

Advanced venous flow dynamics and return mechanisms during
physiological stress and aging

by

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This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis.

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Abstract

Human veins are dynamic vessels responsible for managing return of blood to the heart. Unlike arteries, veins are structurally limited, creating exceptional hemodynamic susceptibility to hydrostatic forces and pressure generating forces. The precise extent of venous flow regulating mechanisms to these forces have implication for disease pathogenesis yet have been historically limited by technological tools. Thus, the purpose of this thesis is addressing the responses to hydrostatic volume stress, muscle pump activation and healthy aging through the piezoelectric lens of vector flow imaging ultrasound through a series of projects. First, progressive hydrostatic volume stress is applied to the internal jugular vein to address regional flow complexity and volume expansion behaviour. The internal jugular vein presented step-wise volume expansion in a trapezoidal shape and increasing flow complexity, increasing the risk for flow stasis and thrombus formation. Second, hydrostatic volume stress is used to compare artery-adjacent and non-adjacent veins to probe the existence of an arterial pump. During relevant hydrostatic driving forces, artery-adjacent veins demonstrated a preservation of venous flow, suggestive of a newly described mechanism of venous and an elegant conservation of mechanical energy within the cardiovascular system. Third, a comparison of younger and older adults leveraged differences in venous compliance to investigate flow complexity features during muscle pump activation during hydrostatic volume stress. Older adults were found to utilize their lower compliance to maintain venous ejection efficiency, whereas younger adults experienced great turbulence and vorticity flow features for the same venous outflow. Finally, venous valves were explicitly investigated during progressive hydrostatic volume stress and muscle pump activation to describe flow complexity features. Healthy valves generated efficient forward jets with downstream disturbed, low-shear zones, and volume stress amplified regional differences, underscoring the role of valve geometry in venous hemodynamics. Together, these studies establish new mechanistic links between venous structure, hydrostatic and muscle pump forces, and aging, advancing understanding of venous flow regulation and its implications for thrombotic risk and cardiovascular health.

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Dedication

This thesis is dedicated to the love and memory of my grandparents whom I lost time with too soon and due to academic pursuits. I hope to have made you proud.

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List of Abbreviations

ANOVA	Analysis of variance
CSA	Cross-sectional area
DBP	Diastolic blood pressure
DFV	Deep femoral vein
FV	Femoral vein
GSV	Greater saphenous vein
HDT	Head-down tilt
HUT	Head-up tilt
IJV	Internal jugular vein
IPC	Ischemic preconditioning
IQR	Inter-quartile range
IVC	Inferior vena cava
MBV	Mean blood velocity
MVC	Maximal voluntary contraction
OA	Older adult
ORE	Office of research ethics
PCO ₂	Partial pressure of carbon dioxide
PO ₂	Partial pressure of oxygen
r	Radius
SBP	Systolic blood pressure
SD	Standard deviation
SFA	Superficial femoral artery
VCC	Vector concentration coefficient
VFI	Vector flow imaging

YA	Young adult
η_p^2	Partial eta-squared
ρ	Density of blood
μ	Viscosity of blood
∂	Partial derivative
θ	Angle

Chapter 1: Introduction

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1.1 Anatomical and biomechanical properties of veins

Carrying ~65% of total blood volume through a large range of pressures (~0 to >200 mmHg) is a challenge, requiring a vast network (~500 km) of highly compliant veins and venules (1, 2). The hallmark feature of veins – compliance, the ability to store volume with little transmural pressure change and typically represented as: $Compliance = \frac{\Delta Volume}{\Delta Pressure}$ – exists largely due to the higher proportion of collagen fibers in veins compared to arteries. In the great arteries, elastin composes a larger percentage of the vessel wall compared to veins to facilitate the Windkessel effect of pressure dampening, rendering arteries ~30 times less compliant than veins (1, 3–5). This paradoxical distinction highlights the importance of wall thickness relative to internal diameter as a determinant of compliance which can also be derived with more specific wall characteristic inputs as $C = \frac{2\pi r^2}{w \times E}$ where r represents vessel radius, w represents vessel thickness and E represents the Young's elastic modulus. Veins have relatively thin walls, defined by thickness-to-diameter ratios ranging from 0.2 – 0.5, whereas the ratios for arteries and arterioles range between 1.0 – 2.0; this thin wall feature of veins results in the 30-50 times greater compliance in veins, dependent on location (1, 6). Regional differences in vein wall thickness are based on their need to handle hydrostatic forces. Veins at or above the level of the heart have thinner walls compared to those of the lower-limbs, where veins can experience pressures higher than arterial systolic pressure during upright posture (7, 8). Veins above the heart often benefit from gravity-assisted drainage, as the arms are frequently positioned above heart level during daily activities. As a result, upper limb veins experience lower hydrostatic pressure and depend less on muscle pump function. Despite similar structural features, they rarely develop chronic venous insufficiency, likely due to these more favorable hemodynamic conditions.

Veins are classified into different systems based on their anatomical arrangement, including superficial, deep, and perforator systems. The superficial veins run beneath subcutaneous adipose tissue, collecting venous outflow primarily from the skin to drain into the deep veins running directly to the right atrium. Communication between superficial and deep systems is supported by perforators, passing through fascia and tissue layers (9). Characteristic differences between venous systems beyond diameters (deep > superficial > perforators) do not seem apparent, with similar compliance in the deep and superficial systems (10, 11). However, venous depth can be determinant for fascial compartment effects, whereby surrounding tissue

pressure can increase the extravascular pressure, contributing to venous transmural pressure. Veins are more susceptible to flow disturbance from fascial compartment pressure changes compared to arteries due to their differing wall structure.

Arteries and veins have the same general wall composition from exterior to interior: 1) the tunica adventitia, containing primarily collagenous structural fibers; 2) the tunica media, where most vascular smooth muscle resides; and, 3) the tunica intima, where endothelial cells are located. The primary difference between arteries and veins is the contribution of the tunica adventitia and tunica media to overall wall thickness: in veins the adventitia composes the majority of the wall due to little media (smooth muscle) present (12). The intima layer differs minimally in thickness except for venous valve locations where intimal thickening is found; however, arterial endothelial cells are typically narrow or ellipsoidal, whereas venous endothelial cells present a shorter, cuboidal arrangement (13, 14). Endothelial cell arrangement is relevant for passage of molecules in and out of the lumen and arteries have notably tight junctions to help withstand higher pressures, whereas veins, particularly small venules, have comparatively loose or disorganized junctions contributing to venous permeability (12, 14). These differences are thought to be representative of the respective shear stress and flow directionality environments demonstrated by phenotypic changes following exposure to laminar, turbulent, or increased flows (14–16).

Venous valves

Venous valves are central to cardiovascular regulation, first functionally described over 400 years ago by Fabricius ab Aquapendente (17). Valves have since been observed throughout the animal kingdom, including in frogs, birds, monkeys, cats, and dogs (18). In humans, venous valves have been identified throughout the body; however, there are fewer valves located above the heart with increasing prevalence towards the periphery, with predictable locations every few centimetres (19–21). Valve prevalence is highest in the lower-limbs, particularly in the deep compared to superficial veins due to higher blood volumes and the need to support venous return without the generation of lengthy hydrostatic pressure columns (18). The distance between valves in deep veins is commonly ~22 mm whereas superficial veins have spacings of ~40 mm. These values align with ~30 valves within the tibial vein and ~16 valves in the saphenous vein in healthy adults and appear independent of sex and height in adults (22). The presence of valves allows for independent, discontinuous mechanisms to aid venous flow, minimizing retrograde flow, often against the force of gravity.

1.2 Functional characteristics of veins

Venous compliance

As mentioned, compliance describes the change in volume per unit change in transmural pressure, a dynamic property describing a vessel throughout a range of pressures (23). The venous compliance curve has a characteristic non-linear shape dependent on transmural pressure (Fig. 1-1); thus, specific capacitance (absolute measure of volume at a given transmural pressure) can vary drastically over the physiological range (24).

At low transmural pressures, the venous compliance curve is steep, passively accepting large volumes with minimal pressure increases, not engaging elastic components of the wall. At this stage, the vein merely undergoes geometric changes from a flaccid to circular shape. This process can be likened to an accordion or folded tube gradually unfolding, where initial volume increases cause the vein to expand without significant elastic resistance. Only once the vein becomes circular are elastic properties engaged and wall stress is >0 (25), describing the pressure distributed across the wall thickness. Wall stress is defined by the Law of LaPlace as: $\sigma = \frac{P \times r}{w}$, where σ is the wall stress, P is the transmural pressure (intraluminal pressure – external pressure), r is the radius of the vein, and w is the wall thickness. However, wall tension (force along the wall length: $T = P \times r$) can exist at any transmural pressure, even before becoming circular. The unstressed volume represents the venous volume where no active elastic tension is exerted on the wall and reflects the normal reservoir function (26, 27). Only after unstressed volume is maximized, when wall stress becomes >0 , additional volume is considered stressed volume and the vein becomes distended (transition of exponential to flattened curve in Fig. 1-1). The maximum unstressed volume is constant, whereas absolute stressed volume can range drastically and represents the volume required to contribute to increasing venous return (1). Depending on external factors, including simple posture and gravity changes, the location and magnitude of stressed volumes will translocate, an important consideration for basic regulation of venous flow (23). As transmural pressure continues increasing, the compliance curve flattens and enters a low compliance range, exhibiting more rigid characteristics. This dual nature of venous compliance is essential for balancing blood reservoir function and circulatory stability. If veins shared the rigid, low-compliance structure of arteries, orthostatic hypotension would likely not occur due to reduced blood volume shifts. However, this would also eliminate the venous blood reservoir, potentially leading to fatal outcomes in hemorrhagic events due to rapid blood loss.

High venous compliance serves two main purposes: at low pressures, it enables blood storage, and at high pressures, greater rigidity prevents excessive volume accumulation and avoids arterial hypotension. Due to the viscoelastic properties of biological tissues, the compliance curve has time-dependent changes termed stress-relaxation or creep, describing further increases in volume with prolonged time at a fixed pressure along the flat portion of the curve (28, 29). Time-dependent creep in compliance may lead to substantial volume shifts and is relevant in scenarios of prolonged standing, possibly contributing to orthostatic hypotension and syncope. Venous elastic strength properties can also be highlighted by their tremendous pressure tolerance, greater than arteries, before mechanical failure. In extracted human vessels, saphenous veins reached pressures >2800 mmHg before rupturing (30), compared to ~1680 mmHg in cerebral arteries (31) and ~1250 mmHg in internal carotid arteries (32). The functional venous responses to different locations and grades of stressed venous volume has been understudied by *in vivo* human physiological investigations as unstressed volumes have been the focus of previous work.

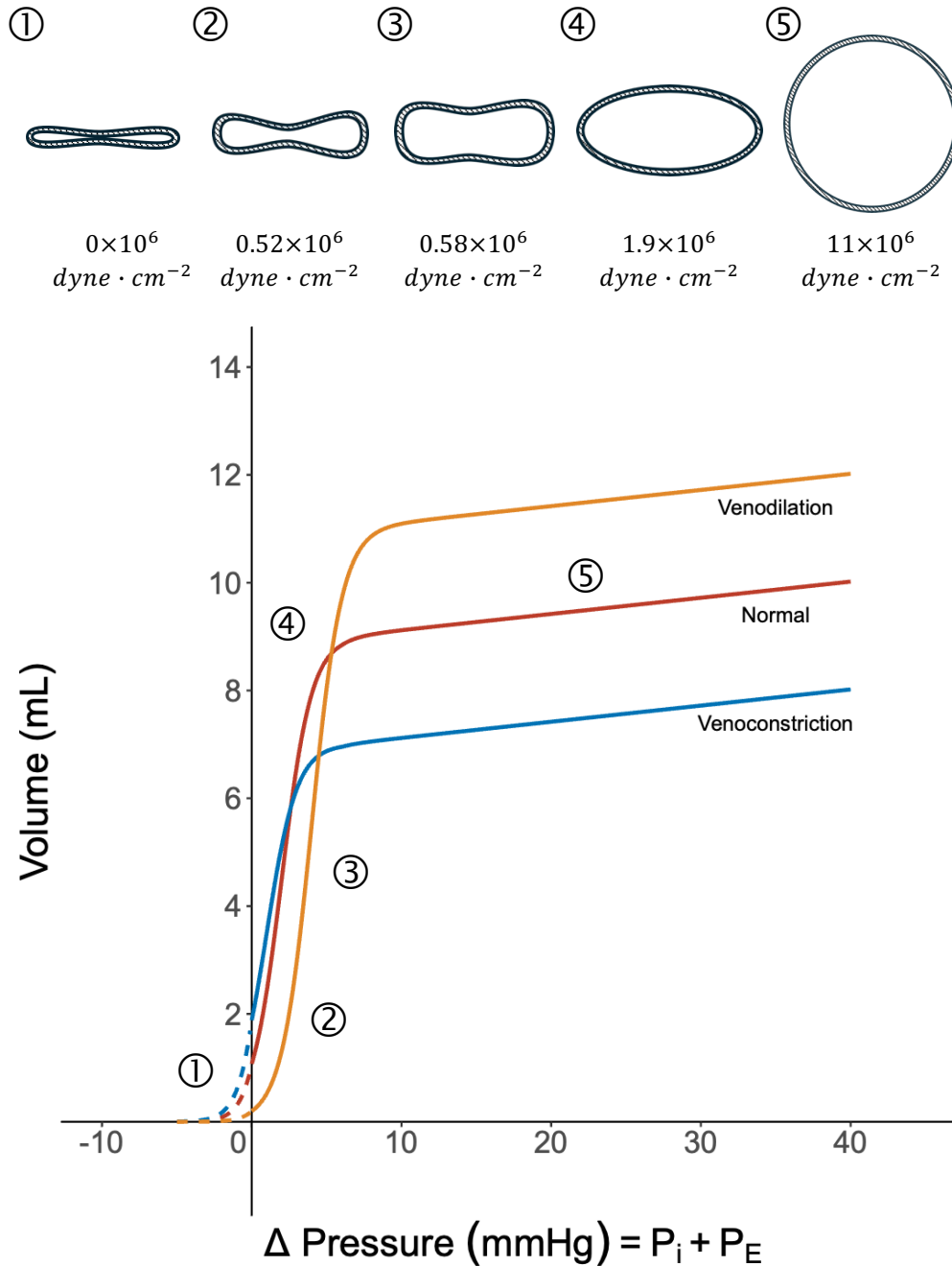


Figure 1-1: Theoretical compliance curve of a collapsible vessel, describing a change in volume for a given change in transmembrane pressure, shown as $\Delta \text{Pressure}$.

The transmembrane pressure is internal vessel pressure (P_i) minus external vessel pressure (P_E). Drawings show the hypothetical vessel conformation and associated tangential modulus of elasticity (derived from (25)) at different points on the curve during different physiological states. Red: Resting conditions; Orange: Venodilation; Blue: Venoconstriction.

Sympathetic activation

Like arteries, veins and venules possess innervated vascular smooth muscle, subject to sympathetic activation generating vascular tone. While the relative allotment of vascular smooth muscle and thus pressure generation capacity of the veins is considerably lower than arteries, sympathetic control still exerts meaningful impact on venous compliance and pressure. Despite lower prevalence, the adrenergic nerve phenotypes to vascular smooth muscle is similar between arteries and veins (33, 34). Additionally, innervation of veins is concentrated near the capillary level and splanchnic circulation rather than large conducting vessels or skeletal muscle veins (35). During scenarios of increased sympathetic outflow (e.g., orthostasis, exercise, hypoxia, post-prandial) venoconstriction is activated, lowering compliance (Fig. 1-1) and promoting venous return to support cardiac output (36). In these conditions, venoconstricted veins may not merely 'push' blood centrally but instead serve as low-compliance conduits that facilitate the transmission of arterial inflow through the capillary network—an effect supported by recent evidence of pulsatile flow across perforator veins during heat and exercise stress (37). During exercise and orthostasis, gravitational and local arterial vasodilation-induced lower body volume shifts occur, considered to be the primary obstacle to arterial pressure maintenance. However, upon baroreflex activation, sympathetic venoconstriction of the highly innervated splanchnic veins can contribute ~25% of total blood volume back to the heart for arterial pressure generation (1, 23). This autonomic input to net cardiovascular regulation is focused in the hepatic venous compartment, possessing a high density and sensitivity of adrenergic nerve terminals (38, 39). By altering the distribution of blood volume between peripheral and central venous compartments, venoconstriction effectively reshapes the stressed-to-unstressed volume ratio, directly influencing preload, stroke volume, and the maintenance of circuit flow at a given heart rate. Conceptually, this autonomic input contributes to central cardiovascular regulation by priming veins to transmit changes in arterial inflow, maintaining the most efficient degree of cardiovascular circuit flow. This effect is highlighted in scenarios of physiological dysregulation including patients with orthostatic hypotension due to autonomic failure, where blood pressure can be better protected by lower abdomen compression, compared to lower-limb compression (40), highlighting splanchnic circulation and autonomic dependence on net circuit flow control. Abdomen compression is also relevant in the post-prandial state as digestion draws considerable blood volume to the splanchnic veins and without adequate venoconstriction, similar hypotension symptoms can develop (41). An additional scenario of sympathetically-mediated venoconstriction occurs at high altitude in response to chronic hypoxia, wherein venoconstriction combats decreases in plasma volume as opposed to regional volume shifts (42). During exercise, sympathetic venoconstriction reduces

venous volume by approximately 10–20% (23), modestly enhancing venous return and preload but with limited impact on vascular resistance. In contrast, sympathetic arterial vasoconstriction can increase vascular resistance by more than 100%, playing a central role in maintaining arterial pressure and redistributing blood flow during exercise (43). This highlights the relatively minor role of venoconstriction in cardiovascular regulation compared to the more functionally dominant arterial response during high sympathetic outflow of exercise.

Sympathetic-induced venoconstriction does not always operate in a feedback loop following arterial blood pressure, as distension of peripheral veins themselves is an independent reflex increasing sympathetic outflow, sometimes termed the venoarterial response (44). Indeed, studies isolating the role of peripheral venous distension by bolus saline infusion to an occluded limb report increases in systemic muscle sympathetic nerve activity and arterial blood pressure (45). The magnitude of this response contributes to orthostatic tolerance, highlighting the importance of peripheral vein afferent innervation (46). In contrast, distension of the central veins (vena cavae and pulmonary veins) by head-down tilt or lower body positive pressure suppresses sympathetic outflow by activation of the cardiopulmonary baroreflex (47). These data highlight the intricate response and input of the peripheral veins to sympathetic activation, phenomena that should not be discounted during investigations of sympathetic control where venous fluid shifting may occur.

Local vaso-responsiveness of veins

The myogenic response is well defined in arteries as a mechanism of local vasoconstriction in response to increased transmural pressure where vascular smooth muscle reflexively contracts thereby increasing pressures and flow resistance (48, 49). It is therefore unsurprising that the vascular smooth muscle-containing venous circulation also presents this reflex. Yet, it remains less considered despite being identified as a similar calcium-dependent reflex (50). Like arteries, the venous myogenic reflex contributes to maintenance of venous tone (51), which may serve to prevent distension and contribute to venous return.

Venodilation is another relevant response of venous vascular smooth muscle susceptible to vasoactive compounds, yet this has been primarily elucidated from dated *in vitro* animal studies. Similar to arteries, common endothelial-derived, metabolic and humoral smooth muscle relaxing compounds including acetylcholine, nitric oxide, P_i , H^+ , and CO_2 produce dilatory effects primarily in small veins and venules of skeletal muscle rather than large conducting vessels (52, 53). Functional sympatholysis causing smooth muscle relaxation facilitates conductance of venous blood flow, similar to the effects seen in small arteries and arterioles, even in the face of

high sympathetic activity (54, 55). While these findings offer valuable mechanistic insight, their functional relevance *in vivo*, particularly regarding the regulation of venous return and capacitance during muscle contractions, and likely minimal next to the pressure generating capacity of skeletal muscle but yet to be fully characterized in the post-exercise hyperemic environment.

1.3 Mechanisms of venous return

Return of blood to the heart is the primary role of the venous system, accomplished through a set of well-described mechanisms, each aiding the movement of blood through a high compliance system. Venous pressures fluctuate drastically in the periphery from $>200 - 0$ mmHg or sometimes negative pressures at the right atrium (56–58), with a strong dependence on orthostatic positioning, muscle activation, fluid status and sympathetic activation. Due to these low pressures, flow is susceptible to the effects of external forces such as gravity and compression. Thus, body posture and the resulting hydrostatic pressure gradient generates the primary perturbation. In the healthy circulation, a combination of mechanisms compete against gravity to continue moving blood in the anterograde direction. In the arterial circulation, an intrinsic continuous powerful pump (i.e., the left ventricle) coupled with pressure buffering mechanisms maintains constant flow to high resistance organs. In the venous circulation, various external ‘pump’ mechanisms act in concert with valves to preserve forward blood flow, most notably the muscle and respiratory pumps, and more recently described, a potential arterial pump.

Muscle pump

The muscle pump has been extensively described previously, essentially increasing transmural pressures of the thin-walled veins to force blood anterograde, guided by valves (23). Muscle pump action exists throughout the body wherever transmural pressure can be imparted onto a vein by surrounding muscles; however, the muscle pump is most important in the lower-limb, specifically the soleus-gastrocnemius complex. In the lower-limb, contracting muscle can generate venous pressures up to ~ 200 mmHg, with an ejection fraction that ranges 40-90%, creating driving forces of ~ 90 mmHg and flow rates up to $4 \text{ L} \cdot \text{min}^{-1}$ toward the heart (23, 59–62). Importantly, these flow rates can be generated instantaneously due to the volume reservoir priming nature of veins which operates outside the cardiovascular circuit flow constraints of matched arterial inflow to venous outflow. These data underline the necessity of competent venous valves to facilitate venous flow against gravity and the prevention of venous hypertension. In healthy individuals, ankle venous

pressure while standing does not differ from patients with valve incompetency (both 80-90 mmHg); however, upon walking and activation of the muscle pump, venous pressure decreases ~65 mmHg in healthy subjects and only 11-37 mmHg in patients with venous incompetence, depending on the disease severity (63). In venous insufficiency, calf muscle pump dysfunction is associated with edema, skin changes and ulceration, with improvements in outcomes following exercise training due to repeated volume clearance (64, 65). During simple walking exercise in healthy individuals, fluctuations in lower-limb venous pressure span a range of ~140 mmHg with direct temporal links to anterior and posterior muscle activation, yet interestingly there is no dependency of mean venous pressure with gait speed and thus muscle pump activation frequency (66). Recent studies have also provided evidence for deliberate directional flow from superficial to deep venous systems via perforators during ambulation, by coordinating muscle antagonist muscle activity and optimizing suction effects (67, 68).

As descriptions of the intrinsic muscle pump behaviour continues to evolve, researchers have also looked to intermittent pneumatic compression (IPC) devices to mimic the muscle pump effect for different scenarios of application. IPC devices apply intermittent pressure to the lower-limbs, imparting cyclical increases in transmural pressure that propel blood toward the heart, augmenting or replacing the intrinsic muscle system with suggested applications ranging from orthostatic intolerance patients to accelerating post-exercise recovery in athletes. In patients with orthostatic intolerance, IPC therapies employ sequential cuff inflations of the lower-limb to augment venous return and cardiac preload (69, 70). Similarly, compression stockings represent a lower-tech intervention that reduces venous congestion and relieves swelling in individuals with venous insufficiency (71). In athletic populations, IPC systems and compression garments have gained popularity as a recovery technique following exercise. Although the marketed performance or metabolic enhancing effects remain equivocal (72, 73); the transient, nominal increases in venous return may help mitigate venous stasis following prolonged activity. Similar external pressure generating devices have also been applied through lower body positive pressure, promoting venous return without active compression of the limbs (74). Together, these external mechanical interventions highlight the central role of the muscle pump in venous return and underscore the therapeutic potential of augmenting venous flow through intermittent compression of veins.

Respiratory pump

Another well-described pumping mechanism results from the spontaneous action of the diaphragm. During inspiration, the diaphragm contracts to lower intrathoracic pressures, generating a pressure gradient, moving fluid into the thoracic compartment towards the heart from tissues above the diaphragm including the head and upper limbs (75, 76). During expiration, the diaphragm relaxes and moves up, re-opening the inferior vena cava and the mounted pressure in the lower-limbs drains toward the heart. Unlike the muscle pump, the respiratory pump is obviously always at work. Although often considered to be a passive mechanism, the respiratory pump is not immutable and is modulated by mechanical factors. An often-overlooked component of the respiratory pump is the liver's reciprocal contribution to the right atrium. The liver acts as a pre-right heart reservoir, or 'sump', modulating venous return during respiration. During inspiration, transdiaphragmatic pressure increases cause the diaphragm to compress the inferior vena cava (IVC), briefly restricting flow. In this moment, the liver empties into the right heart, smoothing inflow much like the Windkessel effect in the arterial system. This mechanism is supported by observations in cirrhosis patients, where reduced liver compliance leads to exaggerated respiratory fluctuations in venous return (23, 77). While respiratory phase alters the pattern of venous inflow, the liver sump helps maintain a stable net return to the heart. During peripheral volume displacement by lower body negative pressure, increased inspiratory load can increase venous return compared to no inspiratory loading, mitigating cardiac preload and arterial blood pressure decrements (78). In contrast, the addition of positive-pressure ventilation during central hypovolemia abolishes the negative pressure gradient into the thoracic cavity and impairs venous return, having downstream consequences on cardiac stroke volume, highlighting the respiratory pump's impact on venous return (79). Positive-pressure ventilation has also been used to highlight the respiratory pump's additive influence on net venous return during graded exercise, working in concert with the lower-limb muscle pump. When intra-thoracic pressure swings are minimized by mechanical ventilation, cardiac stroke volume and preload are reduced compared to spontaneous breathing (80, 81), demonstrating respiratory dependence of venous return. Thus, understanding of the respiratory pump as an actively regulated mechanism with diaphragmatic, hepatic and skeletal muscle influences continues to evolve.

Arterial pump

The arterial pump has been hypothesized to transfer pulsatile energy to adjacent veins, aiding venous return similarly to the muscle pump. While its potential has been recognized, *in vivo*

validation remains limited. Initial evidence from retinal veins linked intraocular pressure oscillations to venous pulsations (82), suggesting localized pressure changes might influence venous flow. A recent *ex vivo* study using bovine forelimbs demonstrated that arterial pulsations can enhance venous outflow against a hydrostatic gradient when valves are competent (83). Notably, with a 30 mmHg pressure gradient, constant arterial flow produced no venous outflow, whereas pulsatile arterial flow increased outflow ~30-fold. Additionally, venous outflow doubled when pulse rate was increased at a fixed pressure gradient, indicating a chronotropic dependency. However, the study's parallel flow design does not fully replicate physiological in-series perfusion pressures, necessitating careful *in vivo* validation. Preliminary human data supports the arterial pump mechanism in leg veins under similar hydrostatic pressure gradients (~30 mmHg) (84). Cohen et al. found that in veno-arterial bundled veins, such as the deep femoral vein, flow was preserved during mild hydrostatic gradients, whereas the unbundled greater saphenous vein showed stepwise flow reductions despite identical gradients. However, pulsatile waveforms have also been recorded in perforator veins and tiny (<1 mm diameter) veins in the skin without adjacent arteries during heating and exercise (37); thus capillary bed cyclical pressure propagation remains a likely contributor. Additionally, the effect of fascial compartment pressure effects on venous transmural pressure with increasing orthostatic gradients remains a limitation when comparing deep and superficial venous networks. Further investigation is needed to fully understand how in-series perfusion pressure transmission from arterioles to venules encased in finite spaces contributes to the arterial pump mechanism. Future consideration of changes in arterial blood pressure, and thus distending radial force, from adjacent arteries should be investigated.

1.4 Technical considerations of measuring venous flow

Since the first descriptions of the 1970's (85), Doppler ultrasound has facilitated cost-effective, non-invasive, and accurate determination of blood flow at rest and during exercise. While other methods exist to estimate venous blood flow, they have significant limitations to either measurement sensitivity or cost. For example, strain gauge plethysmography has poor temporal resolution, the indicator dilution method is too invasive to be practical, and phase-contrast MRI is costly. In the clinic, duplex ultrasound (i.e., simultaneous B-mode and Doppler imaging) is used for diagnosis and surveillance of multiple conditions and offers meaningful patient insight to the clinician through both qualitative and quantitative outcomes. However, for researchers, there are certain assumptions of Doppler ultrasound that must be considered to quantify blood flow in

vessels. Conventional clinical ultrasound is angle-dependent and uses a single fixed insonation angle, limiting velocity assessment to assumed bidirectional flow, often considering the insonation angle θ to estimate the actual velocity component along the true direction of motion. Fixed angle ultrasound estimates the mean blood velocity of the tube by measuring the peak centerline velocity or by an intensity weighted mean velocity, assuming a parabolic blood velocity profile; these assumptions are challenged with non-linear vessel geometry, very low blood velocities, or non-streamlined flow, as discussed below.

Blood flow estimation

Quantification of peripheral venous blood flow is characteristically slow, with resting mean velocities typically ranging from 0-10 $\text{cm}\cdot\text{s}^{-1}$ (37). Thus, veins possess different time domain features that make them unique when compared to arterial flow. Nonetheless, both arteries and veins break numerous assumptions for accurate blood flow estimation based on Poiseuille's law:

$\dot{Q} = \frac{\pi r^4 (P_1 - P_2)}{8\mu L}$, where $\dot{Q}$ is the blood flow rate, r is the vessel radius, $P_1 - P_2$ is the pressure drop along the length of the tube, μ is the blood viscosity and L is the length of the tube (86). Poiseuille's Law is often simplified by vascular research groups to $\dot{Q} = \pi r^2 \times MBV$ (87–96), where MBV is mean blood velocity, assuming the blood viscosity and length of the tube are constants for a given circular vessel, and that fluid is moving as a constant paraboloid with the fastest blood velocities in the center of the vessel and slower velocities near the vessel walls. In reality, the nature of venous circulation breaks a few different assumptions in this approach:

1. Unstressed venous geometry: When venous wall stress is 0 and venous volume is not stressed, the vessel takes a collapsed or elliptical shape, which invalidates the use of πr^2 for vessel cross-sectional area and the paraboloid assumption since vessel walls are not parallel.
2. Venous tortuosity: Poiseuille's Law relies on straight, rigid tubes, which veins deviate from due to inherent tortuosity, flaring at valve sites and high viscoelasticity (venous compliance). These properties break assumptions of laminar streamline flow and vessel rigidity as internal pressure fluctuations directly manipulate vessel cross-sectional area.
3. Low-velocity venous blood: Blood is assumed to be a Newtonian fluid, however at low blood velocities (thus low shear rates), non-Newtonian behaviour exists, requiring velocity-specific viscosity consideration (97).

4. Presence of valves: Assumptions of continuous steady flow (P_1 - P_2 term of Poiseuille's Law) is broken under most physiological flows due to pulsatility; however, venous valves add another element by introducing rapid acceleration and deceleration of blood (Supplemental video 1). Since valves protrude into the vessel lumen, they can also generate multi-directional flow features. This directly underscores the utility of advanced vascular imaging techniques for multi-directional flow description for describing venous blood flow.

In practice, there are few other options to easily estimate blood flow and the above common equations have been applied to approximate blood flow to the best of our ability as an average flow rate per minute based on the mean blood velocity signal and venous diameter. However, poor agreement between Doppler derived flow and thermodilution persists for veins (98) and therefore in non-rested, healthy states (i.e., exercise, external compression, stenosis, valvular insufficiency), a critical lens should be applied to assumptions of blood flow estimation.

Qualitative description of blood flow

The use of Doppler-based blood flow estimates has facilitated multiple discoveries based upon quantitative measurements of blood velocity and vessel cross-sectional area. Duplex ultrasound is the primary diagnostic tool for venous insufficiency, relying on information including anatomy, patency and presence/absence of reflux rather than the quantification of velocities or volumes (99). Doppler information is also used constructively to generally qualify venous blood flow as absent, spontaneous, phasic, disturbed, dampened, congested, retrograde, stagnant, or complex (99–101). Grading or assignment of complex venous flow patterns based on conventional Doppler feedback is understandably an appealing application, yet, there are several physiological scenarios in human veins where qualitative descriptions with conventional Doppler can be misleading. Data reduction to velocities moving towards or away from the probe do not allow a detailed description of multi-directional flow phenomena, for example, during blood stasis, circulation, or oscillatory blood flow; for this reason, fixed-angle Doppler measurements are not recommended in non-laminar blood flow conditions. It's clear that the choice to use conventional Doppler or alternative emerging imaging tools should be dictated depending on the application and clinical or research question.

A different approach to venous imaging: Vector flow ultrasound imaging

Recent advancements in ultrasound technology have tackled some limitations inherent to conventional clinical duplex ultrasound, which are unable to fully capture complex blood flow dynamics *in vivo* (102). Clinical ultrasound units also have relatively low temporal resolution, with frame rates typically 30-100 Hz in the most advanced clinical units today. Initially developed to assess unusual flow patterns in the arteries and heart (102, 103), ultrasound vector flow imaging (VFI) offers a promising approach for studying venous hemodynamics, where low velocities and pressures increase the susceptibility to non-laminar blood flow. VFI is the generic term for beamforming approaches that estimate both magnitude and direction of blood velocity vectors, circumventing the limitations of single-angle Doppler approaches. By eliminating beam-to-flow angle dependence, VFI effectively allows the study of real-time multi-directional or complex blood flow patterns *in vivo* (104). VFI has been used to image large superficial arteries to evaluate multi-directional blood velocity patterns at rest, during physiological perturbations, and to evaluate surgical procedures (105–109). More recently, VFI has been applied to veins to more comprehensively detail venous blood flow in special circulations (110–112). Research application of this tool may complement conventional duplex ultrasound to reveal interesting and novel dynamics of venous stasis, complex flow patterns or venous insufficiency.

