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***In Vivo* MRI Assessment of Blood Flow in Arteries and Veins from Head-to-Toe Across Age and Sex in C57BL/6 Mice**

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Abstract

Although widely used as a preclinical model for studying cardiovascular diseases, there is a scarcity of *in vivo* hemodynamic measurements of the naïve murine system in multiple arterial and venous locations, from head-to-toe, and across sex and age. The purpose of this study is to quantify cardiovascular hemodynamics in mice at different locations along the vascular tree while evaluating the effects of sex and age. Male and female, adult and aged mice were anesthetized and underwent magnetic resonance imaging. Data were acquired from four co-localized vessel pairs (carotid/jugular, suprarenal and infrarenal aorta/inferior vena cava (IVC), femoral artery/vein) at normothermia (core temperature 37 ± 0.2 °C). Influences of age and sex on average velocity differ by location in arteries. Average arterial velocities, when plotted as a function of distance from the heart, decrease nearly linearly from the suprarenal aorta to the femoral artery (adult and aged males: -0.33 ± 0.13 , $R^2 = 0.87$; -0.43 ± 0.10 , $R^2 = 0.95$; adult and aged females: -0.23 ± 0.07 , $R^2 = 0.91$; -0.23 ± 0.02 , $R^2 = 0.99$). Average velocity of aged males and average volumetric flow of aged males and females tended to be larger compared to adult comparators. With cardiovascular disease as the leading cause of death and with the implications of cardiovascular hemodynamics as important biomarkers for health and disease, this work provides a foundation for sex and age comparisons in pathophysiology by collecting and analyzing hemodynamic data for the healthy murine arterial and venous system from head-to-toe, across sex and age.

Keywords

Murine; Cardiovascular; Hemodynamics; Sex; Age; Arterial; Venous

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AUTHOR CONTRIBUTIONS

A Colleen Crouch (ACC), Amos A. Cao (AAC), Ulrich M Scheven (UMS), and Joan M Greve (JMG). ACC conceived and designed the study; AAC wrote the PCMRI pulse sequence; ACC, AAC, UMS developed the in-house image analysis method; ACC collected and analyzed data, performed statistical analysis and interpreted the data; ACC and JMG wrote the paper; ACC and JMG critically revised the paper; all authors gave final approval of the paper.

ELECTRONIC SUPPLEMENTARY MATERIAL

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DISCLOSURE

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INTRODUCTION

Cardiovascular disease is the leading cause of death in the US and worldwide among non-communicable diseases.^{33,41} Cardiovascular hemodynamics are important biomarkers for cardiovascular health.^{9,35} Volumetric blood flow is an important metric because it defines how much blood is delivered to parts of the body per time unit thus impacting the delivery of nutrients and oxygen to those parts of the body. Changes in volumetric flow are achieved by changes in vessel area and/or velocity (Volume flow equals area multiplied by time-averaged mean velocity). It is important to determine whether area or velocity differences account for volumetric flow changes because of their relationship to cyclic circumferential strain of the vessel wall and shear stress on the luminal surface. Alterations in wall shear stress along the vessel tree have been shown to be integral in disease localization and progression in atherosclerosis.¹⁰

Hemodynamic metrics are not just important for cardiovascular health, physiological differences between groups should be considered when investigating the cardiovascular system. Cardiovascular disease is associated with advancing age^{27,34} and differs between the sexes with respect to risk factors,^{28,30,38} symptoms,⁵ incidence rates,^{4,8} and response to treatment.² Therefore, quantitative characterization of the cardiovascular system should include both sex and age groups.

Although murine models are widely valued as a preclinical model for human cardiovascular diseases to reproducibly study disease progression and treatment, *in vivo* hemodynamic measurements of the naïve murine arterial and venous systems across age and sex have yet to be fully characterized. Magnetic resonance imaging (MRI) can be used to non-invasively study vasculature at numerous locations due to high spatial resolution and few limitations on tissue penetration depth. Gated techniques, such as phase contrast MRI (PCMRI), can be leveraged to study processes that change across the cardiac cycle including blood velocity.

Using murine models and MRI, we noninvasively quantified arterial and venous hemodynamics, including blood velocity, volumetric flow, and wall shear stress, *in vivo* in healthy mice at four locations of the body in artery-vein pairs: carotid/jugular, suprarenal abdominal aorta/inferior vena cava (IVC), infrarenal aorta/IVC, and femoral artery/vein. The influence of sex and age on the healthy hemodynamics were also investigated. These geometric and functional data provide a baseline against which disease hemodynamics can be compared and coupled with computational fluid dynamics (CFD) could provide information regarding stresses on the vasculature.

METHODS

All experiments were carried out with local Institutional Animal Care and Use Committee approval. Animals were housed in a room with temperature (22 ± 2 °C) and humidity (~27%) control and an alternate 12 h light/dark cycle.

