Venous Hypoxia: A Poorly Studied Etiological Factor of Varicose Veins

C.S. Lim^{a, b} M.S. Gohel^a A.C. Shepherd^a E. Paleolog^b A.H. Davies^a

^aImperial Vascular Unit, Department of Surgery and Cancer, Faculty of Medicine, Charing Cross Hospital, Imperial College London, and ^bCytokine Biology of Vessels Group, Kennedy Institute of Rheumatology and Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK

Key Words

Hypoxia · Varicose veins · Vein and oxygen

Abstract

Venous hypoxia has long been postulated as a potential cause of varicosity formation. This article aimed to review the development of this hypothesis, including evidence supporting and controversies surrounding it. Vein wall oxygenation is achieved by oxygen diffusing from luminal blood and vasa vasorum. The whole media of varicosities is oxygenated by vasa vasorum as compared to only the outer two-thirds of media of normal veins. There was no evidence that differences exist between oxygen content of blood from varicose and non-varicose veins, although the former demonstrated larger fluctuations with postural changes. Studies using cell culture and ex vivo explants demonstrated that hypoxia activated leucocytes and endothelium which released mediators regulating vein wall remodelling similar to those observed in varicosities. Venoactive drugs may improve venous oxygenation, and inhibit hypoxia activation of leucocytes and endothelium. The evidence for hypoxia as a causative factor in varicosities remains inconclusive, mainly due to heterogeneity and poor design of published in vivo studies. However, molecular studies have shown that hypox-

ia was able to cause inflammatory changes and vein wall remodelling similar to those observed in varicosities. Further studies are needed to improve our understanding of the role of hypoxia and help identify potential therapeutic targets.

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Introduction

Varicose veins are excessively dilated, elongated and tortuous veins, and affect up to a third of the adult population in Europe [1, 2]. The cost of treating varicose veins and associated complications has been estimated at 1–3% of the total annual health care budget in European countries [3]. Despite the unquestionable clinical significance of the condition, the pathogenesis of varicose veins remains unclear. There is increasing evidence that alteration of the vein wall is the primary abnormality in varicose veins [4, 5]. Numerous changes in cellular and extracellular matrix components of the vein wall have been identified, including intimal hyperplasia, smooth muscle cell dysfunction, changes to the collagen and elastin content and imbalances of matrix metalloproteinases and their tissue inhibitors [4–7]. These changes are thought to cause an overall weakening and relaxation of vein wall

leading to venous dilatation, valve incompetence and reflux.

Vein wall hypoxia has been suggested as a contributing factor for varicose vein formation [5, 6, 8, 9]. For several decades, many *in vivo* and *in vitro* studies have assessed the relationship between hypoxia and varicose vein formation. However, opinions remain divided on the evidence evaluating hypoxia as a possible factor contributing to varicose vein formation. The aim of this article was to revisit and review the published evidence for the controversial hypothesis of vein wall hypoxia contributing to the formation of varicose veins.

Methods

PubMed, Embase and Ovid Medline databases were searched using the terms 'varicose veins and blood oxygen', 'varicose veins and vasa vasorum' and 'varicose veins and hypoxia'. No date limitation was used during the search. All available abstracts were reviewed. Of these, articles that discussed the relationships between oxygenation and varicose vein formation were evaluated. Articles that were not related to chronic venous disease were excluded. This review focused on studies assessing the relationship between oxygen tension and varicose vein formation in patients of clinical stages C2 to C6 of Clinical Etiology Anatomical Pathophysiology classification.

Blood Supply to the Vein Wall

Vein wall oxygenation is achieved by diffusion of oxygen from blood in the lumen and vasa vasorum. The lumen of the superficial venous system of the lower limbs contains deoxygenated blood from skin and subcutaneous tissue of the legs. Meanwhile, vasa vasorum of the saphenous veins and tributaries originate from feeding arteries in the surrounding adipose tissue and penetrate the fascia of the veins before opening into the media and adventitia [10]. It has been reported that the main sources of blood in the vasa vasorum for the great saphenous vein (GSV) were the external pudendal artery and descending artery of the knee, while for the small saphenous vein the main sources of blood were the small saphenous artery which is a branch of the popliteal artery [11].

