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BASIC RESEARCH STUDIES

Growth and remodeling of canine common iliac vein in response to venous reflux and hypertension

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Objective: **The passive properties of the venous wall are important for the compliance function of the venous system. The objective of this study was to quantify the passive biomechanical response and structural growth and remodeling of veins subjected to chronic venous reflux and hypertension.**
Methods: **To investigate the effects of venous reflux on venous mechanics, the tricuspid valve was injured in a canine model by disrupting the chordae tendineae. The conventional inflation-extension protocol in conjunction with intravascular ultrasound was used to investigate the passive biomechanical response of both control common iliac veins (n = 9 dogs) and common iliac veins subjected to 8 weeks of venous reflux and hypertension (n = 9 dogs). The changes in vein wall thickness and constituent composition were quantified by multiphoton microscopy and histologic evaluation.**
Results: **Biomechanical results indicate that the veins became less compliant when exposed to 8 weeks of chronic venous reflux and hypertension. The mechanical stiffening was found to be associated with a significant increase in wall thickness ($P < .05$) and collagen-to-elastin ratio ($P < .05$). After 8 weeks of chronic reflux and hypertension, the circumferential vein wall stress was significantly reduced ($P < .05$) because of wall thickening, although it was not restored to control levels.**
Conclusions: **The growth and remodeling of the venous wall reduces the wall stress, but the stress remains higher than at baseline at 8 weeks. The compliance of the veins also decreases because of the increase in wall thickness and remodeling of the microstructure of the venous wall. These findings provide insight into potential adaptations of the venous system in reflux and hypertension. (J Vasc Surg: Venous and Lym Dis 2015;3:303-11.)**

Clinical Relevance: **Chronic venous insufficiency (CVI) affects the venous system of the lower extremities with venous reflux and hypertension. The growth and remodeling of the venous system to the perturbed hemodynamic conditions in CVI are not well understood. The relevance of this study is to provide fundamental data on the morphologic and mechanical properties of the venous system in a translational animal model of CVI. Our findings highlight the adaptive changes of the venous system that may lead to loss of compliance and the various sequelae in CVI.**

Chronic venous insufficiency (CVI) is a condition that affects the venous system of the lower extremities with venous hypertension causing various sequelae, including pain, swelling, edema, skin changes, and ulceration.[1] CVI affects 10% to 35% of the adult population in the United States,[2] with substantial health care costs.[3] Venous hypertension causes the various sequelae associated with CVI.[1]

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It has been experimentally and clinically documented that arteries remodel in response to hypertension.[4,5] Unlike for arteries, there is a dearth of information related to the effects of chronic venous hypertension on venous wall mechanics,[6,7] although the biomechanics of normal vena cava,[5,8,9] femoral vein,[6,10] and saphenous vein[11-16] has been studied. Venous hypertension can be induced by vessel constriction,[10] gravitational loading,[11,17] volume overload,[5] arteriovenous fistulas,[18,19] and vein grafts.[12,20] There is no consensus on how the venous wall responds to chronic venous hypertension.

Reflux due to venous valve incompetence is the prevailing theory in the etiology of venous hypertension.[12,21-23] Venous hypertension is primarily due to incompetent valves, but a secondary cause is obstruction of venous outflow, or failure of the muscle pump.[22] Venous hypertension depends on the hydrostatic and hydrodynamic pressure components.[24] Pascarella et al[18] created a rat model of venous insufficiency by constructing a femoral arteriovenous fistula, whereas Dalsing et al[25,26] developed a canine model of venous insufficiency by disrupting the venous valves of the hind limb. No current animal model, however, is completely representative of the hemodynamic conditions seen in CVI patients.

The primary objective of this study was to quantify the passive biomechanical response and structural remodeling of venous tissue in a canine model of chronic venous reflux and hypertension. Our findings show increased venous stiffness and wall thickness along with remodeling of elastin and elastin-to-collagen ratio in response to venous reflux and hypertension.

METHODS

Canine model of chronic venous reflux and hypertension. To develop a large-animal model of chronic venous reflux and hypertension, nine male mongrel dogs (body weight, 34.1 ± 2.3 kg) underwent disruption of the chordae tendineae with a ∼0.035-inch-diameter custom cutting device (Avulsion wire; Cook Medical, Bloomington, Ind). The dogs were sedated with acepromazine (0.02 mL) and torbugesic (0.7 mL) administered subcutaneously. General anesthesia was maintained with 2% to 3% isoflurane and oxygen. Heparin (1.5-3 mL) was administered every half-hour to prevent coagulation.

