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Effects of Mechanical Properties and Atherosclerotic Artery Size on Biomechanical Plaque Disruption - Mouse versus Human

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Abstract

Mouse models of atherosclerosis are extensively being used to study the mechanisms of atherosclerotic plaque development and the results are frequently extrapolated to humans. However, major differences have been described between murine and human atherosclerotic lesions and the determination of similarities and differences between these species has been largely addressed recently. This study takes over and extends previous studies performed by our group and related to the biomechanical characterization of both mouse and human atherosclerotic lesions. Its main objective was to determine the distribution and amplitude of mechanical stresses including peak cap stress (PCS) in aortic vessels from atherosclerotic, apoE^{-/-} mice in order to evaluate whether such biomechanical data would be in accordance with the previously suggested lack of plaque rupture in this model. Successful finite element analysis was performed from the zero-stress configuration of aortic arch sections and mainly indicated (1) the modest role of atherosclerotic lesions in the observed increase in residual parietal stresses in apoE^{-/-} mouse vessels and (2) the low amplitude of murine PCS as compared to humans. Overall, the results from the present study support the hypothesis that murine biomechanical properties and artery size confer less propensity to rupture for mouse lesions in comparison with those of humans.

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Keywords

plaque disruption; mouse; human; mechanical properties; artery size

1. Introduction

Coronary vulnerable plaque rupture and subsequent thrombosis represent the main cause of coronary events, which are responsible for the majority of cardiovascular deaths (Go et al., 2013; Fleg et al., 2012). From a biomechanical point of view, a relationship has been described between the likelihood of plaque rupture and the amplitude of mechanical peak cap stress (PCS) (Richardson et al., 1989; Loree et al., 1992; Cheng et al., 1993; Finet et al., 2004; Ohayon et al., 2008; Speelman et al., 2011; Akyildiz et al., 2011). Accordingly, Richardson et al. (1989) first suggested the potential role of mechanical stress in plaque rupture by demonstrating the influence of the necrotic core on stress concentration in the fibrous cap of atherosclerotic plaques from patients. Loree et al. (1992) characterized the relationship between fibrous cap thinning and the corresponding increase in PCS whereas Cheng et al. (1993) showed that maximal mechanical stress was significantly higher in unstable vs. stable human coronary lesions. More recently, Ohayon et al. (2008) using structural finite element simulations demonstrated that plaque instability is to be viewed not as a consequence of fibrous cap thickness alone but rather as a combination of cap thickness, necrotic core thickness, and arterial remodeling index. Their study provided clues as to why lesions were more prone to rupture at early stages of positive remodeling, which could explain the fast progression and growth of clinically silent plaques.

Mouse models of atherosclerosis are extensively being used to study the mechanisms of atherosclerotic plaque development (Weber et al., 2008) and the results are frequently extrapolated to humans. However, major differences have been described between the widely used apoE^{-/-} mouse model and human atherosclerotic lesions (Bentzon et al., 2010) and the determination of similarities and differences between these species has been extensively addressed recently (Hansson et al., 2007; Schwartz et al., 2007; Jackson et al., 2007; Jackson, 2007; Falk et al., 2007; Rosenfeld et al., 2007; Rosenfeld et al., 2008; Bond et al., 2011). Specifically, distinct opinions have been expressed regarding the presence and characteristics of murine plaque rupture and the subsequent formation of thrombus. Accordingly, some investigators recommended the replacement of the specific term “rupture” by that of the more general “disruption” when addressing the natural history of mouse atherosclerotic lesion development in comparison with that of humans (Schwartz et al., 2007; Falk et al., 2007). The use of a distinct terminology was motivated by the observation that despite being extremely thin, the cap of murine lesions does not rupture but that plaque disruption is rather generally caused by fissuring of the plaque at the level of lateral xanthomas (Schwartz et al., 2007).

The main objective of the present study was to determine the distribution and amplitude of mechanical stresses including PCS in aortic vessels from atherosclerotic, apoE^{-/-} mice in order to evaluate whether such biomechanical data would be in accordance with the hypothesis of the absence of plaque rupture in mice that was previously suggested by the abovementioned studies. Finite element analysis was used starting from the zero-stress

configuration as obtained using the opening angle technique (Chuong and Fung, 1983; Matsumoto et al., 1995; Rehal et al., 2006; Ohayon et al., 2007) in order to account for residual stresses remaining in the vessel wall in the absence of external load. In addition, differences between murine and human lesions from the point of view of plaque biomechanics will be further discussed in order to examine whether the mechanical properties of murine plaque constituents, the size of atherosclerotic arteries and the resulting amplitude and distribution of mechanical stresses confirmed or infirmed the hypothesis that murine and human lesions might display differential behaviors with regards to atherosclerotic plaque disruption.