Healthy adult (12- to 14-weeks-old, ~20–25 human years) and aged (52- to 58-weeks-old, ~50 human years¹⁷), male and female, C57BL/6 mice, purchased from Charles Rivers

Laboratory, were used in this study ($n = 5$ each, total = 20). Mice were anesthetized with 1.25–2% isoflurane in 1 L/min of oxygen,¹² and eye lubricant applied to prevent eye damage. Animals were imaged in the supine position at 7T using a Direct Drive console (Agilent Technologies, Santa Clara, CA) and a 40 mm inner diameter transmit-receive volume coil (Morris Instruments, Ontario, Canada). Animal core temperature was maintained at 37 ± 0.2 °C using a heater blowing warm air through the bore of the magnet and over the animal, a rectal temperature probe, and a custom-built proportional-integral-derivative (PID) controller (Labview, National Instruments, Austin TX). Heart rate and respiration were monitored (SA Instruments, Stony Brook, NY). CINE phase contrast data was acquired in the neck (carotid artery and jugular vein), torso (suprarenal/infrarenal aorta and inferior vena cava), and periphery (femoral artery and vein).

We previously verified that changes observed in heart rate and vessel area were not due to long exposures to anesthesia¹⁴; here, we tested the effect of anesthesia on volumetric flow by using the same data acquisition and analysis procedures, repeated every 30 minutes with animal's core temperature maintained at 37 °C for 2 h ($n = 2$, adult males). For the carotid artery ($n = 15$ mice), due to the differences in origination site at the aortic arch and location relative to the subclavian arteries, the left and right carotid arteries were compared. The iliac arteries ($n = 5$ mice) were chosen as an additional relevant location to evaluate the flow split, due to the number of murine disease models located in the infrarenal aorta (e.g. aneurysms).

MRI Slice Planning and Parameters

Sagittal 2D and axial 3D acquisitions were used to plan slices perpendicular to the carotid artery and to the jugular vein. Coronal 2D and sagittal 3D acquisitions were used to plan slices perpendicular to the aorta and to the IVC and the femoral artery and vein.

Starting from previously published parameters for PCMRI data acquired in the infrarenal aorta of adult male mice at a lower field strength,¹⁹ during parameter optimization we focused on improving temporal and spatial resolution and reducing partial volume effects.²⁹ Sixteen cardiac-gated 2D CINE phase contrast frames with through plane velocity encoding were acquired perpendicular to each vessel. The PCMRI sequence involved repeated measurements using reversed bipolar gradients. Parameters for arteries were [TR/TE ~ 180/5 ms depending on heart rate, flip angle (α) 60°, FOV (20 mm)², matrix 128² zero-filled to 256², in-plane resolution (78 μ m)², slice thickness 1 mm, NEX 2]. Parameters for veins were [TR/TE ~ 180/5 ms depending on heart rate, α 20°, FOV (25.6 mm)², matrix 128² zero-filled to 256², in-plane resolution (100 μ m)², slice thickness 1 mm, NEX 2]. The difference in parameters (reduced flip angle and resolution, respectively) reflects the slower blood flow and larger size of the veins. TE was minimized to reduce motion artifacts and maintain signal in case of complex flow.

We also focused on optimizing the velocity encoding (VENC) parameter for each vessel. Briefly, multiple datasets were acquired at each location using different VENC's, with the priority being the lowest VENC possible while avoiding velocity aliasing. For each artery/vein acquisition, the phase difference (velocity) from a region of interest located within stationary tissue was calculated at ~ 0 cm/s. Therefore, no baseline corrections were needed. VENC values were: carotid 100 cm/s, suprarenal aorta 120 cm/s, infrarenal aorta 80 cm/s,

femoral artery 40 cm/s, and all veins 20 cm/s. Methods development work for veins included using a VENC of 10 cm/s and a steady flow phantom with volumetric flow to approximate velocities expected in veins (i.e. ~ 40 mL/min as measured by an inline flow meter (40.3 mL/min) and volume + stopwatch (40.4 mL/min), with 6 mm inner diameter tubing results in an estimated mean and max velocity of 2.4 and 4.8 cm/s, assuming fully-developed, laminar flow in a pipe). A total of 40 measurements were made (twenty measurements taken in both flow directions). For $n = 3$ mice per group, the signal to noise ratio (SNR) was estimated at the suprarenal aorta to evaluate the standard deviation in velocity measurements ($\sigma_v = \frac{\sqrt{2}}{\pi} \frac{VENC}{SNR}$,³¹). We chose this location because of its proximity to other organs (e.g. liver and gastrointestinal tract) which could result in susceptibility effects and degrade SNR. In addition, it was the location requiring the highest VENC (120 cm/s).

MRI Analysis: Cross-Sectional Area, Velocity, Volumetric Flow

The CINE images were analyzed for vessel cross-sectional area, mean systolic or diastolic velocity (velocity averaged within the individual systolic or diastolic CINE frame), peak velocity (maximum velocity from any of the 16 CINE frames), average velocity across the cardiac cycle (the mean velocity, averaged across all 16 CINE frames), and average volumetric flow (the mean volumetric flow, averaged across all 16 CINE frames) using an in-house semi-automated MATLAB code. The semi-automated process for area is similar to methods developed and described previously,^{13,36} with the addition of velocity calculations *via* phase subtraction of the two acquisitions. Once the vessel boundary was defined using thresholding, it was verified or adjusted by the user. Volumetric flow of the entire vessel was calculated by Eq. (1):

$$\text{Volumetric Flow} = \sum \text{Area}_{\text{voxel}} * \text{Velocity}_{\text{voxel}} \quad (1)$$

Figure 1 shows a representative magnitude and velocity image from the PCMRI acquisition and demonstrates the reproducibility of PCMRI measurements between animals.