While commercial VFI systems exist on the market (108, 113), there are limited quantitative metrics available to describe the complexity of blood flow *in vivo*, with reports generally relying on qualitative observations of blood flow in different scenarios. Reporting mean velocity from VFI is reasonable, but if velocity vectors are truly multidirectional, reducing fluid motion to a single-dimensional metric oversimplifies the complexity of vascular hemodynamics. To encapsulate this complexity to a metric, Pedersen *et al.* (114) described a vector concentration coefficient (VCC); a unitless description of vector dispersion within a region of interest. For each frame, the VCC is calculated based on the direction of all flow vectors based on Pythagoras' theorem $r = \sqrt{x^2 + y^2}$, where x and y represent axial and lateral vectors; a value of 1 indicates all vectors are aligned in the same direction, and a value of 0 implies complete dispersion in differing directions (29). The VCC metric has garnered interest, offering description of flow uniformity or disorganization in response to an intervention or within a population of interest (105, 106, 111, 115), and is likely related to the mechanical shear stress experienced by local endothelial cells. Moving forward, high VCC outputs can indicate acceptable Poiseuille-based flow estimates in straight vessels, whereas low VCC can indicate flow instability (e.g., flow around curvatures, valves, and stenoses) and require more nuanced flow detection. Until further output

metrics are validated to quantitatively describe the complexities of *in vivo* blood flow in complex vasculature, including venous flow, intra-cycle and average VCC measures are being used to address flow uniformity in regions of interest and many inferences are still being gathered from qualitative descriptions of flow characteristics and biofluid mechanics simulation (109). The future of VFI application in venous physiology is therefore ongoing, with its capacity to shed light on previous unknown properties of venous blood flow.

1.5 Applied venous regulation in physiological systems

Orthostatic stress

Changes in body posture impose gravitational stress on the cardiovascular system, classically described as orthostatic stress (23). Maintenance of blood pressure in this acute scenario is dependent upon sufficient cardiac filling pressures (preload) and adequate autonomic control of resistance arteries. While the venous system has little influence on autonomic regulation of total peripheral resistance, venous return is the primary contributor to cardiac preload and facilitates maintenance of stroke volume during orthostasis. Upon standing, 500-1000 mL of blood is displaced caudally and mean arterial blood pressure can decrease ~15 mmHg in healthy older adults (69, 116), requiring hemodynamic regulation to preserve cerebral perfusion. Rapid preservation of cerebral perfusion pressure can be accomplished by reflex control of resistance arterioles, however beyond ~30 s of orthostasis, venous-dependent mechanisms become relevant for arterial pressure regulation. Contribution of active venoconstriction during orthostasis has been debated (117), yet does not appear to contribute meaningfully (118), likely due to the scant presence of vascular smooth muscle and large orthostatic gradient to be overcome. On the other hand, muscle tone contributes to orthostatic tolerance during head-up tilt tests, whereby non-fainters demonstrate higher tonic intramuscular pressures compared to fainters (119). Thus, external pressure generation from physiologic pumping mechanisms and/or mechanically assistive devices are helpful for orthostatic intolerant individuals, especially when functioning in concert with competent venous valves. Interestingly, this concept is reversed in extreme environments such as microgravity, where the absence—rather than the presence—of gravity leads to fluid redistribution that impairs venous return. The relatively sparse number of venous valves in the upper limbs may further contribute to the understudied challenges of maintaining effective venous return in microgravity. These observations underscore how the venous system's

high compliance and reservoir function demand tight regulation through autonomic control, basal venous tone, and active venous contraction to maintain arterial blood pressure stability.

Acute exercise

Exercise causes local arterial vasodilation increasing blood flow to active muscle vascular beds linearly from rest to maximal exercise, demanding higher cardiac output and therefore greater venous return. During exercise, venous return benefits from a continuously active muscle pump capable of decreasing total calf volume in the face of increased blood flow delivery during dynamic continuous exercise. This occurrence is due to simultaneous activation of the muscle and respiratory pumps helping expel venous volume, increases in interstitial fluid pressures reducing capillary filtration, and superior lymph flow lowering tissue volume (36, 120). As exercise intensity increases, the instantaneous blood volume in a region is re-distributed in a manner that does not necessarily match the changes in delivery rates. For instance, at maximal workloads, 70-80% of cardiac output is directed to the locomotor muscles; however, the instantaneous volume of the limbs are actually 25% lower than baseline, in large part due to the above mentioned pumping and filtration mechanisms (43, 121). Modifying the respiratory pump input to exercising venous return illustrates the importance of its normal function to support exercise. By applying expiratory flow limitation, similar to chronic obstructive pulmonary disease, high intrathoracic pressures during exhalation compress the inferior vena cava severely enough to impair venous return and exercise capacity (122, 123). On the other end of intrathoracic pressure generation, when respiratory muscle pressure swings are severely minimized by mechanical ventilation, the respiratory pump's input is diminished, leading to lower cardiac filling and exercise capacity (80). In contrast, volume and flow rates to the splanchnic circulation are reduced (~80% of baseline volume and ~25% of baseline flow) due to competitive flow re-distribution and active sympathetic venoconstriction. Skin blood flow increases substantially during exercise, especially in the heat, and flow through venous perforators draining the skin to the deep system increases accordingly to accommodate demand (37).

Exercise training

Athlete groups also present interesting venous characteristics which appear to manifest as functional adaptations to exercise training, differing from healthy untrained individuals. Very limited evidence exists to date, but preliminary studies have demonstrated increased resting

venous diameters and compliance in the lower-limbs of endurance trained athletes, indicative of an 'athlete's vein' phenotype (124–126). Interestingly, no differences in muscle pump ejection fraction were found in athletes compared to healthy controls suggesting a well-preserved venous return mechanism in younger individuals. However, other investigations have identified fitness-dependent differences in the venous compliance of endurance trained older adults (127), suggesting, like most cardiovascular phenotypes, the preservative effects of exercise tend to manifest only once the system begins to deviate from optimal function. Though less studied, resistance training presents an interesting environment for the active limb veins, characterized by very high intramuscular pressures (200-300 mmHg during isometric maximal contractions and locomotion) (128, 129). Furthermore, popular training methodology including blood flow restriction which involves the deliberate occlusion of venous outflow (130–132) likely increases venous stress, placing very high loads on venous valves, warranting investigation. Adequate function of the venous system is thus important for maintaining exercise acutely, possibly contributing to performance capacity and normal function during aging.

Healthy aging

Much like arteries, veins undergo age-related declines in structure and function, with consequences for cardiovascular health. Over the lifespan, veins lose elasticity due to fibrosis, increased collagen cross-linking, and vascular smooth muscle cell hyperplasia reducing venous compliance (133). Additionally, the responsiveness of aged veins to local vasoactive stimuli is impaired, as evidenced by reduced dilatory responses to nitroglycerin (134), and compliance responses to blood oxygen manipulation (135, 136). These structural and functional declines contribute to increased vascular resistance and venous hypertension. Typical aging can also lead to vein elongation, dilation, and tortuosity, as seen in the case of varicose veins (137–139). While subclinical adaptations are often observed in aging veins, their impact on venous flow dynamics remains poorly understood. Structural remodeling may precipitate the development of complex fluid flow features which has implications for flow efficiency and net venous return impairments. This can manifest as varicose veins or chronic venous insufficiency, conditions that affect up to 30% of the population (140). Compounding these effects, efficiency of the calf muscle pump declines with regular aging, reducing venous return capacity (141). This reduction is observed both in healthy older individuals and those with venous disease, though fitness appears to play a key role in mitigating these declines. Notably, sympathetic control of venous smooth muscle remains largely intact with aging (142) though a chronic increase in sympathetic activity common

with aging (143), may contribute to a heightened venoconstrictor state. These aging-related venous changes may also impair orthostatic tolerance by limiting the ability to buffer postural blood shifts, reducing preload and stroke volume. Decreased venous compliance has been linked to orthostatic intolerance (144) and is similarly observed in simulated microgravity studies (145), and in astronauts following long-duration spaceflight (146), an accelerated form of aging.

Clinically, the primary pathophysiological concerns for aging veins include chronic venous insufficiency, deep vein thrombosis and ultimately venous ulcerations that develop as a result of valve dysfunction and venous hypertension, requiring medical intervention. While some venous changes are an inevitable part of aging, others may be preventable or attenuated through exercise, particularly endurance training, to maintain venous compliance and muscle pump function. Emerging vascular imaging technologies, including VFI, offer new opportunities to assess how age-related venous remodeling impacts hemodynamic function. These tools could also help clarify how reduced venous compliance and altered flow mechanics contribute to conditions such as heart failure with preserved ejection fraction and hypertension, where venous congestion and impaired volume handling are increasingly recognized (135, 147).

In conclusion, venous physiology remains an overlooked portion of the circulation with numerous unanswered questions. Investigating venous flow behaviours, valve function, responses to physiological stressors, control of bulk venous return, and responses to external stimuli is an ongoing process, highlighting that veins contribute dynamically to circulatory regulation rather than exist merely as passive conduits. The unique anatomical and functional composition of veins presents challenges for estimating venous blood flow, though advanced measurement techniques, including vector flow imaging, are poised to reinvigorate the study of venous function. Resolving the unanswered questions in human venous physiology will enhance our understanding of gravitational fluid shifts, exercise and aging, ultimately elevating the study of veins to the same level of importance as arteries.

Chapter 2: Central thesis

2.1 Rationale

The foregoing status of the literature highlights key principles of venous physiology and its measurement. Despite this, several important research gaps remain. First, the contribution of hydrostatic volume stress to venous return has not been well characterized in humans. Second, the influence of aging on the muscle pump and the interaction with venous return through coordinated contraction and venous compliance remains unclear. Finally, advances in non-invasive imaging have only recently enabled direct visualization of complex venous flow patterns without relying on computational modeling. This foundational evidence provides the rationale for the present work, which addresses these unresolved aspects of venous physiology using direct *in vivo* measurement approaches.

Accordingly, this thesis leverages physiological adjustments of the cardiovascular system during body tilting and muscle contractions to examine venous mechanisms underlying cardiovascular responses in younger and older adults. Across four experiments, advanced ultrasound methods were used to characterize venous structure, flow complexity, and their adjustments to hydrostatic and muscle-induced volume stress. Collectively, this work aims to clarify how venous flow dynamics and return mechanisms interact with aging and physiological stress, advancing both our understanding of venous function and the application of emerging imaging technologies in human research.

2.2 Research questions

1. How does progressive volume distension affect blood flow complexity and vessel structure of the non-uniform shaped internal jugular vein?

H: The irregular shape of the internal jugular vein contributes to greater flow complexity with increasing levels of volume stress

2. Does an arterial pump mechanism of venous return exist in humans and what effect do hydrostatic forces play?

H: Throughout a relevant range of hydrostatic forces, adjacent arterial pulsation contributes to preservation of venous flow, an effect not seen in veins without adjacent arteries.

3. How do lower-limb venous flow responses to muscle contractions respond to different grades of volume stress between younger and older adults?

H: Older adults will demonstrate greater flow complexity during muscle contractions under volume stress, associated with reduced venous compliance.

4. What is the regional distribution of blood flow complexity near venous valves during muscle contractions during progressive volume distension?

H: Blood flow complexity measures will be highest in the area preceding venous valves and progressively disturbed by increasing volume stress during muscle contractions.

Each of these research questions is described and tested in the following chapters 3-6.

Chapter 3: Characterization of internal jugular vein region-specific distension and flow patterns during progressive volume shifting

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3.1 Introduction

In the supine posture, the head-to-foot gravitational vector is abolished and the internal jugular veins (IJV) are patent, providing the primary venous outflow pathway from the brain (148). Like all large veins, the IJV is highly compliant and compressible, distending to accommodate blood accumulation while supine or in a head-down tilt (HDT) position (149) and emptying to the point of collapse due to gravity during head-up postures (150). Consequently, the IJV is highly susceptible to external pressures and distribution of blood volume. Studies redistributing central volume caudally via head-up tilt demonstrate large decreases in IJV diameter and blood flow (151, 152). Conversely, when central volume is shifted cranially during weightlessness or via HDT (despite soft tissue compression while on earth), the total IJV volume increases (153, 154), aligning with changes to intracranial pressure (155–157). How the IJV responds to cephalic fluid shifts is of particular interest due to its unique irregular geometry, presenting a conical shape, widened at the caudal intersection with the brachiocephalic vein and tapering towards the cranium. Relative to other major vessels, the irregular geometry of a distended IJV may contribute to regional differences in blood flow patterns along the IJV length with implication for chronically distended states including microgravity environments (158); however, this has not been assessed to date.

Vessel distension and fluid accumulation typically coincide with blood flow profiles including retrograde flow or flow stasis, which are hallmark characteristics for thrombosis risk as described by Virchow's triad (159, 160). IJV thrombi have recently been identified in astronauts during long-duration spaceflight, where a cephalic fluid shift increases the chronic distension of the IJV (100, 153). Despite no adverse clinical outcomes, the potential health risk of jugular thromboembolism during 0G or partial G exposure remains high, necessitating further investigation and greater understanding of the vascular consequences of cephalic fluid shifts (158).

Current ultrasound-based investigations of astronauts during spaceflight have highlighted changes to IJV blood flow patterns, leading to hypotheses implicating altered IJV flow as a contributing factor to thrombosis development (100). However, this link is confounded by technical

constraints of scanline Doppler methods to assess blood flow patterns. Conventional single-plane ultrasound units are limited by an angle-dependent velocity estimation and poor temporal resolution, which only allow description of blood flow either towards or away from the probe (or, corrected to vessel plane angle relative to the probe) and prevents accurate descriptions of complex blood flow, including recirculation and intra-cycle dynamics (112). Recent developments in biomedical ultrasound can circumvent these limitations with custom beamforming approaches to assess angle-independent blood velocity with high temporal resolution (>750 fps), termed vector flow imaging (161). We have previously employed vector flow imaging to quantify pixel-level complex flow environments *in vivo* (104, 106, 107, 162). Further, vector flow imaging has already been used to characterize fluid flow turbulence in patients with known IJV thromboses, finding chronic thrombosis is co-localized with higher wall stress and flow turbulence metrics (163). Supporting data connecting the spatial complexity and distensibility of the IJV with high temporal resolution of complex blood flow dynamics may offer novel insights into the hemodynamic impact of cephalic fluid shifts and thus pathophysiology of IJV thromboembolism risk during long-duration spaceflight and terrestrial presentations.

The purpose of our study was to investigate the non-uniform volume expansion and complex flow patterns along the IJV during progressive volume displacement, simulating extreme fluid shifts, which can be found in microgravity. As a secondary aim, we tested the short-term effectiveness of low-pressure thigh cuffs in reducing IJV volume and manipulating complex flow patterns. Following links to pathological conditions (i.e., IJV thrombi, spaceflight associated neuro-ocular syndrome), countermeasures to curtail the displacement of blood volume towards the head during microgravity have been proposed and implemented (164). Primarily, usage of low-pressure thigh cuffs, a popular countermeasure amongst cosmonauts (165), or lower-body negative pressure (166) have been applied to shift volume back to the legs. However, clear evidence on the effectiveness of such countermeasures on IJV outcomes, particularly lightweight and non-bulky options such as low-pressure thigh cuffs, remain limited in fluid shifting or simulated microgravity environments. We hypothesized that the irregular, conical geometry of the IJV would produce region-specific differences in distension and blood flow complexity during HDT, as measured by vector flow imaging. Additionally, we posited that thigh cuffs would sufficiently pool blood in the legs to reduce IJV distension and blood flow complexity during HDT.

3.2 Methods

Participants

Eighteen healthy participants between 18 and 35 years of age were recruited (Table 2-1). Participants were recreationally active, free from known cardiovascular (including thrombophilia) or musculoskeletal disease, and not taking medications known to alter the cardiovascular system. Participants reported to the laboratory having refrained from strenuous exercise and alcohol for 24h, caffeine for 12h and large meals for 3h. The study protocol was reviewed and approved by the University of Waterloo research ethics board (ORE# 43670) and conducted in accordance with the Declaration of Helsinki. Each participant provided written informed consent. Based on previous large effect sizes found for short-term IJV distension with -6° HDT (167), we calculated a required sample size of 6 participants ($\alpha=0.05$, $\text{power}=0.80$). To account for potential data loss with vector flow imaging and allow for a sex-balanced study design, we recruited 18 participants (9 females).

Study protocol

We pre-registered the protocol on Open Science Framework (<https://osf.io/na6sp>). Following screening, consent, and anthropometric measurements, participants lay down on an inversion table (Model 75129, LifeGear Taiwan Limited, Taichung City, Taiwan). They rested supine for a minimum of 15 min. The tilt protocol followed a non-randomized progression of HDT, with 5 min of stabilization at each tilt angle before measures were collected. Experimental measures began at supine, followed by -6° HDT, -6° HDT with leg cuffs, -15° HDT, and -30° HDT (Fig. 3-1). At each condition, the order of measures was: 1) clinical duplex ultrasound of caudal (proximal to the thorax) followed by cranial (distal to the thorax) IJV regions, 2) a 3D volume sweep of the accessible IJV from caudal to cranial regions, 3) vector flow imaging of complex blood flow at the caudal and cranial regions, and 4) heart rate and brachial blood pressure at the end of each stage. During each ultrasound scan of the IJV, participants were guided to perform a breath hold at normal end-expiration to minimize respiratory cycle artifact in venous outcomes.

For the -6° HDT with leg cuffs condition, 16 cm wide pneumatic cuffs (ERKA, Bad Tölz, Germany) were secured bilaterally around the uppermost region of participants' thighs and inflated by a sphygmomanometer to 40 mmHg to partially occlude lower-limb venous drainage. Cuff pressure was maintained for a minimum of 5 min prior to imaging and held for the entire sequence of scans. Popliteal vein cross-sectional area (CSA) was also measured in the right leg

following completion of IJV measures in the -6° HDT and -6° HDT with leg cuffs conditions to confirm pooling of venous blood in the lower-limb vessels during the -6° HDT with leg cuffs condition.

Duplex imaging

The right IJV was imaged with a commercially available scanner (LOGIQ E10, GE Healthcare, Chicago, USA) equipped with 3D capabilities (9-4.2 MHz linear array probe, L2-9VN, GE Healthcare). Duplex B-mode and Doppler cine loops (15 s) were collected at the caudal and cranial regions of the IJV, anchored to clavicle and mandible landmarks, respectively. Duplex cine loops of 10s allowed determination of IJV diameter and MBV for conventional estimations of blood flow. The Doppler gate was extended across the entire IJV lumen and held at a 60° insonation angle. Vessel diameter was determined using online calipers by the same observer as an average of 3 cardiac cycles. MBV was analyzed using an open-source analysis software (Flowave.US), extracting the intensity-weighted doppler waveform (168). Flow was estimated in mL·min⁻¹ as: $\pi \cdot (\text{diameter}/2)^2 \cdot \text{MBV} \cdot 60$. The same sonographer performed all imaging and measurements (JNC) with very good to excellent intra-rater reliability for both venous diameter and MBV (ICC(3,1) > 0.90).

3D volumetric imaging

The LOGIQ E10 utilizes a proprietary 3D positioning system to accurately track the probe's spatial location during 3D scans. Three positioning magnets triangulated the spatial location of the probe: one inside the probe housing, one fixed to the participant's shoulder and a third reference electromagnet connected to the ultrasound. The sonographer swept the probe from clavicle to mandible keeping the vessel in cross-sectional view. On-line analysis was performed by manually tracing the CSA of the IJV at 0.3 cm intervals along its length up to the mandible or point of bifurcation. Volume was calculated by multiplying each CSA by a 0.3 cm length, similar to the biplane summated disc method popularized in Simpson's Biplane estimation of left ventricular volume (169). This approach was necessary to account for the irregular geometry of the IJV. To account for differences in neck length between participants and address regional differences of the vein, each 3D IJV scan was divided into thirds: caudal, middle, and cranial regions.

Vector flow imaging

Vector flow ultrasound images were collected using an open platform ultrasound machine (US4US, Warsaw, Poland) optimized for live imaging (104) equipped with a linear array probe (13-3 MHz, SL1543, Esaote, Indianapolis, USA). The B-mode image was navigated to obtain clear longitudinal views of the right IJV at both caudal and cranial regions, the same vein regions collected by the clinical ultrasound. A custom plane wave imaging sequence (transmission angles 10° and -10°; receive angles 10°, 0°, and -10°) was used for acquisition, facilitating multi-angle vector Doppler for angle-independent blood velocity estimations using a least-squares fitting approach (170). Raw data were collected for ~3s to capture one complete cardiac cycle (number of array channels: 192; ultrasound frequency: 5 MHz; pulse repetition frequency: 6 kHz; imaging depth: 5 cm). Data were processed with custom computing (MATLAB R2021; The MATHWORKS Inc, Natick, USA), as previously described (106). In brief, the radiofrequency data were used as input to generate seven unique transmit-receive angle pairs. For each pair, processing was completed on a per pixel basis for clutter suppression (134th order finite-impulse-response high-pass filter 187.5 Hz cut-off frequency), manual dealiasing, and mean Doppler frequency estimation (ensemble size per estimate: 64 samples) to derive final flow vector estimates.

Doppler spectrograms were manually inspected to gate analysis to a single flow cycle (peak to peak). Vector data were then processed for a region of interest using landmarks within the same participant vessel to extract the VCC. The VCC is unitless, ranging from 0 to 1, describing the uniformity of flow vector direction within the region of interest (114). A value of 1 indicates all flow vectors are pointed in the same direction, whereas 0 implies there is equal dispersion of vector directions at the given time-point. The VCC was calculated for each frame using the resultant vector, r , based on Pythagoras' theorem $r = \sqrt{x^2 + y^2}$, where x and y represent axial and lateral vectors respectively, to obtain intra-cycle tracings of flow complexity as previously described (114). Data are presented as the time-averaged value across a cycle. Vector flow images were generated using the vector projectile imaging technique, representing flow vectors as coloured arrows, randomly generating launch points in the vessel lumen and updating pixel vector position each frame until a pre-defined vector lifespan, as previously described (162).

Statistical analysis

All graphing and statistics were completed using R (v4.2.2) (171). To address the primary question of the effect of graded distension, differences in the primary outcome of IJV volume across tilt conditions (supine, -6° HDT, -15° HDT, -30° HDT) were assessed with one-way repeated measures ANOVAs at defined IJV regions (caudal, middle, cranial). Secondly, differences in outcomes for different tilt conditions and measurement sites (caudal vs cranial) were evaluated with two-way (condition x measurement site) ANOVAs. For all ANOVAs, post-hoc Bonferroni-Holm corrected paired Student's t-tests were used to probe significant main effects and interactions. Paired Student's t-tests were used to evaluate the effect of thigh cuffs during the -6° HDT as our secondary research question. Generalized η^2 is reported for one way ANOVA main effects and partial η^2 for two-way ANOVA main effects. The acceptable Type I error rate was set *a priori* at $\alpha = 0.05$.

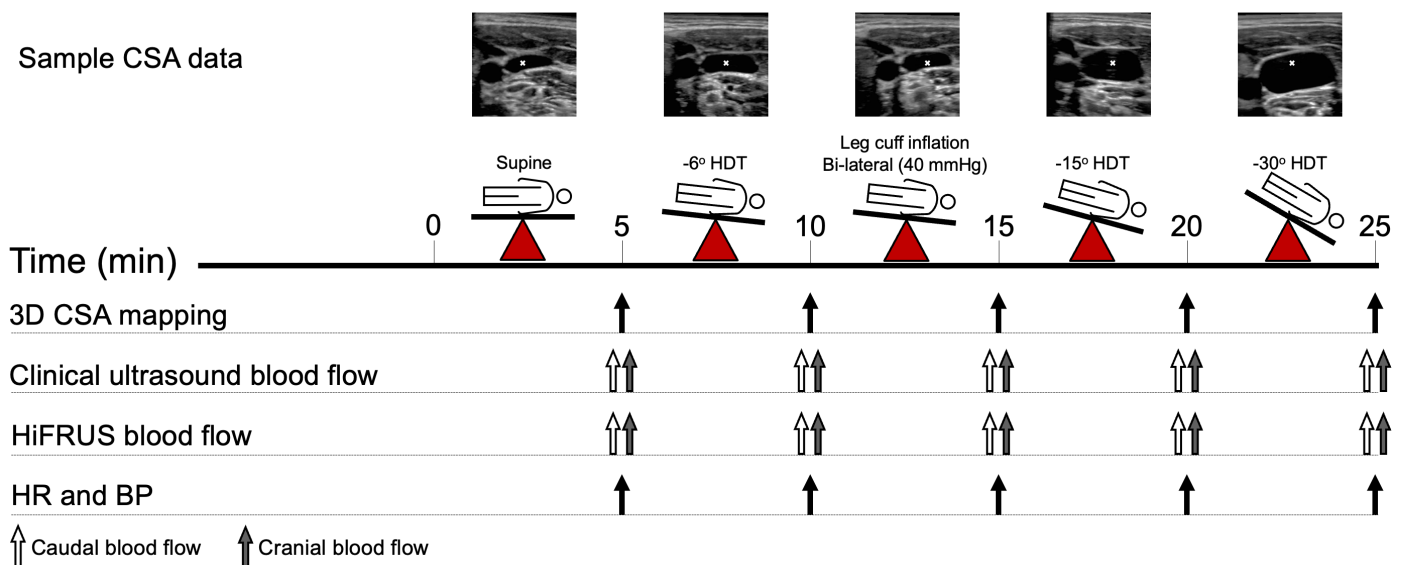


Figure 3-1: Experimental protocol. White “x” marks location of the IJV. CSA; Cross-sectional area, HiFRUS; High-frame rate ultrasound.

3.3 Results

All recruited participants completed all conditions without adverse events. Data are included from all participants, however, Duplex ultrasound-derived blood flow data were removed for n=1 due to clear signs of non-laminar flow, thus breaking the Poiseuille assumptions of streamline flow

calculation. Vector flow data were also removed from $n=2$ due to data loss. Heart rate and arterial blood pressure did not change between conditions (All $P \geq 0.08$, generalized $\eta^2 \leq 0.03$; Table 2-2).

Duplex imaging-derived blood flow

In all conditions, we observed no differences in blood flow between the caudal and cranial measurement sites (All main effects $P > 0.06$; Fig. 3-2). IJV diameter increased with each condition, while MBV decreased at both measurement sites, likely resulting in the absence of blood flow changes with condition and measurement site. IJV diameter increased stepwise from supine to -30° HDT ($P < 0.05$ for all conditions in all sites) and was always larger at the caudal region (all $P < 0.05$) (measurement site $\times$ condition effect $P = 0.03$; partial $\eta^2 = 0.17$). IJV MBV differed as a function of measurement location (main effect $P < 0.001$; partial $\eta^2 = 0.60$) and condition (main effect $P < 0.01$; partial $\eta^2 = 0.64$). MBV was consistently $\sim 2\text{--}3 \text{ cm}\cdot\text{s}^{-1}$ higher at the cranial region compared to the caudal region (all $P < 0.01$). MBV decreased with progressive HDT (all $P < 0.01$) reaching a minimum of $7.5 \pm 2.1 \text{ cm}\cdot\text{s}^{-1}$ at the caudal region during -30° HDT (35% decrease from supine).

IJV Volume

We consistently observed a larger area at the caudal compared to cranial region, owing to the natural taper of the vessel; this geometric observation was maintained during all conditions. Total IJV volume increased stepwise from supine to progressive levels of HDT (Fig. 3-3A-C; Supplemental video 2-1). Volume also increased in stepwise fashion with progressive HDT at all region locations (caudal, middle, and cranial) (All $P < 0.02$). We did not observe the IJV volume plateau, even between the -15° and -30° HDT conditions. In full, the IJV expanded $139 \pm 95\%$ from supine ($4.6 \pm 2.7 \text{ mL}$) to -30° HDT ($10.3 \pm 5.0 \text{ mL}$).

Vector flow imaging

The VCC, describing the directional distribution of flow vectors throughout one cycle, changed as a function of tilt condition (main effect $P < 0.01$, partial $\eta^2 = 0.51$) and scanning location (main effect $P < 0.01$, partial $\eta^2 = 0.40$; Fig. 3-4). Increased distribution of blood vectors (decreased VCC) was found when comparing supine to -15° HDT and -6° HDT to -30° HDT (all $P < 0.05$), suggesting more disturbed blood flow with greater tilt and larger venous volumes. Furthermore, a main effect

of scanning location was found, such that VCC was lower, and therefore flow was more dispersed, within the larger caudal region compared to the smaller cranial region ($P<0.01$).

Qualitative vector projectile images observations

Given the sparse usage of vector flow imaging technology in human physiological testing, quantitative outcome metrics are limited despite rich spatio-temporal resolution of the data. Visual identification of blood flow patterns is useful in the initial characterization of altered hemodynamic environments. Accordingly, we also report qualitative findings from vector projectile images generated from vector flow data: this technique generates updating pixel vector projectiles that allow intuitive tracking of intra-cycle blood flow dynamics (162) (Fig. 3-5, 2-6, Supplemental video 2-2). We observed periods of uniform flow throughout the entire vein early in the cycle in most conditions and participants, followed by increasing dispersion of flow vectors in the second half of the cycle. Non-uniform flow patterns tended to appear closer to vessel walls at the caudal region and during higher degrees of HDT. However, it should be noted that some participants maintained very high levels of flow uniformity throughout all conditions and measurement sites, which is generally captured by the consistently high VCC values in Fig. 3-4.

Thigh cuffing during -6° HDT

Popliteal vein CSA was measured in 16 participants during the -6° HDT and -6° HDT + cuffs condition to confirm the success of the cuffing intervention to induce lower-limb pooling. A significant increase in popliteal vein CSA was found with the addition of low-pressure thigh cuffs (0.74 ± 0.23 vs 0.53 ± 0.24 cm²; $P<0.001$). IJV blood flow did not differ between -6° HDT and -6° HDT + cuffs conditions at caudal (588 ± 246 vs 569 ± 267 mL·min⁻¹; $P=0.76$) or cranial (577 ± 175 vs 545 ± 222 mL·min⁻¹; $P=0.48$) regions (Fig. 3-7C). IJV diameter between -6° HDT and -6° HDT + cuffs did not differ at caudal (1.16 ± 0.3 vs 1.13 ± 0.33 cm; $P=0.37$) and cranial (1.0 ± 0.23 vs 0.98 ± 0.25 cm; $P=0.63$) regions. Likewise, average of caudal and cranial IJV MBV did not differ between -6° HDT and -6° HDT + cuffs conditions (11.1 ± 4.1 vs 11.0 ± 4.0 cm·s⁻¹; $P=0.90$). The caudal IJV volume did not differ between -6° HDT and -6° HDT + cuffs condition ($P=0.20$). Despite the absence of change in IJV blood flow and one-dimensional diameters, the cranial and middle IJV volumes did decrease during -6° HDT + cuffs compared to -6° HDT alone ($P<0.01$; Fig. 3-7A), suggesting some protective effect of the fluid shift countermeasure. There were no differences in

VCC found between -6° HDT and -6° HDT + cuffs conditions at caudal or cranial regions (both $P>0.25$) (Fig. 3-7B).

Table 3-1: Participant characteristics

Outcome	Male	Female
N	9	9
Age (yrs)	26±3	24±2
Height (cm)	177±4	162±4
Body mass (kg)	79.3±7.7	55.8±6.4
BMI ($\text{kg}\cdot\text{m}^{-2}$)	25.2±2.2	21.3±1.5
Resting heart rate ($\text{beats}\cdot\text{min}^{-1}$)	64±6	64±8
Resting mean arterial pressure (mmHg)	82±8	82±7

Data are represented as means±SD. BMI, body mass index.

Table 3-2: Hemodynamic responses to progressive volume shifting

Variable	Supine	-6° HDT	-6° HDT +Cuffs	-15° HDT	-30° HDT
Heart rate ($\text{beats}\cdot\text{min}^{-1}$)	64±8	63±7	63±7	61±7	62±7
Systolic blood pressure (mmHg)	113±9	113±8	112±8	112±8	114±8
Diastolic blood pressure (mmHg)	67±6	65±5	65±7	66±6	68±9
Mean arterial pressure (mmHg)	82±7	81±5	81±6	82±6	84±8

Data are represented as means±SD. No significant differences were found between conditions.

Table 3-3: Internal jugular vein diameters and blood velocities at different tilt conditions

Variable	Supine	-6° HDT	-6° HDT +Cuffs	-15° HDT	-30° HDT
<i>Caudal</i>					
Diameter (cm)	1.02±0.3	1.17±0.3	1.14±0.3	1.37±0.3	1.48±0.3
Mean blood velocity (cm·s ⁻¹)	11.6±5.5	9.4±3.9	9.5±3.3	7.6±2.5	7.5±2.1
<i>Cranial</i>					
Diameter (cm)	0.89±0.2	1.01±0.2	1.0±0.3	1.13±0.3	1.25±0.3
Mean blood velocity (cm·s ⁻¹)	14.5±4.3	12.5±3.5	12.2±4.1	10.6±4.0	8.2±4.6

Data are represented as means±SD.