2. Materials and methods

2.1 Experimental Protocol

Five weeks-old female apoE^{-/-} mice on a C57/BL6J background (n=25) and age-matched wild-type (C57/BL 6J) control mice (n=20) were obtained from Charles River Laboratory. ApoE^{-/-} animals were fed a western-type diet (20% casein milk/0.15% cholesterol - Harlan) whereas control mice remained on chow diet. Control and apoE^{-/-} mice were euthanized by an overdose of pentobarbital at 7, 15, 20, 25 or 30 weeks (n=3–5 apoE^{-/-} and control animals/time point). The aorta was harvested and immersed in ice-cold Krebs-Henseleit containing 0.026 mM EDTA (KH). The aortic arch was carefully dissected free from adhesive tissues under a binocular (Discovery V8, Zeiss) and transferred into a mold containing gelatine (25% in KH, 37°C). The mold was cooled down to 5°C and the embedded arch was fixed on a sample holder immersed in ice-cold KH. A 200 µm-thick section located between the brachio-cephalic and the left carotid arteries ostia was obtained using a vibratom (HM650V, Microm). The cross-section of the aortic ring was rinsed thrice in KH (37°C) and cut radially at a site corresponding to the large curvature of the vessel, i.e. in the healthy portion of the aorta localized at the opposite of the small curvature where atherosclerotic lesions reproducibly develop in apoE^{-/-} mice (Figure 1). The duration of the no-load state from vessel excision to wall vessel incision was reproducible and approximated 60 min. Residual stress/strain (RS/S) dissipation resulted in the opening of the aortic ring. The determination of the opening angle was performed after complete stabilization of the opened vessel (45 min). The aortic arch section immediately adjacent to the 200 µm-thick ring was embedded in paraffin using standard protocols, and 4 µm transversal slices were obtained using a microtom (HM340E, Microm) for histological & immunohistological stainings.

2.2 Histology and immunohistology

Standard trichrome HES staining (Haematoxylin, Erythrosine, Safran) for nuclei, cytoplasm and fibrosis staining as well as Von Gieson staining of elastic lamina, von Kossa staining of calcium carbonate deposits, Vascular Smooth Muscle Cell (VSMC) and macrophage staining were performed on paraffin-embedded sections. VSMC staining was performed using an anti-α-actin antibody (A5691, Sigma) while macrophage staining used an anti-galectin-3 (Mac-2) antibody (CL8942AP, Cedarlane). Briefly, the slices were deparaffinized and rehydrated. Unmasking of tissue antigen was performed for 15 min at 100°C (Vector Laboratories). Following a one hour blocking step at room temperature, the primary

antibody was applied on the tissue sections, either overnight at 4°C (anti-galectin-3, 1:10000) or for 1 hour at room temperature (RT) (anti- α -actin, 1:800). The anti- α -actin antibody was directly coupled with the alkaline phosphatase, whereas a 1 hour incubation with a biotinylated secondary antibody was needed for galectin-3 staining (1 hour at RT). The appropriate chromogen was applied (permanent red or DAB) and the sections were counterstained with haematoxylin.

2.3 Identification of atherosclerotic lesion constituents

HES, von Gieson, VSMC and macrophage stainings were used to subdivide atherosclerotic lesions into 3 distinct constituents. The cellular fibrosis (CeFb) region was defined as a fibrotic area colonized by VSMC; the hypocellular fibrosis (HyFb) region was defined as a hypocellular area with a robust fibrosis staining on von Gieson and trichrome HES images; the lipid-rich (LpRi) region was defined as the area containing either macrophage-derived foam-cells or vacuoles.

Histological and immunohistological stainings could not be performed directly on the opened sector due to the extreme technical difficulty in obtaining paraffin-embedded opened aortic rings that allowed the preparation of histological slices containing the whole length of the opened vessel. We were therefore forced to use the closed section that was immediately adjacent to that used for open angle determination for histo- and immunohistochemical analysis allowing the determination of wall vessel constituents. Once identified, the sectional vessel geometry was mapped back onto the opened sector using the specific and unique anatomical landmarks that were observed on each lesion, with the process being performed by 2 expert observers.

