Characterization of a porcine model of chronic superficial varicose veins

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Objective: Previous animal models of venous disease, while inducing venous hypertension and valvular insufficiency, do not produce superficial varicose veins. In this study, we aimed to develop and characterize a pig-based model of superficial varicose veins.

Methods: Right femoral arteriovenous fistulae (AVF) were surgically fashioned in young adult pigs. Animals were examined at postoperative times up to 15 weeks to determine the development of varicose veins and measurement of both blood pressure and flow velocities within the superficial thigh veins. Histology and vascular corrosion casts were used to characterize the resulting structural venous alterations. Porcine pathophysiological features were compared with those of human primary superficial varicose veins.

Results: Gross superficial varicosities developed over the ipsilateral medial thigh region after an initial lag period of 1-2 weeks. Veins demonstrated retrograde filling with valvular incompetence, and a moderate, non-pulsatile, venous hypertension, which was altered by changes in posture and Valsalva. Venous blood flow velocities were elevated to 15-30 cm/s in varicose veins. Structurally, pig varicose veins were enlarged, tortuous, had valvular degeneration, and regions of focal medial atrophy with or without overlying intimal thickening.

Conclusions: The superficial varicose veins, which developed within this model, have a pathophysiology that is consistent with that observed in humans. The porcine femoral AVF model is proposed as a suitable experimental model to evaluate the pathobiology of superficial venous disease. It may also be suitable for the evaluation of treatment interventions including drug therapy. (J Vasc Surg 2009;49:1554-61.)

Clinical Relevance: Superficial varicose veins do not form spontaneously in lower animals. This represents a considerable barrier to the development and assessment of phlebotropic pharmacological agents. The development and physiological characterization of a reproducible experimental animal model, which develops a vascular pathology that closely mimics human venous disease, may represent a considerable advance in this field.

Varicose veins in the lower extremities have been reported in up to 40% of British men\(^1\) and the disease has a substantial impact on health care resources. Despite the diversity of signs and symptoms of superficial venous disease, a common feature appears to be venous hypertension associated with reflux via incompetent valves.\(^2\) A number of environmental and genetic risk factors have been proposed; however, current theories suggest that these may have a common action via augmentation of vessel wall inflammation. The combination of venous hypertension and localized inflammation results in a pernicious loop resulting in progressive structural degradation. Specifically, varicose veins undergo mural hypertrophy, altered collagen deposition, and dystrophic smooth muscle cells and elastic fibers.\(^3\)

In order to fully evaluate the pathobiology of varicose veins, it is necessary to examine veins early in their pathogenesis and preferably with reference to corresponding normal material. Clearly, it is not readily possible to use human material for such studies and a suitable animal model has been sought. Superficial varicose veins do not occur spontaneously in animals\(^4\) and it has been necessary to induce them experimentally. A number of such animal models have been previously attempted for venous disease but, unfortunately, none develop superficial varicosities. This paper describes how we have established a chronic, large animal model of primary superficial varicose veins.

METHODS

This study was approved by the University of Otago Animal Ethics Committee.

Surgical procedure. This model, of chronic superficial varicose veins, involved the fashioning of a femoral arteriovenous fistula (AVF) in 20 adult pigs. A right oblique groin incision was made through which the femoral artery and vein were dissected and mobilized with preservation of all venous tributaries. The sapheno-femoral junction (SFJ) was typically located along a ~10 mm length of the posterolateral femoral vein wall from the midpoint of the AV anastomosis to 5 mm distal to the fistula. A 10 mm long side-to-side anastomosis was fashioned using a double armed 6-0 prolene (Davis & Geck, Manati, PR) suture to...
form the AVF. The wound was closed in layers using a continuous 3-0 monocryl for the fascia and continuous 3-0 monocryl subcuticular suture for the skin. The patency of each fistula was confirmed immediately after surgery by auscultation for an audible bruit or palpable thrill.