In a study to assess the oxygenation of the vein wall, Taccoen et al. [9] used a microdriven stepwise progression needle probe attached to a computerized polarographic system to measure the *in vivo* oxygen tension across the saphenous vein wall of patients undergoing surgery. The authors found that the oxygen tension of the

saphenous vein wall decreased from the adventitia to the union of the middle and inner third of the media followed by a marked increase in oxygen tension in the intima and lumen. This suggested that the oxygenation of the inner one third of the vein wall was provided by luminal blood and the outer two thirds by vasa vasorum. This was supported by several detailed histological and anatomical studies of vasa vasorum including those in saphenous veins. These studies found that in normal veins, vasa vasorum only opened into the adventitia and outer two thirds of the media but never into the inner one third and intima [10, 12].

The relative oxygenation and nourishment of the vascular wall contributed by the luminal blood, and vasa vasorum may vary depending on several factors including the type and size of the vessel and luminal oxygen tension (PO₂) [10, 13]. Despite having a thicker wall, the overall relative contribution of oxygenation from blood in the vasa vasorum is less in arteries than in veins [13]. It has been postulated that low oxygen content of the luminal blood in the veins is compensated by increased formation of vasa vasorum [13]. Conversely, veins with relatively thinner walls were found to have lower blood flow in the vasa vasorum in comparison to veins with thicker media [12, 13]. It has also been found that vasa vasorum dilate leading to increased blood flow when veins were subjected to acute hypoxia [13].

Pathological changes in the vein wall including intimal hyperplasia and degenerative changes in the innermost layer such as in varicosities and phleboscrosis have also been found to be associated with alterations to the distribution and density of vasa vasorum. In areas of intimal hyperplasia, vasa vasorum were found to penetrate the whole media as compared to only the outer two thirds of the media in normal segments of veins [12]. Similar observations were reported by Bigel and Taccoen [14] in varicose veins where the vasa vasorum were increased close to the intimal layer but not in non-varicose veins. In a study of 22 patients, Kachlik et al. [15] found increased numbers of vasa vasorum in varicose veins, especially recurrent varicose veins and those with thrombophlebitis compared to non-varicose veins. Interestingly, tortuosity and irregular dilatations of adventitial veins were also observed in varicose veins. It has been suggested that degenerative changes and intimal hyperplasia in the innermost layer of the vein wall cause intraluminal and/or intramural hypoxia. This may lead to an increase in vasa vasorum via neovascularization to ensure adequate oxygenation of the entire media [12].

Potential Mechanisms of Vein Wall Hypoxia in Varicose Veins

Inadequate oxygen supply from luminal blood or vasa vasorum or both could potentially cause vein wall hypoxia. Two mechanisms, both related to blood stasis and venous hypertension, have been proposed to cause hypoxia to the varicose vein wall:

(1) Endoluminal hypoxia: stagnation of venous blood flow results in reduced oxygen replenishment in comparison to normal venous flow. Endothelial and inner layers of the vein wall are first affected in endoluminal hypoxia [16–18].

(2) Medial hypoxia: distension of the vein by hydrostatic pressure secondary to blood stasis causes compression of vasa vasorum. Therefore, the media and outer layers of the vein wall are first affected [17, 18].

Measuring Luminal Blood Oxygenation in Varicose Veins

For many decades, researchers have attempted to assess the oxygen content of venous blood carried in varicose and non-varicose veins. However, findings from these studies have been inconsistent as some studies demonstrated a lower oxygen content in blood from varicose compared to non-varicose veins [19–21], whereas others showed no significant differences [22–26]. Paradoxically, some studies even reported higher oxygen content in blood of varicose than non-varicose veins [22, 23, 27–29]. Numerous factors may have contributed to these variations. Clearly, huge inconsistencies in study design and sampling variations existed between studies as contralateral superficial veins, deep veins, femoral veins and antecubital veins had all been used as controls, and the posture of patients during sampling was not consistent [20, 26, 27]. Moreover, in some studies, non-varicose and varicose veins of the same patients were compared while in others, patients without varicosities were recruited as controls [20, 30].