Wall Shear Stress Calculations

Wall shear stress (WSS) was calculated in the arteries using the Hagen–Poiseuille formula (Eq. (2)) with the assumptions that there is laminar flow, blood is a Newtonian fluid, and flow is in a rigid wall cylindrical tube.

$$\text{WSS} = \tau = \frac{8\mu\bar{u}}{d} \quad (2)$$

where μ is the dynamic viscosity (0.04 g/cm/s), $\bar{u}$ is the mean velocity, and d is the inner diameter of the vessel.

Statistical Analysis

Data are plotted as mean $\pm$ standard error (SEM). Velocity and volumetric flow were positive for flow in the inferior to superior direction (i.e. for carotid, IVC, femoral vein) and negative for flow in the superior to inferior direction (i.e. for jugular, aorta, and femoral

artery). To simplify presentation and interpretation, all results are reported and statistically evaluated as the absolute value to be able to compare locations. All data were tested for normality using Shapiro-Wilk test with alpha set to 0.05. To test if velocity (average and peak), average volumetric flow, and WSS (average and peak-systole) differed significantly between sex and age groups in a given vessel, repeated measures two-way ANOVA and Tukey's *post hoc* test to account for multiple comparisons was used. For location differences, groups were treated as categorical and repeated measures one-way ANOVA with Tukey's *post hoc* test was used to determine differences between locations. Significance was set at $p < 0.05$. Data were analyzed using GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA).

RESULTS

A summary of the geometry (diameter for circular arteries, area for irregularly shaped veins), velocity, and volumetric data for all groups at every location is presented in Table 1.

Average body weights increased with age (adult: 21.9 ± 0.6 vs. aged: 32.4 ± 0.9 g, $p < 0.0001$) and were greater in males compared to females (male: 29.8 ± 1.4 vs. female: 24.5 ± 1.1 g, $p < 0.001$). Average heart rate did not change with age (adult: 487 ± 24 vs. aged: 508 ± 16 beats per minute/bpm) but was higher in females compared to males (male: 454 ± 14 vs. female: 551 ± 10 bpm, $p < 0.05$). Similar to heart rate and vessel area,¹⁴ 2 h of isoflurane exposure at normothermic conditions (37 °C) resulted in minimal changes in volumetric flow (largest changes seen in the infrarenal IVC and femoral artery and vein: 6.9, 7.0, and 0.9 %/30 min, respectively, with no statistically significant differences between 30 minute intervals). For both the carotid and iliac arteries, there was no difference between the left and right sides ($p > 0.05$).

MRI parameter optimization resulted in an ~ 33% increase in the number of CINE frames across the cardiac cycle and improvement in in-plane resolution, with a reduction in partial volume effects by reducing the slice thickness by 50%. We were able to achieve an ~ 60% improvement in the VENC for the infrarenal aorta (200 vs. 80 cm/s). Measurements from a steady flow phantom with velocities expected in veins resulted in volumetric flows of 39.4 ± 4.0 mL/min as calculated from PCMRI data, which was within 2.6% of the actual value as measured by flow probe and volume + stop-watch. The average SNR of data acquired at the suprarenal aorta varied little between animals (31.2 ± 2.9), resulting in $\sigma_v = \pm 2$ cm/s.

Influences of Age and Sex on Average Velocity Differ by Location in Arteries

Figure 2 shows the average velocity across the cardiac cycle (left) and the same velocity adjusted for body weight (right) at four locations for the four groups studied here. The average velocity of the suprarenal IVC was smaller in aged females compared to aged males (aged females: 3.3 ± 0.3 vs. aged males: 5.9 ± 0.5 cm/s, $p = 0.04$; Fig. 2a). Average velocity differed significantly along the vascular network for the arteries ($p < 0.0001$). Average velocity of the arteries, when plotted as a function of distance from the heart, decreases nearly linearly from the suprarenal aorta to the femoral artery (adult and aged males: -0.33 ± 0.13 , $R^2 = 0.87$; -0.43 ± 0.10 , $R^2 = 0.95$; adult and aged females: -0.23 ± 0.07 , $R^2 = 0.91$; -0.23 ± 0.02 , $R^2 = 0.99$). Conversely, location had no effect on the venous average velocity

for any animal group. When adjusting for body weight, the average velocity per gram of body weight decreased with age for females at the suprarenal aorta and IVC, infrarenal aorta, and femoral artery. The infrarenal aorta's velocity was larger in adult females compared to males.

