demonstrate this and will now be presented.

3 | FUNCTIONAL PROPERTIES OF THE VASA VASORUM

All the evidence for the physiological properties of the VV comes solely from the animal coronary vessel, which has great significance for extrapolation to the human. Specifically, we refer to studies that investigate the behaviour of the VV in its functional state. Gossl et al. (2003a). studied the functional properties and haemodynamic characteristics of the VV using micro-CT imaging. In two different preparations, 300-micrometre-diameter non–radio-opaque microspheres were injected into the left anterior descending artery of three swine hearts, and the same was done to another three hearts with 100-micrometre microspheres. With three additional control specimens, a radio-opaque polymer was infused at 100 mmHg from the left anterior descending artery ostium to prepare the artery and vasa for imaging. This preparation allowed the vasa to be imaged in their physiological state.

Micro-CT imaging showed that significant occlusion of the VV by micro-embolisation with both sizes occurred. With the proximal VV occluded, the distal vasa became shut off. On imaging, this phenomenon presented as a decrease in the density of VV. Gossl, in another investigation, realised that there were anastomoses among VVI, VVE and VVV which were around 40 micrometres in diameter and affected by injected microspheres. However, if indeed the VV functioned like a plexus with anastomoses, occlusion at any one point of the plexus should not result in an overall decrease in flow. Hence, the author concluded that VV are in fact functional end arteries.

In the second preparation, Gossl and his team filled the left anterior descending artery retrogradely. In this way, although not