An additional four unmanipulated animals were used as controls. One animal was euthanized at the same age when fistulae were fashioned (14 weeks of age, 30 kg); the remaining three animals were euthanised at approximately 30 weeks of age (120-130 kg). These large animals were used due to the need to have superficial veins of sufficient caliber to allow blood pressure catheter insertion and for accurate duplex ultrasound assessments.

Postoperative observations. At weekly intervals post-surgery, each animal was examined for evidence of superficial varicose veins, their size and distribution, and any related cutaneous stigmata of chronic venous disease on the skin overlying the inner and lateral thigh, groin, and abdominal wall. To facilitate this, conscious animals were restrained, and the upper body lifted to bring the animal into a bipedal standing position. This allowed the areas of interest to be closely examined and digitally photographed. The postural effect of lifting the animal to a near vertical position reduced venous return distending the superficial venous system of the groin, thigh, and abdomen. In addition, when the animals vocalized (a loud sustained squeal) in this position, they effected a powerful Valsalva-like response. Pigs were also trained to vocalize when shown food that was placed just out of reach. This response ceased immediately after the food was supplied. This alternative Valsalva-like maneuver was particularly useful in assessing pigs greater than 80 kg, which could not be easily raised into a standing position.

Physiological assessments in anaesthetized animals. Venous ultrasound and intravenous pressure measurements were performed on anaesthetized five animals at 6 and 14 weeks postoperatively. Transcutaneous duplex ultrasound (7-12 MHz) (ATL; Philips Medical Systems, Bothell, Wash) was performed on the ipsilateral thigh veins segments designated A, B, and C (Fig 1). Each vein was examined at three sites; I = 1 cm, II = 5 cm, and III = 9 cm distal to the junction. Intravenous pressures were collected within vein B at sites I and II using a 3F pressure catheter (Millar Instruments Inc, Houston, Tex) introduced through a 20-gauge cannula inserted 15 mm distal to site II. The ability to alter superficial venous pressures and the presence of venous reflux were assessed by abdominal and posterior thigh compressions. Due to the small size of the contralateral superficial veins (<1 mm), it was not possible to collect either pressure or accurate velocity measurements from veins on the left limb at either six or 14 weeks postoperatively. Comparative (anaesthetized) control measurements were therefore performed in the two (120 and 130 kg) unmanipulated control animals. A simple test of superficial venous valve competence (Harvey’s test) was also used in all unconscious animals. While one finger gently occluded the end of a segment of vein, a second finger ‘milked’ the vessel for approximately 40-50 mm followed by release of the second finger. The test was repeated at the same site in the opposite direction producing a simple means of identifying local segmental uni- or bi-directional flow.

Conscious venous blood pressures measurements. Three pigs with AVF had in-dwelling blood pressure radio telemeters (Telemetry Research Ltd, Auckland, New Zealand) inserted into the ipsilateral superficial venous network (segment B,II) under general anesthesia at five weeks post-fistula formation. One animal had an additional radio-telemeter placed in a ipsilateral lateral flank vein arising from an incompetent perforator. A 130 kg unmanipulated pig had radio telemeters placed in both the right and left superficial vein BII sites. All animals with radio-telemeters were allowed to recover for five days before collecting pressure recordings. Conscious supine, standing, walking, and bipedal standing pressures were then acquired and analyzed using the Chart Pro (version 6) software package (AD-
Instruments Pty Ltd, Dunedin, New Zealand). The reported pressures values were means of at least ten measurements in each posture.

Following the final physiological assessment, animals were systemically anti-coagulated with 2000 IU intravenous heparin, then euthanized with an overdose of sodium pentobarbital (30 mg/kg, intravenously).

**Histological assessments.** The skin overlying the nine reference vein sites was marked with a permanent pen. Bilateral thigh/groin skin flaps, including the underlying fascia and saphenous bundle, were excised en mass and pinned, epidermis facing down, to a plastic board. This ensured that the superficial veins were retrieved undamaged. Vessels were fixed overnight in 10% phosphate buffered formalin prior to embedding in paraffin. Vein samples were removed from the distal, mid, and proximal saphenous vein and from the superficial tributaries at standard sites from both limbs. Both longitudinal and transversely orientated specimens were sectioned and stained with either Verhoeff’s elastic stain and counterstained with modified van Gieson or Masson’s Trichrome.

