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Assessment of Shear Stress Related Parameters in the Carotid

Bifurcation using Mouse-Specific FSI Simulations

David De Wilde (1), Bram Trachet (1,2), Nic Debusschere (1), Francesco Iannaccone (1), Abigail Swillens (1), Joris Degroote (3), Jan Vierendeels (3), Guido R. Y. De Meyer (4), Patrick Segers (1)

(1) IBiTech-bioMMeda, iMinds Medical IT, Ghent University, Gent, Belgium

(2) Institute for Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

(3) Department of Flow, Heat and Combustion Mechanics, Ghent University, Gent, Belgium

(4) Division of Physiopharmacology, University of Antwerp, Antwerpen, Belgium

Correspondence to David De Wilde

bioMMeda, De Pintelaan185-blokB5, B-9000 Gent, Belgium

Tel: +32(0) 93324198

Fax: +32(0) 93324159

Email: david.dewilde@ugent.be

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Abstract

The ApoE^{-/-} mouse is a commonly used small animal model to study atherosclerosis, an inflammatory disease of the large and medium size arteries such as the carotid arterial. It is generally accepted that the wall shear stress, induced by the blood flow, plays a key role in the onset of this disease. Wall shear stress, however, is difficult to derive from direct in-vivo measurements, particularly in mice. In this study, we integrate in-vivo imaging (micro-CT and ultrasound) and fluid-structure interaction (FSI) modeling for the mouse-specific assessment of carotid hemodynamics and wall shear stress. Results are provided for 8 carotid bifurcations of 4 ApoE^{-/-} mice. It is demonstrated that accounting for the carotid elasticity leads to more realistic flow waveforms over the complete domain of the model due to volume buffering capacity in systole. The eight simulated cases show fairly consistent spatial distribution maps of time-averaged wall shear stress (TAWSS) and relative residence time (RRT). Zones with reduced TAWSS and elevated RRT, potential indicators of atherosclerosis-prone regions, are located mainly at the outer sinus of the external carotid artery. In contrast to human carotid hemodynamics, no flow recirculation could be observed in the carotid bifurcation region.

Introduction

Mouse models are often used to study the development of atherosclerosis, due to their rapid development of atherosclerotic plaques. In particular, the Apolipoprotein E (ApoE^{-/-}) knockout mouse has been reported to develop spontaneous diet induced plaques, which have morphological resemblance to human plaques (Jawien et al., 2004; Pendse et al., 2009; Whitman, 2004). It is generally accepted that shear stresses, imposed by the blood flow on the endothelial cells, play a crucial role in the onset of atherosclerosis. This hypothesis has been extensively studied and validated in humans (Ku et al., 1985; Moore et al., 1994) and animal models (Peiffer et al., 2013; Thim et al., 2012), as well as in surgically altered flow conditions in vivo (Cheng et al., 2006; Winkel et al., 2015). However, the smaller dimensions and the higher heart rate in mice make the measurements to calculate the shear stress challenging. Although the Hagen-Poiseuille formula may provide an estimate of the order of magnitude of wall shear stress, its accuracy is expected to be low in bifurcation regions (e.g. carotid bifurcation) where blood flow is more complex. The wall shear stress has also been determined based on the shear rate calculated from an MRI measurement of the velocity field in the murine common carotid artery (CCA) (van Bochove et al., 2010). Still, spatial resolution is limited and the temporal resolution of MRI does not allow capturing complex flow oscillations.

In order to overcome these limitations, computational fluid dynamics (CFD) have been used to calculate wall shear stresses rather than measuring them directly. Several derived parameters such as the time averaged wall shear stress (TAWSS), the oscillatory shear index (OSI) (He and Ku, 1996) and the relative residence time (RRT)(Himburg et al., 2004) have been proposed to serve as indicators of regions prone to plaque initiation(Lee et al., 2009). These parameters have been studied in (amongst others) the aortic arch and/or the abdominal aorta (P. Assemat et al., 2014; Pauline Assemat et al., 2014; Feintuch et al., 2007; Hoi et al., 2011; Huo et al., 2008; Trachet et al., 2011, 2009; Vandeghinste et al., 2011) of mice, in relation with both plaque and aneurysm development.