Studies comparing the venous blood oxygen content between varicose and superficial leg veins of patients without varicose vein disease are summarized in tables 1 and 2. On the basis of current evidence, it is not possible to conclude that the blood in the lumen of varicose veins contains less oxygen than blood in non-varicose veins. Interestingly, in non-dependent (lying) positions, the oxygen content of blood from varicose veins appeared to be similar or even higher than that of non-varicose veins. In

the dependent positions, the oxygen content of blood from both varicose and non-varicose veins decreased, but the reduction may be greater in blood from varicose veins [22, 23].

Measuring Oxygenation in Varicose Vein Wall

Our literature search only found one study that measured and compared vein wall oxygen tension between varicose and non-varicose veins in vivo. In this study, Taccoen et al. [9] compared the vein wall oxygen tension of 31 varicose saphenous veins (from 21 patients during venous surgery) with 7 non-varicose saphenous veins in patients undergoing femoro-popliteal bypass surgery. The authors reported that the average minimum oxygen tensions were significantly lower in the media of varicose compared to non-varicose veins (7.9 vs. 13.4 mm Hg; $p < 0.05$). Although study samples were small, this was the only study that measured the vein wall oxygen tension directly, and it reported that the wall of varicose veins may be hypoxic compared to non-varicose veins.

Molecular Studies

Expression Studies

Venous stasis may cause oxidative stress by initiating a series of haemodynamic, metabolic, and nutritional changes including tissue hypoxia [31–33]. In a study of 45 patients with varicosities, downregulation of cytochrome-oxidase C and increased pyruvate expression in varicose compared to non-varicose portions of the veins suggested a reduction in oxidative phosphorylation in varicose veins [34]. Varicose vein wall has also been shown to consume less oxygen and glucose [35], and demonstrates elevated oxidative stress, including increased thiobarbituric acid reactive substances, myeloperoxidase and xanthine oxidase [36], compared to non-varicose veins.

Elevated oxidative stress has been associated with vein wall injury and chronic venous insufficiency (CVI) [31–33, 37]. Moreover, leucocytes in the blood of varicose veins demonstrated increased oxidative stress compared with arm veins in patients with CVI [38, 39]. Leucocyte trapping and oxygen free radical (superoxide dismutase and superoxide anions) retention in lower limbs were also significantly increased in patients with varicose veins compared to those without in gravity-dependent positions [40]. Interestingly, the level of oxidative stress mea-

Table 1. Summary of the designs and findings of studies that measure and compare oxygenation of blood in varicosities of patients and leg veins of controls with no varicose vein disease