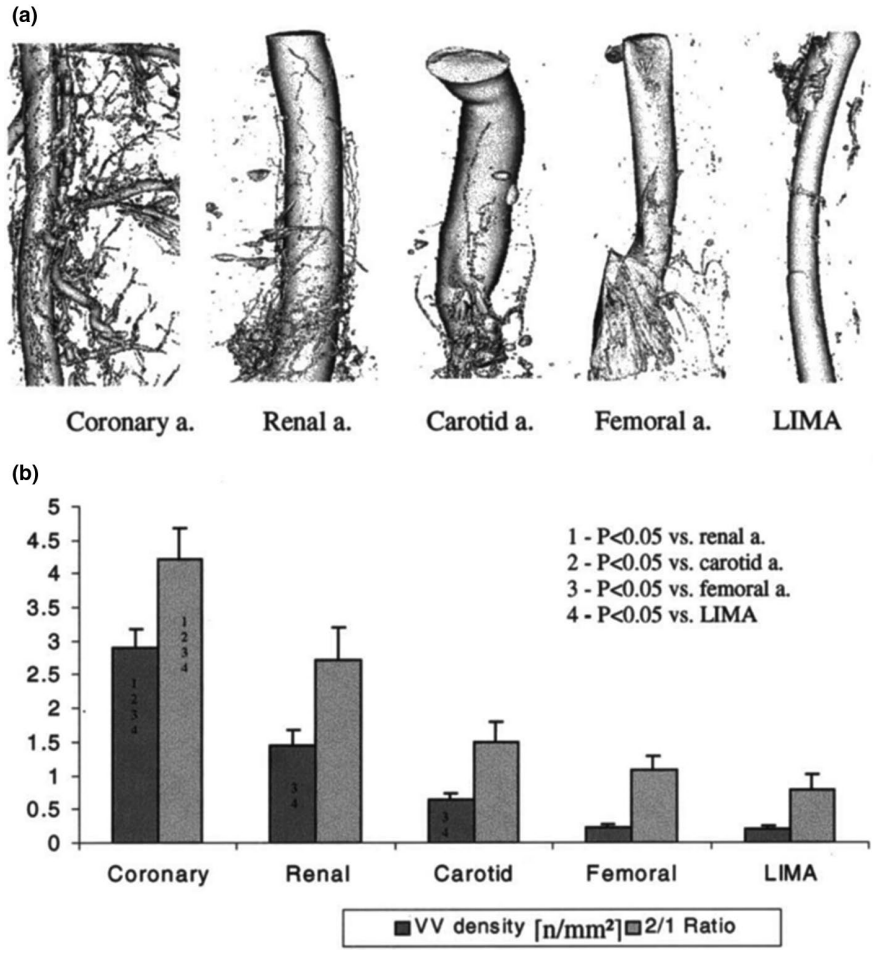

FIGURE 3 Galili et al. (2004) a and b represent the VV density difference between five different vascular beds in the pig. Figure 2 B shows the difference of second-order VV to first-order VV ratio between different vascular beds. From "Adventitial vasa vasorum heterogeneity among different vascular beds." By Galili O, Herrmann J, Woodrum J, Sattler KJ, Lerman LO, Lerman A, 2004, Journal of Vascular Surgery, Volume 40, p. 530. Copyright 2020 provided by the copyright clearance centre. Reprinted with permission

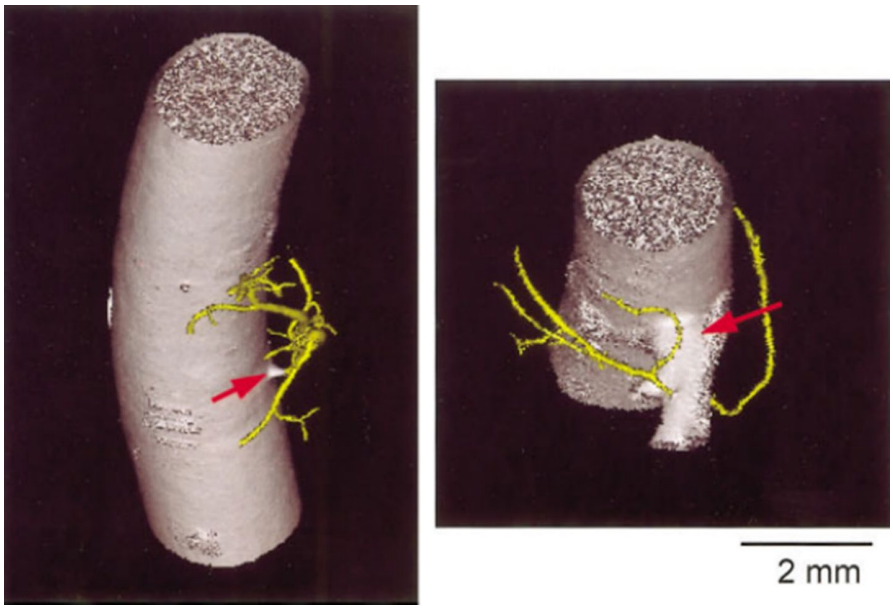

FIGURE 4 Gossl et al. (2003b) on the left the VVI (vasa vasorum interna) are seen and the arrow shows the origin from the main vessel lumen. VVI branches stay within the main vessel wall. On the right, the VVE (vasa vasorum externa) are seen and the arrow shows that the VVE originates form a branch of the main vessel. VVE branches mostly stay outside the main vessel wall. From "Functional anatomy and hemodynamic characteristics of vasa vasorum in the walls of porcine coronary arteries" by Gossl M, Rosol M, Malyar NM, et al. 2003, The Anatomical Record, Volume 272, p. 528. Copyright 2020 provided by the copyright clearance centre. Reprited with permission

mimicking real physiology, they were able to study the effects of blood pressure on the VV. Without external pressure, VV are better perfused and therefore more easily visualised. To further study the effect of pressure on the VV, the coronary vessel was divided into four quadrants in order to differentiate the epicardial portion of the coronary artery from the myocardial portion. Further subgroup analysis showed that VV that exist on the myocardial side of the coronary vessel (left anterior descending artery) had greater dead space and more non-perfused areas of the vessel wall.

The significance of this piece of research cannot be understated. It provides the much needed clarity that VV do not behave like a plexus or a network of blood vessels but are end arteries. However, most importantly, it sheds light on the fact that different vessels, especially coronary vessels that are partly intramuscular, and perfused in diastole, behave dramatically differently from other vessel beds and, therefore, we cannot extrapolate this knowledge to the peripheral arteries. This critical distinction is vital as there are no studies that target the physiological behaviour of non-coronary VV.