Moreover, CFD simulations of the surgically altered shear stress, due to implantation of a tapering cast around the murine right carotid artery, have been reported (Mohri et al., 2014). The main disadvantage of CFD, however, is that it assumes rigid walls and does not allow for volume buffering in the arteries.

Fluid structure interaction (FSI) simulations add the wall movement through combined computational solid mechanics (CSM) and CFD simulations. This approach makes the simulations more complex and time consuming, but leads to more realistic simulations as we recently demonstrated in an FSI model of the abdominal aorta of the mouse (Trachet et al., 2015). In the present study, we have compared CFD and FSI simulations of the carotid bifurcation in mice, based on mouse specific geometries and boundary conditions measured *in vivo*. This was performed for 8 carotid bifurcations (left and right) of 4 ApoE^{-/-} mice. We show that the rigid wall assumption of CFD simulations hampers the application of realistic flow boundary conditions at the carotid bifurcation in mice, and that the main region with low oscillatory shear stress is located at the outer sinus of the external carotid artery.

Methods

Mice

This study was based on data acquired in four female ApoE^{-/-} mice, fed a Western type diet (TD88137, Harlan Teklad, Madison, Wis, USA) *ad libitum* for up to 10 weeks, from the age of 6 weeks until 16 weeks. The mice were housed in groups of 4-8 animals in well-ventilated cages with appropriate cage enrichment. Their cages were kept under controlled environmental conditions, resulting in a 12h normal light/dark cycle, a temperature of 20-23°C and a 50% relative humidity. All the experiments were conducted according to the EC guidelines and were approved by the animal ethics committee of Ghent University. The data represents a subgroup of a larger study, with the complete protocol described in [ref AUROVIST].

Measurements

Ultrasound

Animals underwent an imaging protocol at week 16 consisting of ultrasound examination and a contrast-enhanced μ CT protocol. Ultrasound imaging was performed using a high-frequency ultrasound scanner (Vevo 2100, Visualsonics, Toronto, Canada) equipped with a linear array probe (MS550D, 22-55MHz). A single operator (DDW) performed all measurements. The mice were secured in a supine position while monitoring the ECG, the respiratory rate and the body temperature under 1-1.5% isoflurane anesthesia. The body temperature was kept constant using a heating pad. Flow velocities were assessed using pulsed Doppler measurements proximal to the bifurcation in the CCA and distal to the bifurcation (outflows) at the external (ECA) and internal carotid artery (ICA). Radio frequency (RF) M-mode measurements were performed at CCA, ICA and ECA. Distances of measuring locations relative to the bifurcation were measured to correlate them with the μ CT-geometry.

μCT

After the ultrasound examination, anesthesia was maintained, and the mice immediately underwent a contrast-enhanced μCT scan. First, 100 μl/25g body weight Aurovist (Nanoprobe, Yaphank, NY) was injected in the tail vein as a vasculature contrast agent (Wathen et al., 2013). Afterwards, the μCT scan was obtained using a Triumph-II imaging system (TriFoil Imaging, Chatsworth, CA, USA). The scanner was set to 50 μm focal spot size, 50 μm detector pixel size, 3.5 times magnification, 500 μA tube current, 75kVp tube voltage, 1024 projections and a continuous rotation mode.

Processing of the measurements

Ultrasound

The contour of the Doppler spectrum was tracked using custom written software in Matlab (Mathworks, Natick, Massachusetts, USA). The diameter distension was obtained from tracking RF-data following a previously described algorithm (Rabben et al., 2002). Signals were cycle-averaged (minimally 3 cycles) based on the R-peak of the ECG. Both signals were time-aligned (we detected a non-consistent time-offset in both signals) by shifting the diameter distension waveforms. This was done in an automated way as part of the In(D)U method for the assessment of local wave speed (Feng and Khir, 2010; Swillens et al., 2013). The time shift for which the In(D)U-loop fitted best to the theoretical straight line in the early systolic region was selected by minimizing the L2-norm of the difference between the measured data and the linear fit, divided by the number of sample points in the selected upslope region.

