Hemodynamic Shear Stresses in Mouse Aortas
Implications for Atherogenesis

Jin Suo, Dardo E. Ferrara, Dan Sorescu, Robert E. Guldberg, W. Robert Taylor, Don P. Giddens

Objective—The hemodynamic environment is a determinant of susceptibility to atherosclerosis in the vasculature. Although mouse models are commonly used in atherosclerosis studies, little is known about local variations in wall shear stress (WSS) in the mouse and whether the levels of WSS are comparable to those in humans. The objective of this study was to determine WSS values in the mouse aorta and to relate these to expression of gene products associated with atherosclerosis.

Methods and Results—Using micro-CT and ultrasound methodologies we developed a computational fluid dynamics model of the mouse aorta and found values of WSS to be much larger than those for humans. We also used a quantum dot-based approach to study vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression on the aortic intima and demonstrated that increased expression for these molecules occurs where WSS was relatively low for the mouse.

Conclusions—Despite large differences in WSS in the two species, the spatial distributions of atherogenic molecules in the mouse aorta are similar to atherosclerotic plaque localization found in human aortas. These results suggest that relative differences in WSS or in the direction of WSS, as opposed to the absolute magnitude, may be relevant determinants of flow-mediated inflammatory responses. (Arterioscler Thromb Vasc Biol. 2007;27:346-351.)

Key Words: atherosclerosis • wall shear stress • computational fluid dynamics • micro CT • two-photon microscopy • adhesion molecules

Hemodynamic wall shear stress (WSS) is important in the pathogenesis of atherosclerosis in human arteries,1,2 and interest has focused on observed relationships between low and/or oscillating WSS and plaque localization.3 Recent developments in genetic manipulations, such as the ApoE−/− and LDL receptor−/− mouse lines, have led to an increasing use of the mouse as a model for atherosclerosis.4,5 However, to relate murine studies to human disease, it is necessary to characterize the hemodynamic patterns found in the mouse and to describe differences and similarities between the two species. Given differences in size, heart rate, and ejection fraction, it is expected that the mouse model does not "scale up" to the human and that the levels and distribution of WSS could be quite different. Consequently, we conducted computational hemodynamic investigations in the mouse aorta as a step toward understanding the environment within which atherosclerosis develops in this animal model. In addition, we examined the spatial distribution of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in the mouse aorta. Through this approach, we were able to compute the temporal and spatial distributions of WSS in the mouse aorta and relate these findings to local expression of prototypical proatherogenic proteins.

Materials and Methods
Geometry Data Acquisition of the Mouse Aorta
Four animals from a group of twelve-week-old wild-type mice (C57 Bl/6) purchased from the Jackson Laboratory (Bar Harbor, Me) were scanned using micro CT to obtain images of the thoracic aorta. The animals were housed and cared for according to the guidelines proposed by the National Institutes of Health (NIH) for the care and use of experimental animals. After CO2 euthanasia, the abdominal cavity was pierced to cannulate the abdominal aorta. The thoracic cavity was left unopened to prevent anatomic distortion of the vasculature. The aorta was then pressure-perfused at 100 mm Hg in a retrograde fashion using normal saline, followed by 10% neutral buffered formalin. The vessels were subsequently perfused with microfil (Flow Tech, Inc) to form a rigid cast. This preparation was left in a formalin solution for two days and then decalcified with Cal-EX II (Fisher Scientific). The thorax was carefully dissected and micro-CT imaging was performed (viva CT40, Scanco Medical).6 The scanner was set to a voltage of 55 kVp and current of 109 μA, producing 749 slices in which each slice had 1024×1024 pixels that...
equated to 0.021 × 0.021 mm per pixel. The thickness of each slice was controlled to be 0.021 mm, leaving no gaps between slices, so that the spatial resolution was 0.021 mm in each dimension.

Serial tomograms were reconstructed from the raw data using a cone beam filtered back projection algorithm, while noise was further reduced using a low-pass Gaussian filter. A threshold based on X-ray attenuation was used to segment the vessel lumen from the surrounding arterial wall in the serial slices, and the slices were converted to binary images.

Reconstruction of the Aorta
Reslicing was needed to provide continuous boundary contours of the arterial lumen. A 3D reslicing process was performed in the image data, so that sequential contours of the lumen, which were perpendicular to the local axes of the vessel and covered the region of interest of the aorta, were created. The aortic model was reconstructed using nonuniform rational B-Spline (NURBS) surfaces based on these sequential contours. The NURBS surfaces with C2 continuity characteristics assure continuity for computations in the model.