This identification of atherosclerotic lesion constituents on the opened aortic ring was used for the finite element computation of stress and strain distributions.

2.4 Image analysis and morphometric measurements

The stained atherosclerotic cross-sections were observed using a microscope (Olympus BX41). Digital images were acquired and used for morphometric measurements. From unloaded and closed atherosclerotic cross-sections, neointimal, medial and adventitial thicknesses were measured. These measurements were performed at the level of the small curvature of the aortic arch section where atherosclerotic lesions develop. A planimetric analysis was conducted to determine the area of the lesion and of its constituents. The tortuosity of medial elastic lamina was estimated from von Gieson staining as the arc-to-chord ratio using the formula $\tau = L/C$ with τ : tortuosity, L: length of the elastic lamina, and C: shortest distance between its ends. The stretching index was defined as the inverse of the tortuosity index. The tortuosities of all elastic lamina were calculated for each vessel section and averaged. The opening angle (α) was determined from images of the opened aortic ring acquired 45 minutes following the radial cut. It was defined as the angle formed between the two extremities of the opened ring and the point of the intima crossed by the perpendicular bisector of the segment bounded by these two extremities (Chuong and Fung, 1983).

2.5 Finite element analysis

Starting from the opened stress-free aortic ring configuration we performed the finite element (FE) computations under plane strain assumption using ANSYS 11 software (Ansys, Inc., Canonsburg, PA, USA). The mechanical behavior of each plaque constituent was modeled using incompressible neo-Hookean isotropic strain energy density function: $W=E(I_1-3)/6$ where I_1 is the first invariant of the Cauchy-Green tensor (Holzapfel, 2000) and E the initial Young's modulus of the identified LpRi, HyFb, and CeFb plaque regions. The Young's moduli of these mouse atherosclerotic plaque constituents were recently measured by using the atomic force microscopy technique (Tracqui et al., 2011): $E_{LpRi} = 5.5$ kPa, $E_{HyFb} = 59.4$ kPa and $E_{CeFb} = 10.4$ kPa. The nonlinear anisotropic arterial wall behavior was modeled using the following hyperelastic constitutive law:

$$W_{artery} = \frac{\mu}{2}(I_1 - 3) + \frac{k_1}{k_2} \left[\exp \left\{ k_2 (I_4 - 1)^2 \right\} - 1 \right] \quad (1)$$

which takes into account two families of fibers with symmetrical helicoidal directions (Holzapfel et al., 2005; Baek et al., 2007; Gleason et al., 2008) and where μ is the shear modulus, I_4 is the invariant associated with fiber orientation and k_1 and k_2 two material constants driving the degree of anisotropy. The parameters used for our simulations were determined in a previous work (Ohayon et al., 2012) by fitting this hyperelastic model against the experimental data of Wagenseil et al. (2005) and Carta et al. (2009): $\mu=44.85$ kPa, $k_1 = 32.15$ kPa, $k_2 = 0.85$. First, RS/S distributions were obtained by closing the opened, stress-free configuration of the artery. Second, a systolic blood pressure of 110 mmHg (14.6 kPa) was applied to the closed vessel to simulate the loaded physiological state of the aorta.

2.6 Critical cap thickness and cap stress values

In a study performed on human vulnerable coronary plaques, Virmani et al. (2000) found a threshold cap thickness of 65 μ m below which 95% of plaques were found to be ruptured. Due to technical challenges, the ultimate tensile stress amplitude has never been measured experimentally neither for thin fibrous cap of human vulnerable coronary plaques nor for mouse aortic lesions. Therefore, structural FE analyses - in which RS/S were neglected - were conducted on human plaques (Finet et al., 2004; Cheng et al., 1993), and close ultimate stress threshold amplitudes (~300 kPa) were extracted by considering plaque morphologies with critical cap thickness equal to 65 μ m (Virmani et al. 2000). Moreover, in the present study we assumed a same ultimate stress amplitude of 300 kPa for mouse and human plaques since very little is known about murine cap strength as well.

2.7 Statistical analysis

The correlations between experimental measurements were analyzed by simple regression using a commercially available software package (SigmaStat 3.5, Systat Software, Point Richmond, CA). Results were expressed as mean $\pm$ standard error to the mean (s.e.m.). Student's t-test for paired or unpaired values were used as well as Pearson's moment correlation analysis. The differences were considered significant when $P<0.05$.