μCT

The Iterative Maximum-likelihood Polychromatic Algorithm for CT was applied to reconstruct the CT data at a 0.05 mm isotropic voxel size (De Man et al., 2001). The software package Mimics (Materialise, Leuven, Belgium) was used to segment the contrast-enhanced carotid arteries from the post-contrast datasets. The three branches of the carotid bifurcation, CCA, ECA and ICA, were semi-automatically segmented, based on threshold and region growing steps, combined with manual

121 editing where needed. Afterwards, flow extensions with a length equal to the diameter of the
122 arteries were added in the Vascular Modeling Toolkit (VMTK, www.vmtk.org). In the end the
123 segmented geometry was shrunk to the diastolic radius measured with M-mode ultrasound (a factor
124 of 0.8, 0.84, 0.84 and 0.88 for the four mice). The final result was inspected visually by plotting the
125 contours on the μ CT images.

127 **Creating a volume mesh using the XTM method**

128 The resulting STL-surface was imported in pyFormex (<http://www.nongnu.org/pyformex/>) for the
129 creation of a volume mesh using the XTM algorithm (REF JORIS). This meshing method results in a
130 high-quality unstructured hexahedral mesh, with the cells aligned with the predominant direction of
131 flow. The radial cell size was finest at the bifurcation and linearly coarsened to the in/outlets with
132 factors 2, 1.6 and 1.4 for the CCA, ECA and ICA respectively. A mesh sensitivity study was performed
133 with meshes ranging from 60k to 400k cells. Converged CFD results with respect to low RRT and
134 TAWSS area were obtained with 300 k cells. Meshes of the arterial wall were generated using the
135 XTM algorithm assuming a wall thickness of 0.1 times the local luminal radius (Trachet et al., 2015)
136 and 3 cell layers in the radial direction(Trachet et al., 2015). The structural meshes consisted of 65 k
137 cells. The fluid mesh (white) and structural mesh (grey) are depicted for an example case in figure 1a.

139 **The Wall Mechanics problem**

140 The computational structural mechanics (CSM) simulations were performed with the commercial
141 finite element solver Abaqus Standard using an implicit (backward-Euler) time integration scheme.

142 **Material Model Parameter Optimization**

143 The Arruday-Boyce material model (Arruda and Boyce, 1993) was fitted to the measurements as
144 suggested in (Trachet et al., 2015) and depicted in figure 1b (left). An idealized cylindrical CCA

geometry was constructed in pyFormex using the following dimensions (based on averaging measurements in the 8 carotids): inner diastolic radius of 0.215 mm, wall thickness of 0.1 times the diameter, spring constant of $1e6$ Pa/m. The locking stretch parameter λ_m was set to 1.01 (Trachet et al., 2015). For the shear modulus μ an optimization procedure was performed based on the initial shear modulus μ_0 and the average measured systolic diameter of 0.24716 mm. Applying the axial pre-stress and diastolic inflate backward incremental (BI) method (see below) and a static structural simulation with a systolic pressure load of 119 mmHg, the simulated systolic diameter could be calculated in function of the material parameter guess μ^i . The optimization was performed in Python using the scipy fminbound method with a tolerance on μ of 10 Pa. After 15 iterations a value of 9516 Pa was found for μ^* . The same parameter value was used for all 8 simulations.

External tissue support

The stabilizing influence of external tissue was modeled using springs elements at every node of the outer wall surface (Moireau et al., 2012), as plotted schematically in the bottom of figure 1d. This resulted in 20k springs per bifurcation. Assuming that the tissue pressure of mice and humans is similar ($P_{\text{tissue}} = \text{distension}_{\text{human}} * k_{\text{human}} \sim \text{distension}_{\text{mouse}} * k_{\text{mouse}}$), k_{human} with a value of $1e4$ Pa/m (Moireau et al., 2012) was rescaled to a value k_{mouse} of approximately $1e6$ Pa/m ($\sim \text{distension}_{\text{human}} * k_{\text{human}} / \text{distension}_{\text{mouse}}$) with the $\text{distension}_{\text{human}}$ from literature (Rengier et al., 2012).