**Corrosion vascular casts.** One unmanipulated control and three AVF animals were prepared for corrosion vascular casts (Batson’s #17 resin; Polysciences Inc. War- rington, Pa). Following systemic heparinisation and euthanasia, distal superficial veins were bilaterally cannulated with a 20-gauge needle, flushed with saline, and filled with 150 mL of blue colored resin per limb. The distal abdominal aorta was ligated proximally, cannulated with a 14-gauge needle, and both sets of hind-limb arteries each filled with 150 mL of red colored resin. Tissue was kept moist during resin polymerization (30 minutes) and the thigh/groin region removed en mass. The resin was allowed to harden overnight with the tissue immersed in saline before maceration in 15% sodium hydroxide at 60°C.

**RESULTS**

Animals grew rapidly throughout the study period, from an initial weight of 35 kg to 80-100 kg at the eight to 14 weeks postoperative time points.

**Anatomy of the veins within the saphenous bundle.** In normal controls the superficial venous tributaries of the thigh communicated with the saphenous veins via a perforating junction approximately six to eight cm distal to the junction with the femoral vein (SFJ). The saphenous venous drainage comprises two venous channels, one usually larger than the other, lying on each side of the saphenous artery and communicating with each other via a series of bridging veins. These vessels are surrounded by a distinct fascial condensation forming the saphenous bundle (Fig 2). The saphenous veins remained within the saphenous fascia before penetrating the fascia cribrrosa and joining the femoral vein separately. Each length of saphenous vein consistently contained eight to 10 bicuspid valves preventing reflux from the deep system. In pigs with femoral A-V fistulae, the associated saphenous veins became incompetent approximately one to two weeks postoperatively. Valvular incompetence within the saphenous veins was demonstrated by duplex ultrasound and vascular corrosion casts (Fig 1). While the ipsilateral saphenous veins became enlarged within the fascial sheath, compared with the contralateral saphenous veins, they remained non-tortuous at all time points.

**Development of the superficial veins.** In all, 18 of 20 pigs with AVF developed superficial varicose veins within the ipsilateral limb (Fig 1 and Supplementary Fig 1). In unconscious controls, Harvey’s test and duplex ultrasound demonstrated unidirectional superficial venous blood flow towards the perforating communicating junctions in the thigh region. Following creation of AV fistula,
the direction of flow was reversed with the formation of superficial varices one to two weeks postoperatively. Changes were first consistently noted in vein segment B followed by segments A, C, then the right lateral thigh (Fig 1 and Supplementary Fig 1). Vein segment B was therefore selected for use in subsequent blood pressure assessments.

The superficial varicose veins in the ipsilateral thigh formed in a consistent pattern initially radiating from the communicating junction with the saphenous vein. These veins progressively enlarged (ranging from one to five mm in diameter) before becoming tortuous (Fig 1). Venous distention was most evident post-exercise or during Valsalva-like maneuvers. After approximately three weeks, secondary sites of reflux also appeared through the perforating veins in the medial groin and lateral flank (Fig 1 and Supplementary Fig 1). The varicose venous network eventually extended to include the abdominal epigastric veins (Supplementary Fig 1). Regions of focal varicosities were occasionally observed in association with smaller veins, particularly at the periphery of the incompetent superficial venous network (Fig 1).

Postoperative AVF occlusion occurred in seven animals. Two were relatively acute failures (first two weeks postoperatively), and these animals did not develop superficial varicose veins. The remaining five animals had reduced patency from approximately six weeks postoperatively with an associated reduction in both the development and size of varicose veins.

