Assessing Low Levels of Mechanical Stress in Aortic Atherosclerotic Lesions From Apolipoprotein $E^{-/-}$ Mice—Brief Report

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Objective—Despite the fact that mechanical stresses are well recognized as key determinants for atherosclerotic plaque rupture, very little is known about stress amplitude and distribution in atherosclerotic lesions, even in the standard apolipoprotein E (apoE)$^{-/-}$ mouse model of atherosclerosis. Our objectives were to combine immunohistology, atomic force microscopy measurements, and finite element computational analysis for the accurate quantification of stress amplitude and distribution in apoE$^{-/-}$ mouse aortic atherosclerotic lesions.

Methods and Results—Residual stresses and strains were released by radially cutting aortic arch segments from 7- to 30-week-old pathological apoE$^{-/-}$ (n=25) and healthy control mice (n=20). Immunohistology, atomic force microscopy, and biomechanical modeling taking into account regional residual stresses and strains were performed. Maximum stress values were observed in the normal arterial wall (276±71 kPa), whereas low values (<20 kPa) were observed in all plaque areas. Stress distribution was not correlated to macrophage infiltration.

Conclusion—Low mechanical stress amplitude was observed in apoE$^{-/-}$ mouse aortic atherosclerotic lesions. This original study provides a basis for further investigations aimed at determining whether low stress levels are responsible for the apparently higher stability of murine aortic atherosclerotic lesions. **(*Arterioscler Thromb Vasc Biol*. 2011;31:1007-1010.)**

Key Words: atherosclerosis ■ ApoE$^{-/-}$ mouse ■ mechanical stress

Quantification of mechanical stress represents an essential step for the reliable prediction of vulnerable atherosclerotic plaque rupture.[1–5] Unlike in human atherosclerotic lesions, very few data are available regarding the amplitude and spatial distribution of parietal stresses and strains in apolipoprotein E (apoE)$^{-/-}$ mice despite the common use of this experimental model for the study of biological mechanisms underlying plaque development.[6–8] Therefore, the objectives of the present study were (1) to characterize the amplitude and distribution of parietal stresses and strains in an apoE$^{-/-}$ mouse model of atherosclerosis, (2) to determine whether the computed stresses and strains were correlated with biological processes, and (3) to compare stresses and strains observed in mice with those previously observed in humans. For the first time, this study takes into account and underlines the importance of residual stresses and strains (RSS) in the quantification of mechanical stresses in atherosclerotic lesions.

Methods

Aortic arch were obtained from 7- to 30-week-old apoE$^{-/-}$ and age-matched wild-type C57BL/6 control mice. Fresh 200-μm-thick aortic arch sections were obtained between the brachiocephalic and left carotid artery departures. Following a radial cut, sections opened up to an opening angle for which RSS were dissipated (zero-stress configuration). An adjacent section was used for immunohistological stainings. A subgroup was dedicated to the determination of the mechanical properties of atherosclerotic lesions using atomic force microscopy. Finite element computations were performed to determine RSS spatial distributions by closing the opened artery section from the zero-stress configuration. Stress/strain distribution and amplitude in the loaded physiological state were then obtained by superimposing the effect of internal blood pressure. Details and a glossary are given in the Supplemental Material, available online at http://atvb.ahajournals.org.

Results

Lesion Morphology and Histology

Advanced atherosclerotic lesions were visible at the lesser curvature of apoE$^{-/-}$ mouse aortic arch starting at 20 weeks of age (Supplemental Table I and Supplemental Figure I). These lesions were characterized by a significant increase in medial and adventitial thicknesses and by a significant decrease in the undulation of elastic lamina (Supplemental