Initial stresses

To account for the diastolic in vivo stress state, the backward incremental (BI) method was applied (de Putter et al., 2007; Speelman et al., 2009) (figure 1b). We first applied an axial pre-stress of 100 kPa to the cross section of the CCA (moving all nodes together in the axial direction only) while fixing the ECA and ICA outlets. This is a stress value in between the low and medium extension found in (Gleason et al., 2007). The forward calculation steps of the BI method were performed using the commercial finite element solver Abaqus Standard (Simulia, Johnston, USA). The load was

sinusoidally (de Putter et al., 2007) increased to 100 kPa in 10 steps and kept constant afterwards. For every bifurcation 100 iterations of the BI method were applied.

Secondly, the diastolic pressure of 87 mmHg (Van Herck et al., 2009) was added to the model by performing a second BI method with altered loads and boundary conditions. At the inner surface of the artery, the pressure load was sinusoidally increased in 50 steps from zero to a value of 87 mmHg. This time, only radial movement was allowed at the nodes at the three boundaries: CCA, ECA and ICA. The stress field resulting from the axial pre-stress procedure was applied as an initial condition to the BI Inflate step. Because no axial displacement was allowed, the axial pre-stress could not relax and remained present in the solution. The external tissue was modeled with springs, similar to the FSI – structural solver. The convergence criteria were: 1e-6 m maximum displacement, 1e-11m L2 norm of the displacement and 1 Pa for the L2 norm of the difference in maximal principal stresses between two iterations. This second BI step resulted in the in-vivo stresses in the geometry under a combined axial pre-stress and a diastolic pressure load.

The Flow Mechanics Problem

All flow simulations were performed with the finite volume solver Fluent 14.5 (Ansys, Canonsburg, USA). The density of the blood was set to 1060kg/m³ with a constant dynamic viscosity of 3.5mPas(Chen et al., 2014; Feintuch et al., 2007; Trachet et al., 2011). The SIMPLE algorithm with second-order upwind discretization was used for the pressure interpolation and the momentum equations. For the time integration, a second order accurate discretization was used. Convergence was reached when the continuity and momentum residuals dropped below 1e-6.

Boundary conditions

Mass flow inlets were used at the inlet (CCA) and one outlet (ECA), while a windkessel model is imposed at the ICA. As blood flow velocity is measured with ultrasound rather than mass flow,

rescaling is mandatory. To calculate the instantaneous average velocity over the cross-section, the maximum velocity was divided by a factor 2, assuming a parabolic flow profile at the measuring location (which was confirmed a posteriori by the simulations). As also the cross section varies throughout the cardiac cycle, a structural simulation was performed imposing inflating the pre-stressed geometries to systolic pressure over 200 time steps. The cross-sectional area at the location of the flow velocity measurements was exported. The mean velocity curve was then multiplied with the cross sectional area curve and the density of 1060 kg/m³ to calculate the mass flow profile at these locations.

Due to measuring errors and assumptions in the data processing, the time-averaged mass flow balance in the model (cycle-averaged difference between in- and outflow) is not completely fulfilled. As we estimated the CCA measurement to be the most accurate, a scaling factor c was applied to the ICA and ECA mass flows such that $Q_{CCA-meas} = c \cdot (Q_{ICA-meas} + Q_{ECA-meas})$. Note that only the rescaled ECA flow is directly imposed as boundary condition. The average correction factor for the measured ECA and ICA flow was 1.20 $\pm$ 0.22 (ranging from 0.94688 to 1.6839).

For the FSI simulations, a three element windkessel model (Westerhof et al., 1971) was applied at the outlet of the ICA (figure 1 a). Model parameters were fitted in Matlab (using `fminsearch`) by minimizing the difference between a target pressure curve and the pressure curve predicted by the windkessel, when imposing the rescaled ICA flow. The target pressure curve was approximated by linearly rescaling the measured diameter distension waveform to the assumed in-vivo diastolic and systolic pressures (87/ 119 mmHg) (Van Herck et al., 2009). As such, the global pressure in the model is not a priori prescribed, but will be determined by the imposed inlet and outlet flow, the 3-element windkessel model and the properties of the arterial wall. For the CFD simulations, a pressure outlet (based on the fitted three element windkessel model) was directly applied at the ICA outlet (although imposing pressure in CFD simulations is arbitrary).