**Histological changes in porcine superficial varicose veins.** The superficial veins in the ipsilateral thigh underwent heterogeneous alteration, including fibromuscular intimal thickening, medial atrophy, and medial and adventitial fibrosis (Fig 3). The extent of venous remodeling was reflected in the degree of loss of elastic tissue within the intimal and medial layers and particularly within the adventitia. These changes were consistent with that observed in human primary superficial varices (Supplementary Fig 2).

**Valvular changes and incompetence.** Ipsilateral thigh varicosities displayed valvular incompetence as assessed both functionally (Harvey’s test and duplex ultrasound) and structurally (vascular corrosion casts and histology; Fig 4). In the normal controls, valve cusps were readily observed throughout the superficial venous network, both within the primary channels and at communicating side branch points. In the varicose veins, residual cusps, while remaining attached to the distended commissures, were less than half the length of the luminal diameter and were therefore unlikely to be capable of preventing reflux within the ectatic vein (Fig 4, B and C). Valve leaflets occasionally appeared frayed (Fig 4, B) or showed evidence of fibrotic thickening (Fig 3, B). Such alterations were not observed in controls.

**Superficial venous pressures and flow velocities.** In unconscious supine control animals, the superficial venous pressures in the thigh were 1-4 mmHg. In the conscious controls, pressure ranged from 0-15 mmHg depending on posture (lying to standing) and were modestly elevated by movement of the ipsilateral, but not contralateral, limb. Performing a Valsalva-like maneuver produced a slow rise in venous pressure of less than 10 mmHg, which was followed by a rapid fall in pressure at cessation (Fig 5, A).

In comparison, the ipsilateral varicose superficial thigh veins in pigs with AVF were characterized by retrograde flow...
with a mild non-pulsatile venous hypertension (mean, 24 mmHg; range, 15-35 mmHg; n = 110) in unconscious supine animals. Pressures were not significantly different at either six or 14 weeks. Pressure profiles in conscious lying animals were similar, however, there was a marked postural effect, with pressures approximately doubling upon standing (Fig 5, B). Increased intra-abdominal pressure (via a Valsalva-like maneuver) acutely elevated superficial venous pressures in proportion to the degree of abdominal augmentation (Fig 5, C). Valsalva typically increased superficial venous pressures by approximately 50 mmHg (Table), and exceeded 150 mmHg when Valsalva was performed while in the bipedal standing position. Immediately following a Valsalva, the pronounced inspiratory gasp results in a matching rapid fall in superficial venous pressure (Fig 5, C). Walking resulted in substantially elevated pressures consistent with thigh muscle pump activity (Fig 5, D).

Unconscious supine superficial venous blood flow velocities were elevated from approximately 1-4 cm/s in the un-manipulated control veins to 15-30 cm/s in the ipsilateral thigh superficial varices (Fig 6). As with pressure, abdominal augmentation resulted in a transient increase in retrograde venous blood flow velocity (Fig 6, B). While duplex waveforms within the anastomosed vein, particularly near the fistula, displayed an arterialized pattern, the waveforms within the superficial veins indicated relatively continuous (elevated) flow (Fig 6).

**DISCUSSION**

This study reports the porcine femoral arteriovenous fistula as a novel model of venous disease, as it is associated with the progressive development of superficial varicose veins within the ipsilateral limb. Retrograde flow associated with valvular incompetence occurs following an initial lag period of one to two weeks. We suggest that this delay is due to the presence of numerous valves within the saphenous veins, which must fail sequentially in order for retrograde flow to occur from the deep to superficial compartment.