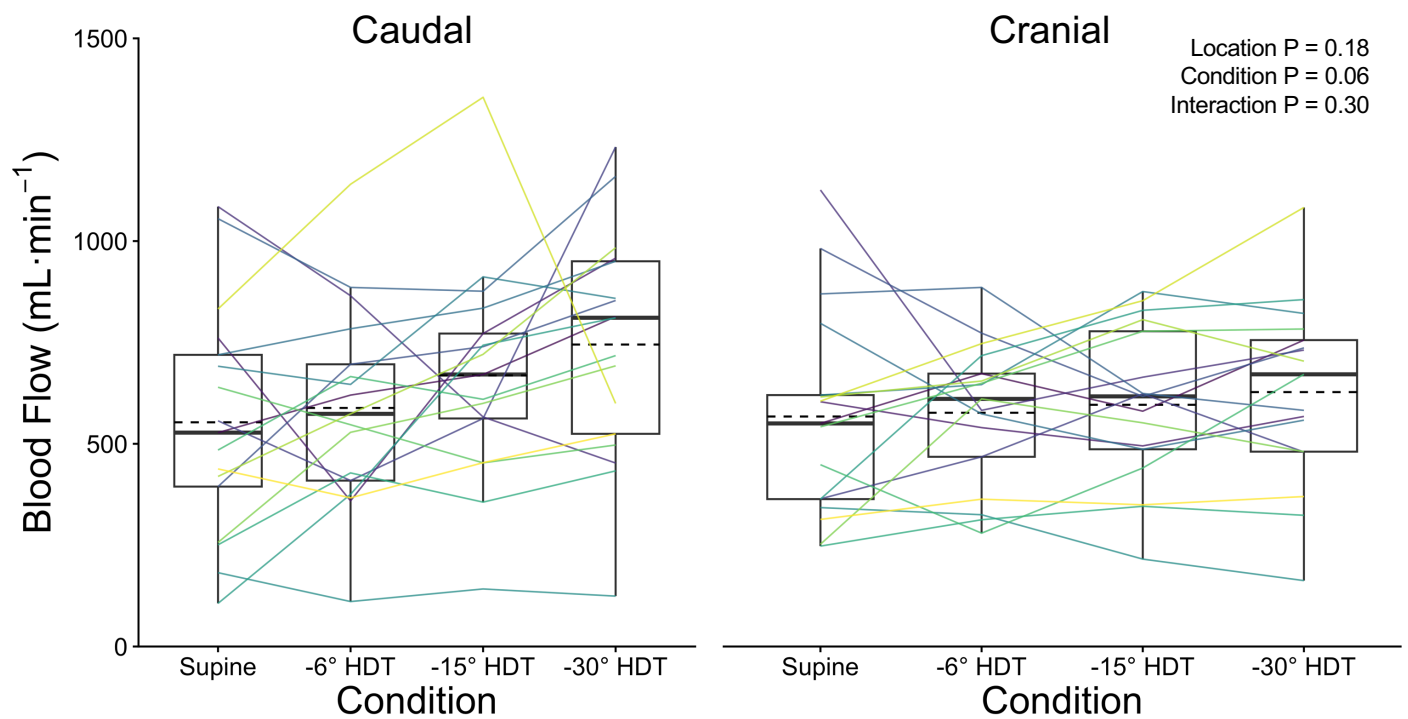


Figure 3-2: Blood flow measured in the internal jugular vein at the caudal and cranial regions.

Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n=17).

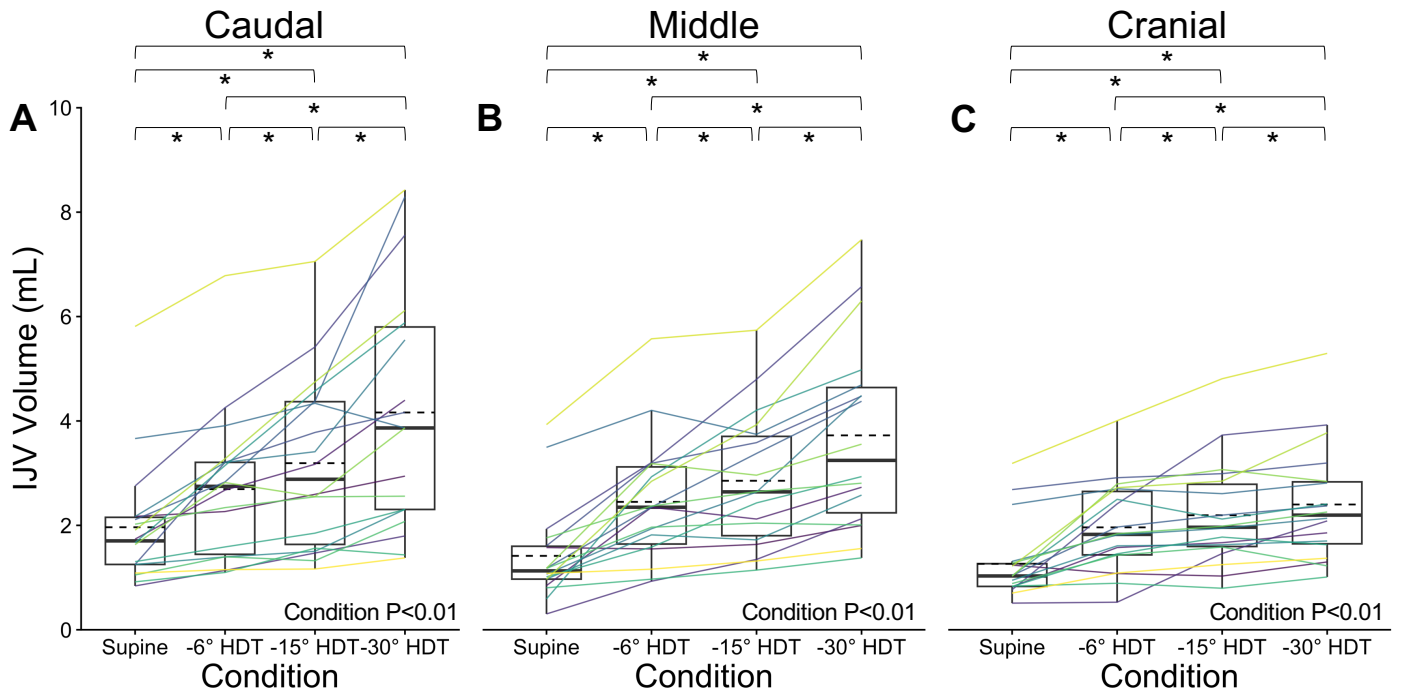


Figure 3-3: Internal jugular vein (IJV) volume (A-C) in three distinct regions (caudal, middle, cranial) during progressive conditions of head down tilt (HDT).

Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (n=17). * All P<0.05 within the specified vein region.

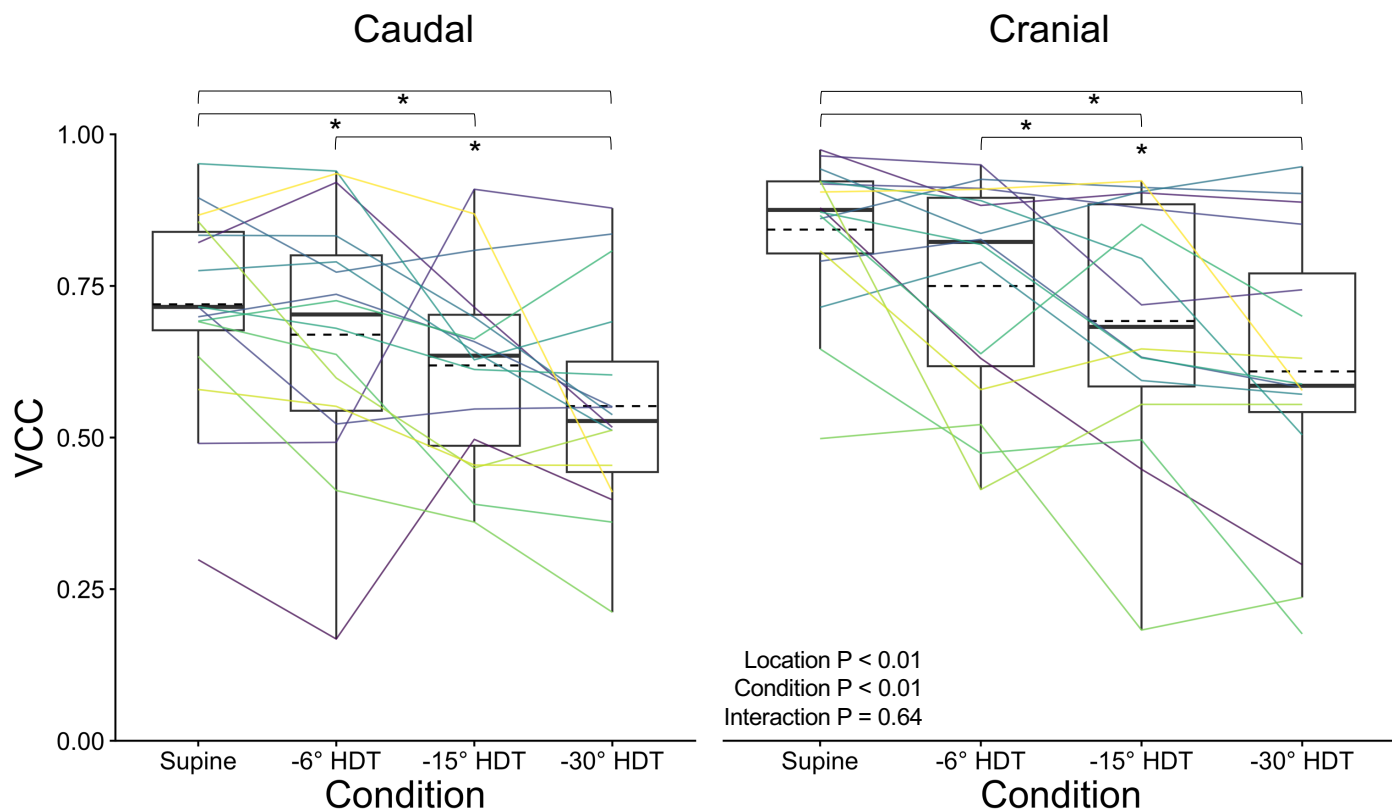


Figure 3-4: Vector concentration coefficient (VCC) derived from vector flow imaging of the internal jugular vein at caudal (A) and cranial (B) regions.

Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within $1.5 \times$ interquartile range beyond the box boundaries. Individual responses shown by coloured lines ($n=17$). * All $P < 0.05$ within the specified vein region.

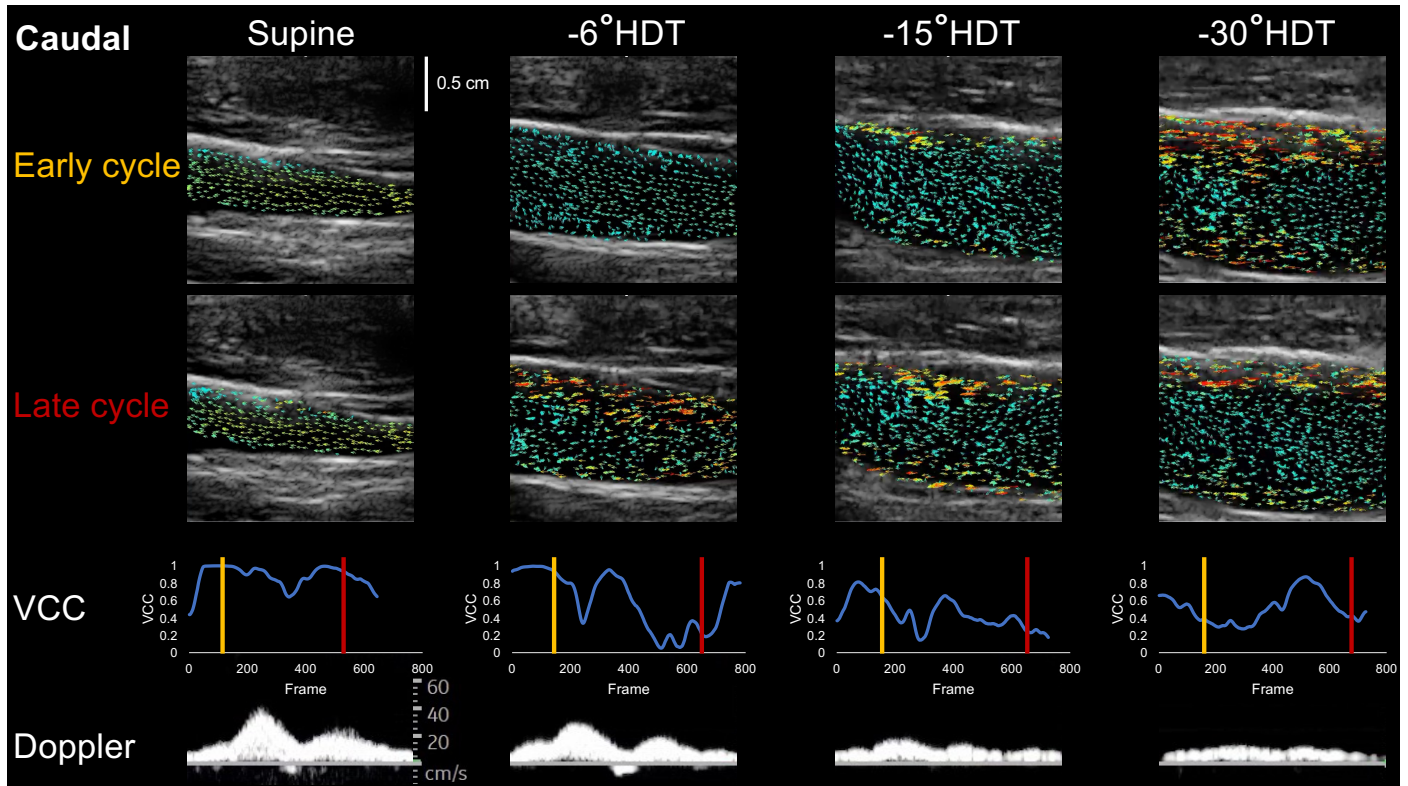


Figure 3-5: Representative data from one participant (male, 26 yrs), depicting vector projectile images during the early and late flow cycle at the caudal region.

The corresponding vector concentration coefficient (VCC) trace (with orange and red bars indicating early and late timepoints) and conventional Doppler spectrogram for the vein region is shown beneath each condition.

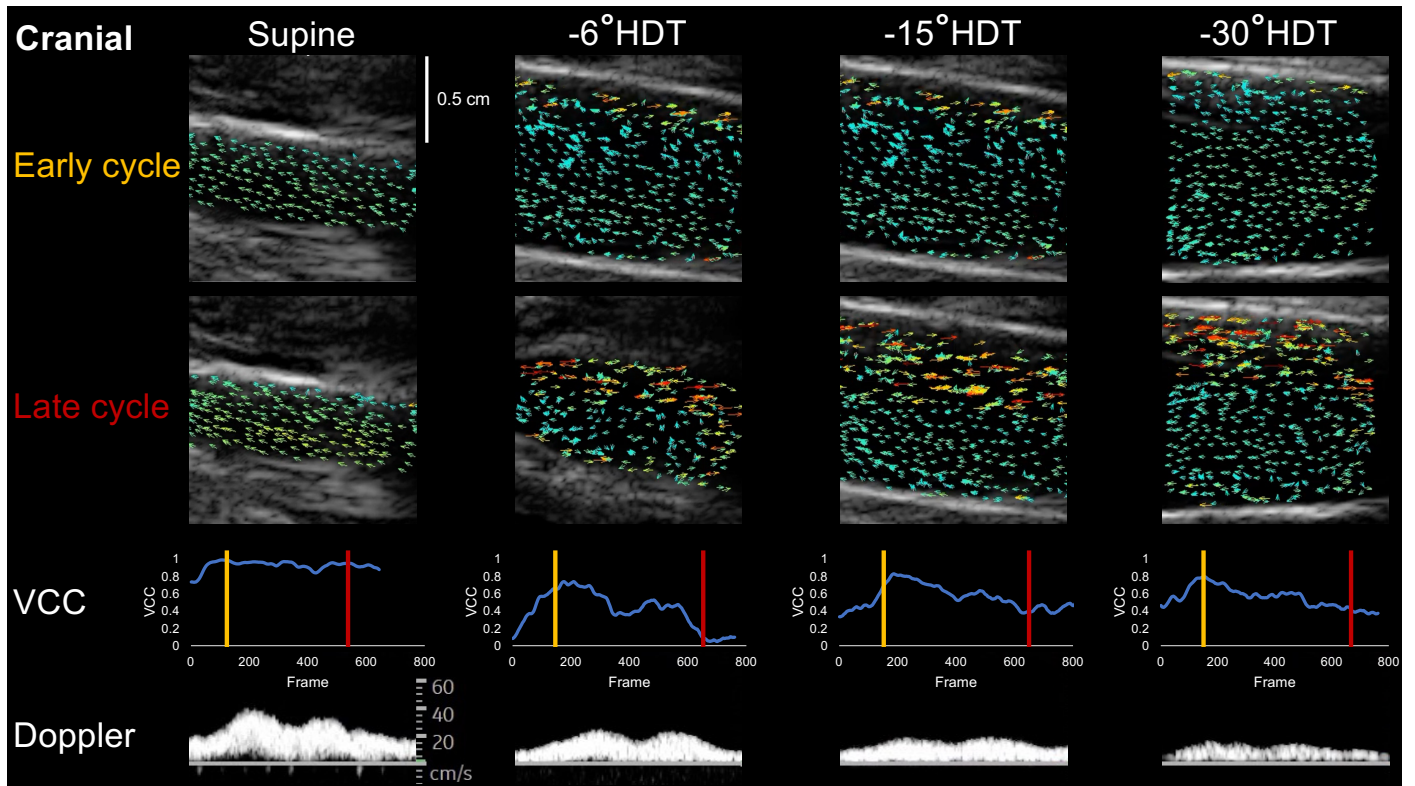


Figure 3-6: Representative data from one participant (male, 26 yrs), depicting vector projectile images during the early and late flow cycle at the cranial region.

The corresponding vector concentration coefficient (VCC) trace (with orange and red bars indicating early and late timepoints) and conventional Doppler spectrogram for the vein region is shown beneath each condition.

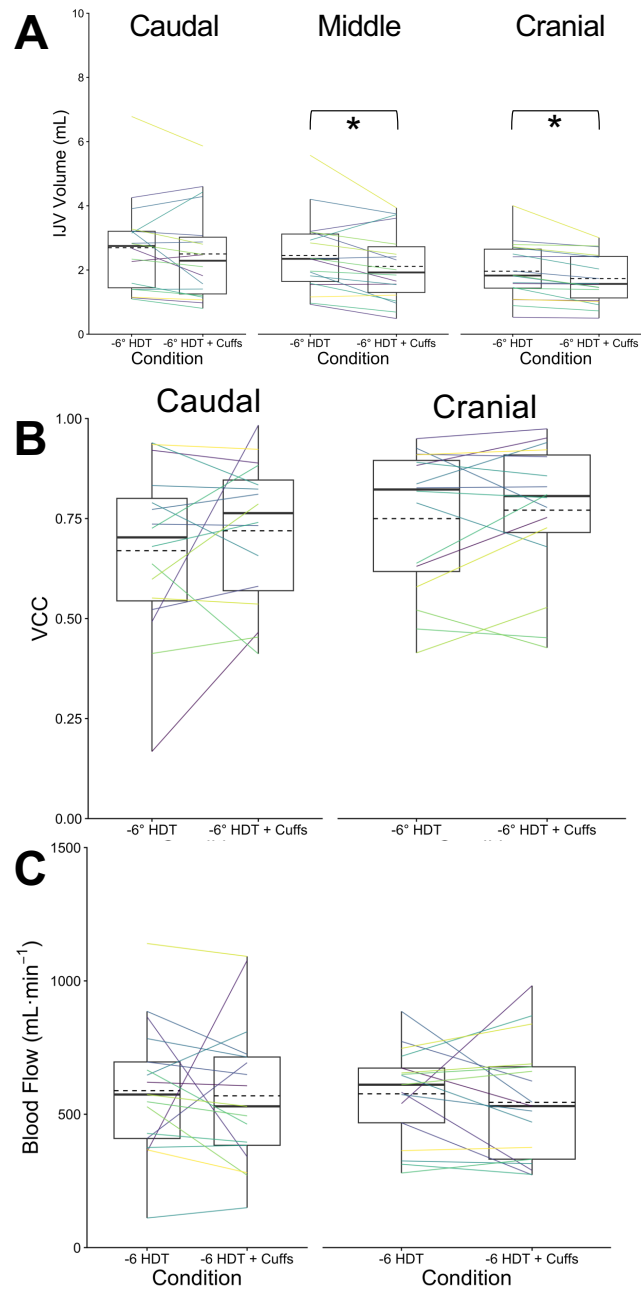


Figure 3-7: Comparison of -6° head-down tilt (HDT) with and without bilateral thigh cuffs on internal jugular vein (IJV) volume (A), vector concentration coefficient (VCC) (B) and blood flow (C) within specified vein regions.

Boxes encapsulate the 1st and 3rd quartile with mean (dashed line) and median (solid black line) shown. Whiskers represent range of data within 1.5*interquartile range beyond the box boundaries. Individual responses shown by coloured lines (Blood flow and volume; n=17, VCC; n=16). * P<0.05 between conditions.

Supplemental video 3-1: Caudal-to-cranial sweep in internal jugular vein cross-sectional area at different levels of head-down (HDT) tilt in a 26-year-old male participant.

<http://doi.org/10.6084/m9.figshare.25215146>

Supplemental video 3-2: Vector flow imaging of the internal jugular vein in the longitudinal view at different levels of head-down (HDT) tilt at the caudal (first half) and cranial (second half) levels in a representative 26-year-old male participant.

<https://doi.org/10.6084/m9.figshare.25215197.v1>

3.4 Discussion

This investigation sought to describe structural and hemodynamic responses of the IJV during progressive cephalic fluid shifting. Conventional and next-generation ultrasound measurement techniques describe the IJV as a highly unique vein with an irregular, conical shape, maintaining blood flow despite regional 3D volume distension. Distension was most prominent at the caudal region of the vein, aligning with more prominent flow dispersion, suggesting higher potential for flow recirculation and thromboembolism risk. The influence of low-pressure thigh cuffs (40 mmHg) during fluid shifting was also tested, which induced decreases in volume of the middle and cranial thirds of the IJV but did not influence blood flow patterns; however, without clinical endpoints it remains unknown whether this fluid redistribution impacts future thromboembolic risk.

Volume expansion

Geometric expansion of the IJV is governed by the distensibility characteristics of the vein wall and internal blood pressures. Given the highly distensible nature of veins, particularly large veins and increases in central venous blood pressure with HDT (172), progressive volume expansion of the IJV was expected, aligning with previous findings (154). Our 3D scanning suggests caudally-dominated expansion of IJV volume supporting previous reports of irregular geometry of the vein using fixed anatomically anchored measurement sites and multiple techniques (167, 173). The IJV has a wide operational range of distension, from collapsed while upright (154) to progressively larger conical dimensions when distended. Prolonged distension of the IJV has been found to occur specifically in astronauts experiencing microgravity for up to 6 months, with volume distension changes similar to those found in our investigation during -6° HDT (153, 174).

Since we did not find a plateau in IJV volume up to -30° HDT, full distension capacity is likely never fully reached under normal 1G, partial G or 0G physiological conditions. Non-maximal distension in 0G conditions is corroborated by recent reports noting similar IJV area during 0G and 1G supine postures (175). Our data support a highly distensible, conical IJV, with the largest volume accommodated at the caudal region, closest to the heart. Regional venous volume changes are likely to impact local blood flow patterns in addition to potential ambulatory conditions which may accentuate flow variability and pro-thrombotic vascular milieus not tested in these controlled conditions. Thus, understanding how non-uniform wall distension coupled with low velocity flow in recirculation-prone environments is needed to fully understand local thrombotic risk (158).

Vector blood flow patterns

Volume distension has been used as a surrogate for blood stasis due to the assumption that wider vessels imply local blood pooling; however, true stasis is rarely observed in circulation (176). While conventional ultrasound can estimate laminar blood velocity, environments with blood pooling are likely to have blood that is moving in multiple directions throughout a cardiac cycle (i.e., recirculating, swirling, or moving perpendicular to the probe), which invalidates the bidirectional sensitivity of single-angle Doppler. Indeed, while backflow of the IJV has been reported (100), it is possible that cyclical flow accounts for negative mean velocities via single-angle Doppler, particularly when simultaneous multi-directional velocities are observed herein. Accordingly, we employed vector flow ultrasound imaging to describe complex flow patterns expected with IJV pooling during acute distension. We describe scanning location-dependent changes in flow uniformity, with stepwise increases in flow dispersion with increasing venous distension. In general, the conical caudal region has greater flow dispersion compared to the cylindrical cranial region of the IJV. When volume loaded, all flow along the IJV becomes more dispersed, indicative of multi-directional blood vectors throughout the cardiac cycle. Based on the expected volume distension with -6° HDT, we anticipated greater flow dispersion consistent with stasis risk theory; however, it appears the magnitude of effect was not sufficient to detect subtle changes in flow dispersion. Large person-to-person variability was noted in the change in VCC between supine and -6° HDT, which might highlight individual susceptibility to flow disturbances. In more extreme tilt conditions (-15 to -30° HDT), greater volume shifting led to further perturbation of blood flow patterns and possible stasis as indicated by the VCC data. While not directly linked to stasis, VCC describes a departure from laminar flow based on Doppler bandwidth changes

which have previously been linked to turbulent nozzle flow (177). Chronic loading of the IJV during spaceflight can increase distension greater than what is observed during 5 min of -6° HDT (153). Evidence of flow stasis with severe or chronic volume shifts may lend to the theory of thromboembolism generation secondary to IJV congestion, though we did not find evidence of stasis in our study. Additionally, jugular venous pressure is increased during HDT (154, 178), which may lower the critical Reynold's number for turbulent flow and explain the increase in flow dispersion denoted by VCC (177, 179). Application of vector flow imaging technology in venous blood flow studies holds considerable potential for understanding the subtleties of blood flow disturbance, especially at low velocities. This can be gleaned from the differences between our conventional Doppler blood flow results (absence of change with HDT and substantial inter-individual variability) with the rich qualitative and initial quantitative insights to blood flow pattern changes during volume shifts with vector flow imaging which holds applicability for both spaceflight and terrestrial medicine. Studies aiming to describe complex blood flow patterns should be implementing vector flow imaging to best interrogate physiological flow during perturbation to overcome the limited inferences of single-angle Doppler methods, which are currently the standard of practice in the field.

Thigh cuffs

As a secondary objective, we tested the effectiveness of a low-pressure thigh cuff countermeasure in attenuating IJV volume expansion during -6° HDT. Previous studies have used low-pressure thigh cuffs during supine and HDT postures for acute and prolonged periods, typically finding reductions in IJV CSA and estimated pressure (164, 180–182), leading to their adoption amongst cosmonauts (183). Adoption of a 40 mmHg cuff pressure was based on standard procedures developed by ROSCOSMOS and further refined recently, demonstrating increases in popliteal vein area and skin contact pressures of 30–40 mmHg, never exceeding cuff pressures of 50 mmHg (180, 184). Thigh cuffs have classically been applied to help manage symptoms and facial oedema associated with the cephalic fluid shift experienced during spaceflight; however, observation of jugular thrombosis in an astronaut on the International Space Station (100) has led to renewed interest in thigh cuffs as a spaceflight countermeasure. Despite the clear applicability of thigh cuffs in microgravity, no study has investigated how they impact region-specific IJV distension characteristics. Our data support previous studies showing decreases in IJV area and volume when thigh cuffs are applied during simulated (-6° HDT, reported here) or real microgravity (165, 185) and, additionally, show that the cuff effect is greater

in the middle and cranial portions of the vein. Using thigh cuffs can reduce IJV volume expansion, but benefits did not extend to measures of flow uniformity. Non-uniform or recirculatory blood flow is a hypothesized risk factor for thrombosis (186), and advanced imaging methods have the potential to identify flow patterns of concern (158). Our data suggest no effect of 40 mmHg thigh cuffs on this outcome, similar to direct measures of intracranial pressure during HDT (187), yet more data is needed in this area. We did note some inter-individual variability in the response, where individuals with the most disturbed caudal region flow had the most improvement in the flow profile; it is unclear whether this reflects a ceiling effect, or regression to the mean response. At a group level the data from progressive volume shifting suggest flow uniformity does not meaningfully deteriorate from supine to -6° HDT in either caudal or cranial regions, thus it is reasonable that 40 mmHg cuff application had no effect on this outcome.

Limitations

A few inherent limitations to this work should be considered when interpreting the data. Primarily, extrapolation of these data to scenarios of partial G or 0G is theoretical since all data were collected on Earth, where the compressive force of soft tissues can influence IJV morphology independent of the cranial fluid shift whereas true microgravity unweights this gravitational force, lowering total venous resistance. (188). Furthermore, our protocol does not include long-duration spaceflight factors such as chronic exposure to fluid shifts, fluid loss, increased environmental CO₂, radiation, and physical inactivity (189). During segmentation of the IJV, interindividual differences in IJV anatomy necessitated common landmarking to identify the caudal and cephalic ends of the IJV, using the clavicular limit (caudal) and the site of bifurcation or mandible limit (cranial) to denote regions of the IJV. Accordingly, in participants who presented 'low' bifurcation sites, volumes are broken into thirds for the length of the vein below that point to eliminate erroneous volume calculation in a bifurcated vessel. Low bifurcations existed in 8/18 participants and resulted in ~1cm of neck length inferior to the mandible removed from those participants' volumetric analyses. Presence and/or insufficiency of the IJV valve was not determined yet may exist in a minority of young, healthy individuals contributing to some variability (190). We also elected to not randomize testing conditions, opening the data to a potential order effect; however, we felt the risk of venous hysteresis outweighed those concerns, aligning with design and results of similar study (154). Another caveat to be noted about the presented vector flow data is that spurious non-uniformities in flow patterns are observed near the vessel wall for some individuals. They may be attributed to flow vector estimation errors stemming from the loss of near-wall

Doppler echoes during clutter suppression. Thus, they should not be interpreted as physiological disturbances in the near-wall flow dynamics. Nevertheless, these image artifacts are simply nuisances in the flow visualization and do not affect the overall inferences that we have drawn about blood volume shifts. Finally, it is worth noting there is limited information on the reproducibility of VCC and direct links to venous thromboembolism risk for VCC outcomes during fluid shifting interventions. Accordingly, we have used the VCC outcome as an index of flow multi-directionality which we infer may contribute to venous thromboembolism risk. Future investigation must ascertain the sensitivity and reproducibility of VCC outcomes and direct relationships with venous stasis before assigning its impact.

Conclusion

We investigated the structural and hemodynamic responses of the IJV during progressive cephalic fluid shifting. Our findings reveal a highly individualized IJV with an irregular, conical shape that maintains blood flow despite substantial volume distension; with the largest volume consistently observed at the caudal region of the vein, expanding up to ~120%. Application of vector flow imaging demonstrated region-dependent differences in flow uniformity, with the caudal region showing more dispersed flow compared to the cranial region and increasing flow dispersion with increasing IJV distension. Low-pressure thigh cuffs showed efficacy in reducing IJV volume distension, particularly in the cranial region, but had negligible effects on flow uniformity, albeit with large inter-individual variability. While these results offer valuable insights to characterize risk of venous stasis with cephalic fluid shifts, their extrapolation to microgravity scenarios remains theoretical, and limitations related to the ground-based microgravity analogue should be considered. This research contributes to our understanding of IJV behaviour during fluid shifting similar to 0G and highlights the potential of next-generation ultrasound technologies for studying complex flow patterns in venous physiology.

Chapter 4: Supporting evidence for an ‘arterial pump’ venous return mechanism in humans

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4.1 Introduction

Pulsatile energy within the cardiovascular system is largely considered an artery-specific phenomenon, with most textbooks describing extensive pressure dampening at the points of venules and large veins (86). Owing to the small pressure gradients between the right atrium and systemic veins, venous return is highly dependent on external pumping mechanisms. The skeletal muscle pump, respiratory pump, and atrioventricular plane displacement generate well-documented venous pressure gradients driving blood toward the right atrium (23, 191, 192). Until recently, little thought had been given to the artery’s pulsatile energy acting in direct contact with veins. LeFevre and Sarvazyan (83) tested the importance of this anatomical arrangement on venous flow using an *ex vivo* bovine model.

The authors elegantly demonstrated a direct effect of arterial pulsation on venous blood flow in the adjacent valve-containing vein segment. This ‘arterial pump’ effect was demonstrated to occur over a range of physiological pressure gradients (25 to 35 mmHg) and pulse rates (70 and 140 beats·min⁻¹), substantially increasing the flow rates in optimal conditions. The importance of arterial pulsatility, heart rate, and state of one-way valves, such that they are actively recruited in the management of blood flow against gravity, was highlighted. Specifically, when the pressure gradient was small, valves were closed, and flow stopped. In contrast, high-pressure gradients and patent valves delivered flow without an additional arterial pump effect. At modest driving pressures (25-35 mmHg), valves were not firmly closed, playing an active role in flow, and an arterial pump effect existed. Confirmation of this phenomenon by vascular ultrasound *in vivo* was considered challenging, however, we postulate that *in vivo* evidence of the arterial pump is possible by investigating veins at the same hydrostatic level, either part of (deep femoral vein, DFV, adjacent to superficial femoral artery, SFA) or not part of (greater saphenous vein, GSV) a veno-arterial bundle.

The aim of this study was to address the presence of an arterial pump contribution to venous blood flow in veno-arterial bundled valve-containing veins in humans. Given the theorized dependence on distending pressure to observe this effect, we manipulated body posture to achieve stepwise changes in the hydrostatic fluid column, similar to the driving pressure

manipulations of LeFevre and Sarvazyan (83). We hypothesized that by manipulating body posture and thus valve status, preservation of venous flow would be observed in veno-arterial bundles compared to non-bundled veins, primarily in semi-upright postures.

4.2 Methods

Participants

A total of 17 healthy adults (11 males) provided informed consent and enrolled in the study. All participants were ostensibly healthy individuals without history of cardiovascular disease, were recreationally active, and not taking medications with known cardiovascular effects. Participants avoided strenuous exercise, alcohol, caffeine for 12h, and large meals for 3h prior to attending the laboratory. Participants were asked to consume ~500mL of water upon waking to ensure hydration. The study was approved by the local ethics committee (ORE43670) and complied with the Declaration of Helsinki concerning the use of human participants. We pre-registered the study prior to data collection (<https://doi.org/10.17605/OSF.IO/85KVD>) with a minimum of 15 participants to align with anticipated medium effects in human physiological studies. Data are presented for 16 of the 17 collected participants; one male participant was removed from the analysis due to inadequate image quality.

