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RESEARCH ARTICLE

In vivo characterization of the murine venous system before and during dobutamine stimulation: implications for preclinical models of venous disease

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ABSTRACT

Although widely used as a preclinical model for studying venous diseases, there is a scarcity of *in vivo* characterizations of the naïve murine venous system. Additionally, previous studies on naïve veins (*ex vivo*) have not included the influence of surrounding structures and biomechanical forces. Using MRI, we noninvasively quantified the cross-sectional area, cyclic strain, and circularity of the venous system in young and old, male and female C57BL/6 mice. We investigated the most common venous locations used to perform venous disease research: the common jugular vein, suprarenal inferior vena cava (IVC), infrarenal IVC, common iliac vein, and common femoral vein. Our results elucidate age-dependent changes in venous cross-sectional area, which varied by location. Maximum cyclic strain, a parameter of lumen expansion, showed 10% change across the cardiac cycle, approximately half the magnitude of arteries. Veins demonstrated noncircular shapes, particularly in the core vasculature. The cardiovascular stressor dobutamine had only a small impact on the venous system. Also, our data demonstrate that the peripheral veins tend to decrease in cross-sectional area and circularity with age. Conversely, the IVC tends to increase in size and circularity with age, with males exhibiting larger variability in response to dobutamine compared to females. This work provides a foundation for drawing age and sex comparisons in disease models, and represents the first *in vivo* characterization of the murine venous system at rest and during the application of a pharmacological exercise surrogate.

1. Introduction

Venous diseases have been designated a national priority by the Surgeon General (Leavitt, 2008) and the Center for Disease Control (Streiff et al., 2014). The troubling statistics on the prevalence of venous disease and data suggesting sub-optimal treatment (Kahn et al., 2014; Prandoni et al., 2002; Puurunen et al., 2016) emphasize the critical need for a more comprehensive understanding of the venous system, including the geometric and biomechanical characteristics of veins in the animal models so commonly used to investigate disease progression and therapeutic options. While the arterial system has been well studied in health and disease (Goergen et al., 2010; Greve et al., 2006), there are far fewer studies regarding venous disease, including a paucity of *in vivo* investigations of the healthy venous system of murine models.

Blood volume, flow, and pressure dictate vessel shape, in conjunction with vessel wall contents. As such, abnormal vein shape can be indicative of an underlying pathology in the vein lumen, such as thrombosis, or an extraluminal structure deforming a vein, such as a tumor (Chen et al., 2005; Liu et al., 2017; Zucker et al., 2016). Alterations in geometry can lead to changes in biomechanical forces, which play a primary role in the location, initiation, and progression of cardiovascular disease (Meissner et al., 2007). Thus, understanding the geometry and biomechanics of the healthy venous system establishes the baseline for investigating disease, enabling better interpretation of research results.

Most geometric and biomechanical investigations of the venous system have acquired data *ex vivo* (Lee et al., 2015, 2013; McGilvray et al., 2010; Sokolis, 2008). Such experiments provide valuable information on material properties and the stress-strain response of the vessel wall. However, *ex vivo* studies are inherently limited

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to a single time-point and subject to misinterpretations. Non-physiological pressures coupled with a lack of blood flow, external tethering from surrounding anatomical structures, and circumferential and axial stresses – all of which influence vessel structure and function – may alter results. While many intuit the relatively motionless nature of veins compared to arteries, *ex vivo* testing may confound some perspectives on the actual motion of veins *in vivo*, potentially compromising research on the venous system. There is little to no data quantifying cyclic strain of the venous system in humans or the preclinical models often used as surrogates.

Murine models are the most commonly used preclinical model for investigating venous diseases. Mice provide a unique biological environment to study disease progression and treatment in a highly controlled manner, with the added availability of genetically manipulated strains to uncover molecular pathways. Recently, the NIH has addressed concerns over lack of sex diversity in preclinical experiments (NIH, 2015). In addition, some venous diseases disproportionally affect the older population (Criqui et al., 2003; Heit et al., 2005; Prandoni et al., 2014). Thus, it is imperative to have an understanding of the naïve venous system that considers age and sex differences, as outlined in this work.

Dobutamine is a β1-receptor agonist that increases cardiac output. As such, it is used clinically in the treatment of acute heart failure and as one of the most widely available pharmacological stress testing agents (Nagel et al., 1999; Yao and Chaudhry, 2005). Pharmacologically induced stress testing is used to diagnose cardiovascular disease in patients presenting with the inability to complete a treadmill test. Preclinically, dobutamine has been used in a similar manner (Calligaris et al., 2013; Wiesmann et al., 2001) and to reveal subtle phenotypic differences in conditional knockout mice (Williams et al., 2001a). The high field magnetic resonance imaging (MRI) used in this work allowed us to non-invasively characterize murine vasculature in both baseline and physiologically stressed conditions *in vivo,* with high spatial and temporal resolution.