4 | ROLE OF VASA VASORA IN INITIATION OF ATHEROSCLEROSIS

The importance of understanding the detailed anatomy and functional behaviour of the VV becomes clear when attempting to study their role in atherogenesis. It is known that certain vessels such as the coronary arteries or carotid arteries are more susceptible to atherosclerosis than others such as the internal mammary arteries (Hildebrandt et al., 2008). Evidence also exists to suggest that atherosclerotic lesions localise in areas of low shear stress, turbulence, and oscillating flow (Glagov et al., 1988; VanderLaan et al., 2004). Certainly, in recent years, the focus of this topic has been strongly focussed on vessel endothelial injury as the cause of arterial atherosclerosis, but research on VV has found that they are closely related to atherosclerotic disease. Recent studies have clearly established the role of VV's in propagating atherosclerotic plaques as well as sudden plaque haemorrhage (Moreno et al., 2004; Sedding et al., 2018; Sun, 2014; Virmani et al., 2005). Mechanisms have been suggested as possible contributing factors such as dysfunction in VV from ageing, smoking and hypertension reducing blood flow in the peripheral arteries and VV as well as leading to VV endothelium damage and consequently extravasation of immune cells, inflammatory markers and plasma cholesterol (Boyle et al., 2017; Erik & Amir, 2007; Gossl et al., 2009; Taruya et al., 2015). However, this hypothesis is yet to be confirmed. Dysfunction of VV as a trigger for the initiation of atherosclerosis in major arteries has a reasonable amount of supporting objective evidence. The research which follows was completed on a variety of different animal species and vessels creating difficulty with extrapolation and generalisability, which highlights the need for further research into this area.

Nakata and Kamiya (1970) were among the first group of researchers to investigate this in a publication from 1970 where the relationship between occlusion of the VV and the development of atherosclerosis was studied in 16 young mongrel dogs. The abdominal aorta was clamped just below the renal arteries and directly above the aortic bifurcation, and a thrombin–gelatine saline mixture was injected into the lumen of the clamped area to achieve occlusion of the VV without mechanical injury of the arterial wall. Postoperatively, the dogs were fed as follows: 5 g of cholesterol and 0.5 g thiouracil (Group A: eight dogs), and 5 g cholesterol only (Group B: five dogs). Three dogs acted as controls for VV occlusion; these animals were injected with saline only followed by 5 g of cholesterol and 0.5 g thiouracil (two dogs) or 5 g cholesterol only (one dog).

Occlusion of the VV brought interesting changes that did not occur in saline injected animals. Histological analysis showed fatty deposition in the inner layers of the abdominal aorta where VV had been occluded at 2 weeks post-procedure. At 4 weeks, accumulation of fat was found in the intima and the inner one third of the media, although not advancing much from 4 to 28 weeks. Intimal thickening, splitting and disappearance of the internal elastic lamina was also seen as well as splitting of the elastic fibres, but interestingly no inflammation was detected around fat deposits in this study. Using micro-angiography, VV were seen to attempt to reconstruct post-occlusion by neovascularisation, but results were poor particularly around the occluded regions compared to the controls. The author concluded that thiouracil did not impact on vascular pathology. Unfortunately, the paper is limited in its study design. The distinction of results between group A and group B dogs were not made very clear. On the same note, there were no comments on the difference in results between cholesterol only and cholesterol- and thiouracil-fed controls. Lastly, a group of dogs that had VV occlusion combined with normal feeding would have formed a useful comparison group which would enable the changes due to VV occlusion alone to be observed. This clearly limits the applicability of the results but nevertheless, this study suggests that VV may have a role in maintaining vessel layer integrity and, if injured or occluded, fatty deposition can occur.

Booth et al. (1989) did a similar experiment in New Zealand white rabbit carotid arteries which were surgically exposed on both sides. A non-occlusive inert silicon collar was used to enclose the left carotid on some rabbits and sterile silk threads were used to indent the left carotid on other rabbits. The right carotid served as control in both series and the subjects with an inert silicon collar were separated into two groups so that one could be fed a high cholesterol diet. Without evidence of endothelial damage, there was massive intimal smooth muscle proliferation on the left side, with foam cells and extracellular lipids present only in the high cholesterol group. Just like Nakata et al. disruption of elastic tissue was observed in both diet groups. Additionally, intimal proliferation was noted, with cells mostly resembling smooth muscle cells. The right carotid artery of both dietary groups was completely free of the above histological features. In rabbits where silk ties were used instead of a collar, similar proliferation was seen but always confined to one region of the circumference.