Influences of Age and Sex on Peak Velocity Differ by Location in Arteries

Figure 3 shows the maximum (max) velocity plotted for each image frame at the four arterial locations for all groups. To better highlight differences at the femoral location, data are plotted with individually scaled y-axes (bottom, right). The maximum velocity during peak-systole, or peak velocity, was larger in aged males compared to adult males for the carotid (aged males: 83.0 ± 8.5 vs. adult males: 55.0 ± 4.3 cm/s, $p = 0.0004$) and the femoral artery (aged males: 14.1 ± 2.3 vs. adult males: 14.1 ± 1.6 cm/s, $p = 0.03$) and was larger in adult females compared to adult males for the carotid (adult females: 85.2 ± 7.3 vs. adult males: 55.0 ± 4.3 cm/s, $p = 0.02$). Peak velocity differed significantly between all locations except carotid and infrarenal aorta (adult males), carotid and suprarenal aorta (aged males), and carotid and both suprarenal and infrarenal aorta (adult and aged females). Conversely, sex, age, and location had no effect on the venous peak velocity.

Influences of Age and Sex on Volumetric Flow Differ by Location and are Body Weight Dependent

Figure 4 shows the average volumetric flow across the cardiac cycle (left) and the same flow adjusted for body weight (right) at four locations for the four groups studied here. To better highlight differences at the femoral location, data are plotted with individually scaled y-axes (bottom). The volumetric flow of the jugular was larger in aged males compared to adult males (aged males: 2.7 ± 0.2 vs. adult males: 1.1 ± 0.2 mL/min, $p = 0.02$). The volumetric flow of the suprarenal IVC was larger in aged males compared to adult males and aged females (aged males: 6.4 ± 0.3 vs. adult males: 3.7 ± 0.8 , $p = 0.003$; and, aged females: 3.7 ± 0.3 mL/min, $p = 0.003$). Similarly, the volumetric flow was larger in aged males compared to adult males for the infrarenal aorta (aged males: 1.7 ± 0.3 vs. adult males: 1.7 ± 0.1 mL/min, $p = 0.003$) and femoral artery (aged males: 0.5 ± 0.05 vs. adult males: 0.3 ± 0.03 mL/min, $p = 0.03$).

When adjusting for body weight, age-dependent differences in volumetric flow were no longer appreciable and sex-dependent differences became apparent. Adjusted volumetric flow was larger in adult females compared to adult males in the jugular (adult females: 0.08 ± 0.003 vs. adult males: 0.04 ± 0.008 mL/min/g, $p = 0.01$), infrarenal aorta (adult females: 0.1 ± 0.01 vs. adult males: 0.07 ± 0.006 mL/min/g, $p < 0.001$), and femoral vein (adult females: 0.01 ± 0.0006 vs. adult males: 0.007 ± 0.001 mL/min/g, $p = 0.01$).

Across all groups, location of the vessel influenced average volumetric flow ($p < 0.0001$). For every animal group, average volumetric flow differed significantly between the suprarenal aorta and all other vessels ($p < 0.0001$). With the exception of adult males, the infrarenal aorta flow was larger compared to the femoral artery ($p < 0.02$). For every group, the suprarenal IVC flow was larger than the femoral vein ($p < 0.0001$). For males and adult females, the suprarenal IVC flow was larger than the infrarenal IVC ($p < 0.03$), and for only

males the suprarenal IVC was larger than the jugular ($p < 0.002$). In aged animals, the jugular flow was larger than femoral vein ($p < 0.02$). In aged males, the infrarenal IVC flow was larger than the femoral vein ($p = 0.01$).

Velocity and Volumetric Flow Differ Between Arteries and Veins

For average velocity (Fig. 2), the arteries had larger velocities compared to their corresponding veins (carotid > jugular; IVC > aorta, $p < 0.01$). Statistical differences were seen between all arteries compared to veins with the following exceptions: for every group, the femoral artery and the suprarenal IVC were not statistically different; for males and aged females, the femoral artery and the infrarenal IVC were not statistically different; and for females and aged males, the femoral artery and the jugular were not statistically different. For peak velocity, the arteries had larger velocities compared to their corresponding veins (carotid > jugular; IVC > aorta, $p < 0.001$). Statistical differences were seen between all arteries compared to veins except between the femoral artery and all the veins for all groups. For volumetric flow (Fig. 4), the artery-vein pairs were not statistically different except in the suprarenal location (suprarenal aorta > suprarenal IVC, $p < 0.0001$).

WSS Differs by Location in Arteries, and Age Influences Average WSS in Males at the Femoral Artery

Figure 5 shows the average WSS across the cardiac cycle (left) and the peak systole WSS (right) at four arterial locations for the four groups studied here. Average WSS in the femoral artery for adult males was 1.7-fold larger than aged males ($p = 0.04$). Average WSS varied by arterial location in adult females (suprarenal < femoral, $p = 0.02$; infrarenal < femoral, $p < 0.05$) and aged males (carotid > suprarenal, $p = 0.02$; carotid < femoral, $p = 0.005$). Average WSS of the arteries, when plotted as a function of distance from the heart, increases from the suprarenal aorta to the femoral artery in the adult animals (adult males: 0.68 ± 0.29 , $R^2 = 0.3$, $p = 0.03$; adult females: 1.23 ± 0.27 , $R^2 = 0.61$, $p = 0.0006$). Peak-systolic WSS also varied by location in adult males (carotid > femoral, $p < 0.05$), in adult females (carotid > suprarenal, $p = 0.02$), in aged males (carotid > femoral, $p = 0.008$), and in aged females (carotid > suprarenal, $p = 0.0006$; carotid > infrarenal, $p = 0.04$). A summary of the average, systolic, and diastolic WSS data for all groups for the arteries is presented in Supplementary Table 1.