A baseline pulse wave velocity (PWV) tracing for the common iliac vein was recorded in the conscious, standing position and the sedated, supine position by duplex ultrasound (L15-7io transducer, iE33 echocardiography system; Philips Healthcare, Andover, Mass) before disruption of the chordae tendineae (baseline) as well as after injury. Once baseline duplex ultrasound data were obtained in the canine, the neck was prepared and draped in a sterile fashion. Access was gained with micropuncture through the jugular vein. A Visions PV .018 intravascular ultrasound (IVUS) imaging catheter (86700; Volcano, San Diego, Calif) in conjunction with the s5i imaging system (S5iVC01; Volcano) was advanced from the jugular vein down to the common iliac vein. The maximum diameter, minimum diameter, and cross-sectional areas were obtained for both common iliac veins.

Hemodynamic measurements were made throughout the injury procedure by a fluid-filled pressure transducer (TSD104A; BIOPAC Systems, Goleta, Calif). The pressure transducer was attached to a catheter connected to a femoral vein.

Once the baseline pressure was obtained, the catheter was advanced into the right ventricle by a percutaneous, over-the-wire technique. The cutting device was subsequently introduced into the right ventricle. Disruption of the chordae tendineae was created by pulling the cutting device retrograde across the tricuspid valve from the right ventricle to the right atrium. Eight weeks after the disruption of the chordae tendineae, the animal was euthanized with a bolus of saturated potassium chloride.

All experimental procedures were performed in accordance with national and local ethical guidelines, including the Institute for Laboratory Animal Research guidelines, Public Health Service policy, and the Animal Welfare Act, and were approved by the Institutional Animal Care and Use Committee at Indiana University−Purdue University Indianapolis.

Measurements of venous reflux. The PWV tracings obtained at each time point were used to calculate the reflux fraction in the common iliac vein. An image analyzer (ImageJ; National Institutes of Health, Bethesda, Md) was used to determine the reflux fraction. The reflux fraction was calculated by integrating the velocity. The portion of the waveform that is above the x-axis represents the displacement of forward flow, whereas the portion of the waveform that is below the x-axis represents the displacement of reverse flow. The reflux fraction can be expressed as the displacement of reverse flow over the displacement of forward flow. Four waveforms were measured per PWV tracing, and the four subsequent reflux fractions were averaged.

Tissue harvest. Immediately after euthanization, the lower extremity venous system was exposed and the common iliac vein of the experimental dogs was harvested for biomechanical testing and histologic examinations. The common iliac vein was optimal for inflation-extension testing because of the diameter (∼10 mm), straight length (∼6 cm), and lack of side branches and valves. A 2-cm portion of the proximal end of the vessel was fixed in 10% formalin for histologic examinations. Vein segments were fixed in formalin for at least 24 hours before any histologic examinations. The remaining segment (∼4-6 cm) was stored in normal saline at 4°C until biomechanical testing. All biomechanical tests were performed within 24 hours of tissue harvest. Before biomechanical testing, the loose adventitia was carefully cleaned under a stereomicroscope (SMZ660; Nikon, Melville, NY). Special care was taken to maintain intact adventitia and to not overstretch or mechanically traumatize the vessel wall.

Control group. The common iliac veins of an additional nine mongrel dogs of either sex weighing 24.8 ± 2.5 kg (mean ± standard deviation) were used as controls in which the chordae tendineae were not disrupted. The animals were used for other unrelated physiologic experiments. The common iliac vein was excised and prepared for biomechanical testing and histologic examination, similar to the experimental group.

Inflation-extension test. The inflation-extension protocol was used to biaxially test the experimental and control veins. Each tubular specimen was mounted in the bath filled with 3 mM ethylene glycol tetraacetic acid solution kept at room temperature to maintain passive mechanical properties of the vein. A manual syringe pump was used to apply internal pressure to the vein segment. The pressure was measured with a fluid-filled pressure transducer (TSD104A; BIOPAC Systems), the force was measured with a force transducer (Fort1000; World Precision Instruments, Sarasota, Fla), and the inner and outer diameters were determined with use of a Visions PV .018 IVUS imaging catheter (86,700; Volcano) in conjunction with the s5i imaging system (S5iVC01; Volcano).

The diameters and length under no load (no axial stretch and 0 mm Hg pressure) were measured and preconditioned by means of three inflation-deflation loops. Once the pressure-diameter curve was reproducible, the vessels were