Study	Patients	Controls	Other veins studied	Positions	Results
Holling et al. [23], 1938	Varicose veins of 13 patients (7 without and 6 with ulcers)	Similarly located veins of 4 patients with no varicose veins.	Nil	'From bed and seated for 2 h with his foot in a dependent position'	No significant difference between oxygenation of blood of varicose and non-varicose veins.
Schraibman [25], 1966	Varicose veins of 62 patients	Leg veins of 14 patients with no varicose veins	Femoral arteries and femoral veins	2 and 10 min of standing from a recumbent position	No significant difference between the oxygen saturation of blood from varicose and non varicose veins.
McEwan and McArdle [19], 1971	Varicose veins of 14 patients with impaired distal skin nutrition	Leg veins of 6 patients without varicose veins	Nil	Sitting with studied legs in dependent position for 10–15 min	PvO ₂ , oxygen saturation and calculated venous oxygen content were significantly lower in blood from varicose than non-varicose veins. Oxygen extraction in tissues was significantly higher in varicose than non-varicose veins.
Blumoff and Johnson [29], 1977	Varicose veins of 20 patients	Saphenous veins harvested for either aortico-renal or femoro-popliteal bypass graft of 18 patients with no varicose veins	Femoral arteries, femoral veins and proximal saphenous veins	Supine (operated with endotracheal anaesthesia)	PvO ₂ was significantly higher in varicose than non-varicose saphenous veins. PvO ₂ of superficial leg veins were significantly higher than that of the femoral veins in patients with and without varicose veins.
Reikerås and Sorlie [21], 1983	Varicose veins of 22 patients (6 were recurrences)	Leg veins of 9 patients with no varicose veins	Popliteal veins	Standing	PvO ₂ was significantly lower in varicose than non-varicose saphenous veins. PvO ₂ was significantly lower in varicose than contralateral non-varicose veins of patients with varicose veins.
Baron and Cassaro [28], 1986	Varicose veins of 49 patients	Leg veins of 43 patients with no varicose veins	Antecubital veins	Recumbent position	PvO ₂ , oxygen saturation and calculated venous oxygen content were significantly higher in blood from varicose than non-varicose veins.
Scott et al. [22], 1990	Varicose veins of 13 patients	Leg veins of 13 patients without varicose veins	Antecubital veins	Laid supine for 20 min and stood for 30 min with arms at the sides	PvO ₂ was significantly higher in varicose than non-varicose veins during supine position. No significant difference was noted in PvO ₂ between varicose and non-varicose veins during standing. PvO ₂ was significantly higher in leg veins than in arm veins in both the varicose and non-varicose vein groups during supine. PvO ₂ in leg veins dropped significantly from supine to standing in both the varicose and nonvaricose vein groups.
Wali [26], 2002	Varicose veins of 21 patients	Leg veins of 21 controls (doctors and nurses) with no varicose veins	Antecubital veins	At least 5 min of standing before venepuncture of leg veins and sitting for antecubital veins	No significant difference in PvO ₂ and oxygen saturation between blood of varicose and non-varicose veins during standing. PvO ₂ and oxygen saturation were significantly higher in veins of the lower than upper limbs of patients with varicose veins. In controls, oxygen saturation and PvO ₂ also seemed to be higher in veins of the lower than upper limbs (only significant in the former).
Murphy and Hands [24], 2008	Varicose veins of 39 patients (C2–C6)	Median cubital veins of 10 patients underwent hernia repair and cholecystectomy	Nil	2 min of lying and 2 min of standing	No significant difference of PvO ₂ between varicose and control veins. Reduction of PvO ₂ in both varicose and control veins during standing compared to supine (significance not reported).

Table 2. Mean values of the oxygen tension and saturation of blood sampled from varicosities of patients and leg veins of controls with no varicose veins in gravity-dependent and non-gravity-dependent positions

Study and group	Number of subjects	Mean O ₂ tension of controls in non-gravity-dependent positions mm Hg	Mean O ₂ saturation of controls in non-gravity-dependent positions %	Mean O ₂ tension of controls in gravity-dependent positions mm Hg	Mean O ₂ saturation of controls in gravity-dependent positions, %
<i>Controls without varicose veins</i>					
Holling et al. [23], 1938	4	42.1	71.4	35.8	63.4
Schraibman [25], 1966	14	not tested	not tested	not tested	57.4 ¹
McEwan and McArdle [19], 1971	6	not tested	not tested	35.2	78.3
Blumoff and Johnson [29], 1977	18	54.0	not tested	not tested	not tested
Reikerås and Sorlie [21], 1983	9	not tested	not tested	35.3	not tested
Baron and Cassaro [28], 1986	43	36.1	64.2	not tested	not tested
Scott et al. [22], 1990	13	34.1	not tested	28.1	not tested
Wali [26], 2002	21	not tested	not tested	31.0	54.8
Murphy and Hands [24], 2008	10	not specified	not tested	not specified	not tested
<i>Controls without varicose veins</i>					
Holling et al. [23], 1938	13	39.3	70.2 ²	31.1 ²	54.0
Schraibman [25], 1966	62	not tested	not tested	not tested	55.9 ³
McEwan and McArdle [19], 1971	14	not tested	not tested	22.7	54.4
Blumoff and Johnson [29], 1977	20	69.0	not tested	not tested	not tested
Reikerås and Sorlie [21], 1983	22	not tested	not tested	27.0	not tested
Baron and Cassaro [28], 1986	49	59.3	87.6	not tested	not tested
Scott et al. [22], 1990	13	45.7	not tested	30.1	not tested
Wali [26], 2002	21	not tested	not tested	31.0	52.3
Murphy and Hands [24], 2008	39	33.8	not tested	29.3	not tested

¹ Only 12 readings out of the 14 patients studied were available in the article.

² Only 12 readings out of the 13 patients studied were available in the article.