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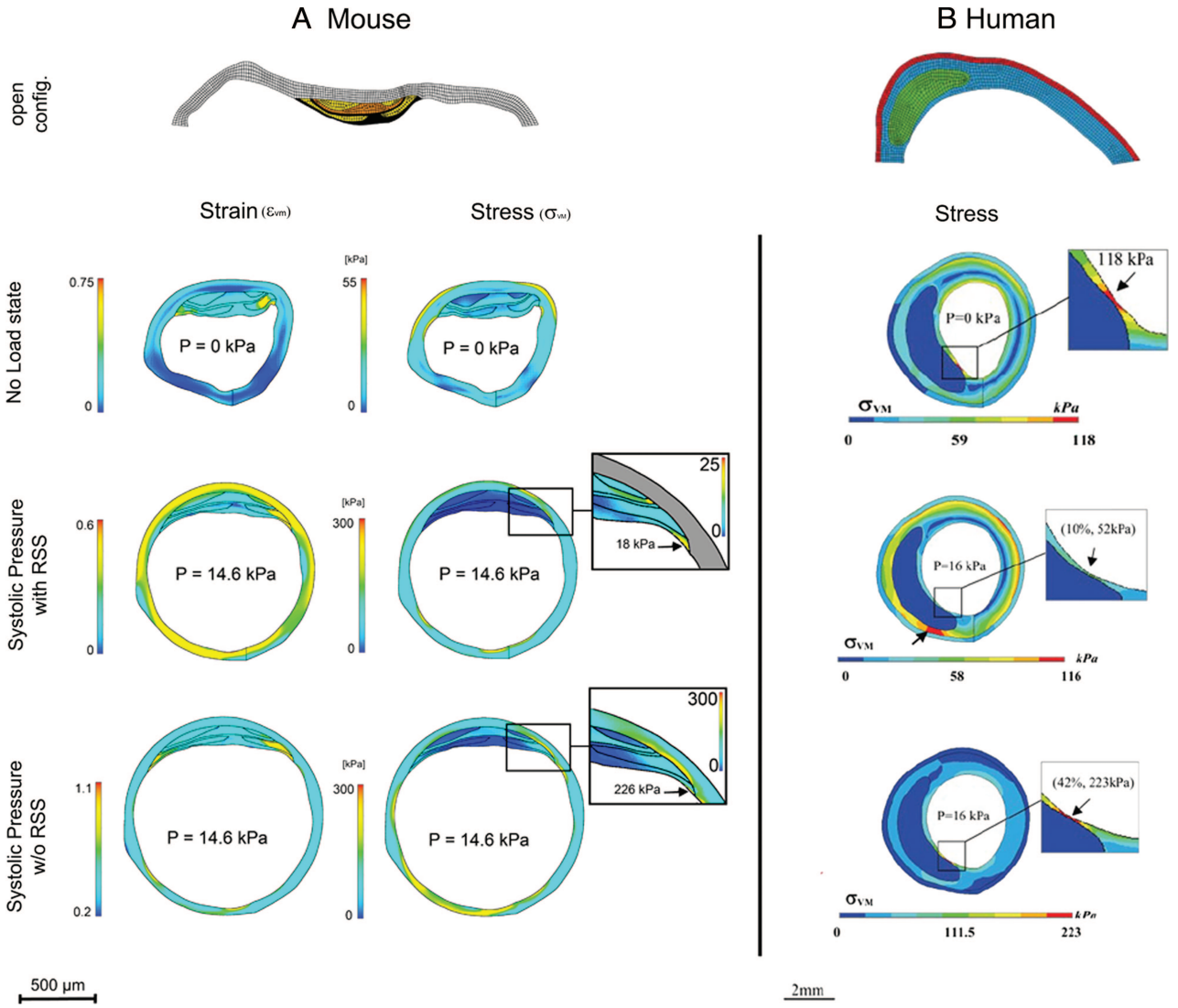

Figure 1. Representative examples of zero-stress, residual (no-load state), and physiological stresses with or without consideration of RSS from mouse (A) and human (B) (reproduced with permission from Ohayon et al[5]) atherosclerotic lesions and demonstrating the lower amplitude of stresses in plaques from apoE$^{-/-}$ mice.

Figure II). The opening angle significantly increased in apoE$^{-/-}$ animals and was significantly correlated to media and adventitia thickness and to elastic lamina undulation but not to the neointimal thickness of atherosclerotic lesions (Supplemental Figure III).