DISCUSSION

The work presented here, to our knowledge, is the first to quantify velocity and volumetric flow in artery-vein pairs at four locations within the same subject, from head-to-toe, across sex and age. We have shown that sex and age influence velocity and volumetric flow, which are also dependent on location in the body. We quantified age-dependent differences because of the influence of age in human cardiovascular health^{27,34} and the growing body of evidence that, similarly, murine models are not immune to the challenges aging imposes.^{13,36} We quantified sex-dependent differences in support of the National Institutes of Health's recent communications regarding lack of sex diversity in preclinical experiments.¹¹ Location-dependent differences are relevant for location specific cardiovascular pathophysiology and clinical interventions where translocation of vessels is used (e.g.

bypass surgery). Initial data for healthy animals in these four groups can be used for computational modeling (e.g. initializing boundary conditions) or to compare future measurements from disease models.

We were able to improve upon many of the criteria known to affect PCMRI measurement errors.²⁹ Compared to previous work,¹⁹ we increased temporal resolution across the cardiac cycle, spatial resolution across the vessels, and reduced slice thickness by half. All of which would minimize underestimations of flow and peak velocity. Optimizing VENC and minimizing noise in our velocity images improves our ability to measure maximum velocity, with lesser impact on volumetric flow, because the latter is calculated using several voxels over which the noise is averaged. The accuracy of PCMRI data acquired using a steady flow phantom with low velocities requiring a reduced VENC supports our measurements acquired from the veins.

Although there is a lack of data in the field regarding hemodynamics for C57BL/6 mice across sex and age, our data is consistent with previously published data acquired in different strains of mice or for only a single location, sex, or age. For example, for the carotid artery, Williams *et al.* reported an average velocity of 12.6 ± 0.2 cm/s for female CD-1 mice using pulse-waved Doppler which is in agreement with our reported data of 12.6 ± 2.2 cm/s for female mice.⁴² For the suprarenal aorta, Amirbekian *et al.* reported an average velocity of 17.2 cm/s for C57BL/6 mice using PCMRI which is in agreement with our data: 18.1 ± 2.3 cm/s for adult mice.¹ For the infrarenal aorta, Greve *et al.* reported an average velocity of 11.9 ± 0.8 cm/s for C57BL/6 male mice using PCMRI which is consistent with our data: 10.7 ± 0.7 cm/s for adult males.¹⁹ For the femoral artery, Song *et al.* reported an average volumetric flow of 0.231–0.35 mL/min for C57BL/6 male mice using optical coherence tomography which is consistent with our data: 0.31 ± 0.03 mL/min for adult male.³⁹ Although careful work using modeling has estimated geometry, velocity, and flow along the arterial tree,^{3,23} on average those estimations are lower than our empirical values and literature values presented here. This may be due to much lower heart rates because of the anesthetic chosen (ketamine-xylazine and 200 bpm vs. isoflurane and ~ 500 bpm), invasive methods (flow probes), and/or inadequate temperature control (no reporting of core body temperature). Given the consistency with previously published data, in addition to improvements in data acquisition parameters, our noninvasively derived empirical values can accurately be used to compare across location, sex, and age. Mice, increase in body weight and size with age.¹⁷ This continuous increase in size affects the cardiovascular system as evident by our data. Average velocity of aged males and average volumetric flow of aged males and females tended to be larger compared to adult comparators. Older animals, and thus larger animals, likely have larger volumetric flow due to greater demand for blood to supply the nutrient requirements of greater mass. This increase in volumetric flow throughout the arterial tree is consistent with previous work showing aged animals had larger cardiac outputs, and thus larger volumetric flow, compared to younger groups of the same sex¹³; however, both results are contrary to previous findings in humans²⁶ and a consideration for future mouse work. This may be due, in part, to our aged mice just bordering senescence without frank declines in cardiac function, which would be consistent with findings in humans suggesting that age-related vascular changes precede declines in cardiac function.²² These age-related vascular declines often first appear during a cardiac

stressor.⁷ However, as with any animal study, one should consider these differences when extrapolating from mice to humans.²⁰

To account for differences in mass, adjusting raw data to body weight is common practice. For the females, in particular, adjusting average velocity to body weight revealed statistically significant age-dependent differences (aged < adult). We hypothesize that this reflects the fact that the older female mice are approaching reproductive senescence. When normalizing average volumetric flow by body weight, differences due to age were diminished or reversed. We can speculate that this is due to age-related decreases in muscle¹⁶ and an increases in fat content, which is less metabolically active than muscle and therefore demands less blood flow per unit mass.¹⁸