³ Only 61 readings out of the 62 patients studied were available in the article.

sured in the blood obtained from foot veins of patients with incompetent GSV were significantly reduced and returned to normal levels following GSV stripping [37], suggesting that venous reflux caused elevated oxidative stress.

Jacob et al. [30] investigated the effect of blood stasis on the expression of 12 biological markers and measured the PO₂ of blood sampled from varicose veins and paired brachial veins (controls) of 22 patients. Several plasma endothelial and leukocyte activation markers including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, angiotensin converting enzyme, pro-matrix metalloproteinases and L-selectin were significantly elevated by postural blood stasis (limbs in the dependent position compared to lying flat) in varicose veins. Such elevations were not observed in blood sampled from brachial veins. Furthermore, upregulation of these markers in varicose veins was inversely proportional to the PO₂ of the blood sampled. This study demonstrated that venous endothelium and leucocytes were ac-

tivated by blood stasis. These changes were associated with reductions in PO₂ in varicose veins.

In vitro Studies

The importance of the endothelium in vein wall remodeling has been increasingly recognized in recent years [4, 5, 8]. Endothelial roles include regulation of coagulation, inflammation, smooth muscle cell function, and synthesis of vasoactive substances and extracellular matrix [41, 42]. The location of the endothelium directly in contact with luminal blood exposes this layer to endoluminal hypoxia in varicose veins.

Venous endothelial responses to hypoxia have often been studied with cell culture including human umbilical vein endothelial cell (HUVEC) culture. Venous endothelial cell culture studies have demonstrated that hypoxia altered venous metabolic regulatory pathways including reduction of adenosine triphosphate (ATP) and oxidative phosphorylation and increase in glycolysis [43, 44]. Increased intracellular calcium concentration has

also been shown in hypoxic venous endothelial cells, leading to activation of phospholipid A₂ and elevated prostaglandin synthesis [45, 46]. Alteration of these cellular processes is thought to cause the release of inflammatory mediators [47]. Activation of venous endothelial cells by hypoxia also increases the release of β -fibroblast growth factors (bFGF) and prostaglandin F₂ α , which are known to stimulate smooth muscle cell migration and proliferation, a feature commonly observed in varicosities [6, 46, 48]. In vitro studies using venous endothelial cell culture have demonstrated that hypoxia increased angiogenesis by upregulating the release of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and bFGF [48, 49].

Venous endothelial cells also responded to hypoxia by upregulating hypoxia-inducible factors (HIFs) [50–52]. HIFs are nuclear transcriptional factors that regulate the transcription of many genes involved in oxygen homeostasis [53]. The transcriptional activity of HIF in venous endothelial cells has been shown to be elevated during hypoxia. Examples of genes regulated by HIF that have been shown to be increased in hypoxic venous endothelial cells include VEGF and an endothelial isoform of nitric oxide synthase (eNOS) [50–52]. Increased prostaglandin I₂ and cyclo-oxygenase-2 expression in hypoxic venous endothelial cells has also been shown to be HIF-dependent [54].

In addition to cell culture, vein organ cultures have also been used to study whole venous tissue behavior in relation to hypoxia. An early study of oxidative metabolism and glucose transport in varicose vein organ cultures suggested that the oxygen consumption of human varicose veins was relatively low and comparable to healthy veins investigated in other studies [55]. However, in the absence of a control group, it is difficult to draw firm conclusions regarding oxygen consumption between varicose and non-varicose veins from this study [55].

Leucocyte infiltration has been found to be more common in histological specimens of varicose than non-varicose veins [5, 56]. Adhesion molecule expressions such as vascular adhesion molecule-1 (VCAM-1), ICAM-1 and von Willebrand factor, which activate leucocytes, were found to be upregulated in varicose compared to non-varicose veins [5, 56]. Activated leucocytes secrete high levels of superoxide anions and proteases which degrade extracellular matrix [8]. To investigate whether such a phenomenon can be caused by hypoxia, HUVECs were exposed to hypoxia and treated with non-stimulated polymorphonuclear nuclear (PMN) cells [41, 42, 57]. This