In the acute phase it is perhaps surprising that the saphenous veins do not occlude due to valve obstruction of blood flow, or that valves are not torn acutely establishing communications with distal venous territories soon after fashioning the fistula. Indeed, this appears to be the case in some AVF models but does not occur in this porcine model. Valves become incompetent as the saphenous vein diameter gradually increases and flow is maintained. We suggest that this may be due to the presence of an extensive network of communicating bridging veins connect the dominant and ancillary saphenous channels (Fig 2, C). Both the dominant and ancillary saphenous veins form distinct SFJs at different distances from the AVF. Proximal versus distal AVF veins have a large pressure differential; we therefore speculate that this may preferentially drive flow into the more distal saphenous vein. Blood flow could then loop back to the femoral vein via the saphenous bridging veins and the proximal saphenous vein. Without this network to allow continued blood flow during the progressive failure of the approximately eight to 10 saphenous valves, the vein would likely occlude prior to establishing refluxing communication with the superficial venous network. In addition, the presence of the saphenous fascia appears to protect this segment of vein from excessive ectasia and tortuosity (Fig 2), during the process of progressive saphenous valve degeneration. Only once outside the saphenous fascia does frank varicosity and tortuosity become apparent.

Acute and chronic fistulae, as created for hemodialysis, are associated with distinct pressure profiles within the anastomosed distal vein, the former having a near arterial pressure profile, which is greatly diminished in the mature fistula, presumably due to outflow remodeling and the formation of collaterals. The consequence of the delay in retrograde filling
Fig 5. Conscious blood pressure profiles in superficial control (A) and varicose (B-D) veins. Blood pressure measurements in right thigh vein segment BII (A, B, and C) or right lateral flank vein (D), acquired via indwelling radio-telemetry. (A) In a normal control vein, standing superficial venous blood pressure of approximately 6 mmHg, a hind limb step, then a sustained (4 second) Valsalva (continuous vocalization). Note the gradual rise in pressure during Valsalva, consistent with increased out-flow resistance (elevated intra-abdominal pressure) rather than reflux. There was no indication of retrograde pressure transmission in the control animals (presumably due to the presence of competent valves). (B) Pressure changes with altered posture, six weeks postoperatively. The animal went from lying on its abdomen to sitting (forequarter raised) to standing (without walking). (C) Effect of Valsalva-like maneuver (loud sustained three-second vocalization) on superficial venous pressure. Notice the pronounced drop in pressure post-Valsalva. (D) A weight shift to ipsilateral limb (at 6-7 seconds) followed by five steps. Note the fall below standing pressures between steps (*) consistent with the actions of a thigh ‘muscle pump.’
Table. Conscious superficial venous pressures (mmHg)

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<tr>
<th></th>
<th>Control n = 2</th>
<th>Varicose Veins n = 3</th>
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<tbody>
<tr>
<td>Lying</td>
<td>2.3 ± 2.4</td>
<td>22.2 ± 6.3</td>
</tr>
<tr>
<td>Standing</td>
<td>5.5 ± 4.2</td>
<td>33.5 ± 8.2</td>
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<tr>
<td>Valsalva (peak pressure)</td>
<td>16.5 ± 6.6</td>
<td>95.8 ± 40.9</td>
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Three varicose veins from eight-week post-AVF animals compared with two veins within a 30-week old control. Varicose veins had significant higher pressures in each posture than the control veins (P < .0001). Pressures are shown as means ± 1 standard deviation.

*P = .005 versus control lying.
†P < .0001 versus control standing.
‡P < .0003 versus varicose vein lying.
§P < .0001 versus varicose vein standing.

of the superficial veins in this model may be significant, resulting in a non-pulsatile modest (20-50 mmHg) venous hypertension rather than direct venous arterialization. In addition, the associated increase in (retrograde) blood flow velocity is likely to significantly contribute to shear stress induced venous remodeling, consistent with the arteriectasis known to be produced by altered shear stress.7

In terms of venous pressure, this study was able to demonstrate that unmanipulated control veins had a low pressure which changed slowly and modestly (<10 mmHg) during Valsalva. In contrast, in animals with AVF induced varicose veins, Valsalva produced a rapid, large (typically >50 mmHg) rise in pressure. We interpret the difference to reflect outflow obstruction, (2) valvular incompetence, (3) augmented hemodynamics, or (4) a combination of these models. Depending on the desired outcome, each class has its own strengths and weaknesses.