Study design

Following anthropometric assessment, participants rested supine on a tilt table (Colin Medical, San Antonio, USA) for 15 min before baseline hemodynamics were recorded. Ultrasonography of the veno-arterial bundled DFV and SFA, and non-bundled GSV was performed in the right leg 20.4±3.2 cm superior to the patella. Following the supine condition, participants were tilted to -6° HDT, 20° head-up tilt (HUT), and 40° HUT in a randomized order using a balanced Latin square design from ChatGPT (OpenAI.com). Tilt conditions were selected based on expected hydrostatic pressure gradients described by LeFevre and Sarvazyan (83), higher HUT was avoided due to large effects on cardiac output and sympathetic activation. Vessel sonography and hemodynamic assessments were repeated for each condition. Upon reaching each posture, participants rested for 5 min before any measurements were recorded. During the HUT postures, participants were instructed to gently brace their body weight using their left leg on the provided footboard to avoid local muscle pump action of the right imaging leg. To eliminate any respiratory pump effects, participants were asked to hold an end-tidal breath during venous flow acquisitions.

The hydrostatic fluid column pressure between the vein site and right atrium was estimated based on body surface measurements, solving for the vertical height component during 20° and 40° HUT and applying pressure estimates for blood: $\Delta\text{Pressure mmHg} = \rho gh \cdot 133.3^{-1}$. Where ρ is the estimated density of blood ($1.06 \cdot 10^3 \text{ kg} \cdot \text{m}^{-3}$), g is the acceleration due to gravity ($9.81 \text{ m} \cdot \text{s}^{-2}$), and h is the column height in m. The estimated hydrostatic blood pressure from the right atrium to leg scanning locations was $16.4 \pm 1.4 \text{ mmHg}$ and $30.8 \pm 2.6 \text{ mmHg}$ during 20° and 40° HUT conditions, respectively, approximating the conditions suggested by LeFevre and Sarvazyan (20-40 mmHg) (5).

Blood vessel sonography

Veins and arteries were scanned in duplex mode using a commercial ultrasound (Vivid iQ, GE Healthcare, Chicago, USA) and linear array probe (12L, GE Healthcare). The imaging sequence was GSV, DFV, and then SFA with at least 30 seconds provided between venous measurements, allowing for a new breath-hold sequence. Scans were optimized during each collection by minimizing wall filter settings to allow low-velocity flow collection. Additionally, care was taken to avoid compressing veins by applying a thick layer of ultrasound gel and using the minimum pressure required to generate an ultrasonic signal. Scanning location was landmarked by ink markings on the skin. Videos were stored in 15 second clips for offline analysis using open-source software (FloWave.US, (168)) programmed to extract the raw intensity-weighted velocity envelope from the Doppler trace for MBV. Vessel diameter was manually acquired at end-diastole from longitudinal images using the ultrasound caliper function. Blood flow was then calculated using the following formula: $\text{Blood flow (mL} \cdot \text{min}^{-1}) = (\pi \cdot r^2) \cdot \text{MBV} \cdot 60$. Sonographer intra-rater reliability of venous diameter and MBV measurements are excellent at $\text{ICC}(3,1) > 0.90$.

Statistics

All numerical analyses and graphing was performed using R (v4.2.2) (171). To evaluate the differential effects of condition (-6° HDT, supine, 20° HUT, 40° HUT) on blood flow-related variables in different veins, a 4×2 (condition $\times$ vein) repeated measures ANOVA was used. To determine the effect of condition on arterial blood flow, heart rate, and blood pressure, one-way repeated measures ANOVAs were used. Significant main effects and interaction terms were evaluated *post hoc* using Bonferroni-Holm-corrected paired Student's t-tests. Corrected P values that approached 1.0 are indicated by $P_{\text{adj}} > 0.99$. The Type I error rate was set *a priori* to $\alpha = 0.05$.

4.3 Results

All participants were young and considered healthy (26.2 ± 3.3 yr, 173 ± 9 cm, 72.8 ± 13.2 kg) with normal supine resting hemodynamics (Table 3-1). All participants completed all testing conditions without adverse events associated with body tilting procedures.

Hemodynamics

Changing body position produced changes in hemodynamics primarily at the 20° and 40° HUT conditions (Table 3-1). From supine, heart rate increased in the 40° HUT condition ($+9 \pm 12$ beats·min⁻¹; $P < 0.01$), but not in the 20° HUT condition ($P = 0.20$). From supine, mean arterial pressure increased in the 20° HUT condition ($+3 \pm 3$ mmHg; $P < 0.01$) and increased a further $+3 \pm 3$ mmHg during the 40° HUT condition ($P < 0.01$). Neither heart rate or arterial blood pressure variables differed between -6° HDT and supine conditions (all $P > 0.20$).

Arterial blood flow

SFA blood flow was highest in the supine condition (144 ± 58 mL·min⁻¹) compared to all other conditions (all $P < 0.01$). No differences were observed between the -6° HDT (101 ± 45 mL·min⁻¹), 20° HUT (84 ± 25 mL·min⁻¹), and 40° HUT conditions (80 ± 25 mL·min⁻¹) (all $P > 0.15$). The primary cause for flow differences was changes in mean blood velocity, decreasing from 7.9 ± 2.5 cm·s⁻¹ while supine to 5.6 ± 2.0 cm·s⁻¹ (-6° HDT), 5.2 ± 1.6 cm·s⁻¹ (20° HUT) and 4.6 ± 1.5 cm·s⁻¹ (40° HUT; all $P < 0.01$). No differences were observed in the SFA diameter ($P = 0.07$, $\eta^2 = 0.01$).

Venous blood flow

Fig. 4-1A is representative ultrasound cross-section of the right thigh, highlighting the SFA and DFV bundle, deep to the GSV. With progressive body tilt, we qualitatively observed greater venous diameter as well as notable valve recruitment in participants who happened to have DFV valves in the same imaging plane, consistent with valve function under greater hydrostatic pressure (Fig. 4-1B-D, Supplemental video 3-1). When evaluating the main question of how vessel pairings respond to tilt, there was a vessel $\times$ condition interaction for blood flow ($P < 0.01$, partial $\eta^2 = 0.31$). From supine, blood flow in both veins decreased similarly at the 20° HUT

condition (-38% and -44% in the GSV and DFV, respectively (both $P<0.01$) (Fig. 4-2). However, a further decrease in blood flow between 20° and 40° HUT was only observed in the non-bundled GSV, decreasing by an additional 20% ($P=0.01$), whereas blood flow did not change in the bundled DFV ($P_{\text{adj}}>0.99$). Neither vein exhibited changes between supine and -6° HDT conditions (both $P>0.30$).

Relevant vessel $\times$ condition interaction effects were also found for mean blood velocity ($P=0.03$, partial $\eta^2=0.27$) and diameter ($P<0.01$, partial $\eta^2=0.77$). Mean blood velocity exhibited stepwise decreases in both veins from supine (GSV: 3.6 ± 2.2 ; DFV: 8.3 ± 3.5 $\text{cm}\cdot\text{s}^{-1}$) through 20° HUT (GSV: 1.1 ± 0.6 ; DFV: 1.8 ± 0.8 $\text{cm}\cdot\text{s}^{-1}$) and 40° HUT (GSV: 0.6 ± 0.4 ; DFV: 1.3 ± 0.9 $\text{cm}\cdot\text{s}^{-1}$) conditions (GSV: all $P<0.01$, DFV: all $P<0.03$). Neither vein experienced changes between supine and -6° HDT conditions (both $P>0.20$). Both veins demonstrated stepwise increases in diameter from supine through 20° HUT conditions (GSV: all $P<0.05$, DFV: all $P<0.01$) (Fig. 4-3). However, only the DFV diameter increased between 20° and 40° HUT conditions ($P<0.01$), while the GSV did not ($P=0.60$). Neither vein changed diameter between supine and -6° HDT conditions (both $P>0.07$).

Table 4-1: Hemodynamic responses to progressive body tilting

Variable	-6° HDT	Supine	20° HUT	40° HUT
Heart rate ($\text{beats}\cdot\text{min}^{-1}$)	57±7	58±7	60±8 ‡	67±12 *
Systolic blood pressure (mmHg)	111±11	111±9	113±8 †	116±9 *
Diastolic blood pressure (mmHg)	63±8	63±8	67±10 †	70±9 *
Mean arterial pressure (mmHg)	79±9	79±8	83±9 †	86±8 *

Data presented as mean±SD. N=16. Changes assessed by one-way repeated measures ANOVA. Pairwise comparisons: Bonferroni-Holm adjustment for multiple comparisons. HDT; Head-down tilt, HUT; Head-up tilt. * $P<0.05$ vs all other conditions. † $P<0.05$ vs all other conditions. ‡ $P<0.05$ vs -6° HDT.

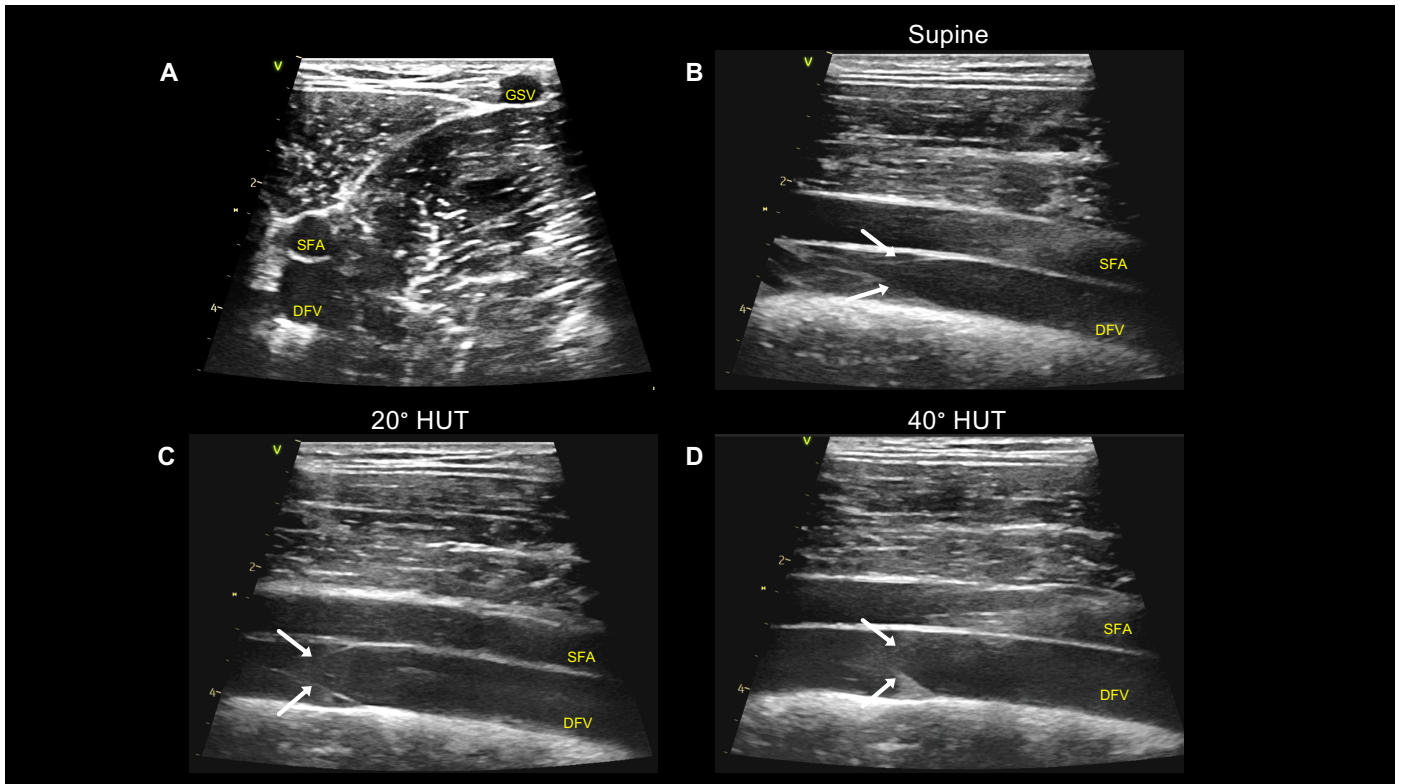


Figure 4-1: Example images of a 24-year-old male participant demonstrating (A) spatial orientation of the SFA-DFV veno-arterial bundle and GSV in a cross-sectional view; and (B-D) presence and recruitment of valves during three experimental conditions.

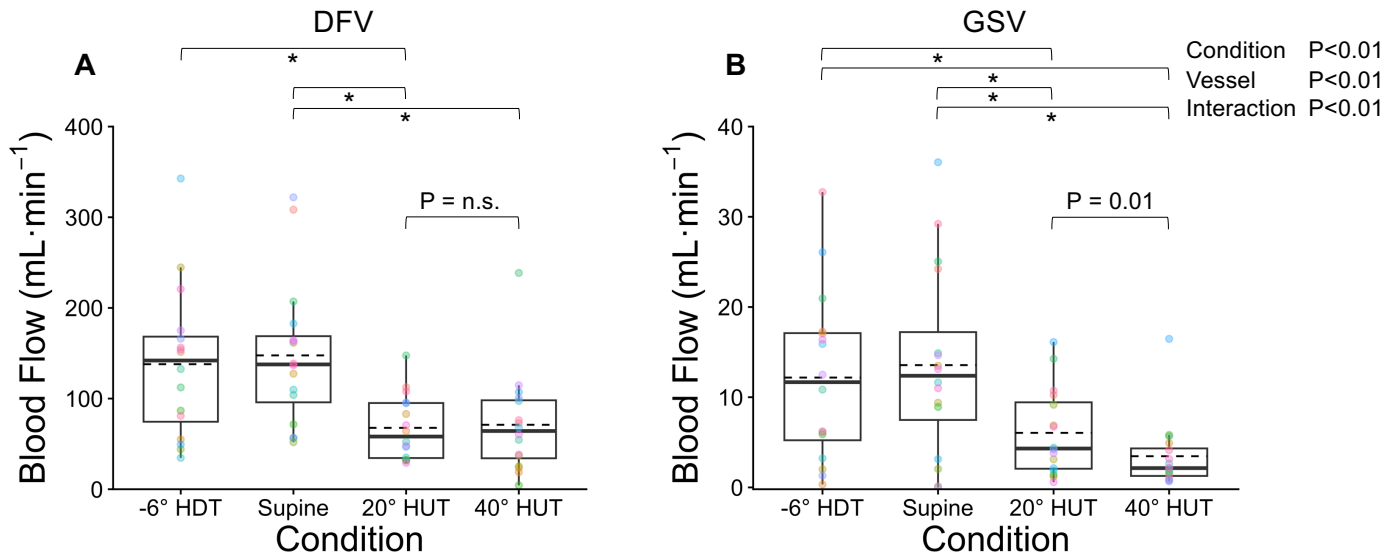


Figure 4-2: Mean blood flow values for separate leg veins in a veno-arterial bundle (DFV) or not (GSV) during progressive body tilting.

Boxes encapsulate 1st to 3rd quartile, with mean (dashed line) and median (solid line). Whiskers extend to data within $1.5 \times \text{IQR}$. Individual data shown ($N = 16$). Changes assessed by 4x2 repeated measures ANOVA. Pairwise comparisons: Bonferroni-Holm adjustment for multiple comparisons. * $P < 0.05$. DFV; Deep femoral vein, GSV; Greater saphenous vein, HDT; Head-down tilt, HUT; Head-up tilt.

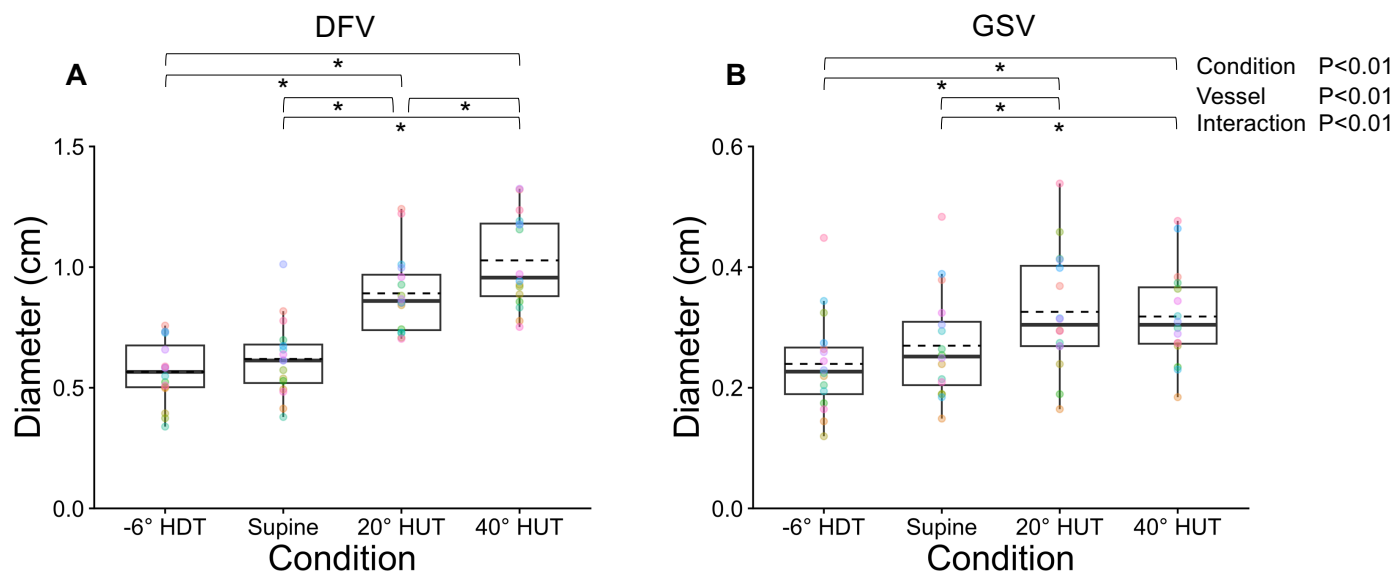


Figure 4-3: Mean vessel diameter for separate leg veins in a veno-arterial bundle (DFV) or not (GSV) during progressive body tilting.

Boxes encapsulate 1st to 3rd quartile, with mean (dashed line) and median (solid line). Whiskers extend to data within 1.5*IQR. Individual data shown (N = 16). Changes assessed by 4x2 repeated measures ANOVA. Pairwise comparisons: Bonferroni-Holm adjustment for multiple comparisons. * P<0.05. DFV; Deep femoral vein, GSV; Greater saphenous vein, HDT; Head-down tilt, HUT; Head-up tilt.

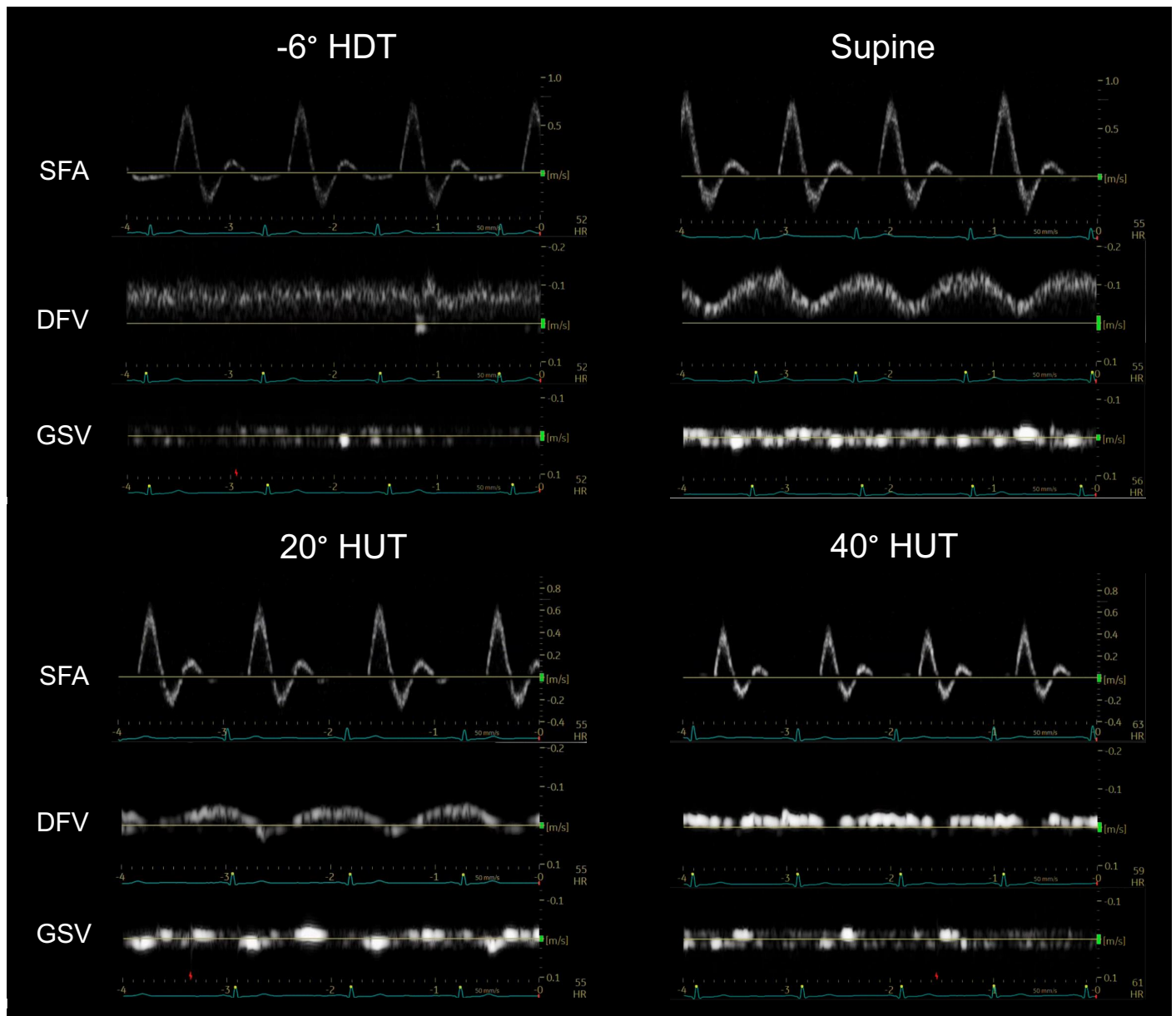


Figure 4-4: Representative Doppler tracings of a 30-year-old male participant. In each condition, tracings are presented for each vessel by aligning electrocardiogram R-waves.

SFA; Superficial femoral artery, DFV; Deep femoral vein, GSV; Greater saphenous vein, HDT; Head-down tilt, HUT; Head-up tilt.

Supplemental video 4-1: Veno-arterial bundles and valve recruitment during head-up tilt.

<https://www.doi.org/10.6084/m9.figshare.23556327>

4.4 Discussion

This investigation sought to provide *in vivo* evidence for the potential role of an arterial pump contributing to venous return within veno-arterial bundles. A previously noted factor controlling this mechanism is the venous driving pressure, and thus valve state. To evaluate the presence of an arterial pump across different driving pressures, we manipulated body position and thus the hydrostatic fluid column to recruit venous valves and examine differences in blood flow changes between veno-arterial bundled and non-bundled veins. Our data support the potential role of an arterial pump mechanism contributing to venous return demonstrated by preservation of venous flow in the DFV (bundled) compared to reduced flow in the GSV (unbundled at the same hydrostatic level) when body position increased to 40° HUT. While the data support the arterial pump theory, our observations are somewhat limited in a human model for investigating an ostensibly small contribution to venous return. Therefore, our data highlight the *plausibility* of the mechanism, which we discuss further below.

Plausibility of an 'arterial pump' in the control of venous return

In the absence of muscle contractions, venous blood flow in the periphery is commonly described as monophasic or phasic with the respiratory cycle (62, 193). However, the concept of venous pulsatility linked to the higher frequency arterial pulse is often discounted, based on original investigations of unvarying pressure waveforms and monophasic ultrasonographic blood velocities (194). Consequently, the prevailing dogma has been a description of steady venous flow by extensive arterial pressure dampening through the microvasculature (23), though this has been challenged in recent years (37). Key to our question, direct mechanical transmission of pulsatile energy in veno-arterial bundles was recently demonstrated to occur *ex vivo* at higher distending pressures, when valves play an active role in venous flow (83). Our results support this hypothesis; we report preservation of bundled venous blood flow between 20° and 40° HUT (estimated 16.4-30.8 mmHg hydrostatic pressure) when venous driving pressure reached values aligned with those reported to be effective for valve recruitment (25-35 mmHg) by LeFevre and Sarvazyan, as well as others who have described pressure gradient dependency for pulsatile limb venous flow (195). This relationship was not present in the non-bundled GSV in our study, showing stepwise reductions in venous blood flow as vertical tilt increased. These findings support the notion of an arterial pump along the lower-limb, where valves are abundant and actively involved in flow regulation, such that veno-arterial bundled veins might receive mechanical energy transmission along their length from arteries. There is not currently evidence for where this energy

transmission may occur. Similar to the muscle pump, upstream coupled venous compression may summate in higher transmural pressure between upstream valve segments, increasing flow within a range of venous driving pressures at the measurement site. Similarly downstream arterial recoil may generate more negative pressures, driving flow from the periphery. Finally, arterial pulsation may non-discriminately displace blood towards recruited valves, loading a venous reservoir to enhance the next venous flow cycle. Regardless, a possible veno-arterial pump effect is temporally displaced from the arterial cycle, as evidenced by a lag between SFA and DFV waveform peaks (Fig. 4-4). Additional replication attempts would be well served to describe the mechanical coupling behind this potential venous return mechanism, which fall beyond the scope of this report.

Other recent investigations have identified pulsatility in veins, specifically perforators of the lower-limb; however, this response was not attributed to an arterial pump (37). Rather, once subjects were heated to an increased core temperature, the authors determined that venous pulsatility was present in non-bundled veins residing near the capillaries in the vascular tree, compared to large veins. Thus, transmission of the arterial pulse through a dilated capillary network was concluded to be the likely candidate for pulsatility observations. These results are informative in our growing understanding of venous physiology; however, it is unlikely that this mechanism was present in our investigation, as we did not intentionally dilate capillary networks and studied large veins far from capillary transmitted forces. In fact, net arterial inflow and peak blood velocities were reduced with increasing levels of body tilt. Given our experimental controls eliminating potential contributors to venous transmural pressures (end-expiratory apneas, strict muscle inactivity), our results support the plausibility of an arterial pump in humans.

Methodological considerations

Given the aim of replicating *ex vivo* results in humans, there are some inherent considerations warranting discussion. Neither increased vessel diameter nor sympathetic outflow were intended effects of our tilting conditions; however, they were expected and a necessary concession to manipulate venous valve status. Both the GSV and DFV diameters increased as these compliant vessels developed larger transmural pressures associated with increasing tilt from supine; however, only the DFV experienced further increases from 20° to 40° HUT. This discordance in diameter changes could be interpreted as an explanatory factor for the preservation of blood flow in the DFV and not the GSV, leading to our arterial pump effect conclusion. However, we caution this interpretation as blood velocity changes must also be considered, and the GSV did experience a 46% decrease in blood velocity, whereas DFV velocity only reduced by 28% at the

40° HUT condition. This diameter observation may also be a direct consequence of the arterial pump effect, since venous diameter will increase to accommodate higher fluid flow rates (196). Due to the difference in vessel sizes, the GSV also experienced lower absolute blood flow. While interpretation of the relative changes in blood flow support our hypothesis, comparing changes in different caliber vessels was a limitation of our *in vivo* design. Different vessels also have different drainage pathways, whereby the DFV primarily drains muscle and GSV drains skin; however, by eliminating active muscle work and maintaining neutral room temperatures, this difference should be negligible. Furthermore, it is possible that a component of the cyclic waves observed in DFV could originate from the right heart; however, this is unlikely due to the distance and passage through the diaphragm, unlike the close relationship between IJV and right atrial pressures.

In terms of sympathetic outflow changes, there were expected increases in blood pressure and heart rate, particularly at the 40° HUT condition. Increased sympathetic activity will cause venoconstriction to accelerate venous velocity by increasing wall tension, which is likely present in our data; however, this reflex venoconstriction signal affects both bundled and non-bundled veins similarly (34, 35). Furthermore, despite the observed changes in heart rate, it is unlikely that cardiac output was increased and causing increased lower-limb venous flow. During HUT, cardiac stroke volume is reduced due to venous pooling and heart rate must increase to maintain cardiac output (23). Measured reductions in lower-limb arterial inflow do not suggest an effect of increased cardiac output. Rather, it is possible that the increased heart rate contributed more of the arterial pump effect, similar to the effect described by LeFevre and Sarvazyan; however, our observed change in heart rate was minimal ($<10 \text{ beats} \cdot \text{min}^{-1}$), compared with the strong effects of $+70 \text{ beats} \cdot \text{min}^{-1}$ indicated by LeFevre and Sarvazyan. Finally, an aim of the pre-registered study design included analysis of SFA radial displacement and blood pressure relationships to DFV flow; however, preliminary analysis suggested a lack of measurement sensitivity to detect differences, given sensitivity of $<0.02 \text{ mm}$ for radial displacement. Acknowledging these limitations associated with human *in vivo* collection under physiological conditions, definitive mechanistic evidence is very challenging, thus we offer support for further investigation.

Perspectives and significance

The arterial pump effect has not been established as a contributor to venous return in humans; however, with these data, we suggest that it may represent a small additional mechanism of venous return through a specific range of driving pressures. The unique anatomical configuration of veno-arterial bundles positions the large veins directly adjacent to pulsating arteries, allowing

mechanical energy transmission and conserving energy in the cardiovascular system. We do note our study intentionally eliminated major energy inputs from respiratory and muscle pumps, thus functional relevance under normal conditions is unknown. Discovery of this mechanism's input to venous return of stressed volume in the lower-limb suggests a comprehensive investigation of contributions by each venous return mechanism *in vivo* is warranted. Accurately attributing the inputs of different venous pump mechanisms (muscle, respiratory, and arterial) is important for a complete understanding of venous physiology, ultimately informing interventions for venous dysfunction.

Further elucidation of the factors contributing to the arterial pump mechanism is also necessary and requires consideration of arterial and venous properties. First, arterial pressure wave properties, including the presence of hypotension, hypertension, and arterial stiffness, could all contribute to the magnitude of energy transmitted within the veno-arterial bundle. It is currently unknown how blood pressure and arterial stiffness influence this mechanism, particularly in clinical populations where clearance of venous volume is important to avoid venous thrombi. Additionally, venous valve competency is critical in the proper functioning of the proposed arterial pump. We did not examine participants with diagnosed venous insufficiency complications because the proposed mechanism relies on the active contribution of venous valves. Thus, it remains to be determined whether this mechanism exists in patients with chronic venous insufficiency or valvular reflux, as they require much higher transmural pressures to drive venous flow toward the heart.

Conclusion

This investigation provides *in vivo* supporting evidence for a plausible arterial pump mechanism contributing to venous return in veno-arterial bundled veins in humans. Support of this effect was found through tilt-induced venous driving pressure manipulation to influence valve state in both bundled and non-bundled lower-limb veins. Our results suggest mechanical energy transmission from artery to vein in veno-arterial bundles along the limb's length, raising transmural pressure like the muscle pump, and preserving venous blood flow in the face of increasing gravitational challenge. The absolute magnitude of this mechanism remains to be determined, and likely depends on venous pressure gradients, fluid status, and vascular disease status, including venous insufficiency. Thus, caution is warranted in the applicability of these findings at this time, yet it remains an interesting area for further investigation, and a comprehensive understanding of venous return mechanisms contributions against stressed volumes is necessary.

Chapter 5: The influence of aging on graded peripheral venous return and complex flow features through human veins

5.1 Introduction

Aging is an unavoidable process with accumulated effects on venous structure and function, which can manifest to clinical conditions (127, 197–199). Decreases in venous compliance and the emergence of valve dysfunction are among the common precipitating factors of venous dysfunction in older adults (OA). These characteristic traits of venous aging likely contribute to the development of venous pathologies, including chronic venous insufficiency and thrombosis (160). Venous function is especially relevant while under orthostatic stress, when blood translocation to the lower-limb veins creates a volume-stressed condition, engaging the compliant structural properties of both small and large veins (7, 200, 201). Normal venous valve function in conjunction with the muscle pump becomes critical to return blood flow to the central circulation and minimize thrombotic risk (202). The muscle pump remains one of the most widely studied mechanisms in human venous physiology for its role in venous return, particularly during exercise (59, 67, 203). Functioning with the basic principle of transmural pressure generation between the surrounding contracting muscle and venous lumen, the absolute force of muscle contraction is meaningful and contributes to the transmural pressure and generation of venous blood movement towards the right atrium (66, 67, 129). The current use of Doppler ultrasound systems and limb volume measurements enables a precise assessment of venous blood flow in response to physiological stressors, including muscle contractions, and can be used to define the muscle pump's capacity under various volume stressors (201). These data have largely been derived from young, healthy individuals, and it remains unclear how aging influences the muscle pump's efficiency under varying volume loads.

Despite this relevance, the venous phenotype of the aging lower-limb, including its response to physiological stress and ability to support muscle pump-mediated return, remains poorly characterized. This gap limits our understanding of whether and how age-related declines in strength and venous compliance compromise venous return and increase susceptibility to insufficiency. Additionally, fluid flow features and endothelial dysfunction have previously been implicated in the pathophysiology of thrombosis development and may precede clinical manifestations (100, 163, 204, 205). However, the description of venous fluid flow features during volume stress has been challenging to acquire due to the technological restrictions of dynamic imaging (201). Vector flow ultrasound imaging (VFI) offers high temporal and spatial resolution,

enabling the resolution of blood flow patterns without insonation angle dependencies (18, 19). This has allowed for detailed descriptions of intra-beat blood flow dynamics, facilitating a better understanding of blood flow regulation and the clinical consequences of vascular disease (206, 207). These works have since described the existence of complex and recirculatory fluid flow patterns in veins of YA during volume stress conditions (111, 208), raising interest to investigate whether these patterns differ in an OA population at greater risk for venous dysfunction with expected differences in venous structure. Thus, the purpose of this investigation was to characterize lower-limb muscle pump activity and venous blood flow dynamics under two volume stress conditions in healthy YA and OA. We hypothesized that increased venous volume stress and older age would interact to produce greater recirculatory or low-flow conditions, indicative of reduced muscle pump efficiency in OA.