Booth went a step further to ensure that the endothelium was not only intact histologically but also functionally by measuring

prostacyclin activity. Between the manipulated region and the control region, there was no difference in the ability of the endothelium to produce prostacyclin. This further confirmed that the changes seen on histology had nothing to do with endothelial damage.

In order to support the model of VV hypoxia-related atherogenesis, Barker et al. (1993) studied specifically the oxygen concentration profiles across the arterial wall of pig's femoral arteries. The first group of four pigs had their oxygen concentration measured to assess their normal oxygenation profiles using Polargraphic oxygen microelectrodes and were immediately euthanised. In the second group of four, the oxygenation profile was recorded as in the first group, then side branches and penetrating branches of both right and left femoral arteries were ligated flush to the main vessel wall to occlude the VV with silk sutures. Immediately after ligation, the oxygenation profile was taken. Segments above and below the experimental artery had their side branches and penetrating vessels isolated but not ligated to use as controls. Each of the four pigs had oxygenation successfully re-measured in one of two femoral arteries. After 3 weeks, pigs in group two had another oxygen concentration measurement and were immediately euthanised for histology assessment.

A consistent trough reading of oxygen concentration was found in the middle to outer media before ligation. In two of four cases where readings were done immediately after ligation of side branches, the mean trough fell a further 20–25%. At 3 weeks, similar findings were demonstrated when compared to Nakata and Booth's experiments. Six of eight cases had increased intimal hyperplasia with smooth muscle proliferation, and this was not seen in any control segments.

Kwon et al. (1998) established that a high cholesterol diet alone would induce VV disorganisation (Figure 5) and increased thickness of the main vessel wall, without classical changes consistent with atherosclerosis. However, Steiner et al. (1949) demonstrated that simply feeding dogs a high cholesterol and thiouracil diet produced atherosclerotic lesions extremely slowly.

Schlichter et al. (1949) cauterised the ascending aorta of eight dogs to damage the VV and simultaneously fed six of them a high cholesterol diet. Two dogs developed atheroma in 20 and 12 weeks, respectively, and the lesions were confined to the areas where the VV were disrupted. Six other dogs that had a sham procedure did not develop any atherosclerosis.

Similar findings were noted by Williams (1961), where six rabbit femoral arteries were subjected to blunt dissection and were freed from surrounding tissue. Rabbits fed a normal diet developed intimal thickening, while those fed high cholesterol diet also developed additional foam cells. These lesions took a maximum of 6 weeks to develop.

Manipulation of the VV together with a high cholesterol diet clearly produced lesions that resemble early atherosclerotic plaques. Together with Barker et al.'s findings, VV dysfunction then became a very plausible factor in the genesis of atherosclerosis.

Of major relevance, is the fact that high VV density correlates well with atherosclerotic lesion development in humans (Hildebrandt et al., 2008). Hildebrandt et al. harvested 38 arteries (coronary, renal and femoral arteries) from 32 patients and used micro-CT to investigate the density of VV. Coronary arteries were the most affected vascular bed and were found to have the densest VV supply. It is more likely that VV density contributes to disease development rather than being a merely reactive phenomenon as most vessels did not have atherosclerotic lesions when examined. It is important to note, however, that a drastic difference was found between coronary and peripheral arteries, but not between different peripheral vascular beds. In a study of only 12 renal arteries and 10 femoral arteries, 2.12 VV per mm^2 were found on the coronary arteries, but only 0.61 and −0.66 per mm^2 on the renal or femoral arteries respectively. Unfortunately, the author did not examine the internal thoracic arteries like Galili et al. (2004) which is known to be resistant to atherosclerotic change (Sisto & Isola, 1989).