FSI- coupling

The in house developed coupling code Tango was applied to couple the flow solver and the structural solver for describing the fluid-structure interaction problem. At the interface a new prediction of the location was made with an IQN-ILS algorithm based on the previous positions calculated by the structural solver (Degroote et al., 2009). At each time step coupling iterations between both solvers were performed to meet equilibrium at the interface. A spring analogy was used to move the interior grid nodes of the fluid domain, with stiffness inversely proportional to the edge length [ref].

Derived Hemodynamic Wall Parameters

Derived hemodynamic wall parameters include the averaged wall shear stress (TAWSS), oscillatory shear index (OSI) and relative residence time (RRT)(He and Ku, 1996; Himburg et al., 2004; Lee et al., 2009).

$$TAWSS = \frac{1}{T} \int_0^T |\bar{\tau}|. dt$$

$$OSI = \frac{1}{2} \left(1 - \frac{\left| \int_0^T \bar{\tau}. dt \right|}{\int_0^T |\bar{\tau}|. dt} \right)$$

$$RRT = \frac{1}{(1 - 2.OSI).TAWSS}$$

$\bar{\tau}$ is the instantaneous wall shear stress vector and T the period of the cardiac cycle. In the case of FSI simulations where the arterial wall is moving, a local coordinate system was constructed at every node of the interface using pyFormex moving with the arterial wall. The wall shear stress was expressed in this local frame of reference.

Results

The fitting of the windkessel model resulted in the following values: $Z = 1603.8 \pm 755.0$ mmHg/ml/s, $R = 8147.4 \pm 1491.1$ mmHg/ml/s and $C = 8.22 \times 10^{-6} \pm 2.61 \times 10^{-6}$ ml/mmHg. The finally obtained diastolic pressure at the inlet is 91.2 ± 2.4 mmHg (averaged for the 8 simulations), while the systolic pressure is 118.3 ± 4.9 mmHg (Table 1). The pressure drop over the carotid artery is small: 1.99 ± 0.40 mmHg between CCA – ECA and 2.52 ± 0.71 mmHg between CCA – ICA. The measured and simulated diameter distensions are listed in table 1. On average, the simulated distension is 2.6% lower compared to the measurements.

Figure 2 displays simulated pressure and flow waveforms along the model for a representative case (mouse2, right bifurcation) as well as the velocity at end diastole and peak velocity of the CCA boundary. The flow profile is highly laminar with no recirculation present around the bifurcation for any of the cases through the whole heart cycle. Only at the ECA outlet, a small recirculation is artificially introduced due to the boundary condition, but it stays very local. In diastole only a small amount of mass flow is present in the ECA, resulting in low flow velocities. The highest velocities are visible in the ICA at both time points, due to the flow split being in favor of the ICA while the diameter is smaller than the CCA. Furthermore, figure 2a shows that the pulsed inflow at the CCA (CCA-in), is buffered and loses part of its pulsatility as it moves towards the outlets. The displacement (figure 2c) is smooth over the entire bifurcation with the highest displacement being at the bifurcation itself. A little outward bending is present.

The added value of the FSI approach and the volume buffering is illustrated in Figure 3. The flow at the ICA is a result of the simulation, and agrees very well with the flow directly derived from the measurements (ICA-measured). In a CFD approach, the ICA-flow would equal the difference between inflow and outflow via the ECA, which is clearly much more peaked due to the lack of buffering in systole.