Blood Flow Measurement
Velocity measurements in the aortas of isoflurane anesthetized mice were acquired with Doppler ultrasound using a 2-mm-diameter 14 MHz pulsed-Doppler probe with a focal distance of 5 mm. The probe was placed just right of the sternum and angled to record velocity in the ascending aorta.

Blood Flow in Branches
Measuring flow velocities accurately in the arch branches was not feasible because of their small size, so we assumed the flow waveforms in all aortic branches are similar to the waveform in the ascending aorta. Although this assumption is an approximation, the fact that the arch vessels are close to the aortic root implies that flow into these branches may have a similar temporal variation. The blood flow fractions in the major branches of the aortic arch—the innominate artery (IA), the left common carotid (LCCA), and the left subclavian artery (LSCA)—were estimated from the literature. If flow into the ascending aorta is set as 100%, the flow into the IA was taken to be 13% and those into the LSCA and the LCCA were 7% and 6%, respectively. All boundary conditions for the branches were assumed to have uniform velocity over the lumen with a waveform similar to the ascending aorta. The boundary condition in the descending aorta was assumed to be zero velocity gradient along the axial direction of the outlet section.

Governing Equations
Using the above flow conditions, the Navier-Stokes equations of laminar and incompressible flow were solved in the aorta model of each mouse, following procedures used previously in a study of human aortas.

Ex Vivo Immunohistochemistry and Two-Photon Excitation Microscopy
Animals were euthanized by CO2 inhalation and the aortas were pressure-perfused at 100 mm Hg with a cold 0.9% NaCl solution followed by pressure-fixation with a 10% formalin solution. The aortic arches were carefully dissected in situ and the aortas were opened. Finally, a two-step immunohistochemical protocol was performed using Qdot-biocongjugated secondary antibodies (Invitrogen). En face images were collected using a Zeiss LSM 510 META two-photon microscope equipped with a Ti:Sapphire femtosecond laser (Mira 900 Ti:S, Coherent) tuned and mode-locked at 750 nm. A 40× (Plan-Neofluor, NA 1.3) oil immersion objective connected to an inverted Zeiss Axiovert 100 microscope was used. The beam pathway consisted of only one beam splitter (HFT KP 680). Separation of the emission signals was performed by selecting narrow (40 nm) windows, based on the Qdots spectra, using the META detector. Fluorescence intensity and percent surface area were quantified using the frequency histogram function of the LSM-510 examiner software as previously described. For the epifluorescence images of the proximal mouse aortas, 655-nm Qdot bioconjugates were excited at 360 nm and their emission was detected with a 500-nm LP filter. Images were captured with a 4× objective attached to a Zeiss Axioscop 2 microscope. We targeted two adhesion molecules, VCAM-1 (rat-anti mouse, 1:50, BD) and ICAM-1 (rat anti-mouse, 1:100, Fitzgerald), known to mediate monocyte adhesion during the development of early lesions. Three to five animals were studied for each experimental group.

Statistical Analysis
Quantitative confocal microscopy results were expressed as mean±SEM. Differences among groups were analyzed with an unpaired t test using a commercially available software package (GraphPad Prism 4). A probability value <0.05 was considered statistically significant.

Results
The top panel of Figure 1 presents the geometry of a representative mouse aorta acquired with high resolution micro-CT after image segmentation. This high resolution geometry, along with aortic flow data (lower panel of Figure...
1), formed the basis for the computational model, which was restricted to the ascending aorta, aortic arch, and a portion of the descending aorta. There were strong similarities in the shape and dimensions of the four aortas. Mean measurements of vascular diameters were: proximal ascending aorta 1.17 ± 0.06 mm, mid ascending aorta 1.27 ± 0.06 mm, proximal descending aorta 1.09 ± 0.04 mm, innominate artery 0.63 ± 0.03 mm, left common carotid artery 0.49 ± 0.05 mm, and the left subclavian artery 0.56 ± 0.02 mm. We computed both the instantaneous WSS vectors and the mean WSS magnitudes averaged over the cardiac cycle in these aortas. Figure 2 presents the distributions of the mean WSS in two aortas. As would be expected, there are variations in the magnitudes of mean WSS between the animals, attributable to differences in local geometry and the measured flow conditions. However, there are also strong similarities. The inner curvature of the arch tends to have lower mean WSS, as does the region approaching the orifice of the IA. However, the region in the neighborhood of the second branch, the left common carotid artery, appears to have higher WSS values, probably arising from a reduction in aortic diameter and effects of local geometry. Note that the diameter of the left common carotid artery is the smallest of the arch vessels.