Since chronic venous hypertension is widely accepted as a key pathological mechanism of chronic venous disease, venous occlusion models have been employed in an attempt to raise venous pressures by limiting outflow. Despite an acute elevation, venous occlusion models, in and of themselves, do not appear to maintain chronically elevated venous pressures.5,6 Burnand concluded that this was due to the formation of collateral drainage, which he demonstrated on phlebograms.8

Valvular incompetence is a common feature of chronic venous disease. Lalka, Dalosing, and colleagues10 described a simple, reproducible model of hind-limb valve disruption in the greyhound, which represents a useful model of venous valvular insufficiency, particularly in the evaluation of valve reconstruction procedures. Animals developed an immediate increase in segmental venous pressure that persisted for as long as 14 weeks. While phlebography demonstrated reflux in the disrupted valve segments, there was no indication of extension into tributaries and no evidence of varicose veins, even at the most chronic time point. Other aspects, which should be considered when assessing this model’s suitability, include the acute nature of the valve degeneration, which abruptly exposes the vein to increased pressure, and the relatively short hydrostatic column present in the quadruped hind-limb.

Finally, Dart and colleagues formed acute AVFs in the dog but noted an arterialized pressure profile within the distal veins. Burnand produced a more mild chronic venous hypertension in the greyhound associated with a greatly reduced exercise induced pressure decline.8 However, these models appear to be associated with sustained venous hypertension, along with an acute phase arterial pressure profile, which is not particularly consistent with chronic venous disease. Moreover, these previous AVF-based models have not reported the formation of associated superficial varicose veins.

Models combining the above mechanisms have also been developed. Most notably, the combination of AVF and outflow obstruction has been used in order to produce sustained venous hypertension. Van Bemmelen applied this
model to rats in order to study valve remodeling. Although valve incompetence and remodeling was noted, as early as 24 hours postoperatively, no pressure profiles were recorded and the animals did not develop varicosities. More recently, a detailed series of studies conducted by Bergan’s group has utilized the same model and reported arterial-like pressure profiles with mean venous pressures in the order of 100 mmHg. Vein alterations reported included media atrophy, wall fibrosis, increased protease expression, progressive valve degeneration, and dilation of the commissures. Using this rat model, valvular inflammation has been suggested to be the key pathologic feature in the development of reflux in this model. None of these previously described models develop obvious superficial varicose veins and, while not suitable to model this venous disease phenotype vein, it should be noted that they may still have utility in the study of other venous conditions, such as acute venous obstruction, thrombophlebitis, or valvular insufficiency.

In contrast, the principal feature of the pig femoral AVF model was the formation of chronic superficial varicose veins. The heterogenous pathohistology of this model is consistent with the range of alterations observed in human superficial varicose veins, including focal medial atrophy, intimal thickening, connective tissue degeneration, fibrosis (Supplementary Fig 2), and chronic valvular degeneration. These changes appear to be due to physiological alterations that are not simply that of acute arterialization. While the pathophysiology of this model clearly has distinct features from that of humans – indeed, we do not believe it is possible to create a perfect mimic of the human state – the venous alterations nevertheless appear consistent with chronic varicose veins in humans.

The use of the pig hind limb does have some limitations as a model of venous disease. Assessment of venous reflux was limited to Valsalva since the absence of significant calf musculature precluded standard calf augmentation. Similarly, it was not possible to assess the hind limbs for any significant length of time in a bipedal posture. The use of indwelling blood pressure radio-telemetry and training to produce a quadrupedal Valsalva did, however, significantly aid in overcoming many of these issues.

While the use of a human sized experimental animal, such as the pig, is costly, it does allow for a greater range of physiological evaluations to be performed, which would not be possible in smaller animals.

In conclusion, we suggest that the porcine femoral AVF model represents a suitable experimental system to study the pathobiology of primary superficial varicose veins, incorporating the key components of modestly elevated (non-pulsatile) venous pressure, ectasia, valve distortion, altered shear stress, and inflammation. We propose the use of this model to advance the development of phlebotropic pharmacological agents for the treatment of superficial venous disease.

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