3. Results

3.1 Evolution of the morphologic indexes during plaque progression in mouse

Neointimal thickening corresponding to plaque formation was observed from 20 weeks of age in apoE^{-/-} animals with subsequent stabilization (Figure 2A). Medial thickness did not change significantly over time in control animals whereas a significant increase was observed at 20, 25, and 30 weeks in apoE^{-/-} animals ($P < 0.01$ vs. 7 weeks and $P < 0.05$ vs. Control at 25 and 30 weeks, Figure 2B). Adventitial thickness was also significantly higher in apoE^{-/-} than in control animals at 25 and 30 weeks ($P < 0.05$) ($P < 0.05$ vs. 7 wks at 15, 20, 25, and 30 wks, Figure 2C). The circumferential stretching of elastic lamina significantly increased in apoE^{-/-} mice at 20, 25, and 30 weeks ($P < 0.05$ vs. 7 weeks and $P < 0.01$ vs. Control at 25 and 30 weeks), whereas no significant variation was observed in control animals over time (Figure 2D). Finally, a significant increase in opening angle (OA) was observed in aortas from apoE^{-/-} animals from 20 weeks of age whereas no significant variation was observed in control animals over time (Figure 2E).

3.2 Relationships between morphologic indexes and the opening angle in mouse

Control animals were not included in the analysis of the potential relationship between neointimal and residual stress development since the term neointima only applies to animals undergoing atherosclerotic plaque development. Neointimal thickening was not correlated with the increases in opening angle that were observed in apoE^{-/-} animals ($P = 0.59$ and $R^2 = 0.03$, Figure 3A) whereas medial (Figure 3B) and adventitial thickenings (Figure 3C) were both significantly correlated with the observed increase in OA amplitude as was the sum of medial and adventitial thicknesses (Figure 3D). Finally, circumferential stretching of the medial elastic lamina was also correlated with variations in OA (Figure 3E).

3.3 Spatial stress and strain distributions in mouse atherosclerotic lesions

Figure 4 presents the resulting physiological stress and strain distributions from finite element analysis of three atherosclerotic plaques from 25 weeks-old apoE^{-/-} mice. Physiological stress amplitudes were found higher in the arterial wall (with maximal values close to 350 kPa) than in neointimal regions (with maximal values close to 25 kPa in sites distinct from plaque cap, see paragraph 3.5 below). Although high strain areas were observed in atherosclerotic lesions from apoE^{-/-} animals, stress values remained at low levels (< 25 kPa) at these sites due to the low values of Young's moduli that were determined for constituents of murine aortic atherosclerotic plaques (see case p-wk25-s3 from Figure 4 for a representative example).

3.4 Relationships between regional residual stress amplitude and the opening angle in mouse

The results presented in Figure 5A indicated that mean residual stress as determined from FE analysis was significantly increased in the media of apoE^{-/-} animals as compared with normocholesterolemic animals. In addition, mean neointimal residual stress in apoE^{-/-} animals was significantly lower than residual stress values that were observed in the media of similar vessel sections. Shown in Figure 5B are the linear regression analyses that were

performed in order to investigate potential correlations between the OA amplitude and the mean computed regional residual stresses. The results demonstrated a strong and significant correlation ($P < 0.05$, $R^2 = 0.96$) between the amplitude of medial residual stresses and the OA of corresponding vessels. The correlation between the OA and neointimal residual stress was significantly lower ($P = 0.02$, $R^2 = 0.56$) than that observed between OA and medial residual stress.

3.5 Residual and physiological PCS amplitude in mouse atherosclerotic lesions

FE simulations performed on all atherosclerotic apoE^{-/-} mouse sample geometries allowed the quantification of residual and physiological mean PCS amplitudes (Figure 6). The mean residual and physiological PCS values were found lower than 10 kPa, with mean values of 3.4 ± 0.9 kPa and 8.4 ± 2.6 kPa, respectively.

4. Discussion

The main objective of the present study was to determine the distribution and amplitude of mechanical stresses including PCS in aortic vessels from atherosclerotic, apoE^{-/-} mice in order to evaluate whether such biomechanical data would be in accordance with the previously suggested lack of plaque rupture in this model. Successful finite element analysis was performed from the zero-stress configuration of aortic arch sections and mainly indicated (1) the modest role of atherosclerotic lesions in the observed increase in residual parietal stresses in apoE^{-/-} mouse vessels and (2) the low amplitude of murine PCS as compared to humans (Ohayon et al., 2007). The combination of experiments and modeling described in the present study forms a valuable asset for the evaluation of animal models of atherosclerosis from a mechanical perspective.