Comparisons of the instantaneous WSS at various times during the cycle show that WSS exceeds 600 dynes/cm² at some locations in the mouse, values that are rarely encountered in normal human vessels. Because flow is pulsatile and three-dimensional, the WSS vector at any given point on the surface changes in both magnitude and direction during the cardiac cycle. This is illustrated for a third aorta (Figure 3) at three locations of interest: the inner curvature of the aortic arch (area b), has instantaneous magnitudes of WSS up to approximately 150 dynes/cm², whereas values exceeding 600 dynes/cm² were found along the lateral wall of the ascending aorta (area c).
the arch branches. This distribution correlates with the lower
values of mean WSS (Figures 2 and 3). A quantitative assess-
ment of VCAM-1 induction at areas exposed to different flow
conditions is presented in Figure 5 using results from five
images obtained per area of interest from three mice. For this
comparison, we studied previously well-defined areas of the
mouse ascending aorta and arch.8 The lateral walls of the
ascending aorta are areas where there is less VCAM-1 expres-
sion, which coincides with a relatively high mean WSS in the
computational model. Conversely, the inner curvature in the
aortic arch is an area of higher VCAM-1 expression and where
WSS is, for the mouse, relatively low. Similar results were found
for ICAM-1, another surface protein known to be regulated by
shear stress (Figure 6). As shown in Figure 3, these low WSS
areas are regions where the WSS vector is also highly oscillatory
in direction during the cardiac cycle, whereas the high WSS area
coincides with relatively unidirectional WSS.

Discussion

We have shown that the magnitude of WSS present in the
normal mouse aorta is overall much higher than in humans,
consistent with concepts of allometric scaling.9,10 Indeed,
peak, mean, and low shear stresses are approximately an
order of magnitude higher in the mouse aorta, and at the peak
levels WSS can be well above values reported to cause
endothelial cell disruption in acute studies in dogs11 and
significantly larger than those computed for healthy human
arteries.3,7,12–14 It is instructive to consider fluid dynamic
principles in interpreting the results. The heart rates, aortic
diameters, and peak velocities of the mouse and human result
in peak Reynolds numbers (Re_{peak}) and Womersley numbers
(\alpha) of: Re_{peak} = 250 (mouse) and 6350 (human); \alpha = 2
(mouse) and 17 (human). Thus, the mouse aorta is within a
viscous-dominated flow regime, very similar to the coronary
arteries of humans, whereas human aortic flow is in an

Figure 4. The differential localization of VCAM-1 protein expression is
evident in the epifluorescence image of a whole aortic mount (center). Note
the intense distribution of the fluorophore along the inner curva-
ture of the arch and in the orifices of the arch vessels, areas that cor-
respond to relatively lower values of mean WSS in the CFD results
(Figure 3). The central image corresponds to a mouse treated with
LPS to achieve fluorescence enhancement. However, the peripheral
confocal pictures were taken from specific areas of a non-LPS treated
mouse, which correspond to areas denoted in Figure 3. The quantum
dot methodology coupled with confocal microscopy allowed ade-
quate visualization of VCAM-1 expression without the need to use
LPS treatment. The arrow in panel c indicates the direction of flow in
this region of unidirectional flow.

Figure 5. Data for the low wall shear stress (LWSS) area were obtained from
the inner curvature of the aortic arch whereas the lateral ascending aorta was
used for a prototypical high wall shear stress (HWSS) area. The bottom panels
show en face representative images of these two areas. The red fluorescent
staining (655 nm Qdot bioconjugate) indicates surface VCAM-1 immuno-
reactivity, whereas the blue color represents endothelial cell nuclei stained with
Hoechst stain. VCAM-1 expression was quantified using both total florescence
intensity and the percentage of the area with positive fluorescence staining, and 5
to 7 images were scanned per area. Results are representative of 4 different
experiments. The arrow indicates the direction of flow in the area of unidirec-
tional HWSS.
inertia-dominated regime. This has several implications, but perhaps the most significant are that (1) pulsatility is less important in the mouse than in the human because the Womersley number is much lower; (2) turbulence would not occur in the mouse aorta because of the low Reynolds number; and (3) secondary flows are less significant for the mouse. On the other hand, flow in the mouse aorta does vary over the cardiac cycle, and the high heart rate of the mouse leads to extremely rapid changes in WSS by comparison to humans. Mouse aortic endothelial cells may experience WSSs that vary over a range as great as 600 dynes/cm² in less than 50 ms, a temporal gradient that is roughly two orders of magnitude greater than in human aortas.