5.2 Methods

Participants

A total of 29 participants were enrolled in this study (14 YA (7 females) and 15 OA (9 females)). One OA was excluded prior to beginning the protocol based on presentation of stage II hypertension, leaving 28 participants for analysis. Previous investigations comparing venous outcomes in YA and OA have considered 8-15 participants per group, finding large effect sizes (Cohen's $d > 1.5$) (127, 197). Based on these results, simulation-based power calculations for a multifactorial design with age (YA vs OA) and volume stress (loaded vs unloaded) indicated that enrolling 10 participants per age group would provide >80% power at $\alpha=0.05$ to detect interactions in muscle pump efficiency across volume stress conditions. An additional 4 participants per group were collected to account for data loss with VFI.

Healthy YAs were <35 years old, whereas OAs were >65 years old and recruited from the local community. All participants were screened for cardiovascular disease, including venous insufficiency or history of thrombosis. Physical activity history was collected through self-report; participants achieving more than 30 minutes of moderate-to-vigorous activity per week were included to avoid any accentuated effects of aging associated with extreme inactivity. Presentation or report of stage II hypertension, diagnosed venous insufficiency, or previous venous thrombosis were exclusionary. All participants were asked to avoid strenuous exercise, alcohol, caffeine and recreational drugs for 12h and large meals for 3h prior to attending the laboratory. Adequate fluid status was enforced by drinking ~500mL of water upon waking on the

testing day. The study design underwent review by the University of Waterloo Office of Research Ethics (#46018) and complies with the Declaration of Helsinki for data collection involving human participants. The study design was pre-registered: <https://osf.io/nzh6a>.

Study design

Following anthropometric assessment, participants rested quietly on a tilt table (Colin Medical, San Antonio, USA) positioned at -3° HDT to induce mild gravitational assist to venous flow for 15 min ahead of baseline limb volume, lower-leg muscle mass, blood pressure, and heart rate assessment. During this time, landmarking of lower-limb vessels was also completed including mapping the femoral vein (FV) in the right thigh (15 ± 3 cm superior of the patella). Following landmarking and collection of resting measures, participants performed a unilateral maximal voluntary calf contraction (MVC) with adequate rest between three attempts until a peak was determined using a custom isometric plantarflexion device capable of relaying live force data to the participant and researchers. Participants were coached to refrain from activating quadriceps muscles and only generate force through the ankle and toes. A practice period was then allowed to perform a 1s 50% MVC plantarflexion contraction. Experimental plantarflexion contractions were then performed as individual contractions separated by a minimum of 90s to allow venous refilling to occur. The 50% MVC condition was performed four times to allow for repeated measures with both the conventional ultrasound and the VFI unit. The target force was then changed to 8 Kg and the plantarflexion protocol was repeated. For participants with sufficient strength, a third force condition was included of 20 Kg; however, only 4 younger males completed this condition. Participants were then tilted to 20° HUT to induce mild venous pooling in the lower-body without generating significant central hemodynamic effects for a minimum of 5 min before baseline measures were collected and the plantarflexion protocol was repeated with the same force targets. For all ultrasound measures, participants were asked to perform an end-expiratory apnea to eliminate confounding effects of the respiratory or splanchnic pumps. Ultrasound collection began 1 s after apnea initiation and lasted 4-10 s. Representative time-aligned data and study design features are captured in Fig. 5-1, demonstrating time-aligned relationships of muscle force, ultrasound and limb volume measurements.

Valvular reflux was assessed in the supine position using a simple Valsalva maneuver to confirm healthy valve function. Reflux in the FV was defined as retrograde flow lasting more than 1 second during Valsalva (209). Clinical reflux was not identified in any recruited participants. Finally, ultrasonographic calf muscle thickness was recorded at a distance 30% between the

fibular head and lateral malleolus, following previous guidelines (210). Muscle thickness was measured using online calipers by measuring the distance from the muscle-adipose interface to the tibia.

Blood vessel sonography

FV blood flow was collected with a conventional commercial ultrasound machine (Vivid S70N, GE Healthcare, Chicago, USA) using a linear array probe (9L, GE Healthcare). Blood velocity and vessel diameter were collected in duplex mode for 10s for each measurement condition (~6cm depth, ~19 fps). Scanning location was kept consistent using washable ink markings on the skin surface and deliberately avoided the presence of valves. Settings for each scan were optimized by minimizing wall filters and maximizing velocity scales when appropriate to capture flow under baseline and contraction conditions. The velocity sample gate was extended to capture both superior and inferior vessel walls. Additionally, care was taken to minimize pressure application to the vein by using a thick layer of ultrasound gel and minimal external pressure. Offline analysis of blood flow used open-source software (FloWave.US) to extract raw intensity-weighted Doppler information for the determination of MBV (168). Venous diameters were determined prior to the contraction phase, using online ultrasound calipers by the same observer. As previously described, blood flow through a linear portion of the vein was quantified by $\dot{Q} \text{ (mL} \cdot \text{min}^{-1}) = \pi \cdot r^2 \cdot \text{MBV} \cdot 60$ (111). From $\dot{Q}$, the volume pumped was determined by calculating the area under the curve for the contraction phase to achieve a volumetric change. Additionally, the time to 95% peak flow rate and the flow rate of change between 25-75% of the peak flow were determined to account for any differences in the rate of force production between individuals or conditions. The sonographer's intra-rater reliability for repeat venous diameter and MBV measures is excellent ($\text{ICC}(3,1) > 0.90$) (84, 111).

Vector flow imaging

All ultrasound scans were repeated with a vector flow imaging platform (US4r ultrasound, US4US, Warsaw, Poland) using a 13-3 MHz linear array probe (Esaote SL1543). Data were collected in duplicate, 4s sequences during an end-expiratory apnea, using a custom plane wave imaging sequence with transmission angles of 10° and -10° and, receiving angles of 10°, 0°, -10° facilitating multi-angle vector Doppler at 6000 Hz. Custom beamforming analysis with a least-squares fitting approach facilitated angle-independent velocity estimates for axial and lateral

components for the full-field of view (170). Custom radiofrequency processing follows methods previously described in detail elsewhere (106, 111). The resulting datasets yielded pixel-level estimates of blood velocity magnitude and direction at an effective frame rate of 750 frames per second.

One muscle contraction cycle was identified from the velocity information, and a region of interest was identified along the walls of the FV, where the flow signal was adequately detected. Flow features were processed as means and peaks over the period of muscle force generation using axial and lateral velocity components, computing mean blood velocity, flow divergence, flow vorticity, shear rate, kinetic energy, Reynold's stress, and VCC. VCC is an established VFI outcome, representing the dispersion of velocity vectors in a region of interest, where a value of 1.0 indicates all vectors are moving in the same direction, and a value of 0.0 indicates all vectors are perfectly dispersed. With the exception of mean blood velocity and VCC, which have been previously measured by our group (106, 109, 111), additional quantitative outcomes were exploratory and described in Table 5-1.

Vector videos were rendered by applying the vector projectile imaging technique (104) to add qualitative substance to the data and were manually inspected. Inspection of projectile images was completed by the same observer, inspecting each contraction cycle for recurring flow profiles not identifiable with quantitative outcomes. Expected patterns included evidence of flow recirculation (retrograde), flow swirling, and extremely low-flow states (defined by areas of low power). Upon extraction of qualitative flow characteristics, observations were compared to quantitative outcomes to generate flow profile insights.

Muscle pump efficiency testing

To assess the efficiency of the muscle pump venous return mechanism, participants were instrumented with a strain gauge plethysmography system utilizing indium-gallium strain gauges (EC6, Hokanson, Bellevue, USA) to track changes in lower-leg volume. Muscle pump efficiency was interpreted as the volume of blood expelled per unit of force produced. The maximum circumference of the right calf muscle belly was measured using a surface tape measure, and an appropriately sized strain gauge was then individually selected to be 2 cm smaller than this measurement, following manufacturer recommendations. The strain gauge was double-looped and secured with medical tape, and the lower leg was positioned to ensure that no external pressure was applied against the strain gauge. Limb volume changes were continuously

measured as a percent change throughout the protocol. Baseline anthropometric limb volume estimates were made using a three-point circumference cylinder model for the thigh and shank individually, adapted from previously validated models (211).

When processing limb volume data, the time period of plantarflexor force generation was identified and a change in limb volume following the contraction was calculated. Programmatic curve fitting was also applied to address the rate of venous refilling within the lower leg in the first six seconds following the limb volume nadir. Briefly, data were inverted for power and linear decay model fitting and whichever model reported a higher R^2 value was selected, and lambda estimates were recorded to describe the rate of venous refilling observed. Shank volume estimates from anthropometric measures were used to determine ejection volume and ejection fraction to describe the muscle pump effectiveness in absolute volume units (mL).

Statistical analysis

Since all contraction conditions were collected in duplicate, repeated data were averaged, unless mean blood velocity or change in limb volume were >30% different, then the higher value was recorded for that condition. Quantitative results of this investigation were analyzed in R v4.4 (171) by 2x2 repeated measures ANOVA with factors of volume stress (-3° and +20° levels of tilt) and group (older and younger) separately for each contraction condition (8 Kg and 50% MVC) for strain gauge muscle pump efficiency metrics, blood flow, and VFI derived quantitative outcomes. Significant main effects were probed for differences using paired Student's t-tests with Bonferroni-Holm *post hoc* corrections for multiple comparisons. The magnitude of change from HDT to HUT was also evaluated via independent samples Student's t-tests where appropriate. Additionally, Pearson correlations were used to relate measures of muscle pump capacity and VFI outcomes. Statistical significance was set *a priori* at $\alpha = 0.05$. The qualitative outcomes from VPI ultrasound videos are described and representative images are presented as supporting evidence in our arguments.

5.3 Results

All participants completed the study procedures without adverse events. Younger adults were 50 yrs younger and 7 cm taller ($P=0.03$) compared to OA with no differences in body mass or BMI ($P=0.73$ and $P=0.09$, respectively). There were no differences between groups for estimated shank, thigh, or leg volume ($P>0.34$). There were also no group differences in foot length

($P=0.18$), which could have influenced the moment arm of force application onto the plantarflexor force transducer. Younger adults had a stronger MVC (+10 Kg; $P=0.01$) and thicker calf muscles (+13 mm; $P<0.01$) (Table 5-2). Younger adults achieved 16.4 ± 5.4 Kg and OA achieved 11.2 ± 4.1 Kg forces during the 50% MVC trials, whereas YA and OA pushed 8.9 ± 1.2 Kg and 8.4 ± 1.4 Kg, respectively, during 8 Kg force targets.

Resting blood pressure and heart rate

There was a main effect of group for resting systolic and mean arterial blood pressure (both $P<0.01$; $\eta_p^2=0.43$ and 0.33), whereby OA presented with +22 mmHg and +11 mmHg, respectively. Comparison of diastolic blood pressure only revealed a main effect of tilt such that participants decreased 3 mmHg from HDT to HUT postures ($P<0.01$; $\eta_p^2=0.36$). There were no group differences or changes in heart rate, where HDT and HUT mean heart rates were 62 ± 8 and 62 ± 8 beats $\cdot\text{min}^{-1}$, respectively.

Clinical ultrasound blood flow

FV diameters were not different between groups in the HDT position (YA: 0.64 ± 0.19 cm, OA: 0.66 ± 0.23 cm) and both groups increased in response to the HUT position (YA: $+41.3\pm22.7$ %, OA: $+23.0\pm10.9$ %; $P<0.01$, $\eta_p^2=0.85$); however, YA increased to a greater extent compared to OA ($P=0.01$, $\eta_p^2=0.20$), consistent with reduced venous compliance in OA (Fig. 5-2). Peak blood flow for both contraction intensities only demonstrated main effects of tilt position, increasing from 586 ± 613 to 1693 ± 1011 mL $\cdot\text{min}^{-1}$ during 8 Kg contractions and 764 ± 712 to 1954 ± 1111 mL $\cdot\text{min}^{-1}$ during 50% MVC contractions (both $P<0.01$, $\eta_p^2>0.69$). Following the same trend, ultrasound-derived volume of blood pumped for each contraction intensity increased as a function of tilt position (both $P<0.01$, $\eta_p^2>0.25$) increasing from 11.2 ± 16.2 to 35.0 ± 30.6 mL during 8 Kg contractions and 16.2 ± 18.2 mL to 33.5 ± 29.4 mL during 50% MVC contractions. Alternative descriptors of blood flow properties increased during HUT positions at both contraction intensities ($P<0.05$), including the time to 95% peak flow and the rate of change in blood flow between 25–75% of peak flow. The only exception was the time to 95% peak flow at 50% MVC, which remained unchanged across tilt conditions (0.35 ± 0.27 s vs 0.41 ± 0.24 s; $P=0.43$, $\eta_p^2=0.02$). There were no group level differences for any alternative blood flow related outcomes.

Muscle pump efficiency

The muscle pump's contribution to limb volume change as measured by strain gauge plethysmography is described by a percent change in cross-sectional limb volume (Fig. 5-1). For each contraction intensity, there was a main effect of body position, increasing the limb volume change observed from HDT: 0.92 ± 0.56 % to HUT: 1.64 ± 0.70 % for 8 Kg and HDT: 1.04 ± 0.61 % to HUT: 1.76 ± 0.83 % at 50% MVC condition (both $P < 0.01$, $\eta_p^2 > 0.54$), consistent with more fluid to be pumped with increased venous pooling. After converting to absolute volumes, these changes aligned with estimated volume changes of 59.5 ± 36.8 mL and 68.7 ± 44.3 mL at HDT to 108.0 ± 55.7 mL and 116.0 ± 69.0 mL at HUT for 8 Kg and 50% MVC conditions, respectively (both $P < 0.01$, $\eta_p^2 > 0.50$) (Fig. 5-3). A group by tilt interaction was detected for percent change and estimated limb volume change ($P = 0.02$ and $P = 0.04$, $\eta_p^2 = 0.17$ and $\eta_p^2 = 0.15$) for 50% MVC, such that OA demonstrated a greater increase in volume change compared to YA from HDT to HUT positions. No changes were detected in the rate of refilling as defined by the lambda estimate (All $P > 0.30$, $\eta_p^2 < 0.04$).

A subset of young males ($n = 4$) additionally completed a fixed 20 Kg load condition, providing supposition to the effect of stronger contraction forces on limb volume changes. Despite not having statistical inferences, a positive relationship between force production and change in limb volume was observed for these individuals. These data were not addressed statistically due to their lack of statistical power; however, they offer supplementary support for the primary findings by reinforcing the relationship between contraction intensity and venous volume shifts observed across the full cohort (Fig. 5-4).

Selected vector flow imaging output

A significant interaction effect was observed in the 8 Kg condition ($P = 0.03$, $\eta_p^2 = 0.18$), where mean VCC increased from 0.37 ± 0.20 in YA-HDT to 0.60 ± 0.11 in YA-HUT, while values in OA remained unchanged (0.55 ± 0.19 in OA-HDT to 0.60 ± 0.19 in OA-HUT). In contrast, at 50% MVC, no interaction effect was observed ($P = 0.58$); both groups showed similar increases in mean VCC with tilt: YA from 0.40 ± 0.15 (HDT) to 0.56 ± 0.18 (HUT), and OA from 0.44 ± 0.19 to 0.56 ± 0.18 . Peak VCC also increased with tilt ($P = 0.04$, $\eta_p^2 = 0.16$) suggesting greater flow uniformity with increased volume stress, with no group effects ($P = 0.92$) or interactions ($P = 0.41$).

Mean Reynolds stress during 8 Kg contractions was unaffected by tilt ($P = 0.12$) or group ($P = 0.32$) and no interaction was observed ($P = 0.57$). Similar patterns were seen at 50% MVC.

Peak Reynolds stress increased with tilt ($P<0.01$, $\eta_p^2=0.34$), with no group differences ($P=0.55$) at the 8 Kg intensity, whereas only a significant interaction effect was found at 50% MVC ($P=0.02$, $\eta_p^2=0.21$) such that OA decreased peak Reynold's stress at HUT and YA increased the metric.

Mean vorticity showed no group ($P=0.32$) or tilt ($P=0.12$) effects at 8 Kg. Similar trends were observed at 50% MVC. Peak vorticity decreased with tilt ($P=0.02$, $\eta_p^2=0.22$), with values at 8 Kg ranging from $100.52\pm52.70\text{ s}^{-1}$ in YA-HDT to $55.06\pm26.00\text{ s}^{-1}$ in YA-HUT. Similar trends were observed at 50% MVC, and no significant interaction effects were observed ($P=0.08$ and $P=0.54$). Additionally, we found a moderate correlation between peak vorticity and volume pumped for both OA and YA. The strength of the association was relatively greater in OA compared to YA (Pearson $R = -0.43$ vs Pearson $R = -0.32$; both $P<0.05$) (Fig. 5-5).

Mean shear rate increased with a main effect of tilt ($P<0.01$), whereas mean axial and lateral shear rates were higher in OA only during 8 Kg contractions (both $P=0.04$). Mean divergence and kinetic energy demonstrated no main effects or interactions (all $P>0.14$).

All mean and peak data are found in Table 5-3. Nearly all vector data outcomes demonstrated only a main effect of tilt for their peak values.

Qualitative descriptions of flow

Despite the range of quantitative VFI outcome metrics presented, the data also contains rich spatiotemporal information that is dynamic and not easily captured by mean values. To complement this, we qualitatively assessed vector projectile videos to characterize flow patterns across conditions and groups. During HDT, particularly in YA, flow signals were often weak during plantarflexion contractions, leading to signal dropout near vein walls and reflected in lower VCC metrics. Although recirculation was only observed in a subset of participants, HDT generally produced more prolonged antegrade flow with lower peak velocities, likely due to reduced volume stress. In contrast, HUT induced clear venous distension, and pre-contraction flow signals were nearly absent in both groups, consistent with low-flow conditions under increased volume stress. Notably, OA participants exhibited more homogeneous flow features during HUT, whereas YA maintained a more distinct boundary layer (Fig. 5-7). These patterns align with expected differences in venous compliance and suggest age-related variations in venous wall behaviour in response to pressure changes during plantarflexion and can be visualized in Supplemental Video 5-1.

Table 5-1: Description and calculation of vector flow imaging quantitative output metrics

Metric	Description	Calculation	Unit	Example
Vector Concentration Coefficient	Coherence of flow direction	$\sqrt{[(\text{mean}(\cos\theta))^2 + (\text{mean}(\sin\theta))^2]}$	Unitless (0 to 1)	Close to 1 in laminar, directionally consistent flow; lower in disturbed or complex flow fields
Divergence	Flow expansion or contraction	$(\partial v_x/\partial x) + (\partial v_y/\partial y)$	s ⁻¹	Positive in regions where fluid spreads outward (e.g., vein expansion)
Vorticity	Rotational or swirling motion	$(\partial v_y/\partial x) - (\partial v_x/\partial y)$	s ⁻¹	Elevated near curved vessel segments, junctions, or recirculating zones
Shear Rate	Velocity difference between adjacent fluid layers	$\sqrt{[(\partial v_x/\partial y - \partial v_y/\partial x)^2 + (\partial v_x/\partial x + \partial v_y/\partial y)^2]}$	s ⁻¹	Increases in zones of rapid velocity change, such as narrowing vessels or dynamic muscle contractions
Axial Shear Rate	Shear along flow direction	$(\partial v_x/\partial y) + (\partial v_y/\partial x)$	s ⁻¹	High in segments where forward flow gradients dominate
Lateral Shear Rate	Shear perpendicular to flow direction	$(\partial v_x/\partial x) - (\partial v_y/\partial y)$	s ⁻¹	Prominent in zones of transverse mixing or where vessel wall deformation redirects flow
Kinetic Energy	Flow energy	$0.5 \times \rho \times \text{area} \times (v_x^2 + v_y^2)$	g·cm ⁻¹ ·s ⁻² (dyne)	Increases during high-velocity inflow phases (e.g., muscle contractions)
Reynolds Stress	Turbulence via velocity fluctuation	$(v_x - \text{mean}(v_x)) \times (v_y - \text{mean}(v_y))$	cm ² ·s ⁻²	Peaks in disturbed or transitional flows, (e.g., rapid volume shifts or venous valve closure)

vx: Axial velocity component, aligned with the primary flow direction; **vy**: Lateral velocity component, perpendicular to the axial direction; **x**:

Axial spatial coordinate; **y**: Lateral spatial coordinate; ∂ : Partial derivative symbol; **ρ**: Density of blood (1.06 g·cm⁻³).

Table 5-2: Participant characteristics

Variable	Male	Female
<u>Younger adults</u>		
N	7	7
Age (yrs)	27 ± 4	25 ± 4
Height (cm)	176 ± 5	166 ± 7
Body mass (Kg)	75 ± 8	60 ± 4
Body mass index (Kg·m ⁻²)	24.2 ± 2.2	21.7 ± 1.8
Mean arterial blood pressure (mmHg)	87 ± 8	81 ± 11
Heart rate (beats·min ⁻¹)	59 ± 5	63 ± 13
Calf muscle thickness (mm)	68 ± 8	67 ± 10
Shank volume (L)	6.9 ± 1.0	6.2 ± 1.1
Plantarflexor strength (Kg)	36.2 ± 10.4	27.4 ± 10.4
50% plantarflexor strength (Kg)	18.1 ± 5.2	13.7 ± 5.2
<u>Older adults</u>		
N	5	9
Age (yrs)	74 ± 6	77 ± 6
Height (cm)	170 ± 10	161 ± 5
Body mass (Kg)	83 ± 20	62 ± 8
Body mass index (Kg·m ⁻²)	28.4 ± 5.0	23.9 ± 4.1
Mean arterial blood pressure (mmHg)	98 ± 4	95 ± 7
Heart rate (beats·min ⁻¹)	63 ± 6	63 ± 7
Calf muscle thickness (mm)	61 ± 11	51 ± 6
Shank volume (L)	7.1 ± 1.8	6.0 ± 0.9
Plantarflexor strength (Kg)	27.9 ± 10.4	18.7 ± 5.9
50% plantarflexor strength (Kg)	14.0 ± 5.2	9.3 ± 2.9
Data are represented as means±SD.		

Table 5-3: Quantitative mean and peak output metrics from vector flow imaging obtained during plantarflexion contraction cycles

Outcome	Contraction	YA		OA		Age	Tilt	Interaction
		<u>HDT</u>	<u>HUT</u>	<u>HDT</u>	<u>HUT</u>			
Mean blood velocity ($\text{cm}\cdot\text{s}^{-1}$)	8 Kg	23.91 $\pm$ 11.57	36.81 $\pm$ 6.37	27.98 $\pm$ 13.52	35.42 $\pm$ 10.41	0.701	<0.001*	0.266
	50% MVC	25.88 $\pm$ 8.74	37.69 $\pm$ 9.36	26.92 $\pm$ 12.81	33.44 $\pm$ 11.52	0.594	<0.001*	0.249
Mean VCC (a.u)	8 Kg	0.37 $\pm$ 0.20	0.60 $\pm$ 0.11	0.55 $\pm$ 0.19	0.60 $\pm$ 0.19	0.128	0.001*	0.031*
	50% MVC	0.40 $\pm$ 0.15	0.56 $\pm$ 0.18	0.44 $\pm$ 0.19	0.56 $\pm$ 0.18	0.662	<0.001*	0.580
Mean divergence (s^{-1})	8 Kg	0.63 $\pm$ 1.59	0.54 $\pm$ 1.29	1.17 $\pm$ 2.40	1.97 $\pm$ 2.18	0.050	0.532	0.441
	50% MVC	0.13 $\pm$ 1.58	1.53 $\pm$ 2.01	4.23 $\pm$ 10.18	2.30 $\pm$ 3.16	0.055	0.875	0.362
Mean vorticity (s^{-1})	8 Kg	-2.59 $\pm$ 6.56	-4.88 $\pm$ 2.56	-3.43 $\pm$ 11.88	-8.37 $\pm$ 8.54	0.320	0.124	0.563
	50% MVC	-2.48 $\pm$ 10.29	-3.07 $\pm$ 4.82	10.15 $\pm$ 14.61	-1.92 $\pm$ 9.64	0.034*	0.029*	0.047*
Mean shear rate (s^{-1})	8 Kg	3654.47 $\pm$ 804.93	2584.32 $\pm$ 601.84	3152.61 $\pm$ 1100.65	2661.52 $\pm$ 1185.94	0.526	<0.001*	0.086
	50% MVC	3591.41 $\pm$ 651.54	2947.12 $\pm$ 670.94	3625.00 $\pm$ 1045.49	2903.09 $\pm$ 1059.01	0.922	0.003*	0.764
Mean axial shear rate (s^{-1})	8 Kg	0.52 $\pm$ 1.50	0.46 $\pm$ 1.16	1.16 $\pm$ 2.45	1.81 $\pm$ 1.94	0.038*	0.584	0.513
	50% MVC	0.17 $\pm$ 1.52	1.38 $\pm$ 1.82	3.89 $\pm$ 9.31	2.17 $\pm$ 2.93	0.055	0.868	0.380
Mean lateral shear rate (s^{-1})	8 Kg	4.15 $\pm$ 7.82	5.49 $\pm$ 2.46	8.69 $\pm$ 9.73	10.37 $\pm$ 8.80	0.046*	0.453	0.933
	50% MVC	4.68 $\pm$ 12.43	5.82 $\pm$ 5.92	7.04 $\pm$ 15.70	4.30 $\pm$ 11.16	0.930	0.822	0.507
Mean kinetic energy ($\text{g}\cdot\text{cm}^{-1}\cdot\text{s}^{-2}$)	8 Kg	0.22 $\pm$ 0.04	0.20 $\pm$ 0.04	0.21 $\pm$ 0.08	0.21 $\pm$ 0.07	0.914	0.476	0.201
	50% MVC	0.22 $\pm$ 0.04	0.23 $\pm$ 0.04	0.23 $\pm$ 0.06	0.21 $\pm$ 0.05	0.608	0.799	0.139

Outcome	Contraction	YA		OA		Age	Tilt	Interaction
		<u>HDT</u>	<u>HUT</u>	<u>HDT</u>	<u>HUT</u>			
Mean Reynolds stress (cm ² ·s ⁻²)	8 Kg	-16.06 ± 33.76	3.17 ± 37.17	-21.87 ± 19.26	-12.80 ± 45.44	0.322	0.118	0.566
	50% MVC	-6.56 ± 32.35	14.08 ± 46.64	1.27 ± 26.82	-13.44 ± 37.25	0.488	0.900	0.081

Outcome	Contraction	YA		OA		Age	Tilt	Interaction
		<u>HDT</u>	<u>HUT</u>	<u>HDT</u>	<u>HUT</u>			
Peak velocity (cm·s ⁻¹)	8 Kg	45.62 ± 19.48	66.34 ± 11.30	50.10 ± 23.24	63.31 ± 12.16	0.888	<0.001*	0.407
	50% MVC	52.40 ± 17.24	72.55 ± 12.62	54.92 ± 23.28	61.18 ± 17.69	0.414	0.004*	0.106
Peak VCC (a.u)	8 Kg	0.98 ± 0.02	1.00 ± 0.00	0.99 ± 0.02	0.99 ± 0.01	0.915	0.040*	0.409
	50% MVC	0.99 ± 0.01	1.00 ± 0.00	0.99 ± 0.01	1.00 ± 0.01	0.610	0.038*	0.900
Peak divergence (s ⁻¹)	8 Kg	41.40 ± 29.01	23.75 ± 14.37	34.02 ± 24.39	28.16 ± 19.64	0.819	0.068	0.347
	50% MVC	42.78 ± 37.66	30.00 ± 20.17	46.19 ± 47.88	28.85 ± 14.96	0.900	0.049*	0.783
Peak vorticity (s ⁻¹)	8 Kg	100.52 ± 52.70	55.06 ± 26.00	66.42 ± 31.87	58.92 ± 37.51	0.189	0.017*	0.077
	50% MVC	105.95 ± 65.98	69.31 ± 32.61	117.75 ± 92.31	63.42 ± 24.78	0.882	0.005*	0.542
Peak shear rate (s ⁻¹)	8 Kg	4948.97 ± 305.06	4934.83 ± 312.19	5167.02 ± 452.11	4993.00 ± 514.66	0.283	0.326	0.403
	50% MVC	5003.13 ± 325.97	4988.11 ± 314.89	5190.75 ± 454.72	5061.58 ± 419.38	0.366	0.481	0.387
Peak axial shear rate (s ⁻¹)	8 Kg	38.20 ± 26.72	21.75 ± 13.05	31.33 ± 21.81	26.21 ± 17.91	0.838	0.066	0.322
	50% MVC	39.06 ± 33.86	27.49 ± 18.23	42.40 ± 43.89	26.40 ± 13.58	0.891	0.050*	0.769

Peak lateral shear rate (s ⁻¹)	8 Kg	112.76 ± 55.33	65.91 ± 30.45	92.14 ± 32.19	81.61 ± 41.29	0.848	0.009*	0.087
	50% MVC	121.09 ± 93.12	86.58 ± 39.54	122.44 ± 91.84	73.44 ± 31.50	0.786	0.018*	0.652
Peak kinetic energy (g·cm ⁻¹ ·s ⁻²)	8 Kg	0.30 ± 0.02	0.32 ± 0.07	0.34 ± 0.07	0.33 ± 0.06	0.147	0.684	0.286
	50% MVC	0.31 ± 0.03	0.36 ± 0.07	0.36 ± 0.08	0.33 ± 0.05	0.865	0.290	0.005*
Peak Reynolds stress (cm ² ·s ⁻²)	8 Kg	79.34 ± 54.18	147.02 ± 68.59	84.84 ± 51.21	118.93 ± 63.28	0.550	0.002*	0.255
	50% MVC	104.25 ± 47.55	185.10 ± 109.83	151.52 ± 86.09	119.66 ± 79.68	0.919	0.373	0.019*

Data are represented as means±SD. ANOVA output represents the Age x Tilt output within each contraction intensity. VCC, Vector concentration coefficient.

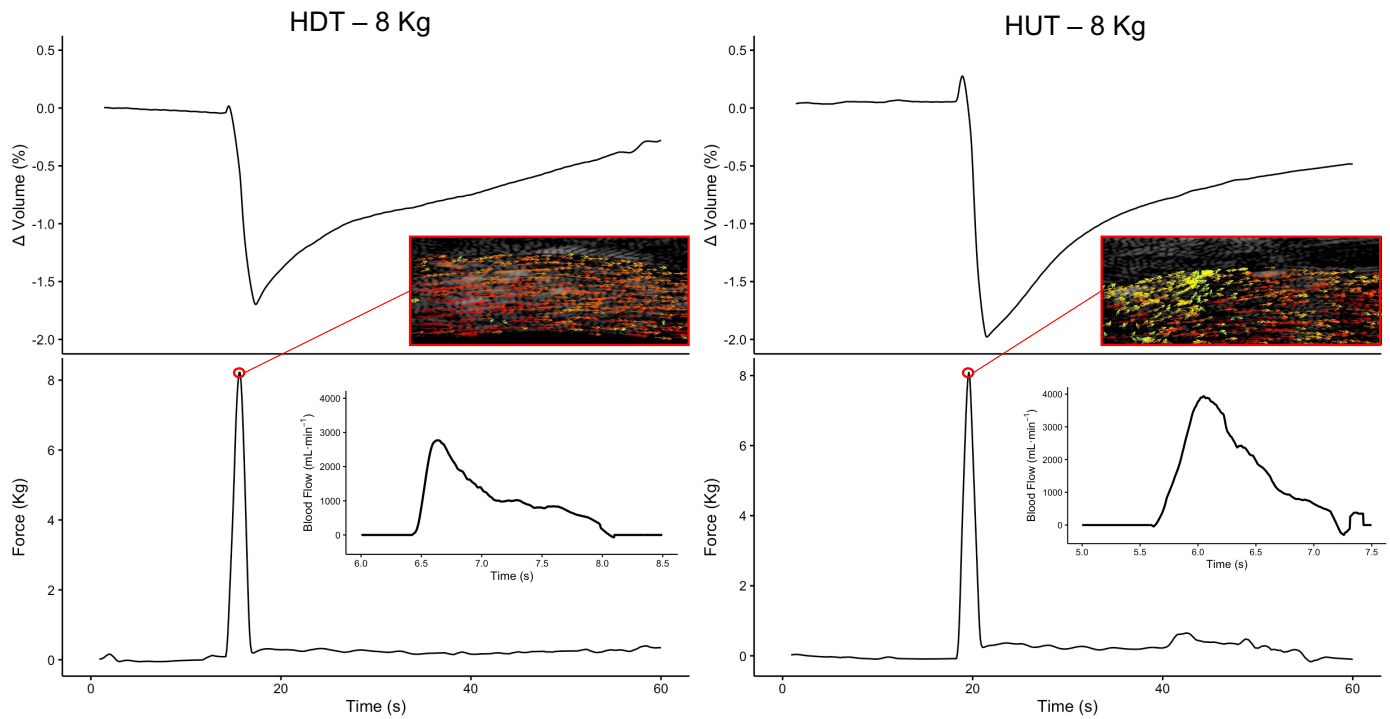


Figure 5-1: Representative data from a 33-year-old male demonstrating the relationship between calf volume change (top) and a 8 Kg plantarflexor contraction (bottom) during head-down tilt (left) and head-up tilt (right).

The corresponding blood flow tracing using conventional ultrasound is overlaid in the bottom panel and a snapshot of vector-projectile imaging demonstrating vector velocity and direction distribution during peak contraction is shown.