For sex comparisons, females had significantly higher heart rates compared to males consistent with previous murine data¹³ and in agreement with human data.²¹ Differences in the sexes for both adult and aged animals were location dependent. Statistically significant sex-differences were seen at the suprarenal IVC for average velocity and volumetric flow. Previous work in characterization of venous murine vasculature found the cross-sectional area of the suprarenal IVC in males was significantly larger than females and attributed sex-differences in aged animals to changes in reproductive capacity for aged females, alluded to above.³⁶ Although only this location had significant sex differences, trends were seen across groups and locations. For the carotid, jugular, infrarenal aorta, femoral artery and vein, adult females had larger velocities and volumetric flow compared to adult males. The larger volumetric flow can be attributed to greater velocities and not larger vessel areas.^{7,36}

When considering sex and age, the carotid and jugular of aged males tended towards having larger velocities but similar volumetric flow compared to aged females, in contrast to adult animals where females have larger velocities and volumetric flow, suggesting differences in brain demand due to sex are age dependent. Research in humans has shown greater cerebral blood flow in females at all ages; however, although not statistically significant, aged males had slightly larger volumetric flows compared to aged females in Fig. 4.³⁷ In the infrarenal aorta, aged males had slightly larger volumetric flows compared to aged females in contrast to adult animals (adult females > adult males), further supporting the effect of reduced reproductive capacity and impact on vessel area.³⁶

Adjusting for body-weight revealed statistically significant sex differences in volumetric flow in adult animals for the jugular, infrarenal aorta, and femoral vein (female > male in all cases). The relative differences between males and females (either no difference, males larger than females, or females larger than males) differed in adult animals for: average velocity in suprarenal aorta and IVC, and volumetric flow in both suprarenal and infrarenal IVC. The relative differences between males and females differed in aged animals for: average velocity in carotid and suprarenal aorta, and volumetric flow in carotid, suprarenal and infrarenal aorta, and femoral artery. This indicates that sex related differences in velocity and volumetric flow are not simply based on body weight alone but also autonomic control of cardiovascular system.^{15,24}

Volumetric flow between the arteries and veins are similar in all pairs except the suprarenal location. Based on the principle of mass conservation, we would expect the larger arteries/veins to have similar volumetric flow (flow in= flow out). Although not quantified in this study, the hepatic vein could account for the larger volumetric flow in the suprarenal aorta compared to the suprarenal IVC.^{6,25} The differences in arterial and venous hemodynamics were also investigated in this study. In the arteries, there is high-pressure flow, and thus higher blood flow velocity, compared to the low-pressure/slower velocity in the veins.³²

Area and/or velocity differences account for volumetric flow changes and contribute to cyclic circumferential strain of the vessel wall (area) and shear stress on the luminal surface (area and velocity). For this work, alterations in shear stress along the arterial tree were quantified and can provide baseline information for comparison to pathological conditions since alterations in WSS have been implicated in disease localization and progression in atherosclerosis.¹⁰ WSS baseline data for each group is more important than the baseline comparison between groups because the relative difference in WSS or directional changes between baseline and pathological states has been shown to be a stronger determinant of flow-mediated inflammatory response compared to absolute magnitude differences between groups.⁴⁰ WSS in veins was calculated; however, because of the non-circular nature of veins,³⁶ the approximation of a circular cross-section is violated. If we assumed veins were circular and used cross-sectional area to calculate diameter, WSS did not exceed 15 dynes/cm² in any vein; therefore, not reported in this work.

Although not statistically compared in this work because the differences in sex and age are captured in the other three measurements (i.e. average velocity, peak velocity, and average volumetric flow), mean systolic and mean diastolic values for velocity are presented in Table 1. With these values, shear stress at both systole and diastole were calculated; however, the study lacked adequate power and an additional 8 animals in each group are necessary for systolic comparison across sex and age. Future work includes calculating the shear stresses at both systole and diastole and comparing across sex and age and creating CFD models to compare stress along the arterial tree.

This study was not without limitations. For the veins, the non-circular geometries and vessel curvature made planning data acquisition perpendicular to the vessel more challenging, which could introduce a source of uncertainty into the data due to partial volume effects. However, using 3D acquisitions which were optimized in previous work, with parameters for slower blood flow to enable anatomical landmarks for planning, we are confident our work is reproducible.³⁶ We are also limited in the physiological state of the animals due to the use of anesthesia. Although, studies show a slight reduction in cardiac output under anesthesia using isoflurane, our heart rates are closer to those recorded in conscious mice.¹²

To our knowledge, this is the first time that the velocity and volumetric blood flow of the murine arterial and venous systems have been quantitatively characterized non-invasively, at multiple locations across age and sex. The results demonstrate that there are differences in velocity and volumetric flow across locations and between vessel types. Furthermore, age and sex are relevant to consider when drawing conclusions from disease models. Future work incorporating pressure measurements and/or computational fluid dynamics (CFD)

would be useful for examining stress-strain properties and luminal biomechanical forces such as wall shear stress *in vivo*. Our data can provide physiologically-relevant parameters to CFD models and provide baseline data from the healthy murine vasculature to use as a benchmark for investigations of a variety of physiological and pathophysiological conditions of the cardiovascular system.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

(a) Representative anatomical images from magnitude (left) and velocity (right) images from the PCMRI sequence (arrow, aorta; arrowhead, vena cava). (b) Volumetric flow measurements from the suprarenal aorta calculated for every adult female animal at 37 °C demonstrates that PCMRI measurements were reproducible between animals.