model demonstrated a clear increase in PMN cell adherence to HUVECs when the PO₂ decreased below 25 mm Hg [8]. Both HUVECs and PMN cells were activated by hypoxia with upregulation of adhesion molecules including CD-18-dependent adhesion molecules and ICAM-1, superoxide anions, and leukotriene B₄ [42, 58–60]. Similar observations were also observed in whole-vein explants exposed to hypoxia. Michiels et al. [61] described an in vitro model that assessed the effect of hypoxia on saphenous vein explants in the presence of non-stimulated PMN cells. Non-varicose segments of GSV incubated in hypoxic and normoxic environments were perfused with radiolabeled PMN cells. This study demonstrated an increased number of activated PMN cells retained in veins incubated in hypoxic compared to normoxic conditions. PMN cells in hypoxia were also found to produce more superoxide anions and leukotriene B₄. Such in vitro models and varicose veins in vivo revealed similar histological appearances and behaviors, hence prompting the authors to postulate that hypoxia, as a result of blood stasis, was the trigger for endothelial cell responses that could cause alterations of the venous wall and varicose vein development [8].

Hypoxia and Treatment of Varicose Veins

Few studies have investigated potential pharmacologic agents that may protect the vein wall from hypoxia. The most commonly studied group of pharmacological agents in this context has been the venoactive drugs such as flavonoids [19, 41, 55, 57, 62]. Venoactive drugs are often derived from plant extracts, and their therapeutic actions include increased venous tone, reduced vein wall inflammation and decreased capillary permeability [6, 63].

In vitro models have been used to investigate the effects of venoactive drugs on hypoxia-induced endothelial and PMN activation [41, 57]. In a HUVEC model, aescine was shown to inhibit hypoxia-induced activation of endothelial cells, leucocytes, and the interactions of these cells in a dose-dependent manner [41, 57]. Venoactive drugs were also shown to target complexes I and III of the mitochondrial respiratory chain or adenine nucleotide translocase, reduce oxidative stress, and increase ATP synthesis during hypoxia [8, 41, 64]. A further study used an ex vivo vein explant model and found that flavonoids significantly reduced the oxygen consumption of varicose veins [55].

One clinical study reported that patients who took hydroxyethylrutosides (ParovenTM) 250 mg, a flavonoid,

4 times a day for 4 weeks showed significant improvements in the PO₂ and saturation of blood sampled from varicose veins compared to initial levels [19]. These improvements in varicose vein oxygenation were also associated with symptomatic relief in this patient group [19]. However, the findings of this study should be interpreted with caution as patients were not randomized, neither patients nor clinicians were blinded, and the timing of venepuncture was not matched between the study groups. It was also unclear whether the patients in the study received any other treatment such as compression which might have positive effects on the oxygenation of the luminal blood. Some randomized controlled trials have also reported that venoactive drugs such as flavonoids and horse-chestnut extracts improved symptoms of CVI [63, 65–67]. These drugs are likely to increase venous tone and decrease vein wall inflammation and capillary permeability [6, 63]. Flavonoids such as micronized purified flavonoid fraction, containing 90% diosmin and 10% hesperidin, may also protect the vein wall from hypoxia and inhibit the expression of adhesion molecules on endothelium and leucocyte [6, 17, 67, 68].

Discussion

Hypoxia has often been proposed as a factor contributing to or even causing varicose vein formation [61]. Although varicose vein wall hypoxia has been investigated for several decades, the evidence supporting or refuting this theory remains debatable. Measuring luminal blood oxygen content in varicose and non-varicose veins might appear to be an obvious way to assess if varicose veins are hypoxic, and this approach has been used repeatedly. However, results from these studies have been inconsistent and inconclusive. Such inconsistency was likely to be due to the heterogeneity of controls used, timing and site of venepuncture, subject posture and method of oxygen measurement. Although the luminal blood oxygen content in varicose veins may not be lower than that in non-varicose veins, the evidence suggests that postural changes from gravity-independent to dependent position induce luminal blood hypoxia in both varicose and non-varicose veins, possibly through blood stasis. Furthermore, the drop in luminal blood oxygen content during such postural changes seems to be greater in varicose veins. One possible explanation may be that the varicose vein wall suffers more stress due to a drop in endoluminal oxygen ten-

sion when moving to an upright position compared to non-varicose veins [30].