Distribution Pattern and Quantification of Parietal Stresses and Strains

Shown in Figure 1A are representative spatial distributions of parietal stress and strain as determined from biomechanical modeling. Starting from RSS computation in the absence of blood pressure (no load state, upper line), the amplitude and distribution of parietal stresses and strains was determined in the presence of a physiological blood pressure (middle line). Neglecting the influence of RSS strongly biased the estimation of overall stresses and strains (bottom line). Shown for comparison in Figure 1B are previously reported data in humans. Image quantification showing maximal and mean parietal stresses and mean strains is presented in Figure 2A, 2B, and 2C, respectively. This regional quantitative analysis confirmed the crucial impact of RSS when determining physiological stresses in atherosclerotic lesions. Indeed, with the exception of the front area, a significant increase in maximal and mean physiological stresses in all regions of atherosclerotic lesions was observed when RSS were neglected. Specifically, there was a 14-, 2.9-, and 3.6-fold increase in maximal stress in the shoulder, center, and back areas of atherosclerotic lesions, respectively. Similarly, there was a trend toward higher mean strain in atherosclerotic plaques and the media when RSS were not considered, with statistical significance reached for the center and back areas of lesions, as well as for the media. When RSS were included, the maximal physiological stress within atherosclerotic lesions was observed in the shoulder area (19±3 kPa). Importantly, maximal and mean physiological stresses in the lesion were significantly lower than those observed in the normal arterial wall.

Macrophage infiltration in the front, center, shoulder, and back areas of atherosclerotic plaques was compared with mean physiological stresses and strains, including RSS, as

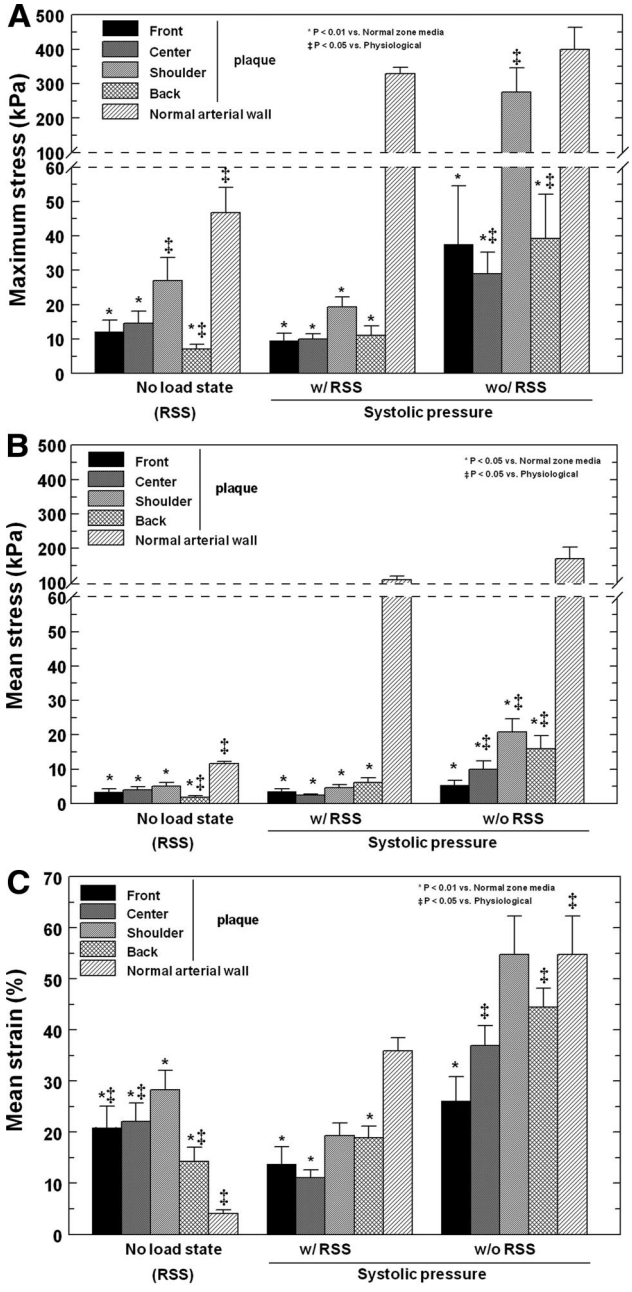

Figure 2. Quantification of residual and physiological maximal (A) and mean (B) stresses and mean strains (C) in the front, center, shoulder, and back areas of atherosclerotic lesions from the aortic arch of apoE$^{-/-}$ mice.