FIGURE 2.

Average velocity across the cardiac cycle (mean $\pm$ SEM) at four locations in the arterial and venous system of C57BL/6 mice (a), and average velocity adjusted to individual gram (g) body weight (b). Velocity varied by location and across sex and age. Average velocity varied by sex and age with significant age differences denoted for males (m*) or females (f*); and, sex differences for adult (Y†) or aged (A†) with significance at $p < 0.05$. IVC inferior vena cava.

FIGURE 3.

Maximum (max) velocity for each CINE frame in the carotid artery, suprarenal and infrarenal aorta, and femoral artery for all adult and aged, male and female mice ($n = 5$ each). Aged male animals had larger maximum velocities across the cardiac cycle in the carotid and femoral. In the carotid, adult females had larger maximum velocity across the cardiac cycle compared to adult males. Peak velocity varied by sex and age with significant age differences denoted for males (m*) or females (f*); and, sex differences for adult (Y†) or aged (A†) with significance at $p < 0.05$.

FIGURE 4.

Average volumetric flow across the cardiac cycle (mean $\pm$ SEM) at four locations in the arterial and venous system of C57BL/6 mice (left), and volumetric flow adjusted to individual gram (g) body weight (right). Bottom panel expanded view of femoral artery and vein. Volumetric flow varied by location and across sex and age. Significant age differences denoted for males (m*) or females (f*); and, sex differences for adult (Y†) or aged (A†) with significance at $p < 0.05$. IVC: Inferior Vena Cava.

FIGURE 5.

Average wall shear stress across the cardiac cycle (left: mean $\pm$ SEM) and wall shear stress during peak-systole (right: mean $\pm$ SEM) at four locations in the arterial system of C57BL/6 mice. WSS varied by location and across age. Significant age differences denoted for males (m*) or females (f*) with significance at $p < 0.05$. WSS wall shear stress

TABLE 1.

A summary of the geometry (arteries: vessel diameter; veins: cross-sectional area), velocity and volumetric data for all groups at every location.

		Velocity (cm/s)		Volumetric flow (mL/min)		Velocity (cm/s)		Volumetric flow (mL/min)	
		Average	Peak velocity	Average	Peak velocity	Average	Peak velocity	Average	Peak velocity
Carotid	Adult male	11.6 ± 2.6	55.0 ± 4.3	1.4 ± 0.2	1.1 ± 0.1 mm ²	2.6 ± 0.6	6.0 ± 0.7	1.1 ± 0.2	1.1 ± 0.2
	Avg. diameter								
	0.5 ± 0.03 mm								
	Adult female	12.6 ± 2.2	30.6 ± 3.0	1.7 ± 0.3	1.0 ± 0.1 mm ²	3.2 ± 0.6	1.5 ± 0.5	2.2 ± 0.4	2.2 ± 0.4
	Avg. diameter								
	0.5 ± 0.02 mm								
Aged male	Adult male	85.2 ± 7.3	42.4 ± 4.2	2.2 ± 0.3	1.0 ± 0.07 mm ²	8.2 ± 1.4	4.7 ± 0.9	2.7 ± 0.2	2.7 ± 0.2
	Avg. diameter								
	0.5 ± 0.003 mm								
	Adult female	3.7 ± 2.8	15.0 ± 2.1	2.2 ± 0.3	1.0 ± 0.07 mm ²	2.4 ± 0.4	2.5 ± 0.3	2.5 ± 0.5	2.5 ± 0.5
	Avg. diameter								
	0.5 ± 0.008 mm								
Suprarenal aorta	Adult male	13.4 ± 1.0	89.5 ± 4.3	2.0 ± 0.2	1.6 ± 0.2 mm ²	9.8 ± 0.6	6.4 ± 0.8	3.7 ± 0.8	3.7 ± 0.8
	Avg. diameter								
	1.0 ± 0.02 mm								
	Adult female	5.7 ± 2.3	43.1 ± 2.8	9.5 ± 1.4	1.5 ± 0.2 mm ²	5.6 ± 0.4	3.5 ± 0.5	3.7 ± 0.2	3.7 ± 0.2
	Avg. diameter								
	1.2 ± 0.02 mm								
Suprarenal IVC	Adult male	18.1 ± 2.3	80.4 ± 15.3	7.4 ± 0.7	1.4 ± 0.2 mm ²	13.6 ± 2.1	7.9 ± 1.4	6.4 ± 0.9	6.4 ± 0.9
	Avg. diameter								
	1.0 ± 0.02 mm								
	Adult female	9.5 ± 1.9	44.9 ± 8.7	14.2 ± 1.5	2.0 ± 0.2 mm ²	2.5 ± 1.4	1.4 ± 0.5	5.9 ± 0.5	5.9 ± 0.5
	Avg. diameter								
	1.2 ± 0.02 mm								
Suprarenal IVC	Adult male	16.6 ± 1.6	95.3 ± 3.9	7.4 ± 0.7	1.4 ± 0.2 mm ²	4.6 ± 0.5	11.4 ± 1.6	6.4 ± 0.9	6.4 ± 0.9
	Avg. diameter								
	0.9 ± 0.01 mm								
	Adult female	48.8 ± 2.1	3.4 ± 2.8	14.2 ± 1.5	2.0 ± 0.2 mm ²	5.9 ± 0.5	15.2 ± 1.3	6.4 ± 0.9	6.4 ± 0.9
	Avg. diameter								
	1.2 ± 0.02 mm								