Unfortunately, only one study has assessed and compared varicose and non-varicose vein wall oxygen content directly and demonstrated reduced PO₂ in varicose veins [9]. Such observations need to be confirmed by further studies before we can conclude that varicose veins are hypoxic. Studies on the anatomical distribution and physiology of vasa vasorum demonstrated variations between varicose and non-varicose veins [10, 12]. It is likely that the increased blood supply from vasa vasorum in varicose veins is associated with endoluminal hypoxic stress, a major cause of neovascularization. Increased angiogenesis in response to hypoxia is part of an adaptive response aimed at achieving increased delivery of oxygen and nutrients to tissues. In vitro studies have clearly demonstrated that hypoxia upregulates the formation of new vessels through activation of the HIF pathway, leading to the release of pro-angiogenic factors such as VEGF and bFGF [48, 49].

In vitro studies have demonstrated that venous tissues responded to the hypoxic environment by upregulation of inflammatory processes, similar to varicose veins in vivo [69]. Increases in adhesion molecules and leucocyte activation and adherence to endothelium have also been shown experimentally to be associated with postural blood stasis, more so in varicose compared to non-varicose veins [30], with similar changes seen in cell and organ cultures subjected to hypoxic conditions [8, 16, 42, 70]. These findings support the hypothesis that hypoxia secondary to blood stasis may contribute to the inflammatory processes observed in varicose veins. Figure 1 summarizes the potential mechanisms that induce hypoxia and lead to varicose vein formation and possible pharmacological targets.

It should be recognized that hypoxia may be a contributing factor to varicose vein formation, but other possibly related factors, including mechanical stretch and low shear stress, may also cause similar inflammatory processes [71–73]. Venous hypertension stretches the vein wall which could then cause venous hypoxia secondary to increased oxygen demand and compression of vasa vasorum. On the other hand, venous hypoxia also causes vein relaxation, leading to blood stasis, venous hypertension, increased vein wall tension and low shear stress. These stresses are also known to cause injury to the vein wall, leading to inflammation, which is a known feature of varicose veins [6]. Therefore, studying the role of an individual factor is reductionist. It is likely that a combination of several factors contributes to the pathogenesis

Fig. 1. Schematic diagram of potential mechanisms that induce hypoxia and lead to varicose vein formation, and possible pharmacological targets. Potential targets are numbered with Arabic numerals with their corresponding group of drug pharmacological agents shown in the grey box. eNOS = Endothelial nitric oxide synthase; VEGF = vascular endothelial growth factor; bFGF = basic fibroblast growth factor; PDGF = platelet-derived growth factor; MMP = matrix metalloproteinase; TIMP = tissue inhibitor of matrix metalloproteinase; COX-2 = cyclo-oxygenase-2; NSAID = non-steroidal anti-inflammatory drug.

of varicose veins. The heterogeneity of the disease including the various associated risk factors and huge variation of severity of the skin changes may also contribute to the complex pathogenesis.

We recognize that our review is limited by the heterogeneity of studies published between 1938 and 2009. Techniques and patients assessed varied hugely between reports. Patients with skin changes and ulceration may be more likely to show inflammatory activity and reduced venous oxygenation compared to patients without these

complications. Despite the uncertainty, there are suggestions that tissue hypoxia may contribute to the remodeling seen in the varicose vein wall, and further investigation seems worthwhile. Furthermore, improving venous oxygenation, for example using flavonoids, may lead to symptomatic relief in CVI [17, 19, 66, 67]. Interestingly, these drugs have been shown to improve venous oxygenation most likely by elevating venous tone, increasing ATP production, reducing vein wall inflammation and decreasing capillary permeability [35, 64, 66]. Moreover,

improving our understanding of the role of hypoxia in varicose vein formation may lead to the discovery of further therapeutic targets for drug development in this disease.

Future research may need to move away from flawed traditional study designs measuring luminal blood oxygen. Newer molecular techniques including assessing the cellular responses and molecular changes in varicose veins to hypoxia may be more useful [74]. Understanding

the molecular changes including the cellular adaptations and responses to hypoxia in varicose veins could help to develop a pharmacologic target.

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