4.1 Adventitial and medial remodeling in apoE^{-/-} mouse

In the present study, advanced aortic arch atherosclerotic lesions were clearly visible in all mice starting at 20 weeks of age, in accordance with previous studies by Nakashima et al. (1994) and Agianniotis and Stergiopoulos (2012). The fact that no significant increase in lesion size was observed from 20 to 30 weeks of age (see Figure 2A) might be due to the intrinsic variability in lesion severity among age-matched animals that has been observed previously by others in either chow diet- or high-fat diet-fed animals (Maeda et al., 2007 and Nakashima et al., 1994). Finally, considering that no variations were observed between 20, 25, and 30 wks-old animals for the parameters of neointimal thickness, medial thickness, adventitial thickness, lesion area, lesion composition and opening angle as described above, all animals at these ages were pooled for the analysis of stress amplitude and distribution in atherosclerotic lesions.

4.2 Does mouse neointimal lesions contribute to the increase of global RS/S?

The appearance of aortic arch lesions at 20 weeks was also concomitant to significant changes in the vessel wall at the site of lesion development. Specifically, significant increases in medial and adventitial thicknesses together with a significant increase in elastic lamina circumferential stretching were observed at this time point in apoE^{-/-} but not in control animals. Importantly, the OA of aortic arch sections also significantly increased in

20 weeks-old apoE^{-/-} animals when compared with controls and no significant changes in the OA were observed between 20, 25, and 30 wks. In addition, the lack of correlation between neointimal thickness and OA and the strong relationships between OA amplitude and adventitial and medial thicknesses indicated that atherosclerotic lesions from apoE^{-/-} mice do not significantly participate to global RS/S. This finding was further confirmed by FE analysis results which indicated significantly higher correlation between the OA and the amount of medial residual stresses than that observed in the neointima of apoE^{-/-} animals, together with significantly lower neointimal residual stress amplitude when compared to the media. Overall, regional residual stress quantification using FE analysis confirmed the modest role of neointimal atherosclerotic lesions and the major contribution of medial remodelling to the overall amount of parietal residual stresses.

4.3 Effect of RS/S on physiological PCS amplitude in human and apoE^{-/-} mouse lesions

In a recent study, Broisat et al. (2011) investigated the influence of residual stress/strain (RS/S) on the physiological PCS amplitude. The results obtained on apoE^{-/-} mouse were similar to those found previously on human (Ohayon et al., 2007) and showed that neglecting the RS/S resulted in the overestimation of physiological PCS amplitude by a factor of ~2. As a result, the human critical CTh of 65 μ m (Virmani et al., 2006; Ohayon et al., 2008) would be reached for a computed ultimate tensile stress value of ~150 kPa and not ~300 kPa as numerically estimated when RS/S were neglected (see Figure 7, curves A and B). Interestingly, this ultimate stress value of ~150 kPa is close to the lower circumferential ultimate stress amplitude found experimentally by Holzapfel et al. (2005) for nonatherosclerotic intima layer of human coronary arteries (i.e. 394 ± 223 kPa).

4.4 Effect of plaque mechanical properties on physiological PCS amplitude in human and apoE^{-/-} mouse lesions

The Young's moduli of the mouse aortic atherosclerotic plaque components (Tracqui et al. 2011, Hayenga et al. 2011) were found much lower than those of human (Lee et al. 1991): 10.4 ± 5.7 kPa vs 510 ± 220 kPa for CeFb and 59.4 ± 47.4 kPa vs 900 ± 220 kPa for HyFb. As a direct result of such findings, the residual and physiological PCS amplitudes were found lower in mouse lesions than in human lesions (Figure 6). The isolated impacts of mechanical properties of the atherosclerotic vessel on physiological stress distribution were investigated by Ohayon et al. (2012). In this previous study, intraparietal stress distributions were computed by using the geometries of mouse lesions while assuming the mechanical properties of human plaque constituents and arterial wall instead those of the mouse. Notice that similar cross substitution of apoE^{-/-} mouse and human plaque material properties were investigated by Weinbaum's group (Vengrenyuk et al., 2010). It was found that by substituting mouse mechanical properties by those of human drastically increased the physiological PCS amplitudes with values that could be twenty-fold larger (8.4 ± 2.6 kPa vs 182.3 ± 55.9 kPa) (Ohayon et al., 2012). Such differences in PCS amplitude may represent a key feature for a better understanding of lesion stability between human coronary and mouse aortic atherosclerotic plaques.