	Velocity (cm/s)		Volumetric flow (mL/min)		Velocity (cm/s)		Volumetric flow (mL/min)	
	Mean diastolic	Average	Mean diastolic	Average	Mean diastolic	Average	Mean diastolic	Average
Infrarenal aorta	Aged female	15.6 ± 1.2	Average	9.5 ± 1.0	Avg. area	3.0 ± 0.3	Average	3.7 ± 0.3
	Avg. diameter	89.9 ± 9.3			2.0 ± 0.2 mm ²	Peak velocity		
	1.1 ± 0.03 mm	43.7 ± 4.0				Mean systolic		
		4.9 ± 1.0				Mean diastolic		
	Adult male	10.7 ± 0.7	Average	1.7 ± 0.1	Infrarenal IVC	5.2 ± 1.0	Average	1.6 ± 0.2
	Avg. diameter	56.3 ± 9.6			0.4 ± 0.1 mm ²	Peak velocity		
	0.6 ± 0.02 mm	32.3 ± 5.2				Mean systolic		
		4.8 ± 1.7				Mean diastolic		
	Adult female	12.5 ± 0.4	Average	2.6 ± 0.2	Avg. area	3.9 ± 0.6	Average	1.5 ± 0.1
	Avg. diameter	71.1 ± 4.5			0.6 ± 0.1 mm ²	Peak velocity		
Femoral artery	0.6 ± 0.02 mm	36.9 ± 2.0				Mean systolic		
		5.1 ± 0.2				Mean diastolic		
	Aged male	12.8 ± 1.8	Average	3.2 ± 0.3	Avg. area	4.7 ± 0.6	Average	2.6 ± 0.4
	Avg. diameter	75.8 ± 3.8			0.8 ± 0.2 mm ²	Peak velocity		
	0.7 ± 0.02 mm	39.7 ± 3.1				Mean systolic		
		3.6 ± 1.2				Mean diastolic		
	Aged female	14.2 ± 1.4	Average	3.0 ± 0.3	Avg. area	3.7 ± 0.7	Average	1.9 ± 0.3
	Avg. diameter	71.3 ± 3.3			0.8 ± 0.2 mm ²	Peak velocity		
	0.7 ± 0.008 mm	36.0 ± 2.3				Mean systolic		
		6.4 ± 1.1				Mean diastolic		
Femoral vein	Adult male	7.2 ± 0.8	Average	0.31 ± 0.03	Femoral vein	1.5 ± 0.1	Average	0.18 ± 0.03
	Avg. diameter	14.1 ± 1.6			0.2 ± 0.02 mm ²	Peak velocity		
	0.3 ± 0.004 mm	11.5 ± 1.5				Mean systolic		
		4.8 ± 0.7				Mean diastolic		
	Adult female	8.7 ± 0.9	Average	0.36 ± 0.03	Avg. area	1.6 ± 0.2	Average	0.26 ± 0.01
	Avg. diameter	20.4 ± 0.8			0.2 ± 0.01 mm ²	Peak velocity		
	0.3 ± 0.008 mm	17.1 ± 0.8				Mean systolic		
		4.5 ± 0.6				Mean diastolic		
	Aged male	5.8 ± 0.5	Average	0.49 ± 0.05	Avg. area	1.6 ± 0.3	Average	0.27 ± 0.05

	Velocity (cm/s)		Volumetric flow (mL/min)		Velocity (cm/s)		Volumetric flow (mL/min)	
	Peak velocity		Peak velocity		Peak velocity		Peak velocity	
Avg. diameter	26.2 ± 2.3		0.3 ± 0.01 mm ²		4.9 ± 0.4		0.33 ± 0.03	
0.4 ± 0.02 mm	15.2 ± 1.0				2.0 ± 0.4			
	1.9 ± 0.4				1.2 ± 0.3			
Aged female	7.9 ± 1.3		Avg. area		Average		Average	
	25.0 ± 2.9		0.2 ± 0.02 mm ²		5.1 ± 0.4		0.33 ± 0.03	
Avg. diameter	18.7 ± 3.3				2.8 ± 0.2			
0.4 ± 0.03 mm	3.4 ± 0.8				1.7 ± 0.2			