Flow derived indices are displayed in Figures 4 and 5 for all eight simulated cases. Despite differences in absolute values of TAWSS between the different bifurcations, a similar spatial distribution of the lowered and elevated TAWSS is present in all cases (Figure 4). The high velocity in the ICA results in an elevated TAWSS compared to the CCA, with the highest values in the order of 15 Pa. A region with a lowered TAWSS (in red) is present at the outer bend of the ECA close to the bifurcation but spreads out over the complete ECA. The OSI is highest mainly at the outer bend of the ECA zone but stays more localized compared to the TAWSS (figure not shown). This trend continues in the RRT pattern shown in figure 5. In mice 2 and 3, the higher RRT value at the outer bend of the ECA is most pronounced, but only in mouse 3R this spans the whole ECA.

Discussion

In this study, an FSI model of the carotid bifurcation was developed for the left and right carotid of 4 ApoE^{-/-} mice, providing, to the best of our knowledge, the first estimates of wall shear stress distributions along the mouse carotid artery. The model was animal-specific in terms of carotid geometries and imposed flow boundary conditions; the material model was tuned to averaged diameter distension measurements in the animal cohort.

In order to determine hemodynamic wall parameters, a realistic simulation of the blood flow is important. We are convinced that accounting for the fluid-structure interaction is of added value as it allows for a volume buffering in systole and a much more natural application of the boundary conditions. This is best illustrated in Figure 3, in which it is shown that merely using the difference between CCA inflow and ECA outflow to retrieve the ICA outflow (as is the case in CFD) leads to waveforms differing much more from the measured waveform than the one retrieved in the FSI simulations (L2norm difference of 0.1330+/-0.0317 ml/s vs. 0.0111+/-0.0039 ml/s). Both approaches can also be compared with the pulsatility index (PI), defined as $(V_s - V_d)/V_{mean}$ with V_s the systolic, V_d the end diastolic and V_{mean} the average outflow velocity. The PI at the ICA obtained by CFD (1.86+/-0.300) is considerably higher than the measured value of 0.777+/-0.164, which again is much better replicated by the FSI simulations (0.555+/-0.180).

In humans, flow separation and recirculation in the carotid bifurcation has been reported in 99 out of 100 subjects (Schuierer and Huk, 1990). In none of the 8 simulated murine carotid bifurcations, however, flow separation was present (neither in the FSI, nor in CFD simulations). It has been reported that the critical Reynolds number, below which no recirculation is present, is geometry dependent (Motomiya and Karino, 1984). The Reynolds numbers in mice are much lower than in humans, and are lower in the carotid than in the aorta. Our simulations resulted in a Reynolds number of 12.7+/-2.9 (peak systole). Womersley numbers were 0.46+/-0.03 at the CCA inlet. Note, however, that simulations were done in animals not showing any signs of plaque. As demonstrated in

296 the aorta [ref 18], the presence of plaque may lead to geometrical discontinuities that can induce
297 flow recirculation zones.

298 Overall, the patterns of TAWSS and RRT were quite consistent between animals, and between the
299 left and right sides. Only for the CCA of mouse1 L/R and mouse3 R, the global TAWSS is lower
300 compared to the other mice. This is consistent with the calculation of the TAWSS based on the
301 Poiseuille flow assumption (Katritsis et al., 2007), which is lower for these CCA (between 3.3 and 4.7
302 Pa) compared to the other CCA (between 6.1 and 9.2 Pa). However, the locations where TAWSS is
303 lowest (red in figure 4) are similar in all mice: the outer bends of the ECA branch of the bifurcation. It
304 is also observed that TAWSS in the ECA is lower compared to the ICA and CCA, due to the lower flow.

305 Setting up and tuning the animal specific model is, obviously, largely dependent on the
306 hemodynamic measurements that are much more challenging in mice than in humans, due to the
307 higher heart rate and the smaller size. Direct measurement of the flow in the murine CCA using MRI
308 is possible but results in limited spatial (50x50x500 μm) and temporal (27 timeframes/cardiac cycle)
309 resolution (van Bochove et al., 2010). We determined the flow by multiplying the pulsed Doppler
310 velocity with the cross-sectional assuming a parabolic flow profile. As can be seen from Figure 2, the
311 parabolic flow profile is a reasonable assumption for the CCA and for the side branches when
312 measuring far enough downstream. Probably the highest source of error is in the angle correction of
313 the pulsed Doppler velocities, especially for the ICA and ECA measurements. To guarantee a correct
314 mass balance over the bifurcation, we decided to apply a rescaling factor at the outflow velocity
315 waveforms of the ECA and ICA outlets.