Several possible mechanisms of VV disruption have been speculated. As VV are functional end arteries, they are vulnerable to systemic insults such as hypertension, nicotine in cigarette smoking and general ageing (Jarvilehto & Tuohimaa, 2009). Local anatomical factors also play a role as plaque formation occurs preferentially at regions of flow separation and low and oscillating wall shear stress as summarised by Glagov et al. (1988). These regions include artery bifurcations and branching points. Lawrence-Brown et al. (2011) furthered Glagov's research and specifically focused on the carotid bifurcation. By studying a multilayer model subjected to pulsatile pressure, the inner flow divide of the common carotid artery as it bifurcates was found to have the most interlayer movement. This region is also subjected to major vessel wall stress and correlates with the localisation of plaque in this area.

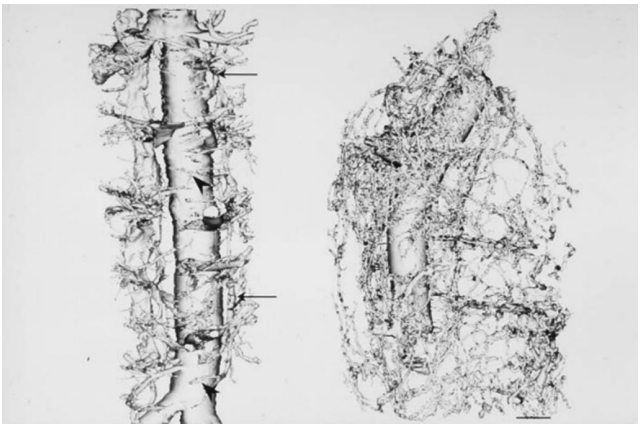

FIGURE 5 Kwon et al. (1998) on the left is a micro-photo of a section of normal coronary artery. On the right is a micro-photo of a section of coronary artery after high cholesterol diet for 12 weeks. There is clear disorganisation and proliferation of the VV in the latter. From ''Enhanced Coronary Vasa Vasorum Neovascularization in Experimental Hypercholesterolemia'' by H. M. Kwon, G. Sangiorgi, E. L. Ritman, C. McKenna, D. R. Holmes Jr, R. S. Schwart, A. Lerman, 1998, Journal of Clinical Investigation, volume 101, p. 1551. Copyright 2020 by the American Society for Clinical Investigation provided by the copyright clearance centre. Reprinted with permission