4.5 Effect of artery size on physiological PCS amplitude in human and apoE^{-/-} mouse lesions

Mechanical properties may not be the only factors explaining the apparent stability of atherosclerotic apoE^{-/-} mouse lesions. Specifically, the small size of mouse vessels have prompted interrogations as to whether the small dimensions encountered in murine models of atherosclerosis might be expected to reproduce a human pathology (Schwartz et al., 2007). The small size of mouse arteries might actually be held partially responsible for such stability. Indeed, structural computations performed on VP geometries showed that for fixed mechanical properties and blood pressure, the intraparietal stress distribution remains invariant whatever the geometrical size-factor used. In other words, if we assume that a mouse aortic lesion is obtained by reducing approximately three-fold a human coronary lesion, then the PCS amplitude will be the same despite the fact that the mouse cap thickness (CTh) has been also decreased three-fold. As a result, the human critical CTh of 65 μm (Virmani et al., 2006; Ohayon et al., 2008) (see Figure 7, curve B) would become close to 22 μm for mouse geometry lesions when assuming human material properties (see Figure 7, curve C).

4.6 Are plaque mechanical properties and artery size responsible for the apparent stability of atherosclerotic apoE^{-/-} mouse lesions?

Knowing that mechanical properties of mouse lesions decrease approximately twentyfold the physiological PCS amplitude (see section 4.4) (Ohayon et al. 2012), and assuming that human and mouse have a similar ultimate tensile stress threshold of 150 kPa, we corrected the previous mouse critical CTh amplitude estimation of 22 μm (estimated when human material properties were ‘abusively’ considered, see section 4.5). As a result, we found that the mouse critical CTh could be lower than 1 μm (see Figure 7, curve D). Such small critical CTh value could explain the apparent stability of mouse lesions since it might rarely be reached over the course of murine atherosclerotic lesion development.

In addition to those of the present study, previously published results have suggested that mechanical plaque disruption is unlikely in mice due to mechanical stress distribution resulting in low stress values in neointimal murine lesions (Broisat et al., 2011, Ohayon et al., 2012, Campbell et al., 2013) as compared with the local maxima of stress observed on the thin fibrous caps of human lesions (Cheng et al., 1993, Ohayon et al., 2007). More specifically, atherosclerotic lesions located on the mouse brachiocephalic artery and supposedly prone to rupture demonstrated a stress distribution pattern similar to that observed in aortic lesions (Campbell et al., 2013, Broisat et al., 2011). Biological rather than biomechanical disruption of lateral xanthomas has been implicated in turn by Schwartz et al. (2007) on the basis of results published by Gough et al. (2006) and showing that increased proteolytic activity could induce acute plaque disruption in mouse in accordance with previous (Lendon et al., 1991) and more recent studies (Seneviratne et al., 2013).

4.7 Study limitations

This study highlights original and potentially promising concepts for improving our understanding on vulnerable plaque rupture of mouse and human. However, several limitations need to be pointed out:

1. The opening angle technique was the experimental approach used to approximate the zero-stress geometrical opening configuration of the diseased artery. Such assumption of an open configuration of the atherosclerotic vessel that is free of stress has been defined by Matsumoto et al. (1995) as the “Macroscopic stress-free configuration”, although admittedly other lower amplitude RS/S patterns might still be at work in the plaque even after the radial cut of the pathological artery (Matsumoto et al., 2004).
2. The full three-dimensional structures of the vulnerable atherosclerotic plaques have not been considered since our FE simulations were conducted under plane strain assumption. This assumption is reasonable insofar as (i) plaque length is large with regard to the radial dimension, and (ii) neighboring cross-sectional morphologies remain similar (Ohayon et al., 2008).
3. Blood pressure was not assessed in animals included in our study and similar values were assumed for control and ApoE^{-/-} animals. Indeed, conflicting results have been published regarding differences in blood pressure between normocholesterolemic and hypercholesterolemic animals. Accordingly, Hartley et al. (2000) did not find a significant difference between the systolic pressure of isoflurane-anesthetized WT and ApoE^{-/-} mouse aged 13 months, while Yang et al (1999) observed a ~10% increase in systolic, diastolic, and mean blood pressure in 7.5 mo-old vs. 6wks-old conscious and unrestrained ApoE^{-/-} mouse. In our study, including a hypothetical 10% increase in blood pressure in apoE^{-/-} versus control animals in the finite element analysis would have resulted in a corresponding small increase in physiological stress values. However, such an increase would not have changed the conclusions of our study, i.e. that parietal stresses are lowered by an order of magnitude or more in mouse lesions in comparison with human lesions.
4. The ultimate tensile stress threshold for fibrous cap of human atherosclerotic coronary arteries was extracted from numerical simulations (see section 2.6) and not from experimental studies, and moreover the ultimate tensile stress threshold for fibrous cap of mouse vulnerable aortic plaque was assumed to be equal to the one estimated numerically for human coronary plaques.