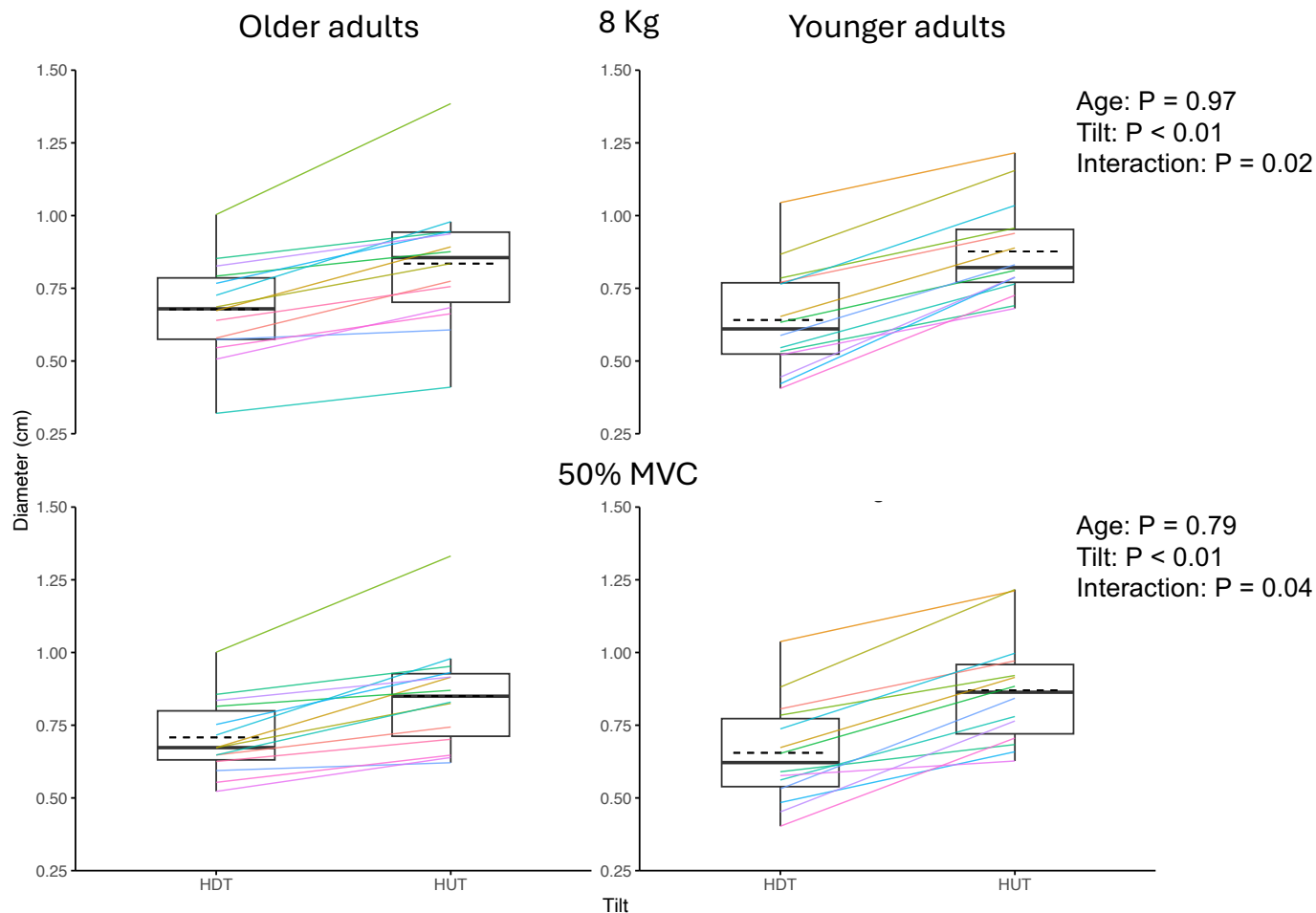


Figure 5-2: Femoral vein diameters prior to a single plantarflexion of 8 Kg (top) and 50% maximum voluntary contraction (bottom) in older (left) and younger (right) adults.

Box plots display the inter-quartile range, mean (dashed line), median (solid line) and whiskers display 1.5 times inter-quartile range. Coloured lines show individual participant responses to head-down and head-up tilt. ANOVA output represents the Age x Tilt output within each contraction intensity.

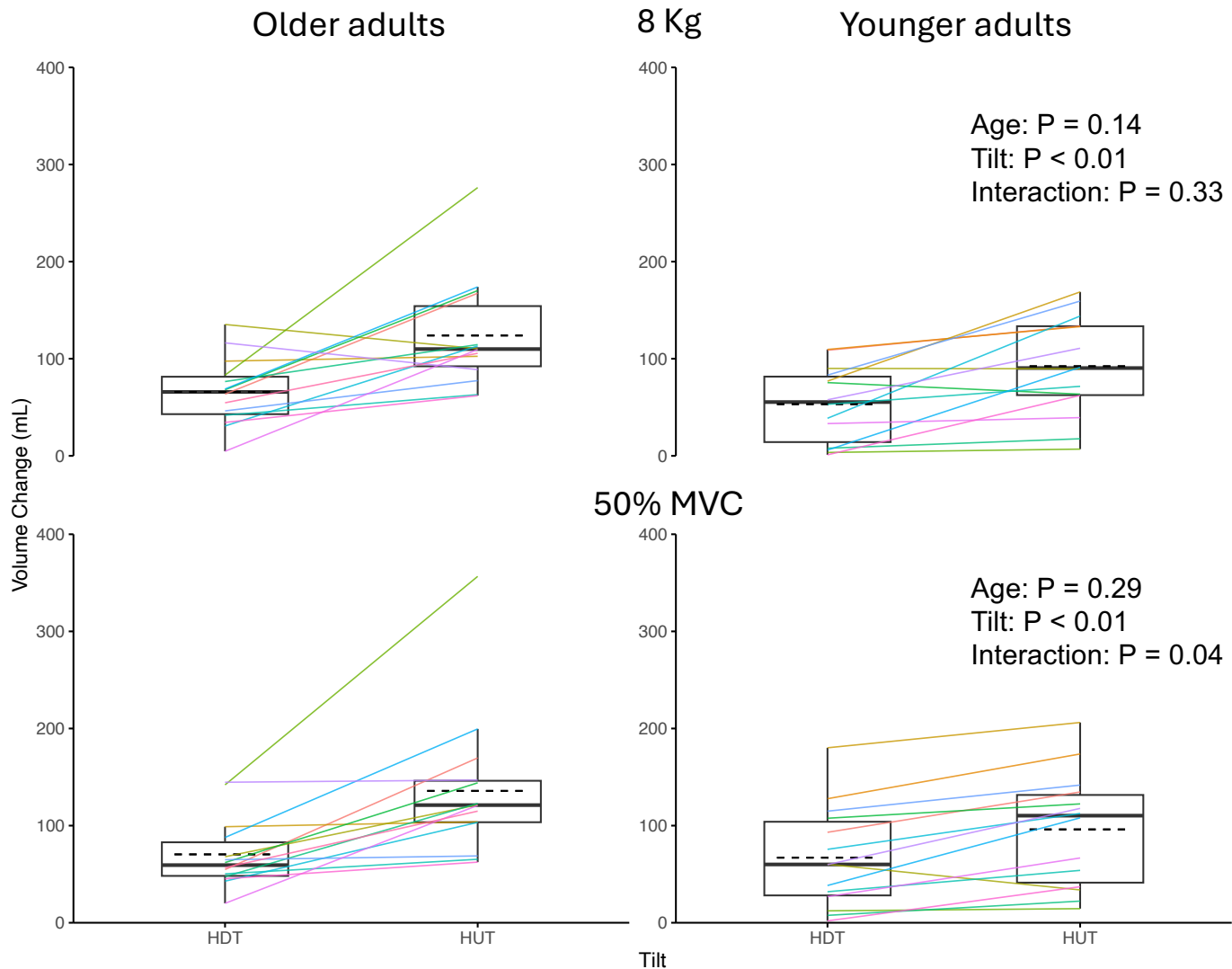


Figure 5-3: Strain-gauge plethysmography-based changes in shank volume following a single plantarflexion of 8 Kg (top) and 50% maximum voluntary contraction (bottom) in older (left) and younger (right) adults.

Box plots display the inter-quartile range, mean (dashed line), median (solid line) and whiskers display 1.5 times inter-quartile range. Coloured lines show individual participant responses to head-down and head-up tilt. ANOVA output represents the Age x Tilt output within each contraction intensity.

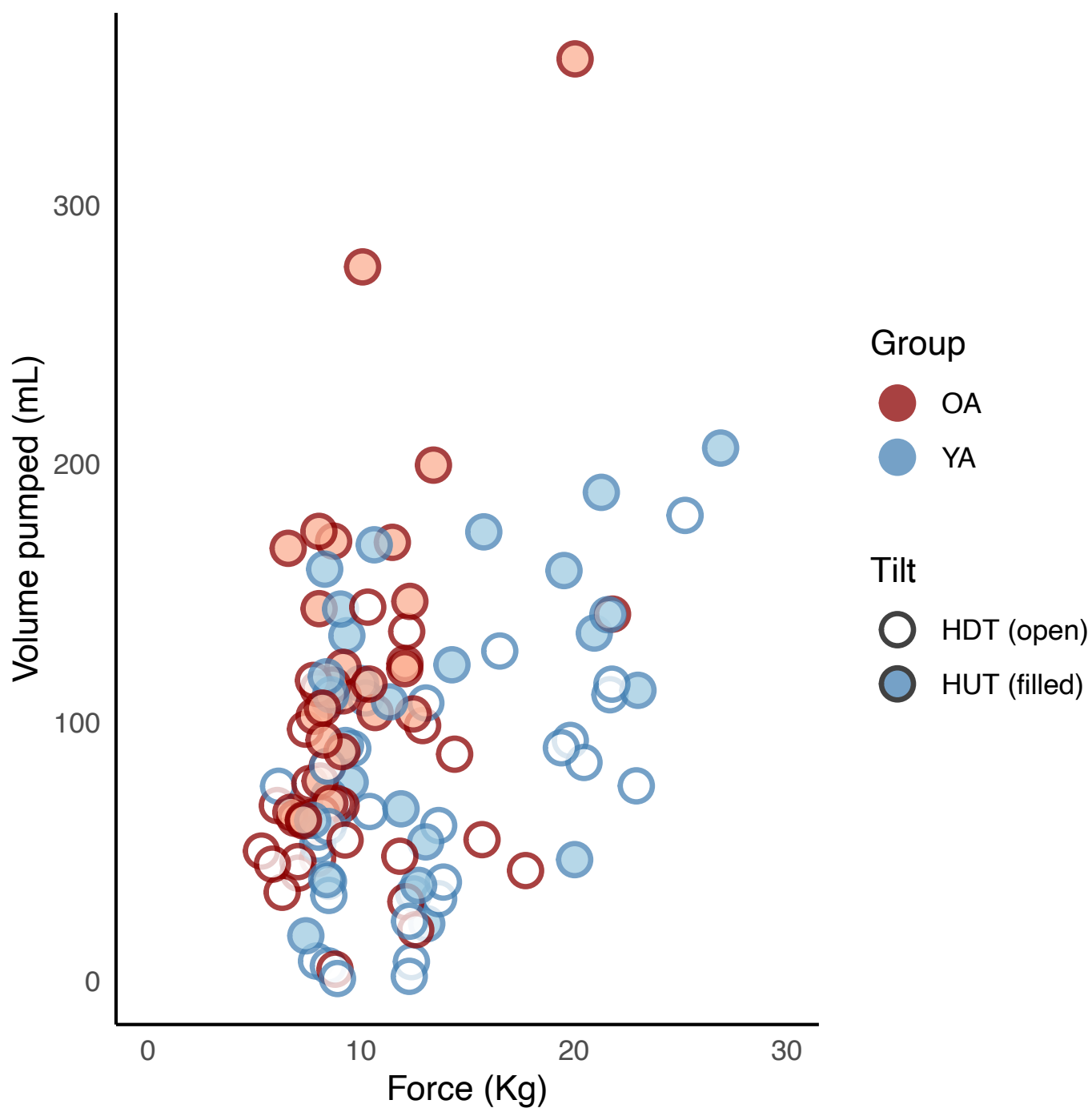


Figure 5-4: Scatterplot of plantarflexion contraction force and volume pumped for all conditions, including 20 Kg. Datapoints are designated by group and level of body tilt.

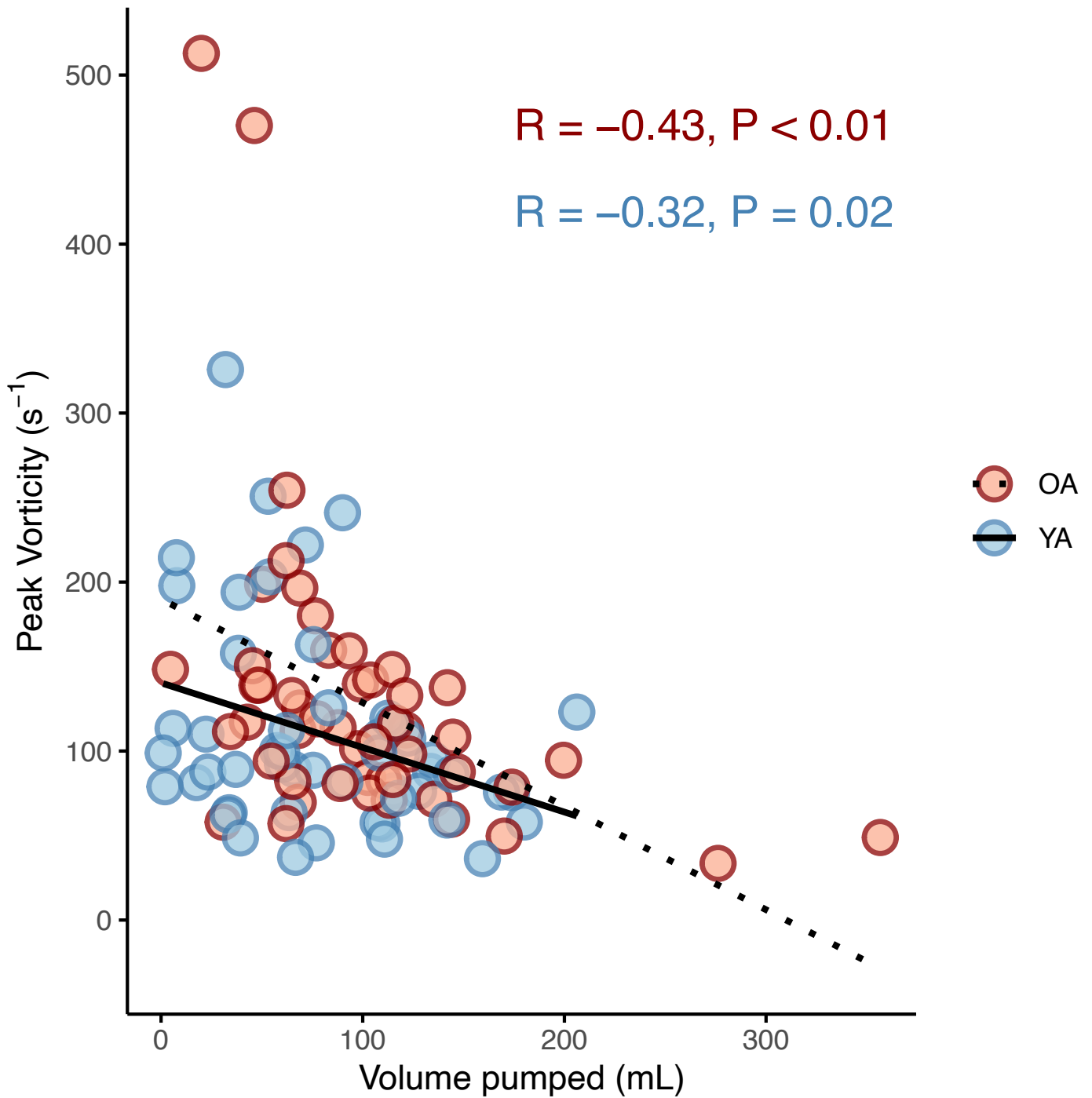


Figure 5-5: Relationship between volume pumped during a single plantarflexion contraction and the corresponding peak vorticity measure in older (red) and younger (blue) adults at all contraction intensities and body tilt conditions. Pearson correlation values are shown for older and younger adults.

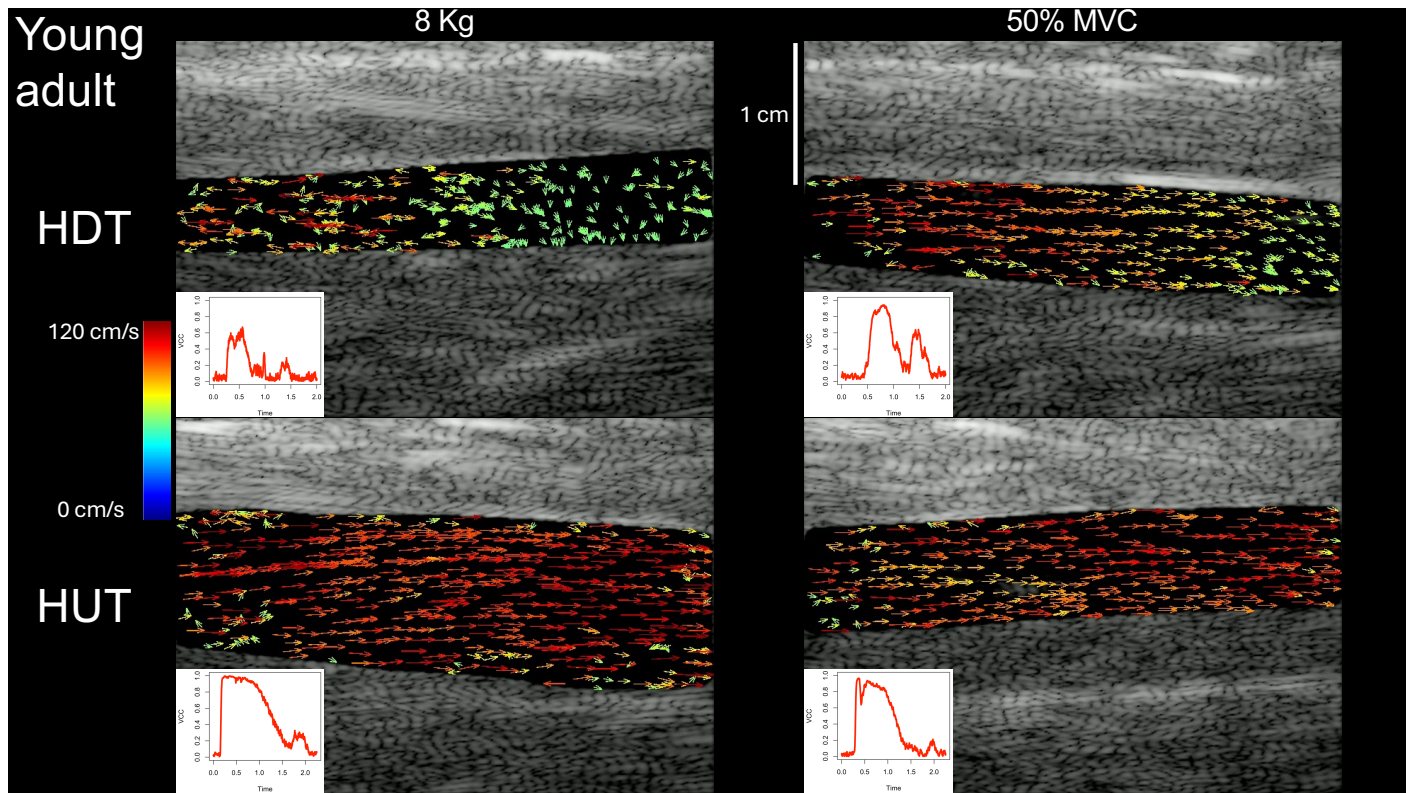


Figure 5-6: Representative stills from vector projectile videos during HDT and HUT and both contraction intensities in a 24yr old male.

The image is captured slightly past the peak of outflow velocity following muscle contraction. The corresponding vector concentration coefficient (VCC) tracing is displayed for each contraction.

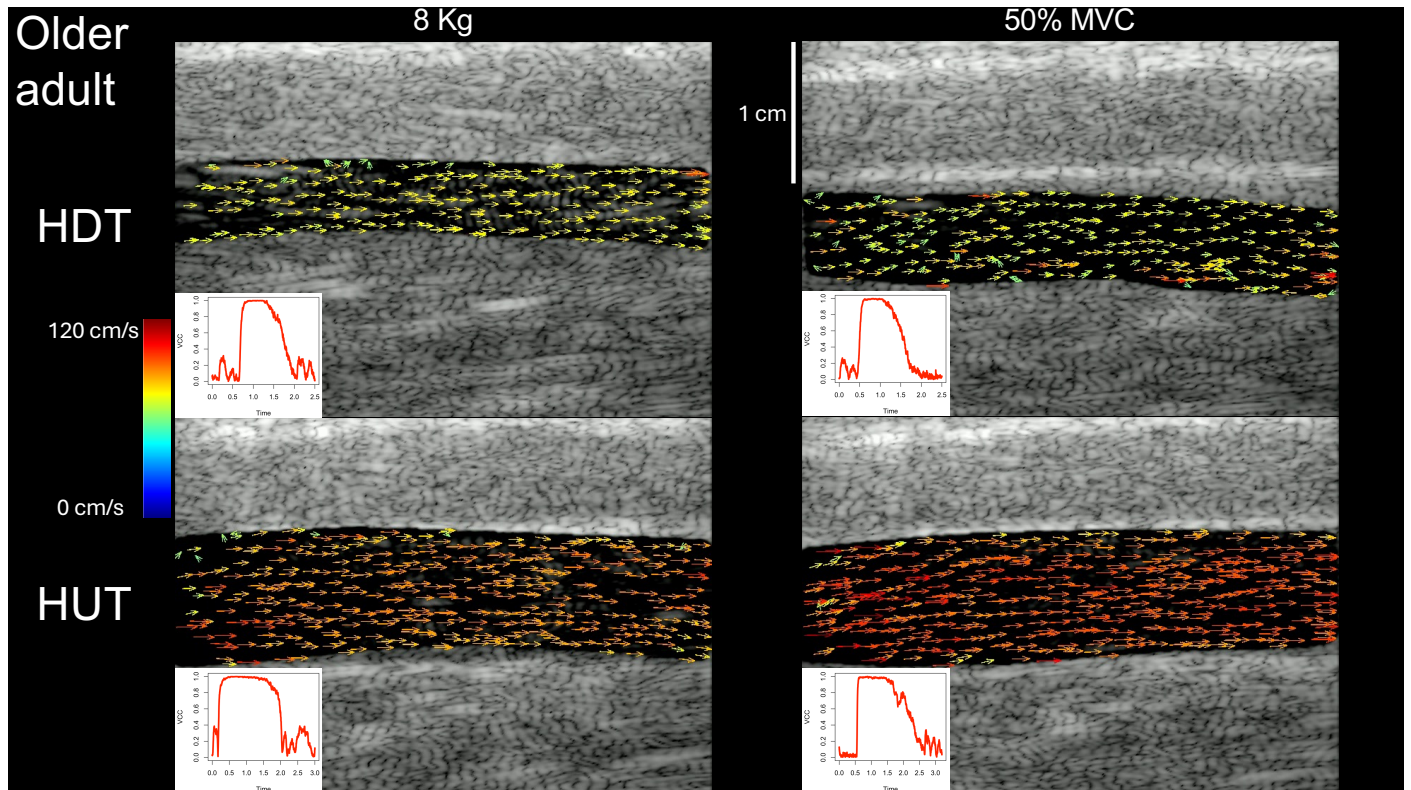


Figure 5-7: Representative stills from vector projectile videos during HDT and HUT and both contraction intensities in a 70yr old male.

The image is captured slightly past the peak of outflow velocity following muscle contraction. The corresponding vector concentration coefficient (VCC) tracing is displayed for each contraction.

Supplemental video 5-1: Representative vector projectile videos during -3° HDT and $+20^{\circ}$ HUT and both contraction intensities in a 24yr old male followed by a 70yr old male. The corresponding vector concentration coefficient (VCC) tracing is displayed for each contraction.

<https://doi.org/10.6084/m9.figshare.30247282.v2>

5.4 Discussion

We investigated the inter-dependence of chronological age and volume stress to describe lower-limb muscle pump efficiency with controlled levels of force production. Application of conventional Doppler and advanced VFI ultrasound techniques revealed expected indicators of reduced

venous compliance in OA, and new links between blood flow vorticity and muscle pump-induced blood flow are established for YA and OA. Contrary to our hypothesis, healthy OA demonstrated preserved muscle pump capacity under venous volume stress and YA generated higher velocities at the expense of more recirculatory or complex flow conditions under venous volume stress. These findings contribute to a more nuanced understanding of how muscle pump function is maintained with healthy aging alongside vascular structural changes.

Venous structural characteristics

To evaluate age-related differences in venous structural adaptations to orthostatic stress, we compared changes in FV diameter from HDT to HUT positions. Younger adults experienced a greater change in venous diameter ($+41.3 \pm 22.7\%$ vs. $+23.0 \pm 10.9\%$) upon reaching the HUT posture. While direct measurement of transmural pressure was not feasible, the upright posture imposes a predictable hydrostatic gradient, and assuming comparable upstream venous collapse, these diameter changes reasonably reflect group differences in venous compliance and align with previous reports of compliance differences of similar magnitude between OA and YA (127, 199). While the mechanisms of this characteristic vascular stiffening with aging have been described (212, 213), the minimized volume translocation to the lower-limbs during HUT may serve a functional purpose. Decreasing the maximum stressed volume through vascular wall stiffening may help preserve arterial blood pressure, compensating for the blunted arterial baroreflex responsiveness observed in older adults (214, 215). Albeit with mild orthostasis in our investigation ($+20^\circ$ HUT), OA regulated blood pressure and heart rate to the same degree as the HDT condition, supporting the possibility that venous stiffening may act as a compensatory mechanism to maintain hemodynamic stability during postural transitions. However, whether this 'adaptive compensation' remains effective under more pronounced orthostatic stress or contributes to circulatory limitations in more severe levels of tilt or advanced aging warrants further investigation.

Blood flow and volume pumped

Venous volume stress increased both the velocity and volume of blood outflow from the lower-limb upon activation of the calf muscle pump, with little discrimination between age categories. During both contraction intensities, peak blood outflow rates in the FV increased $\sim 3x$ owing to the greater volume present in the lower-limb veins. With veins operating on the flatter portion of their

compliance curve under volume-stressed conditions (HUT), muscle contractions generated a greater increase in transmural pressure rather than vessel distension, as would occur in a non-volume-stressed state. Muscle force generation and blood volume pumped were related and modulated by volume stressed conditions, whereby HUT always produced higher lower-limb volume outflow, aligning with previous work highlighting the volume stress effect on volume outflow to a fixed contraction intensity (216). Our study is the first to address muscle pump efficiency between healthy YA and OA; contrary to our hypothesis, gross impairment in OA muscle pump capacity compared to YA was not found, irrespective of the absolute or relative forces studied. Maintenance of muscle pump capacity in OA, particularly during the 50% MVC condition, where OA must produce a greater relative volume per absolute unit force to achieve the same absolute venous return and contend with the higher strength of YA, is owed to reduced venous compliance. Venous stiffening shifts the compliance curve towards the flatter portion at a lower absolute volume (199), possibly allowing superior transmural pressure generation. In healthy OA, venous stiffening may be a compensatory mechanism with positive functional outcomes; however, the progression of stiffening to the valvular sinuses or cusps may present different challenges, limiting valve function and leading to venous insufficiency. Studying OA with these clinical manifestations may reveal insight to the pathological processes between vascular stiffening and maladaptation to muscle pump efficiency.

The trade-off between reserve capacity, greater in YA, and efficiency, more pronounced in OA, underscores distinct hemodynamic strategies. Younger adults leverage greater compliance and muscle pump reserve for rapid flow increases, though possibly at the cost of flow disturbances. Older adults, despite lower absolute flow capacity, maintain efficient venous ejection through stiffer, pressure-generating veins, mitigating flow disturbances but potentially limiting adaptability to higher demands such as during exercise.

Vector flow dynamics: Velocity, turbulence, and vorticity

Implementation of VFI in veins has facilitated the exploration of blood flow outcome variables *in vivo*, allowing for a more comprehensive understanding of fluid flow features. Through the expansion of typical VFI-associated outcome variables (velocity and VCC), we provide additional descriptors to capture venous phenotypes of YA and OA veins under different conditions. At rest in both HDT and HUT conditions, VFI revealed minimal features of interest for blood flow, owing to extremely low flow velocities nearing stasis, a phenomenon we have previously highlighted in veins under volume stress (111). During contraction phases, a sharp increase in blood velocity

facilitates calculation of advanced output metrics describing the (dis)organization, turbulence, and vortical characteristics of blood flow. In general, increased volume stress has a prominent effect on increasing the organization of blood flow, whereby VCC, divergence, and vorticity changed in a direction suggestive of more laminar, organized flow. These findings align with previous studies where lower velocities increase susceptibility to flow disruption (111) and high velocities can generate highly organized flow when the critical Reynold's number is not exceeded (179). Previous studies using 4D flow MRI to examine flow in a valve-dependent system (cardiac) have established links between higher flow velocities and increased flow turbulence (217). Our results suggest that while younger adults can generate higher venous outflows, which may transiently increase flow dispersion, the consequences of this disorganization remain unknown, and volume stress remains the dominant factor promoting organized flow across ages. At 8 kg with no volume stress, YA showed lower mean VCC than OA, indicating greater velocity dispersion. This difference disappeared at HUT, suggesting venous compliance organizes vector patterns during muscle pumping. Reduced compliance in OA limits venous distension, thereby promoting antegrade flow and reducing recirculation. In YA, once veins distend and compliance falls at HUT, this organizing effect is diminished, likely due to volume stress or lower vein capacitance.

High Reynolds stress reflects velocity fluctuations from eddies or swirls that drive momentum transfer through inertial rather than viscous mechanisms, contributing to flow disorganization. Younger adults may exhibit greater velocity fluctuations due to higher venous compliance and associated vein expansion during muscle pump activity. Vorticity, a measure of rotational blood flow, decreased from HDT to HUT across both groups; however, OA showed a stronger inverse relationship between vorticity and ejected volume, suggesting that reduced compliance limits their ability to dampen vortical amplification and preserve efficient outflow. While vortices can enhance momentum in veins, excessive rotational flow may promote stagnation or retrograde motion (218), particularly when damping is impaired (219). This may explain why YA, despite achieving higher outflows, also experience more disorganized flow, whereas OA, with lower compliance, show a tighter link between vorticity and muscle pump inefficiency. Nevertheless, OA demonstrated similar relative efficiency under equal force conditions, while YA achieved greater flow capacity at the cost of increased turbulence.

Clinical implications

Given that OA exhibit reduced venous compliance and increased efficiency of the muscle pump under non-volume-stressed conditions but diminished reserve capacity under volume stress,

interventions should prioritize maintaining venous compliance and optimizing muscle pump function under orthostatic stress. Endurance exercise training has been shown to maintain venous compliance (127) and muscle pump function in the case of venous insufficiency (64), potentially mitigating the progressive stiffening that occurs with aging, as seen combatting arterial stiffness (220). Regular exposure to postural changes, such as orthostatic stress training or exercises that encourage shifts in venous volume distribution (e.g., tilt-tables), may help preserve venous compliance and reduce dependence on stiffening mechanisms for maintaining hemodynamic stability (221, 222). In cases where venous stiffening progresses to involve valve structures, OA may face an increased risk of chronic venous insufficiency and thrombosis. Identifying early markers of maladaptive stiffening through advanced flow features and implementing targeted interventions, including exercise or external compression therapy, could help preserve venous function. Further studies should explore the mechanistic links between turbulent flow patterns, vorticity, and the development of chronic venous insufficiency.

Limitations

There are some limitations to our data that should be considered when assessing the results. Firstly, despite recruiting a sex-balance cohort, we were not adequately powered for sex-based analyses, which was secondary to the primary aims of the research. Second, ultrasound resolution through aged tissue is poor (223), compared to younger, leaner tissue and led to some data dropout in the OA groups due to inadequate image and data quality. However, this was mitigated by completing all contraction conditions in duplicate, ensuring there was at least one useful collection. Blood volume also contributes to venous volume stress and subsequent venous diameter changes, and the lower blood volume typically observed in OA may have contributed to the smaller venous diameter changes seen during the HUT condition. Furthermore, OA also presented with an expected higher arterial blood pressure which may contribute to forms of venous hypertension; however, this could not be investigated with the current data. Nevertheless, given that all participants were healthy and free of overt cardiovascular disease, and that the primary outcomes were relative changes within individuals rather than absolute hemodynamic values, the influence of baseline differences in blood volume and pressure is unlikely to have significantly confounded our interpretations of the data. Another significant limitation to the impact of our study findings is the lack of a venous insufficiency-diagnosed group of OA. While we aimed to study the effects of healthy aging, a clinical arm would have added greater insight to the aging process and its links to blood flow dynamics. Finally, owing to the recency of VFI application in

human physiological study, blood flow dynamics outcomes remain exploratory and without widespread validation, particularly from single-plane data and the inability to fully resolve complex 3D vortical flows. Accurate 3D flow reconstructions require assumptions about out-of-plane motion, limiting our ability to characterize multi-directional flow patterns that may be relevant to venous hemodynamics. Future work integrating VFI with complementary imaging modalities or advanced computational modelling may help address these gaps.