316 A drawback of our study is the absence of pressure measurements, which is still extremely
317 challenging in mice. Non-invasive (tail-cuff) pressure data show large variability and we are probably
318 not accurate enough, while invasive pressure measurements imply sacrificing the animal. We
319 therefore decided to generate a pseudo pressure waveform based on rescaling the M-mode
320 diameter distension to a pressure range that was previously reported (Van Herck et al., 2009).

Although the high correlation between the measured and simulated distension curves justifies this choice, a mouse specific measurement would have further improved the simulations.

As described in the methods, we applied a shrinking step after the initial segmentation based on the micro-CT images. This was necessary to account for the arterial pulsation/movement during the μ CT measurements, and the partial volume effect, resulting in a gradual intensity transition from artery to surrounding tissue. With the extra shrinking step to the measured diastolic diameter (M-mode), we assured that these artifacts did not artificially inflate the geometry. The final segmentation was visually inspected on the micro-CT image. Future cardiac gated micro-CT (Badea et al., 2004) might circumvent the necessity of this step.

The material model used in these simulations is fairly simple. It does not include anisotropy, neither does it account for the layering of the arterial wall (which was not visible from the ultrasound or CT images). We also assume a fixed ratio between local radius and wall thickness. Given the focus of the study on flow-derived parameters, any material model providing a realistic degree of wall distension over the physiological pressure range would suit our purpose. Our choice for the Arruda-Boyce model is mainly driven by the model simplicity, with hyper-elastic behavior achieved by fitting only one model parameter. We did, however, include the supporting effect of surrounding tissue in the simulations. This aspect, together with implementing the axial pre-stress, improved both the realism and the stability of the simulations compared to previous FSI simulations of the murine vasculature (Trachet et al., 2015).

In the present study, we have focused on advancing our computational biomechanics approach, and we have implemented a methodology that allows assessing carotid hemodynamics and derived flow-related parameters in a fairly realistic way. The next step will be relating the fluid mechanical parameters with plaque development and progression in these models. Overall, the zones of low TAWSS corresponded with the areas in which plaque development was found, as recently reported [ref je paper]. Subsequently, an in depth analysis of the association between parameters derived

from computational biomechanics simulations as presented here and plaque development and growth will be performed. Moreover, the inclusion of the plaque mechanics (and more advanced constitutive models) would allow for a detailed view on the plaque rupture through the cap stresses.

Conclusion

We have presented mouse specific FSI simulations of eight carotid bifurcations of 4 ApoE^{-/-} mice. A realistic wall movement was obtained by including the axial pre-stress, the *in vivo* stress state and the influence of the surrounding tissue. Accordingly, the buffering capacity of the carotid bifurcation was taken into account. This approach opened the possibility to use realistic, measurement-based boundary conditions for the CFD part. The hemodynamic wall parameters showed that the primary region of interest from a hemodynamic point of view is the sinus of the ECA at the bifurcation.