We have also shown that expression of VCAM-1 and ICAM-1 is high in areas of the aorta with low WSS despite the relatively large (compared with humans) magnitudes of WSS. Although we used lipopolysaccharide (LPS) to facilitate the visualization of these differences in the intact aorta, quantitative assessments were made using untreated samples. A number of other investigators have studied the expression of VCAM-1 and ICAM-1 in mouse aortas. Nakashima et al demonstrated upregulation of these adhesion molecules at atherosclerosis-prone sites (inner curvature of arch and orifice of the IA) in ApoE-deficient mice and suggested that the upregulation of ICAM-1 may be associated with areas of apparent decreased shear or disturbed/turbulent flow.

Because the magnitude of WSS in the mouse is much larger than that observed in humans, our findings suggest that the absolute magnitude of WSS may not be the primary determinant of atherogenic gene expression and subsequent atherosclerosis. Many shear stress studies using cultured mouse aortic endothelial cells (MAECs) typically reproduce WSS values in vitro that are characteristic of those found in human arteries, such as the carotid bifurcation. For example, investigators have exposed MAECs to steady mean WSS values of 15 dynes/cm² and to oscillatory values of ±5 dynes/cm² about a mean value of zero in studies of VCAM-1 and ICAM-1 expression. In contrast, the present investigation suggests that MAECs are exposed to much higher WSS values in their native environment, and yet the relative responses in VCAM-1 and ICAM-1 expression are similar. Furthermore, the spatial localization of VCAM-1 and ICAM-1 is consistent with the distribution of atherosclerotic lesions in humans. Taken together, these observations suggest that the relative differences in WSS or possibly the direction of WSS, as opposed to the absolute magnitude, may be a more relevant determinant of flow-mediated inflammatory responses. An in vitro study of MAECs using the relatively high in vivo WSS values of the mouse might elucidate this issue, although it could be technically challenging to retain a confluent monolayer of endothelial cells in culture under such elevated shear conditions.

Several previous investigations have suggested that a change in the magnitude of WSS may be an important mechanical stimulus, and others have questioned the idea that shear rate is relevant. Numerous studies have reported the effects of laminar WSS oscillations on various aspects of endothelial cell function, and the expression of VCAM-1 and ICAM-1 is increased when endothelial cells are exposed to a WSS that oscillates about a low mean value. There have been suggestions that flow disturbances may be involved in increased expression. However, although there are rapid changes in the WSS vector attributable to the high heart rate in the mouse, no turbulence would be present because of the low Reynolds and Womersley numbers, which imply a viscous-

Figure 6. Using a similar approach as presented in Figure 4 and 5, ICAM-1 expression was analyzed in the mouse aorta. Panel A shows a qualitative epifluorescence display of ICAM-1 expression at different areas of a mouse ascending aorta, arch, and its main branches (LPS-treated). In panel B, quantitative en face ICAM-1 expression compares low and high WSS areas using the same methodology as in Figure 5. The HWSS area has low levels of protein expression and cell nuclei that are aligned in the same direction, consistent with the WSS vector directions in that region, as shown in Figure 3. The arrow indicates the direction of flow in the area of unidirectional HWSS. On the other hand, LWSS areas were associated with higher ICAM-1 expression and less cell alignment.

dominated flow. Although we are not able to distinguish between
the potential importance of changes in WSS magnitude
versus direction on atherogenic proteins, both of these factors
may well be more important than the absolute magnitude of
WSS. Others have commented on the relatively high WSS
values in the mouse and on the high temporal gradients in
WSS. 21,26 The spatial distribution of lipid deposits around aortic
branches of mice lacking LDL receptors and apolipoprotein E
appear to differ from the distribution observed in ostia of
branches in the human aorta, suggesting either species differ-
dences or differences on local hemodynamics that might affect
localization. 27 Finally, it is also possible that differences in the
fundamental responses of MAECs and human endothelial cells
in response to WSS may be a factor.

Future work is needed to investigate in greater detail the
relationship between low and oscillating WSS and other
markers associated with atherogenesis in mice using this
methodology. Additionally, our imaging techniques may be
improved, for example, by using micro phase contrast MR to
obtain the in vivo blood velocity distributions in arterial
sections at a high resolution so that the boundary conditions
can be specified more accurately in the CFD simulations.
Further, it is desirable to include motion of the aorta in the
computational modeling, 7,28 although this presents significant
technical challenges because of limitations in spatial and
temporal resolution of imaging modalities. However, the
findings that WSS values in the mouse aorta greatly exceed
those in humans and that, despite this, there is a striking
correlation between the spatial distribution of endothelial
inflammatory markers and the inverse of the mean WSS
distribution are intriguing and may have implications for the
interpretation of studies of atherogenesis in the mouse model.

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Disclosures
None.

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