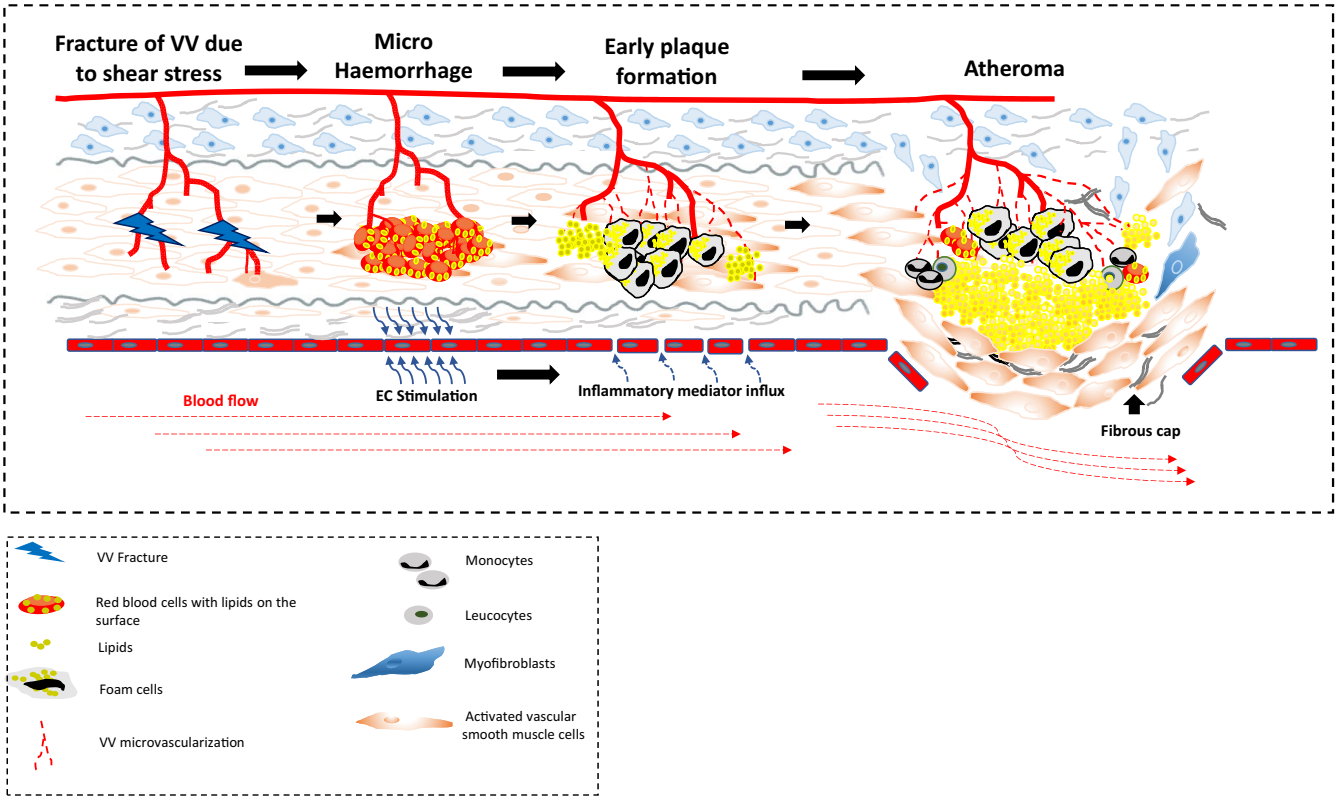

FIGURE 6 Atherosclerotic plaque initiation process from VV fracture and microhaemorrhage. This figure shows plaque initiation starting from an injury to VV in the medial layer due to differential interlayer movement caused by shear stress followed by microhaemorrhage in the site of injury which activates endothelial cells. Lipids from red blood cell membranes and the leaked plasma accumulate at the injury site with macrophages and inflammatory mediators inducing inflammation. A recurrent injury/healing cycle may potentially lead to early plaque formation which develops into an atheroma

In the human, VV have been shown to penetrate from the adventitial layer to the medial layer in major arteries (Billaud et al., 2017; Moulton et al., 2003; Ritman & Lerman, 2007). Interlayer movement could easily disrupt these fragile vessels, leading to micro-haemorrhage. The haemorrhage will contain cholesterol from plasma and red cell membranes and lead to inflammation and an on-going cycle of injury and healing (Figure 6). This is certainly speculative in the context of current available evidence and more

TABLE 1 Summary of previous studies on VV manipulation to initiate atherosclerosis

Author	Animal and vessel area manipulated	Method of manipulation	Results
Nakata and Kamiya (1970)	Mongrel dog aorta	Thrombin-gelatine saline occlusion	Fatty deposition, intimal thickening, splitting and disappearance of internal elastic lamina
Booth et al. (1989)	New Zealand whiter rabbit carotid artery	Non-occlusive silicon collar and sterile silk in two experiments	Intimal smooth muscle cell proliferation and disruption of elastic lamina. Foam cells and extracellular lipids present in the high cholesterol group
Barker et al. (1993)	Pig femoral artery	Ligation of side and penetrating branches	Increased intimal hyperplasia and smooth muscle cells proliferation. Some evidence of reduced trough oxygen level in the middle to the outer media of the vessel
Schlichter et al. (1949)	Dog Aorta	Cauterisation of ascending aorta	Development of atheroma in the vessel confined to areas where VV were disrupted
Williams (1961)	Rabbit femoral artery	Blunt dissection	Intimal thickening and foam cells in the group that was fed high cholesterol diet

research is needed to understand the connection between VV shear stress in the wall and interlayer movement to explain the localisation of atherosclerotic plaque at predictable sites in the arterial tree.