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Figure Legends

Figure 1: Overview of the followed methodology to setup a numerical model for a reference bifurcation. (A) The fluid mesh (white) and structural mesh (grey) constructed with the XTM algorithm. A refinement of the cell size from the in/outlets (CCA, ECA and ICA) to the bifurcation (BIF) is visible in the cross-sections. For the CFD model a mass flow inlet is used for the CCA and ECA. For the ICA, a windkessel model relates the local pressure to the local flow. For the solid mechanics, the external tissue is modeled by springs with a stiffness k_s in every surface node (Moireau et al., 2012) and in the boundaries only radial displacement is allowed. (B) Schematic representation of the methodology. First a material parameter optimization loop was followed to fit the μ parameter of the Arruda-Boyce material model to the measured distensions, similar to the material fitting proposed in (Trachet et al., 2015). In short this loop executes the loading of an average CCA cylindrical geometry (X_{cyl}) with the axial prestress ($\sigma_{load\ axial}$) and the systolic pressure P_{sys} and optimizes the material model parameter so that the systolic distension (d_{sys}) equals the measurement. Using the optimized parameter μ^* , the *in vivo* stress state under an axial stress load ($\sigma_{load\ axial}$) and a diastolic pressure (P_{dia}) was calculated for the actual bifurcations with two BI steps (BI-axial and BI-inflate). The areas of the lumen boundaries were approximated by inflating the bifurcations according to the pressure profile. Based on these areas (A_b) and the measured PD-velocity(U_b), the massflow ($\dot{m}_b$) at the boundaries was determined. Finally the ICA windkessel was fitted to the pressure (P_{ICA}) and flow profile($\dot{m}_b$) and the FSI model was setup based on all the previous results according to the boundary conditions shown in panel A, still keeping the external tissue into account with springs with a stiffness k_s . (C) The maximal principal stress in the carotid bifurcation under an axial pre-stress with the original geometry, calculated with a first BI step. (D) The *in vivo* stress state, under a combined load of an axial pre-stress and a diastolic pressure. In the second BI step, BI-inflate, no axial displacement is allowed at the boundaries, so the axial pre-stress of the previous steps cannot relax and will remain present in the final result. The resulting stress field is used as an initial condition for the following CSM step and the final FSI calculations.

508 Figure2: (A) The calculated pressure and flow profiles in an example bifurcation at three different
509 locations close to the bifurcation (CCA, ECA and ICA) and at the CCA-inlet (CCA-in). The pressure
510 profile is very similar through the whole bifurcation. The CCA buffers the flow, resulting in a less
511 peaked flow profile at the CCA compared to the CCA-in. The flow in the ECA is small compared to the
512 flow through the ICA. (B) The velocity fields at end diastole and peak velocity. Laminar flow, without
513 recirculation at the bifurcation is visible. Only the ECA boundary artificially induces local flow
514 recirculation. (C) The displacement at peak pressure is biggest at the bifurcation.

515 Figure3: Comparison of the different flow profiles at the boundaries. For the CCA and ECA, the
516 imposed flow profiles are plotted. The ICA measured flow, is the measured flow after the rescaling
517 for correcting the flow imbalance, this is the target flow profile for the simulations. The FSI
518 simulation resulted in an ICA flow profile (ICA simulation) that corresponds well with this profile. CFD
519 simulations would neglect the buffering of the bifurcation and would result in an ICA flow equal to
520 $Q_{cca}-Q_{eca}$.

521 Figure4: The TAWSS result from the FSI simulations of all the different bifurcations. Differences in
522 absolute values between different bifurcations are present. However, the qualitative patterns
523 between the several bifurcations are similar.

524 Figure5: An overview of the RRT values based on the FSI simulations for the different carotid
525 bifurcations. The zones most prone to development of atherosclerosis are characterized by high RRT
526 values and indicated in red. The outer bend of the ECA close o the bifurcations is the most important
527 one for all the bifurcations.

Figure 1	overview-methodology.png
Figure 2	overview-flow-vel-disp.png
Figure 3	flow_calculations.tiff
Figure 4	TAWSS05-5-inverse-overview.png

Figure 5

RRT04-overview-labels.png

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Table Legends

Table1:

An overview of the pressures [mmHg] and distensions [%] at the boundaries of the FSI simulations.

The pulse pressure, diastolic pressure and systolic pressure are similar for the three boundaries. The

distensions of the simulations are 2.6% lower compared to the measurements. Moreover, in the

measurements there is a higher non-uniformity with the CCA having a higher distension.

537 **Tables**

538 Table1:

	CCA	ECA	ICA
Pulse Pressure - FSI	27.2+/-7.0	27.3+/-6.8	27.1+/-6.9
Pdia - FSI	91.2+/-2.4	90.5+/-2.3	89.8+/-2.5
Psys - FSI	118.3+/-4.9	117.8+/-4.8	117.0+/-4.7
Distension - FSI	9.4+/-1.5	10.1+/-1.3	8.2+/-1.3
Distension - Measured	16.2+/-2.8	11.7+/-4.1	8.4+/-3.2

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