Herein, we present *in vivo* quantification of the healthy venous system of murine models in the most common veins used to perform venous disease research: the common jugular vein, suprarenal inferior vena cava (IVC), infrarenal IVC, common iliac vein, and common femoral vein. Using MRI, we analyzed cross-sectional area, cyclic strain, and vessel circularity across age and sex. Additionally, we administered the cardiac stressor dobutamine to evaluate the venous response. These novel data provide foundational knowledge in the characterization of the naïve murine venous system.

2. Methods

2.1. Mice

All experiments were performed at the University of Michigan with approval from the University of Michigan's animal care and use committee. Adult male and female C57BL/6 mice aged either 3–4 months or 15–18 months were used in this study. These ages are the human equivalent of a young adult or adult over the age of 50, respectively (Flurkey et al., 2007a, 2007b). Data was acquired in 50 mice: n = 15 young males, n = 15 old males, n = 10 young females, and n = 10 old females; within this cohort, dobutamine was administered in n = 5 mice per group. For imaging procedures, mice were anesthetized using 2% isoflurane, 1 L/min O_2 carrier gas. Heart rate and respiration were monitored with ECG electrodes and a respiratory pillow, respectively (SA Instruments, Inc., NY). A PID controller was used to maintain the animal's temperature at $37 \pm 1\,^{\circ}C$ via warm air circulation through the bore of the magnet. Mean total imaging time was 1.5 h, or 2.5 h when administering dobutamine (approximately 1 h with dobutamine).

2.2. Magnetic resonance imaging

Imaging was performed on a 7 Tesla horizontal bore magnet using a Direct Drive console (Agilent Technologies, Santa Clara, CA). All animals were imaged supine with the legs taped into an extended position. 3D time-of-flight (TOF) gradient echo sequences were used to acquire volumes at each of five locations: common jugular, suprarenal IVC, infrarenal IVC, iliac vein, and femoral vein (repetition time/echo time [TR/TE] 20/2 ms, field of view [FOV] $30 \times 30 \times 30$ mm, flip angle [α] 20°, matrix $128 \times 64 \times 64$ zero-filled to $256 \times 128 \times 128$, number of excitations [NEX] 2, slab thickness 12 mm, imaging time 3 minutes). Coronal and sagittal maximum intensity projections (MIPs) of the venograms were used to plan cardiac-gated 2D TOF slices perpendicular to each vessel location (TR equal to the period of the cardiac cycle—approximately 180 ms, TE 6 ms, FOV $(25.6\,mm)^2$, α 30°, matrix 512×256 zero-filled to 512^2, resolution $50 \times 50\,\mu m$, slice thickness 1 mm, NEX 6, imaging time 6 min per location). These parameters resulted in approximately 25 voxels across the jugular vein, 31 across the suprarenal IVC, 25 across the infrarenal IVC, 17 across the iliac, and 12 across the femoral vein. The imaging parameters were chosen to enhance signal in moving blood, while maintaining a flip angle adequate to prevent saturation of the slower venous blood flow at the edge of the vessel. Twelve CINE frames were acquired across the cardiac cycle to quantify lumen expansion for each location.

2.3. Dobutamine administration

In a subset of n = 20 mice (5 of each sex and age group), a 30-gauge needle connected to a polyethylene catheter (PE10; Braintree Scientific) initially filled with saline was inserted into the tail vein for dobutamine infusion. Dobutamine infusion was initiated following acquisition of baseline (i.e. resting heart rate) CINE images at each of the five locations. A dosage of 40 μg/kg of body weight was infused by the tail vein catheter at a rate of 2 μL/min. Once the increased heart rate reached a steady state, CINE images were acquired using the same slice planning as the baseline acquisitions.

2.4. Image quantification

Each of the five locations was analyzed for cross-sectional area, cyclic strain, and circularity using a semi-automated in-house MATLAB script. The k-space data acquired at 512×256 and zero-filled to 512×512 points in the frequency and phase encode direction were multiplied by a broad Gaussian weighting function to produce slight image blurring with a k-space attenuation chosen to have a root mean squares width of 0.8 voxels (voxel = three-dimensional pixel). This blurring step served to reduce random noise at a small cost of spatial resolution, due to the sub-voxel spatial width of our filter. The filtered data was then Fourier transformed, and the blurred image was up-sampled to obtain an image with 1024×1024 voxels for improved threshold searches. The added up-sampling step (implemented in MATLAB using 2D interpolation of the image) introduces information about the local derivative of voxel intensities at a vessel boundary, to better constrain the spatial location of a point identified to be on the vessel boundary by the thresholding algorithm.

To produce a reference image at a given vessel location, the 12 images acquired over the cardiac cycle were averaged. Then, a region of interest containing the vessel was defined and the voxel of maximum signal intensity was identified, allowing for 50% of the maximum signal intensity within the ROI to be used as a cutoff threshold in that frame. Starting at the maximum intensity voxel, an iterative radial search, subject to the thresholding condition, was used to find a connected patch of voxels surrounding the brightest voxel. The patch of voxels delineated the mean vessel shape. We