Conclusion

Our findings reveal age-related differences in venous compliance, muscle pump dynamics, and flow characteristics, highlighting a trade-off between greater reserve capacity in YA and a more pressure-driven, yet equally efficient, ejection strategy in OA. Despite signs of reduced venous compliance, OA maintain venous ejection efficiency comparable to YA, likely through enhanced transmural pressure generation. Vector flow imaging provided novel insights into turbulence and vorticity, suggesting distinct hemodynamic strategies between age groups. Future research should explore the pathophysiological links of these flow patterns and interventions to preserve venous function, such as exercise training and orthostatic stress conditioning.

Chapter 6: Characterization of blood flow features through lower-limb venous valves with graded volume stress in humans using vector flow imaging

6.1 Introduction

While blood flow is often described as laminar and parabolic, this characterization primarily applies to idealized arterial conditions. When blood flow achieves very high velocity (e.g., aorta) (224), very low velocity (e.g., veins), or encounters vessel curvature and/or obstructions to internal vessel diameter (e.g., stenosis, valve leaflets) (179, 208, 225), there is a loss of laminar flow conditions and possible turbulence generation. Flow turbulence is physiologically unfavourable because it requires greater pressure gradients to maintain flow, which reduces efficiency (225) and diminishes wall shear stress, both of which promote pro-atherogenic states (15, 226). Unlike arteries, valvular regions of veins often operate under low-flow, high-compliance conditions that are predisposed to complex flow patterns. Despite the high prevalence of pro-atherogenic shear stress profiles, venous valves are critical to manage orthostatic tolerance (202). However, it remains unclear how valves manage increased volume stress, particularly in the lower-limb veins where orthostasis imposes pronounced hydrostatic loads. Given these unique hemodynamic challenges, healthy venous valves provide a valuable model to study how physiological volume changes influence flow complexity and efficiency. Previous studies have identified flow recirculation and stagnation in the valvular sinus region under mild, static volume stress (seated) but did not include comparisons to other physiological conditions, including muscle contractions which occur during activities of daily living (208). Volume stress conditions resembling daily activities (e.g., standing) induce greater lower-limb venous distension and may mimic the responses of large veins in the neck during progressive volume stress (111), with implications for Virchow's triad and deep-vein thrombosis (159).

Valve dysfunction is considered a risk factor for thrombosis formation when blood becomes trapped between vein wall and valve leaflets. This region, known as the valvular sac, is a region of interest, characterized by lower blood PO_2 and increased hematocrit (227), and has been a noted thrombosis formation site *in vitro*, *in vivo* and *post-mortem* (228, 229). Valve dysfunction is also associated with impaired muscle pump efficiency in venous return (230). Effective propulsion of blood from the lower-limbs against gravity relies on competent valves; when leaflet closure is incomplete, reflux occurs. As a result, less blood returns to the right atrium while excess blood pools in the lower-limb veins during prolonged orthostasis, promoting edema

and blood trapping. The relationship between muscle pump activation and blood ejection during volume stressed conditions such as orthostasis is not only critical for normal blood pressure control (69), it also may include links to thrombotic risk, relating to incomplete blood expelled from valvular regions. The driving pressure for venous blood flow can vary drastically based on volume stress conditions and activation of pump mechanisms (muscle, respiratory pumps), accordingly changing the forces exerted on venous valve leaflets. As volume stress increases, the back-pressure increases, requiring greater pressure generation to open each valve segment. Despite this physiological interplay, the *in vivo* flow dynamics through and around venous valves in a human during combined volume stress and muscle pump activation are not well defined. Examining flow complexity under increasing venous driving pressures may clarify how these conditions alter regional shear and vorticity (local spinning motion of fluid), providing linking insights into valve geometry, venous return capacity, and potential thrombosis risk. Using an ultrasonographic approach to this problem provides greater temporal resolution and flexibility in data collection, allowing measurements of activities relevant to daily living at the bedside.

Conventional clinical ultrasound is technologically limited for investigating venous complex flow features in humans *in vivo*; emerging techniques in non-invasive VFI may serve to enhance the study of dynamic venous valve function. VFI reconstructs full-field velocity vectors using multiple transmit–receive angle pairs, providing angle-independent flow quantification with high spatial and temporal resolution (>1000 fps) implemented through open-platform ultrasound approaches (161). Currently, VFI remains largely confined to technical development within biomedical engineering, with little application in integrative human physiology. Its use for assessing venous valve function represents a novel approach with strong potential to advance the field (231).

Given the lack of data surrounding complex flow characteristics through venous valve segments in functional situations, we aimed to describe flow responses during graded levels of volume stress and in response to the muscle pump using VFI and conventional Doppler ultrasound. These data offer quantitative descriptions of flow uniformity, region-specific shear stresses and qualitative accounts of flow recirculation to characterize venous valve flow behaviour under volume stress. While this study is exploratory, we hypothesized that increasing volume stress would reduce flow uniformity near venous valves. Additional quantitative and qualitative analyses explored how hydrostatic loading influenced shear rates and recirculatory flow patterns around venous valves as identified by VFI.

6.2 Methods

Participants

Inclusion criteria for participation included adults 18-35 years old without history of venous disease in addition to screening for potential contraindications to normal cardiovascular function including hypertension, diagnosed vascular disease, current smokers and prescription medication with cardiovascular effects. All eligible participants had their right leg screened with standard duplex ultrasound for visible venous valves and absence of valvular reflux to confirm study inclusion.

The total sample was 20 participants, equally balanced for sex owing to the dearth of evidence for VFI outcomes. Based on the small existing literature on this topic, strong effect sizes (Cohen's $d = 0.8-1.7$) associated with venous valve flow measurements during muscle contractions and volume stress manipulation were expected (112, 208). This sample size also accounts for expected data dropout due to the difficulty of accurately scanning venous valves (expected 75% success rate). A simulation-based power analysis with $n=2000$ simulations for a repeated measures design with two-tails, $\alpha=0.05$, estimated >90% power to detect venous velocity changes associated with muscle contractions in 15 included participants (232).

Study design

Participants refrained from food for 3h, caffeine for 12h, strenuous exercise, alcohol and recreational drugs for 24h prior to all experiments. Participants were positioned on a self-supported tilt table beginning in the supine position. The right femoral vein at a valve site was landmarked on the skin using conventional clinical ultrasound. After baseline measures, participants completed MVC repeats of a single plantarflexion with their right foot to determine strength. Thereafter, a standardized 8 Kg contraction was practiced to activate the calf muscle pump. The calf was targeted given its dominant role in the muscle pump and to reduce motion artifacts that are more pronounced in the upper thigh. Conventional Doppler ultrasound and VFI were acquired at resting baseline and during plantarflexion contractions. Conventional Doppler was used to quantify blood velocities before, through the valve channel, and after the identified valve, and was repeated three times per contraction to capture each of the three regions of interest. VFI imaging was then captured in duplicate with full-field velocity encompassing all three regions of interest simultaneously. The testing battery (baseline and calf muscle pump) was repeated in randomized order under 20° and 40° HUT to induce graded venous volume stress.

Each measurement was separated by 5 min of quiet rest to allow normalization of blood pressure and arterial inflow. Heart rate and brachial arterial pressure (Carescape V100 Dinamap, GE Healthcare, Chicago, USA) were recorded at each timepoint to account for sympathetic activation associated with increasing HUT.

Muscle pump protocol

The calf muscles generated a single plantar flexion movement for each measurement, generating an absolute load of 8 Kg. A custom-built plantar flexion device equipped with a continuously recording load cell was interfaced with a PowerLab system (ADInstruments Inc., Colorado Springs, USA) providing live feedback to the participant via LabChart (ADInstruments Inc.). Participants were instructed to reach and hold the 8 Kg target force for a single 1-s contraction. Time alignment of force output was confirmed during analysis by identifying the peak contraction to synchronize with ultrasound measures. Absolute force measurements, standardized across participants of similar characteristics has been used in previous investigation of muscle pump activation (62).

To evaluate the effectiveness of the muscle pump in generating venous return, participants were equipped with a strain gauge plethysmography system (EC6, Hokanson, Bellevue, USA) using indium-gallium strain gauges to monitor lower-leg volume changes. The maximal circumference of the right calf at the muscle belly was measured with a surface tape measure, and a strain gauge approximately 2 cm smaller than this measurement was selected according to manufacturer guidelines. The gauge was double-looped, secured with medical tape, and positioned to avoid external compression. Continuous recordings of leg volume were expressed as percent change over time. Baseline limb volumes were estimated for shank using a three-point cylindrical model adapted from validated anthropometric methods (211). During analysis, periods of plantarflexor contraction were identified, and the resulting change in limb volume was calculated; using shank volume estimates, ejection volume and ejection fraction were then derived to provide absolute measures of muscle pump activity in mL.

Conventional Doppler ultrasound

Conventional pulsed-wave Doppler ultrasound was used to collect blood velocity similar to that described previously in our lab (84). A linear array probe (9L, GE Healthcare) connected to an ultrasound (Vivid S70N, GE Healthcare) was used to measure blood velocity in the same three

regions of interest as VFI. The pulsed-wave Doppler sample gate was maintained at 0.3 cm for all collections and positioned to capture flow velocities before (upstream to the valvular orifice), at the channel (through the valve orifice) and after (downstream to the valvular orifice) the venous valve (Fig. 6-1). The regions of interest were placed relative to the venous valve leaflet, with the before and after regions placed at least 2 mm beyond the path of valve leaflets and the channel region centred upon the valve orifice. A standardized sample gate ensured consistency between participants and ensured capture of jet-like flows, given the known intra-lumen regional dependence of blood velocities surrounding valves (208). Offline blood flow analysis was performed using the open-source software FloWave.US, which extracted raw intensity-weighted Doppler data (168) to calculate MBV within the sample gate and estimated blood flow ($\text{mL} \cdot \text{min}^{-1}$) following: $\pi \cdot (\text{diameter}/2)^2 \cdot \text{MBV} \cdot 60$. In data processing, the maximum MBV was identified to describe responses to plantarflexion contractions as peak blood velocity.

Vector flow imaging

A 13-3 MHz linear array probe (Esaote SL1543) interfaced with an open-source ultrasound machine (USPlatform, US4US, Warsaw, Poland) was optimized for live imaging (104). The B-mode image was navigated to obtain clear longitudinal views of a valve segment, consistent with the skin markings from the conventional ultrasound assessment. An adequate valve segment required evidence of movement from two valve leaflets correctly oriented relative to the probe and <6 cm depth. A 2-angle sequence (transmission angles 10° and -10° ; receive angles 10° , 0° , and -10°) was used for acquisition, facilitating multi-angle vector Doppler for angle-independent blood velocity estimations as described previously in detail (111, 170).

Raw data was collected for 4 s to capture an entire muscle contraction cycle. Doppler spectrograms were manually inspected to identify relevant frames for one cycle, defined by the time between velocity upstrokes. Vector data was then processed for one muscle contraction cycle, and three regions of interest were identified: before, channel and after, following the same approach as the conventional Doppler system (Fig. 6-1). For each region of interest, quantitative metrics were calculated resultant from axial and lateral velocity components, including peak blood velocity, and the VCC. The VCC is unitless, ranging from 0 to 1, describing the uniformity of flow vector direction within the region of interest (114). A value of 1 indicates all flow vectors are pointed in the same direction, whereas 0 implies there is equal dispersion of vector directions at the given time-point. The VCC was calculated for each frame to obtain intra-cycle tracings and data are presented as the cycle average value. We have previously described the strong reliability

of VCC metrics for vascular assessment in our laboratory (109). Additionally, shear rate, vorticity and Reynold's stress were calculated as advanced metrics based on measured axial and lateral velocities to describe shear forces, rotational flow rate and turbulence-driven momentum transfer.

$$\text{Shear rate} = \sqrt{[(\partial v_x / \partial x - \partial v_y / \partial y)^2 + (\partial v_x / \partial y + \partial v_y / \partial x)^2]} ;$$

$$\text{Vorticity} = (\partial v_y / \partial x) - (\partial v_x / \partial y) ;$$

$$\text{Reynold's stress} = (v_x - \text{mean}(v_x)) \cdot (v_y - \text{mean}(v_y));$$

Where v_x : Axial velocity component; v_y : Lateral velocity component; x : Axial spatial coordinate; y : Lateral spatial coordinate; ∂ : Partial derivative. To maintain consistency and avoid bias, a single observer (J.C.) recorded flow features and generated representative videos to be used for communicating findings.

Statistical analysis

All graphing and statistics were completed using R (v4.2.2) (171). Differences were tested using a 3x3 repeated measures ANOVA with factors: region of interest (before, through, after valve) and volume stress (0°, 20°, 40° HUT). Where appropriate, significant main effects were tested with paired Student's t-tests with Bonferroni-Holm post-hoc corrections for multiple comparisons. Statistical significance was set *a priori* at $\alpha = 0.05$.

Qualitative flow descriptions

Quantitative VFI outcomes remain largely exploratory in this domain, and qualitative descriptions of flow profiles provide meaningful insight into physiological blood flow features. Structured qualitative observations were performed for each participant, visually assessing flow before, at the channel and after the valve site during each condition. These observations focused on overall patterns, organization, and striking or unusual features, highlighting characteristics such as laminar versus turbulent, vortical, disorganized flow, or evidence of stasis. Representative examples are presented in Fig. 6-5 and supplemental video 6-1 to illustrate these features.

6.3 Results

The study protocol was completed by all participants without adverse events. Participants characteristics are presented in Table 6-1, including their maximum plantarflexor force generated. The 8 Kg force corresponded to a mean 23 ± 5 %MVC in males and 34 ± 7 %MVC in females.

Blood pressure and heart rate

There was a main effect of tilt angle for diastolic and mean arterial blood pressure (both $P<0.05$; $\eta_p^2>0.18$), increasing from 63 ± 8 mmHg and 79 ± 10 mmHg to 69 ± 14 mmHg and 83 ± 12 mmHg at 20° HUT and 70 ± 10 mmHg and 84 ± 11 mmHg at 40° HUT, respectively. Systolic blood pressure was not affected by tilt angle, presenting 111 ± 14 mmHg supine and 113 ± 14 mmHg at 40° HUT ($P>0.4$). Heart rate increased with a main effect of tilt angle ($P<0.01$; $\eta_p^2>0.49$), increasing to 65 ± 9 beats $\cdot$ min $^{-1}$ at 20° HUT and 71 ± 11 beats $\cdot$ min $^{-1}$ at 40° HUT. These observed blood pressure and heart rate effects are consistent with the known hemodynamic effects of HUT in young, healthy adults.

Clinical ultrasound blood flow

Clinical ultrasound flow rates are calculated using a fixed 0.3 cm vessel diameter for standardized velocity assessment. Under resting conditions, femoral vein blood flow decreased with more volume stress during tilt, from 50.4 ± 33.9 mL $\cdot$ min $^{-1}$ while supine to 13.0 ± 7.8 mL $\cdot$ min $^{-1}$ at 20° HUT and 7.8 ± 4.9 mL $\cdot$ min $^{-1}$ at 40° HUT (main effect $P<0.01$, $\eta_p^2=0.71$). A tilt angle by valve region interaction was detected ($P=0.02$, $\eta_p^2=0.13$), however *post hoc* testing revealed no significant differences between valve regions. Identical trends were found for peak MBV measurements during the 8 Kg contraction, demonstrating no effect of tilt ($P=0.08$, $\eta_p^2=0.14$) nor valve region ($P=0.21$, $\eta_p^2=0.09$; Fig. 2), with a mean value of 56.8 ± 27.7 cm $\cdot$ s $^{-1}$ (Fig. 6-2).

Muscle pump capacity

The estimated volume ejected from the lower-limb during 8 Kg contractions increased with tilt angle ($P=0.01$, $\eta_p^2=0.21$; Fig. 6-3). The estimated volume increased from 96 ± 63 mL while supine to 119 ± 61 mL and 119 ± 48 mL at 20° HUT and 40° HUT, respectively.

Selected vector flow imaging output

Peak blood velocity measured by VFI differed across valve regions during the 8 kg contraction and revealed velocity heterogeneity not observed by conventional Doppler (Fig. 6-2). Mean velocity increased from $39.0 \pm 11.9 \text{ cm} \cdot \text{s}^{-1}$ before the valve opening to $41.7 \pm 12.7 \text{ cm} \cdot \text{s}^{-1}$ through the valve channel, then decreased to $37.6 \pm 12.2 \text{ cm} \cdot \text{s}^{-1}$ after the channel, while peak velocities rose from $66.4 \pm 16.1 \text{ cm} \cdot \text{s}^{-1}$ before the valve to $73.3 \pm 17.9 \text{ cm} \cdot \text{s}^{-1}$ through the channel and fell to $68.6 \pm 17.1 \text{ cm} \cdot \text{s}^{-1}$ after. This pattern demonstrates how valve leaflets accelerate blood through the channel and moderate flow downstream. Body tilt increased peak velocity ($P=0.01$, $\eta_p^2=0.22$), from $62.1 \pm 18.1 \text{ cm} \cdot \text{s}^{-1}$ supine to $74.7 \pm 16.3 \text{ cm} \cdot \text{s}^{-1}$ and $71.4 \pm 14.8 \text{ cm} \cdot \text{s}^{-1}$ at 20° and 40° HUT, indicating that orthostatic venous distension contributes to accelerated venous flow during contractions, with consistent effects across valve regions (no tilt by region interaction, $P>0.80$, $\eta_p^2<0.13$).

Flow alignment, quantified by the VCC, varied by valve region ($P<0.01$, $\eta_p^2=0.27$) and body tilt ($P=0.03$, $\eta_p^2=0.19$; Fig. 6-4). VCC was highest through the valve channel (0.75 ± 0.18), reflecting streamlined, unidirectional flow, and lower before (0.69 ± 0.20) and after the channel (0.71 ± 0.18) indicating more heterogeneous flow patterns. Body tilt enhanced flow organization, with VCC rising from 0.64 ± 0.26 supine to 0.75 ± 0.11 and 0.76 ± 0.13 at 20° and 40° HUT, paralleling the increase in peak velocity and supporting a role for orthostatic venous distension in promoting aligned flow patterns during contractions.

Shear rate mirrored VCC patterns, showing main effects of tilt ($P<0.01$, $\eta_p^2=0.41$) and valve region ($P<0.01$, $\eta_p^2=0.36$; Table 6-2). Shear rate was highest supine ($3046 \pm 1511 \text{ s}^{-1}$) and decreased with tilt ($1934 \pm 772 \text{ s}^{-1}$ at 20° HUT, $1644 \pm 765 \text{ s}^{-1}$ at 40° HUT), consistent with reduced velocity gradients. Regionally, shear was highest before the valve ($2389 \pm 1221 \text{ s}^{-1}$), lowest through the channel ($1933 \pm 1179 \text{ s}^{-1}$), and increased after the channel ($2303 \pm 1257 \text{ s}^{-1}$), illustrating localized acceleration–deceleration dynamics imposed by valve geometry.

Peak vorticity demonstrated sensitivity to both tilt ($P<0.01$, $\eta_p^2=0.49$) and valve region ($P<0.01$, $\eta_p^2=0.49$) with a tilt by region interaction ($P=0.04$, $\eta_p^2=0.14$; Table 6-2), indicating that rotational components of flow are shaped by both gravitational load and regional geometry. Most comparisons differed, highlighting that the swirling motion of blood is concentrated immediately before and after the channel and modulated by posture.

Mean Reynolds stress varied across valve regions ($P=0.03$, $\eta_p^2=0.17$; Table 6-2), with the greatest stress after the valve (mean: $31.1 \pm 76.7 \text{ s}^{-1}$), reflecting the combined effects of flow acceleration through the channel and subsequent turbulence propagation downstream.

Qualitative descriptions of flow

Visual assessment of vector projectile videos revealed consistent evidence of vortical and rotational flow patterns in regions directly surrounding venous valve leaflets. These features were most apparent in the period following peak antegrade flow during the plantarflexion-induced contraction. Flow vectors frequently organized into circular trajectories adjacent to the leaflet base, within the valve sinus. In some cases, interaction between these recirculatory zones and the outflow jet emerging from the valve channel produced shearing flows. Following the dissipation of antegrade flow, regions of minimal or absent flow signal were also observed, suggesting transient stasis, particularly within the sinus region. These features were more pronounced during 20° and 40° HUT compared with the supine condition, consistent with the greater venous volume load. Representative examples of these flow dynamics are shown in Fig. 6-5 and in the supplemental video 6-1.

Table 6-1: Participant characteristics

Variable	Male	Female
N	10	10
Age (yrs)	28 ± 3	24 ± 4
Height (cm)	177 ± 7	164 ± 9
Body mass (Kg)	78 ± 9	60 ± 11
BMI ($\text{Kg} \cdot \text{m}^{-2}$)	25.0 ± 2.7	22.2 ± 2.5
Supine MAP (mmHg)	85 ± 7	74 ± 8
Supine heart rate ($\text{beats} \cdot \text{min}^{-1}$)	64 ± 8	61 ± 5
Calf muscle thickness (mm)	68 ± 7	66 ± 6
Shank volume (L)	7.3 ± 0.9	6.0 ± 1.2
Plantarflexor strength (Kg)	35.4 ± 7.1	24.9 ± 5.8
50% plantarflexor strength (Kg)	18.1 ± 5.2	13.7 ± 5.2

Data are represented as means $\pm$ SD. BMI, body mass index; MAP, mean arterial pressure.

Table 6-2: Quantitative vector flow imaging outputs acquired during the plantarflexion contraction flow phase

Outcome	Supine			20° HUT			40° HUT			Tilt	Location	Interaction
	<u>Before</u>	<u>Channel</u>	<u>After</u>	<u>Before</u>	<u>Channel</u>	<u>After</u>	<u>Before</u>	<u>Channel</u>	<u>After</u>			
Peak blood velocity (cm·s ⁻¹)	59.5 ± 18.4 *	65.6 ± 19.2 *†	61.3 ± 17.2 *	71.1 ± 14.4	79.6 ± 16.7 †	73.6 ± 17.4	68.6 ± 13.7	74.9 ± 15.5 †	70.9 ± 15.1	0.02	<0.01	0.86
Mean blood velocity (cm·s ⁻¹)	37.5 ± 15.9	41.4 ± 16.9 †	37.6 ± 16.0	40.9 ± 9.6	42.7 ± 10.3 †	38.6 ± 10.9	38.6 ± 9.5	40.9 ± 10.2 †	36.8 ± 9.3	0.70	<0.01	0.87
Mean VCC (a.u)	0.61 ± 0.27	0.67 ± 0.27 †	0.63 ± 0.25	0.74 ± 0.13	0.78 ± 0.09 †	0.74 ± 0.12	0.74 ± 0.14	0.80 ± 0.12 †	0.74 ± 0.13	0.05	<0.01	0.92
Mean shear rate (s ⁻¹)	3228 ±1465*	2748 ±1625 *†	3164 ±1472 *	2157 ±931	1677 ±601 †	1969 ±710	1781 ±657	1374 ±472 †	1776 ±1026	<0.01	<0.01	0.85
Peak vorticity (s ⁻¹)	111.6 ± 49.0 *	147.9 ± 57.4 *	159.5 ± 62.3 *	79.3 ± 37.6	149.5 ± 46.4 †	118.6 ± 52.4	57.3 ± 14.6	119.7 ± 24.6 †	86.7 ± 29.3	<0.01	<0.01	0.04
Mean Reynolds stress (cm ² ·s ⁻²)	-2.7 ± 18.7	-2.5 ± 36.9	28.1 ± 54.1 †	6.0 ± 70.0	8.9 ± 90.4	29.2 ± 90.7 †	18.3 ± 49.9	23.7 ± 73.1	35.9 ± 84.4 †	0.38	0.03	0.89

Data are represented as means±SD. ANOVA output represents the Tilt x Location output within each outcome. VCC, Vector concentration coefficient. † Different from other two locations of interest P<0.05; * Different from other two tilt conditions P<0.05.

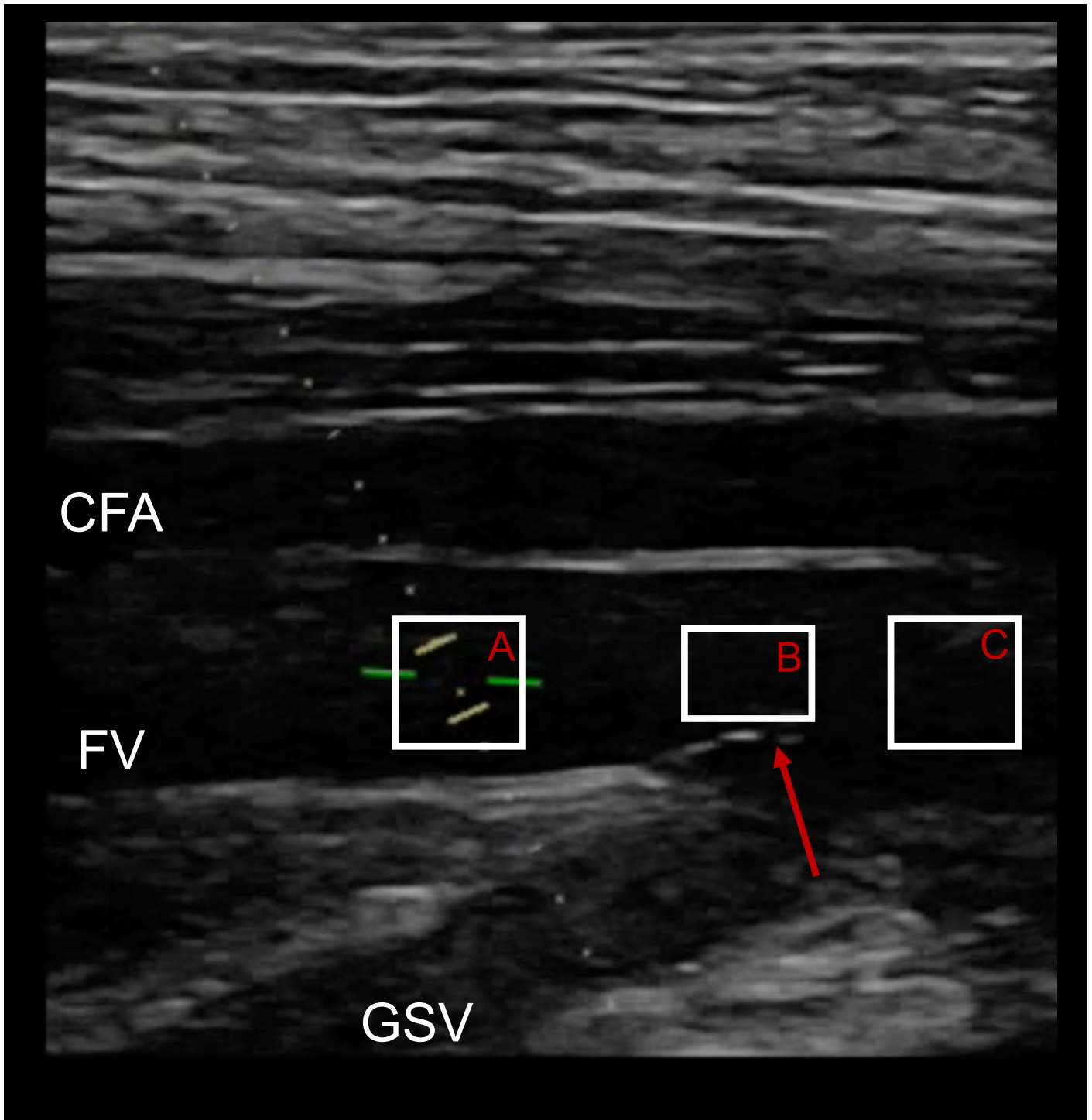


Figure 6-1: Representative B-mode image of a femoral vein in a 33 year old female, with valve leaflets highlighted.

The three regions of interest (before, channel, after) are denoted by the A, B, C box markings, corresponding with the areas used to derive region specific flow data.

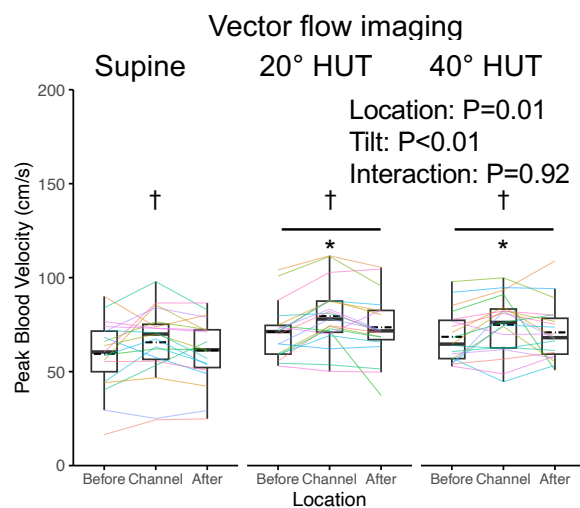
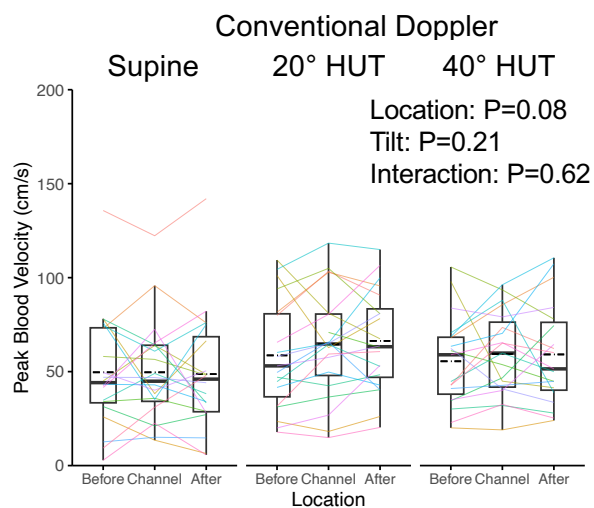


Figure 6-2: Peak blood velocity during 8 Kg plantarflexion contractions measured from three regions of interest surrounding a venous valve at three levels of body tilt, captured by conventional Doppler and vector flow imaging.

Box plots display the inter-quartile range, mean (dashed line), median (solid line) and whiskers display 1.5 times inter-quartile range. Coloured lines show individual participant responses.

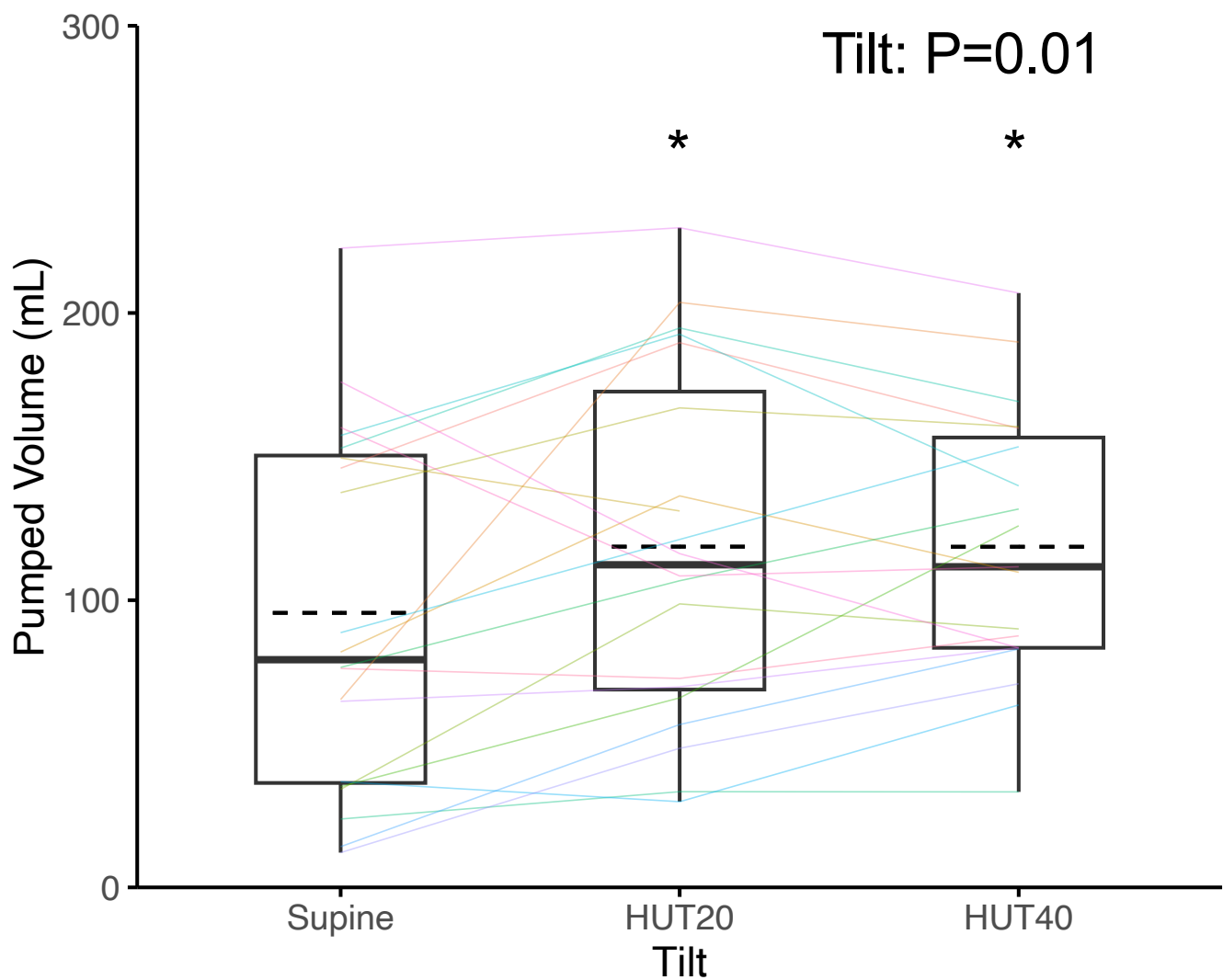


Figure 6-3: Shank volume changes following a single 8 Kg plantarflexion contraction at three levels of body tilt.

Box plots display the inter-quartile range, mean (dashed line), median (solid line) and whiskers display 1.5 times inter-quartile range. Coloured lines show individual participant responses. * $P < 0.05$ vs Supine.