5 | CONCLUSION AND FUTURE PERSPECTIVES

The evidence we present would suggest that VV could be involved in the initiation of atherogenesis (Table 1). While endothelial dysfunction and lipid retention is a very accepted theory, it is possible that ultimately, atherosclerotic disease is a multifactorial process, as the relationship between plaque location at predictable sites, and wall stress cannot be ignored.

There are, however, two major problems when looking at current evidence regarding VV, namely, the lack of human or cadaveric research and the differing opinions regarding organisational structure. Most of the current evidence is based on animal studies, and all the functional evidence is based on coronary vessels. These are major limitations to interpretation of the role and function of VV in the periphery.

As discussed previously, Wolinsky and Glagov's research supported that human and animal vessels are very different. Rabbit aortas contain too few medial lamellar units to need medial VV, so does that mean Barker and Booth's publications need to be re-examined for relevance and are probably not applicable to humans? Wolinsky and Glagov only studied the aorta, so no data on medial VV exist for other vascular beds. Furthermore, most of the pig research used vascular beds other than the aorta. Barker et al. clearly mentioned that they only counted 20-25 lamellar in the porcine femoral artery. What then is the relevance of the trough oxygen concentration? Clearly vascular beds are structured differently and cannot be easily compared.

Additionally, most pig studies were done on coronary arteries. Coronary vessel VV behave very differently from other vessels as they are semi-intramuscular and perfused during diastole, thus, anatomy and function cannot be extrapolated to the peripheral vessels easily. Gossl et al. presented this phenomenon perfectly as we have described.

Even within the animal research on this topic, there are discrepancies in the presented evidence. While some authors describe the VV as a plexus of blood vessels that run circumferentially around the vessel they supply, others found that they are structured in a tree-like pattern and are functional end arteries. This vital difference in structure impacts how VV dysfunction can lead to atherosclerosis as end arteries are much more vulnerable to compression and injury.

Sparse research in human and non-coronary arteries makes it hard for us to extrapolate animal studies to human peripheral vascular disease, especially when considering the relevance of lamellar units. Less lamellar units will exist in smaller vessels perhaps those of animals, merely as a function of vessel size. Furthermore, many species do not develop atherosclerosis probably as a result of lifespan; indeed, mice do not develop atherosclerosis unless genetically mutated.

In addition, there is no research investigating the functional properties of human non-coronary VV, and lastly, there would appear to be strong evidence that occlusion of the VV lead to early signs of atherosclerosis in the absence of intimal injury.

Fortunately, modern imaging and other experimental techniques can be very helpful in furthering our understanding of the human VV. Micro-CT imaging provides three-dimensional images of the VV without disrupting the integrity of the specimen. This technique has already been used extensively on animals in recent years as presented in this study. However, human experiments utilising micro-CT remains rare. Taruya et al. (2015) adopted optical coherence tomography (OCT) to study the VV in atherosclerotic human coronary vessels. This technique allows for in vivo insertion of intra-arterial catheters that can be used to study microstructures of the vessel in detail. Importantly, human micro-CT studies or OCT does not focus on the fundamental structures of VV and evidence in this specific area is severely lacking. Also, advances in fresh tissue histology staining using markers such as CD31, CD34 or CD105 can help further characterise the microscopic environment.

The authors are conducting extensive experimental research of the human VV. Harvesting of major arteries from fresh frozen cadavers followed by micro-contrast CT imaging and tissue staining with markers to identify diseased and non-diseased sections of the vessel are currently been carried out. In addition, a composite pipe flow model is planned to demonstrate the interlayer movement which could be responsible for VV injury, as we suspect micro-haemorrhage of the VV maybe an important initiating factor for atherosclerosis rather than a late event in advanced plaque.

Although the most accepted theory regarding atherogenesis is endothelial dysfunction and lipid retention, there is sufficient evidence to support further research into the role of stress injury on human VV in the initiation of atherosclerosis.

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None.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Sophie Zhao research and write up. Hanane Belhoul-Fakir research and write up. Shirley Jansen planning, design, research and edit. Juliana Hamzah design and edit. Siamak Mishani edit. Michael Lawrence Brown planning, design and edit.

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