4.8 Conclusion

Residual stress/stain, artery size and plaque mechanical properties play a major role in the biomechanical stability of vulnerable atherosclerotic plaques. Overall, the results from the present and abovementioned studies strongly suggest that biomechanical rupture of aortic atherosclerotic lesions is unlikely at any site in the apoE^{-/-} mouse model of atherosclerosis.

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Figure 1.

Schematic view of the mouse aortic arch anatomy. The 200 μm thick rectangle indicates the location where aortic rings were sampled along the aortic arch of the ApoE $^{-/-}$ mouse. The gray area represents an atherosclerotic plaque. Schematic views of typical aortic ring after sampling (closed geometry in the top rectangular frame) and after a radial cut that releases the residual stresses are presented (open geometry in the top rectangular frame). The two extremities of the open ring are used to determine the opening angle α of the aortic ring sample. ZSC: zero-stress geometrical configuration. Adapted from Figure 1, Ohayon et al., 2012.

Figure 2.

Time-course evolution of neointimal thickness (A), medial thickness (B), adventitial thickness (C), elastic lamina stretching (D), and opening angle (E) of arterial sections from the aortic arch of control and apoE^{-/-} mice aged 7 to 30 weeks. * $P < 0.01$ vs. 7 weeks; † $P < 0.05$ vs. Control. Adapted from Supplemental Figure II, Broisat et al., 2011.

Figure 3.

Results from linear regression analyses performed between the opening angle of aortic arch sections from both control and apoE^{-/-} mice and neointima thickness (A), media thickness (B), adventitia thickness (C), adventitia+media thickness (D), and elastic lamina stretching (E). Adapted from Supplemental Figure III, Broisat et al., 2011.

Figure 4.

Computation of the *in vivo* von-Mises stress and effective strain distributions in atherosclerotic arterial wall submitted to physiological internal pressure of 14.5 kPa by taking into account residual stress/strain RS/S (b and c). a) Zero stress configurations of 3 atherosclerotic lesions (pathological samples # 2, 3 and 4 for mice of 25 weeks) (yellow: LpRi regions, orange: CeFb regions, red: HyFb regions, white: arterial wall). Rectangular frames highlight intraplaque peak stress locations only.

Figure 5.

Quantification of mean residual stresses from finite element analysis in the media of control and apoE^{-/-} mice as well as in the neointimal area of apoE^{-/-} animals (A). Linear regression analyses between the opening angle of aortic arch sections from both control and apoE^{-/-} mice and mean neointimal residual stress (triangles) and mean media residual stress (closed circles: apoE^{-/-} animals, open circles: Control animals) (B). * $P < .001$ vs. media from ApoE^{-/-} animals.

Figure 6.

Comparisons between mean residual and physiological peak cap stresses obtained on apoE^{-/-} mouse samples by Ohayon et al. (2012) and those found on human based on the study of Ohayon et al. (2007).

Figure 7.

Effects of residual stress/strain (RS/S), artery size and mechanical properties on the relationship between peak cap stress (PCS) and cap thickness (CTh). Shift from curve A to curve B illustrates the effect of RS/S on human atherosclerotic lesions. Shift from curve B to C results from the small size of the mouse artery while shift from curve C to D accounts for the effect of the lower mouse plaque components stiffness compared to that of humans. The shaded area corresponds to the domain with high probability of plaque rupture whereas the clear area corresponds to the domain of plaque biomechanical stability.