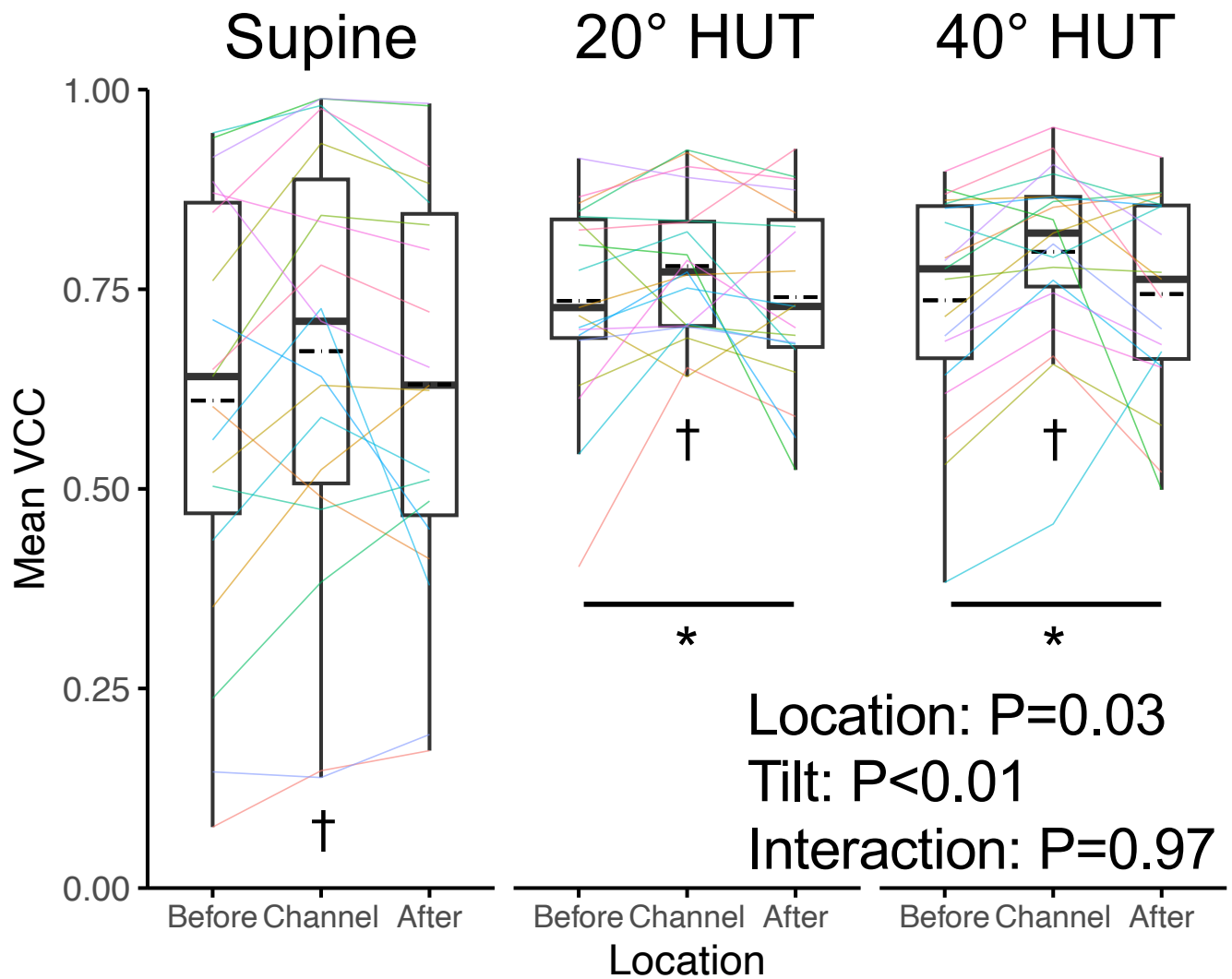


Figure 6-4: Mean vector concentration coefficient (VCC) during a single 8 Kg plantarflexion contraction captured from three regions of interest surrounding a valve at three levels of body tilt.

Box plots display the inter-quartile range, mean (dashed line), median (solid line) and whiskers display 1.5 times inter-quartile range. Coloured lines show individual participant responses.

* $P<0.05$ vs Supine. † $P<0.05$ vs Before, After.

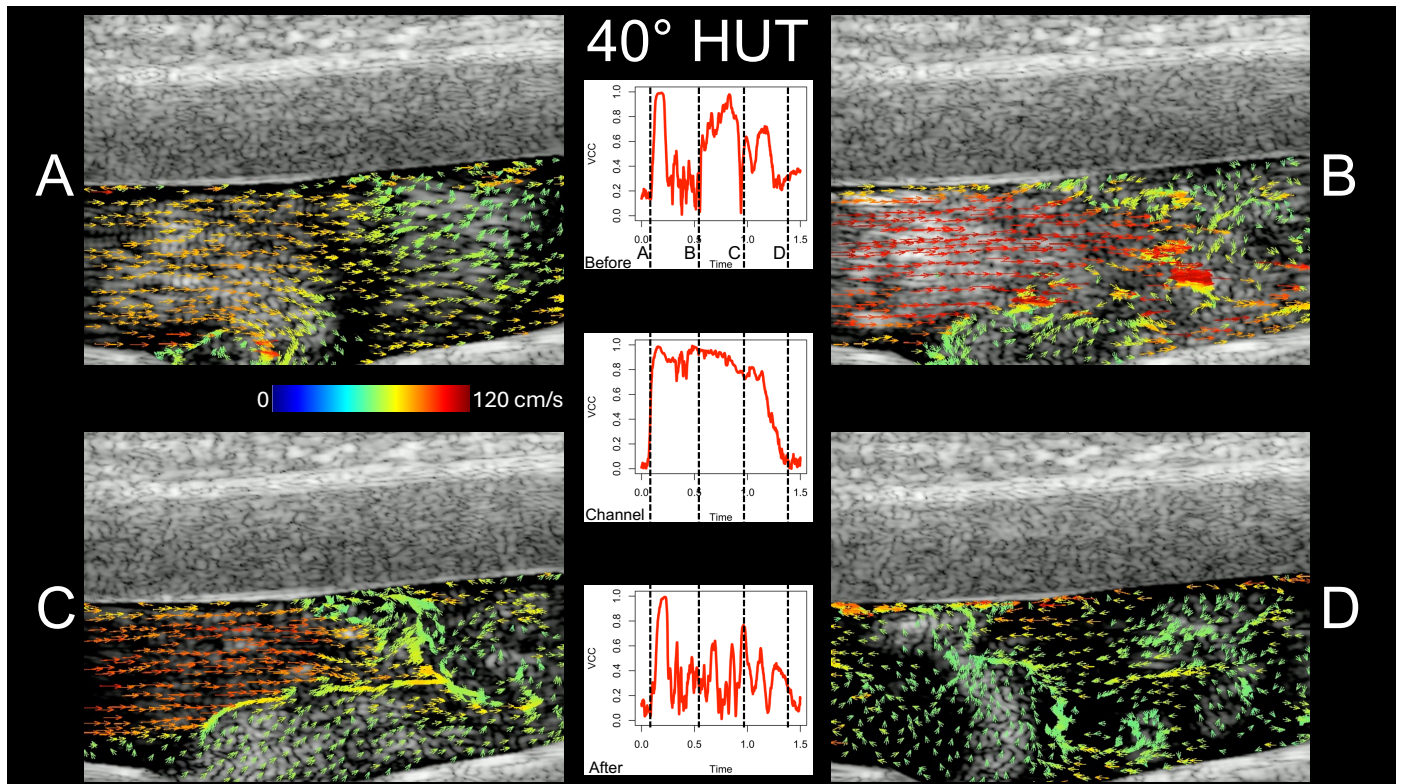


Figure 6-5: Representative vector projectile still frames of a 27 year old male throughout an 8 Kg plantarflexion contraction at 40° HUT.

Frames A-D are time linked to the contraction period and aligned with plots of vector concentration coefficient over time in the three regions of interest.

Supplemental video 6-1: Vector projectile videos of an 8 Kg plantarflexion contraction at 40° HUT in two young adults.

<https://doi.org/10.6084/m9.figshare.30324361.v1>

6.4 Discussion

This study addressed the regional distribution of blood flow features around and through lower-limb venous valves throughout a series of volume stress conditions and during active muscle contractions. Implementation of VFI was critical for identifying flow feature heterogeneity, demonstrating greater blood velocity and flow uniformity through the valve channel, whereas vorticity and Reynold's stress tended to prevail downstream to the venous valve during active muscle contractions and with increasing volume stress. We discuss the potential implications of these findings through the lens of ultrasound technology deployment and physiological outcomes linked to these complex, differential blood flow features surrounding lower-limb venous valves.

Regional valve flow

Venous valve flow characteristics have been hypothesized and experimentally described for decades (18, 138, 233), yielding estimates of jet-like features and recirculation risks. Our data affirms these descriptions, with clearly evidenced flow feature heterogeneity relative to venous valve leaflets as identified by VFI. Both blood velocity and blood flow uniformity increased through the valve leaflets, generating a flow jet. Relative to the upstream and downstream regions of interest, the valve leaflets are responsible for these expected increases in velocity and uniformity. Increased flow velocity has been previously associated with higher levels of flow uniformity by VFI (106, 109) however, this has not been explicitly demonstrated in the case of human venous valves *in vivo*. Interestingly, venous volume stress only generated a measurable velocity and flow uniformity effect (+15-19%) from supine (no volume stress) to 20° HUT (mild volume stress) with no further increase in blood velocity or flow uniformity at 40° HUT despite a theoretical hydrostatic loading pressure increase of ~15 mmHg (84). This lack of further increase could be due to a plateau effect, supported by near equal muscle pump induced volume ejection between HUT conditions. Thus, the velocity and flow dynamics features appear linked to the fixed muscle force production under our mild HUT conditions, rather than small changes in venous volume stress.

By facilitating jet-like flow, the valve leaflets optimize antegrade propulsion during contraction phases and minimize retrograde leakage, highlighting a previously known stabilizing role at the leaflet itself (234). However, this effect comes at the cost of disturbed flow patterns in adjacent regions, as blood vorticity and Reynold's stress were both increased in the area immediately downstream to the venous valve, implicating features linked to recirculation and/or pro-atherogenic/thrombotic conditions (218, 235). This duality of stable jet formation at the leaflet

followed by disturbed flow beyond the valve describes a physiologically functional yet mechanically imperfect design. These disturbances are unlikely to pose risk in healthy young adults, where thrombotic events are rare (236). Yet, in conditions of venous valve dysfunction, aging, or venous insufficiency, such disturbed flow regions may amplify stasis and thrombogenic risk. Importantly, thrombi most often originate in the immediate vicinity of venous valves (228, 237), making accurate *in vivo* descriptions of these flow features essential to understanding thrombotic pathophysiology. The ability of VFI to capture both the stabilizing jet and the downstream (and upstream) disturbances positions it as a translational tool for non-invasively identifying mechanistic drivers of venous pathology at the bedside.

Volume stress effects

The physiological implications of upright posture are well described, with noted influence on venous volume distribution. In the absence of external pumping mechanisms, venous blood activates vein wall compliance features and alters the driving pressures surrounding venous valves (201). This effect was evident from our measured MBV during baseline which demonstrated stepwise decreases from supine to 40° HUT, implicating a higher degree of volume stress. The increased volume stress was matched by an increased volume pumped from the lower-limb compared to supine despite identical contraction magnitudes. With increasing volume stress, a higher critical pressure was required to sustain flow, imposing greater mechanical strain on the vein walls and valve leaflets. (234). Though we lack direct venous pressure recordings, previous investigations have demonstrated venous blood pressure decreases during ambulatory muscle contractions under volume stress and is associated with increased blood velocities (68). Here, we note the systematic increase in peak blood velocity during lower-limb contractions using both conventional and VFI ultrasound techniques. Notably, only the VFI-based measures revealed a valve region dependency in peak velocity during muscle contraction, with higher channel velocities aligning with greater vector uniformity through the leaflets compared to upstream and downstream regions. Thus, assessment of venous blood velocities, particularly in valvular regions, by conventional Doppler should be cautioned due to error susceptibility under non-laminar flow conditions (201). These findings highlight that volume stress amplifies regional differences imposed by valve geometry, extending earlier reports of venous valve function (208) and underscoring the importance of studying posture-induced hemodynamic shifts with high-resolution imaging.

Conventional Doppler vs VFI

Use of conventional Doppler ultrasound remains the field standard for venous physiology research and clinical diagnostics (101, 201). However, single-angle dependence and minimal spatial resolution limit its utility for detection of complex flow features which may hold insight for venous pathophysiology (201). This limitation was evident in our data: while conventional Doppler successfully captured the expected increases in blood velocity during muscle contractions and decreased velocity with orthostasis, it failed to capture the spatially motivated valve region differences in velocity. Comparatively, use of VFI captured the same expected features as conventional Doppler and owing to the high spatial resolution and full-field velocity information available we were able to selectively discern velocity profiles around venous valves. Furthermore, since VFI offers angle-independent velocity measurements, interpretation of axial and lateral velocity components allowed for the advanced flow complexity analysis providing information regarding jet and recirculation features within each valve region. Prior studies have emphasized the importance of capturing these features involving multidirectional flow, particularly under venous volume stress conditions in areas prone to recirculation, including venous valves (111). Our data reinforces this importance as VFI generates similar primary findings as conventional Doppler and extends to describe vector concentration coefficient, shear rates, vorticity and Reynold's stress as well. Availability of these measures allowed for the description of heterogenous flow features around venous valves during muscle contraction-driven increases in blood velocity. Future *in vivo* study should prioritize the implementation of VFI and similar technologies to adequately address the intra-luminal hemodynamics of veins.

Physiological and clinical implications

Healthy venous valves appear capable of generating efficient flow jets even under increasing volume stress, reinforcing their role in facilitating effective muscle pump function. However, the persistence of downstream flow disturbances, despite this efficiency, suggests that valve-mediated propulsion does not normalize venous flow features but rather prevents normal flow. The reduction in shear stress we observed during HUT may carry clinical significance, as venous wall shear is a key regulator of vascular health. Low-shear environments are more permissive to thrombosis, with evidence that venous pooling reduces wall shear rates and impairs endogenous antithrombotic signalling, including nitric oxide and prostacyclin release (238, 239). This framework may explain why prolonged standing is associated with increased thrombotic risk in

otherwise healthy individuals (240), and aligns with observations that immobility or reduced calf pump function increases susceptibility to deep vein thrombosis (230, 241).

Our findings indicate that even in young, healthy valves, elevated vorticity and Reynolds stress under volume loading reveal that hemodynamic efficiency during valve jetting does not ensure optimal downstream mixing. Rather, the acceleration through the valve channel to sustain forward propulsion appears to generate complex rotational and shear interactions after the valve, fostering localized zones of low shear. These dynamics suggest that valve geometry and venous distension together create a trade-off between efficient ejection and spatially heterogeneous flow dynamic distributions. This interpretation aligns with prior observations that venous valves act not only as conduits of unidirectional flow but also as focal points for disturbed flow patterns that can evolve into thrombogenic niches under specific conditions (242). The capacity of muscle pump activity to mitigate but also produce shear and vorticity disturbances underscores the physiological importance of regular limb movement and calf muscle activation during orthostasis.

Limitations

There are some limitations of this work that warrant contextualization. First, our cohort of young, healthy adults with no evidence of venous disease limits the applicability of our findings to clinical populations, including those with venous insufficiency. However, investigating a healthy system provides critical mechanistic insight into flow patterns that may be altered in disease and allowed us to capture robust and consistent effect sizes. Second, without direct measurement of venous pressure we are unable to comment on more specific fluid flow features as fluid pressure governs much of fluid dynamics (179). Spatially precise pressure measurements surrounding venous valves should be prioritized in future studies. Third, a subset of valve sites were situated at the saphenofemoral junction which may have impacted post-valve flow, via a secondary flow stream from the saphenous vein, an effect visible in the supplemental video. However, the overall flow organization and qualitative valve-related dynamics remained consistent across participants, suggesting that this mixing did not substantially alter the primary findings. Fourth, our use of 20° and 40° HUT represents mild to moderate venous volume stress and may not capture the extremes of posture or activity (e.g., full upright standing, ambulation, hypergravity, or microgravity). Nonetheless, these angles reflect physiologically relevant orthostatic conditions commonly encountered in daily life. Finally, the experimental design involved single-leg, single-contraction plantarflexion, which limits direct extrapolation to dynamic activities such as walking. This approach, however, reduced inter-individual variability and enabled precise regional

assessment of valve-related flow, providing a controlled framework for future studies to extend to bilateral or repetitive contractions.

Conclusion

Our study provides a detailed *in vivo* characterization of blood flow through and around lower-limb venous valves under graded volume stress and during active muscle contractions. Healthy valves generate efficient, jet-like flow through the leaflets; however, downstream regions exhibit disturbed flow, low shear, and increased vorticity. These findings highlight the dual role of venous valves in facilitating forward propulsion while generating localized hemodynamic heterogeneity that becomes more pronounced under volume stress. Through the application of VFI, this study provides a novel, spatially resolved depiction of valve hemodynamics not previously achievable with conventional Doppler methods, revealing complex patterns of vorticity and shear that deepen our understanding of valve flow behaviour and its response to physiological loading. Our observations extend previous descriptions of valve function and provide mechanistic insight into conditions that may predispose to thrombosis during orthostasis or impaired calf pump activity. These findings emphasize the importance of accounting for both regional flow characteristics and overall volume stress when assessing venous physiology and pathophysiology, providing a foundation for future translational studies exploring valve dysfunction, aging, and venous disease.

Chapter 7: General discussion

7.1 Overview

Control of venous blood flow is complex from both systems physiology and fluid dynamics perspectives, integrating multiple overlapping mechanisms that ultimately preserve venous return to the heart. The structural and functional characteristics of veins, marked by thin walls and high compliance, expose them to the effects of hydrostatic forces and pressure-generating mechanisms governing their flow. The determinants of venous return have been described for decades, forming the foundation for understanding both healthy and pathological conditions (1). Understanding these mechanisms remains critical for vascular health, supporting orthostatic tolerance, characterizing age-related adaptations, and assessing thrombotic risk. Despite this longstanding interest, the specific determinants of venous flow in vivo have remained difficult to define, limited by decades of reduced research focus and a lack of technological advancement. To address these gaps, this thesis examined advanced venous flow dynamics (Chapters 3, 5, and 6) and mechanisms of venous return (Chapters 4, 5, and 6) under the common theme of hydrostatic volume stress. The overall aim was to better understand the interaction between venous blood flow dynamics and venous return mechanisms in healthy younger and older adults. Through a series of four studies, venous properties were characterized during progressive volume shifting in major veins, both with and without active muscle contractions, to uncover mechanisms of venous return, the influence of aging, and the dynamic flow features generated within veins.

7.2 Summary of findings

Manipulation of hydrostatic volume stress was applied in each study to impose venous distension and probe specific changes in venous behaviour. In Chapter 3, progressive fluid shifting in the IJV revealed a relationship between increasing venous volume and reduced flow alignment. Flow alignment was further linked to vessel geometry, as irregular vein shape corresponded to lower flow organization at the caudal, most distended region compared to the narrower cranial region. These results were contextualized through a lower-limb cuff countermeasure protocol that trapped venous volume against a hydrostatic gradient, directly reducing IJV volumes and demonstrating how regional venous distension and hydrostatic constraints modulate central venous flow. Together, these findings provide evidence that recirculatory venous flow occurs during cranial volume shifts, supporting the proposed link between recirculatory flow and thromboembolic events observed during prolonged cranial fluid redistribution (100).

Building upon this central-to-peripheral perspective, Chapter 4 investigated the concept of an arterial pump mechanism across physiologically relevant distending pressures. Supporting the hypothesis that veno-arterial bundles facilitate the conservation and transmission of arterial mechanical energy, this study found that bundled veins maintained higher venous flow than non-bundled veins under identical hydrostatic conditions. These findings provide *in vivo* evidence for mechanical pressure transmission through valve-competent veno-arterial bundles as a mechanism of venous return in humans, consistent with recent *ex vivo* observations (83). While alternative explanations involving vasoactive reflexes cannot be fully excluded, methodological controls minimized their influence. Collectively, these findings refine understanding of venous-arterial coupling, suggesting a direct mechanical contribution to venous return during orthostatic stress.

To further explore venous return under hydrostatic load, Chapter 5 examined muscle pump function and associated flow dynamics in healthy younger and older adults. This comparative design leveraged age-related venous stiffening (127) to test the role of venous compliance in modulating dynamic blood flow responses to plantarflexor muscle activation. Contrary to the initial hypothesis, older adults exhibited preserved muscle pump capacity across volume stress conditions, whereas younger adults generated higher outflow velocities accompanied by more complex and recirculatory prone flow patterns. These results suggest a physiological trade-off: younger adults maintain a more compliant venous reservoir, enabling greater volume displacement but at the cost of reduced flow efficiency, while older adults compensate for reduced compliance through higher transmural pressures that sustain effective venous ejection for functional activities such as walking. This balance between compliance, pressure generation, and flow organization may represent adaptive hemodynamic strategies that evolve with healthy aging. Importantly, this study marked the first application of new exploratory quantitative measures of blood flow dynamics using VFI in humans, offering novel insights into venous flow organization, turbulence, and vorticity.

Extending the investigation of muscle pump dynamics, Chapter 6 addressed the regional distribution of blood flow features surrounding lower-limb venous valves under graded volume stress conditions with active muscle pump contractions. Use of VFI and the development of new quantitative outcome metrics were critical for resolving the hypothesized spatial heterogeneity in blood velocity around venous valves. Results revealed the valve channel to consistently exhibit greater velocity and flow uniformity, while regions immediately downstream were characterized by elevated vorticity and Reynolds stress, features indicative of disturbed, recirculatory flow.

These effects were notable during muscle contractions and under higher volume stress, underscoring the interaction between valve geometry, flow organization, and hydrostatic loading. The findings emphasize a double-edged role of venous valves: facilitating efficient antegrade propulsion through jet-like flow while simultaneously generating localized regions of disturbed flow that may, under certain conditions, contribute to stasis or thrombotic risk. Importantly, this study provides the first *in vivo* characterization of valve-specific hemodynamics in humans using VFI, bridging theoretical and measurable aspects of venous valve function and demonstrating the translational potential of advanced ultrasound technologies for studying venous physiology.

In summary, these four studies tested multiple hypotheses related to the control of venous flow in healthy humans, revealing nuanced relationships between venous return mechanisms and flow dynamics across a spectrum of hydrostatic distending pressures. Together, these findings establish a framework linking structural, mechanical, and functional determinants of venous return, forming the basis for physiological integration and conceptual significance.

7.3 Significance and integration

This body of work advances understanding of venous return and local flow regulation by synthesizing findings across four complementary studies and situating them within the existing literature. While each experiment tested a distinct hypothesis, together the results reinforce and expand the physiological framework linking venous anatomy and compliance to the maintenance of venous return across postural and age-related gradients. Collectively, the studies show that venous return emerges through interacting systems that dynamically redistribute volume and energy across vascular compartments, underscoring the integrated and adaptive regulation of the circulatory system.

Venous volume stress due to hydrostatic gradients emerged as a key determinant of venous behaviour, with the greatest distension observed in regions and individuals exhibiting higher venous compliance indicators. This pattern was most evident in younger adults and in the caudal IJV region, where higher venous compliance during HUT and HDT, respectively, allowed greater volume expansion but was associated with more disorganized flow features. These findings demonstrate a functional trade-off of a compliant reservoir: while it enables greater capacity to buffer hydrostatic volume stress, it introduces flow disorganization, increasing recirculatory behaviour. This understanding is supported by the tendency of highly compliant veins to change conformation under hydrostatic volume stress, altering fluid flow features (201)

and evidenced physiologically by venous maladaptation and flow abnormality during prolonged cephalad volume shifts (100, 174, 180). Such distension-induced flow disorganization may contribute to the higher incidence of lower-limb deep vein thrombosis in gravity-dependent regions (18), as well as venous thrombosis observed in astronauts when hydrostatic gradients are inverted (100). These features appear largely passive, secondary responses to the vessel's role in dissipating hydrostatic energy, highlighting the physiological importance of venous compliance in hemodynamic stability.

Venous valves were identified as regulators of flow organization within the system. The arterial pump mechanism described in Chapter 4 relied on active engagement of venous valves to translate arterial pulsations into venous propulsion, a process operating most efficiently within a specific range of distending pressures. Within these conditions, venous valves appear to serve a dual mechanical role: facilitating energy transfer from adjacent arterial pulsations while simultaneously shaping local flow organization. Across 20° and 40° HUT, this interaction produced marked post-valve flow disorganization during active muscle contractions, suggesting that valve motion imposes both order and fragmentation of venous flow depending on the prevailing hydrostatic load, geometric alignment and external pumping activation. The recurrence of similar flow disorganization patterns in the caudal IJV, often near a valve (243), suggests that these dynamics are not isolated to the lower limb but reflect a broader systemic response to hydrostatic loading. In both regions, compliant venous segments deform under pressure, altering how blood volume and kinetic energy are distributed along the vascular column. These concepts are reinforced by inverse evidence of IJV structural and flow collapse during upright posture (75, 244), identifying a primary role of relative position in the vascular hydrostatic column. This pattern indicates that local mechanics including vessel shape or valve motion scale to influence whole-body venous return, reinforcing a unified mechanical framework that contributes to circulation under gravity.

Age-related differences further revealed adaptive strategies in the regulation of venous return. In Chapter 5, older adults maintained venous return during upright posture and active muscle contraction despite reduced venous compliance, relying on increased transmural pressures rather than reservoir expansion. While sufficient for everyday activities, this compensatory strategy likely limits their ability to accommodate the large volume shifts required during high-intensity exercise, contributing to the well-documented decline in exercise capacity with aging. In contrast, younger adults retain a more compliant venous reservoir that supports greater volume displacement and superior orthostatic tolerance, albeit with increased flow

disorganization. These findings align with prior work demonstrating reduced limb venous compliance in older adults diminishing their ability to buffer hydrostatic loads (127). Conversely, endurance-trained individuals exhibit venous adaptations that enhance volume storage and ejection efficiency (124), underscoring the plasticity of the venous system and its contribution to hemodynamic regulation. Collectively, these patterns illustrate an age-dependent trade-off between efficient, pressure-driven venous ejection in the aged and flexible, high-capacity venous storage in the young, linking venous mechanics directly to functional limits of exercise tolerance.

The findings also invite broader consideration of arterial–venous coupling beyond the lower limbs. The arterial pump mechanism in Chapter 3 describes lower-limb veins which may have parallels in the head and neck circulation, particularly within the IJV, where the mechanical transmission of the carotid artery pulsatile energy could assist in clearing venous blood during cephalad fluid shifts. In conditions such as microgravity or prolonged head-down tilt, where hydrostatic gradients are altered and venous pooling occurs in the upper body (153, 167), this mechanism could partially compensate for impaired passive drainage, reducing cranial venous congestion and potentially mitigate associated thrombotic or intracranial pressure-related risks. Understanding the functional limits of this arterial–venous interaction could also clarify why some individuals are more susceptible to cephalad venous distension and its consequences, including spaceflight associated neuro-ocular syndrome (245). Moreover, these insights provide a rationale for designing targeted countermeasures in spaceflight or clinical settings, including interventions targeting mechanical energy transfer to support venous drainage under conditions of extreme or sustained volume redistribution.

Throughout the thesis, the potential link between flow disorganization and thrombotic risk was considered within the context of hydrostatic volume stress and aging. While younger adults exhibited greater recirculation and flow irregularity, they rarely experience thrombotic events (236), implying that robust anti-thrombotic mechanisms including endothelial responsiveness, and efficient fibrinolysis offset transient flow disturbances. In contrast, older adults experience a shift in this balance: reduced venous compliance limits wall motion and attenuates the shear-driven endothelial protection that typically mitigates clot formation (246). Qualitative VPI evidence from Chapter 6 reinforces this concept, revealing recirculatory flow zones in the valvular sinus following muscle contractions. These regions, characterized by low shear, prolonged residence times and increased hematocrit (227), are not only theorized sites of blood stasis but also correspond to known anatomical locations where thrombi commonly originate (228), suggesting that age or compliance-related alterations may shift these regions from physiologically benign to

pathologically thrombogenic. Importantly, the arterial pump mechanism described in Chapter 4 may serve an underappreciated anti-thrombotic function in this area by mechanically transmitting arterial pulsations to intermittently disrupt pockets of stagnant blood during relevant hydrostatic loads, minimizing blood stasis. In older adults, diminished arterial–venous coupling due to vascular stiffening (247) could therefore reduce this protective effect, facilitating venous pooling and increasing susceptibility to thrombosis. These links highlight that thrombotic risk may also arise from an age-related loss of the dynamic mechanical energy conservation system which may prevent stasis in veins. Translationally, these insights underscore the effort to prioritize countermeasures that enhance mechanical veno-arterial coupling or maintain venous wall compliance, ranging from intermittent pneumatic compression (69), resistive and aerobic exercise (87, 124), healthy diets (248) to targeted strategies for maintaining venous health in aging populations and special environments including microgravity (164).

7.4 Limitations

Specific limitations should be considered when interpreting the findings of this thesis. First, VFI data were not collected during resting conditions in the lower limb due to signal intensity constraints, preventing assessment of baseline flow features and potentially underestimating the range of venous adaptation across hydrostatic conditions and age groups. Although flow disorganization and recirculation were discussed in relation to thrombotic risk, no clinical populations were studied, and no biomarkers of coagulation or endothelial activation were collected, limiting direct relations of observed flow features to thrombus formation. Additionally, the arterial pump mechanism remains speculative, as observed veno-arterial coupling could reflect passive volume transmission within a closed tissue circuit. Arteriolar expansion in fascia-encased compartments may displace venous blood, making it unclear how much flow is actively propelled versus passively redistributed through the circuit. Further, velocities measured during muscle pump activation were not normalized to rate of contraction force generation, which can influence venous outflow characteristics. Extrapolations to microgravity and cephalad fluid shifts should also be viewed cautiously in the absence of direct data from spaceflight or prolonged headward fluid loading models. Technical constraints, including the use of two-dimensional VFI rather than three-dimensional imaging techniques, may have limited detection of additional rotational flow structures near valve sinuses. Finally, all studies only included healthy adults in sex-balanced designs powered only for primary outcomes, restricting the generalizability of the results to clinical or sex-based differences. However, the equitable recruitment of males and

females provides a foundation for exploratory comparisons and the generation of sample size estimates to inform future studies specifically targeting sex differences. Together, these limitations define important directions for future work.

7.5 Future directions

The body of work contained here provides the foundation for various launch points into future investigations of venous physiology, hopefully ushering a renewed interest from the field. Future work should begin by integrating VFI with invasive venous pressure measurements, experimental gravity analogs, and biomarkers of thrombus formation to strengthen mechanistic interpretations. The proposed arterial pump mechanism requires further testing to determine whether increased pulse pressure amplifies effects, or arterial stiffening attenuates the transmission of pulsatile arterial energy to adjacent veins. Valve regional flow study should be expanded to include resting conditions following methodological refinement and extended to older adults to evaluate how reduced venous compliance and valve stiffness influence flow organization and local stasis. Longitudinal investigations are also warranted to follow venous compliance and flow dynamics relationships throughout aging or interventions including exercise, bedrest, or pharmacologic treatment. To explore the opposite end of the venous function spectrum, future studies should examine how enhanced venous compliance relates to flow dynamics by expanding athlete phenotyping beyond preliminary work (124). Finally, computational modeling incorporating vessel geometry, compliance, and valve kinematics could further quantify how veins and venous blood respond under different physiological conditions. Collectively, these directions can further advance the field toward a more integrated and mechanistic understanding of venous function, defining whether the observed flow features represent adaptive physiology, early dysfunction, or modifiable targets to optimize venous return and protect against thrombotic risk.

7.6 Thesis conclusion

This thesis advances understanding of human venous function by integrating physiological and imaging evidence to describe how compliance, hydrostatic stress, and local flow features interact. Visualization of *in vivo* flow organization and vessel deformation by advanced ultrasound techniques demonstrated how distension, collapse, and valve motion modulate the transmission of volume and cardiovascular circuit energy. Venous compliance consistently emerged as a key determinant of flow organization, linking mechanical properties to circulatory effects and exercise.

Together, these results position the venous system as a dynamic and mechanically responsive facet of the circulation, integral to hemodynamic regulation.

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Appendix A

Unsung signals: Bits, crystals, and the Doppler effect

Much like atomic bonds hold together the matter of the universe, this thesis is bound by the imaginary connections of logic between 1s and 0s deep within the CPU's. Every datapoint, figure and paragraph reflects not only my intellectual input but also the relentless computational power behind it: ~1700 cine loops collected, ~25 terabytes of data stored and analyzed, 256 gigabytes of RAM pushed to their limits, and GPUs running countless iterations to make sense of complexity.

I am particularly indebted to the 6 hard disk drives who housed the data necessary for completion of this work. The rhythmic clicking noise of tirelessly spinning platters became the background noise to the lab for years. The persistent audible tone of scientific progress often followed me too into my dreams, linking dream states to flow states.

I am equally grateful to the piezoelectric crystals of the 5 separate ultrasound probe heads used to complete this work, endlessly excited to convert voltage into physiology to reveal what cannot be seen, millions of times over. Their unwavering precision and endurance made it possible to explore physiology where only the highest reaches of the sound spectrum can go.

And to the Doppler effect itself, a phenomenon that has come to govern more of my life than I ever anticipated. From the Doppler shifts that shape the flow patterns in my studies, to the weather radar that dictates when I can ride my bike, to my car radar that quietly protects me while I ponder Doppler shifts on those rides; the same principle echoes through it all. It is a fitting reminder that frequency, motion, and reflection are not only physical concepts but guiding forces that link work, life, and curiosity together.