well as with cyclic strain variation between systolic and diastolic pressures. The results indicated that none of these parameters was significantly correlated with macrophage content (Supplemental Figure IV and Supplemental Table II).

Discussion

The main objective of the present study was to characterize the amplitude and distribution of physiological parietal stresses and strains in an apoE$^{-/-}$ mouse model of atherosclerosis. Our analysis included for the first time stresses and strains persisting in the arterial wall in the absence of blood pressure, ie, RSS. RSS are usually neglected when the role of parietal stresses and strains in atherosclerotic plaque rupture is investigated. However, it has recently been suggested that neglecting RSS in human coronary lesions may have a significant impact on overall parietal stress amplitude and distribution, which may therefore potentially affect the estimation of plaque vulnerability.[5] In the present study, the amplitude of the opening angle, which reflects the overall RSS level, was correlated to adventitial and medial thickness. On the other hand, the lack of correlation between the neointimal thickness of aortic atherosclerotic lesions and the opening angle amplitude indicated that atherosclerotic lesions from apoE$^{-/-}$ mice do not significantly participate in global RSS. Our results further indicated that including RSS strongly affected the amplitude of maximal stress because a 14-fold increase was observed in the shoulder when RSS were not considered. Interestingly, such an overestimation of maximal stress was also reported for human atherosclerotic lesions when RSS were neglected.[5]

At the cellular level, macrophages were preferentially observed in the shoulder area of lesions. However, macrophage distribution was correlated neither to mean stresses and strains nor to the amplitude of cyclic strain variation between systole and diastole. Therefore, parietal stresses and strains under physiological loading imposed by blood pressure do not seem to influence macrophage distribution in aortic lesions of apoE$^{-/-}$ mouse.

Importantly, the distribution of stress and strain in apoE$^{-/-}$ mouse atherosclerotic lesions differed from that of human lesions. In the murine model, the lowest parietal stresses were located in atherosclerotic lesions, with the normal arterial wall being subjected to higher stress. On the contrary, human lesions exhibit peak parietal stresses in shoulder areas that are usually higher than those observed in the normal vessel wall.[5] Two parameters may be responsible for such species differences. First, human and mouse plaque components have distinct properties, as illustrated by their respective elastic moduli (see Supplemental Material and Lee et al.[9]). Second, mouse plaque morphology also differs from that of humans. Coleman et al[10] previously noticed that, as observed in the present study, mouse aortic arch lesions developed in a dome-like manner, whereas human lesions exhibit positive remodeling without initial protrusion of the plaque into the vessel lumen. It should also be noted that although mouse lesions were pooled for biomechanical analysis, buried fibrous layers potentially indicative of previous plaque rupture were occasionally observed in aortic atherosclerotic lesions included in the present study.

In conclusion, the histological and biomechanical analysis performed in the present study provides an accurate reconstruction of RSS and therefore a realistic quantification of parietal stresses and strains under physiological conditions. The amplitude of stress in apoE$^{-/-}$ mouse atherosclerotic plaques was found to be lower than that observed in normal portions of the vessel and lower than that previously described in coronary arteries from patients. Taken together, these results might have important implications as a quantitative basis for further investigations on apoE$^{-/-}$ mouse atherosclerotic plaque stability.

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Disclosures

None.

References

1. Richardson PD, Davies MJ, Born GV. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet*. 1989;2:941–944.
2. Loree HM, Kamm RD, Stringfellow RG, Lee RT. Effects of fibrous cap thickness on peak circumferential stress in model atherosclerotic vessels. *Circ Res*. 1992;71:850–858.
3. Cheng GC, Loree HM, Kamm RD, Fishbein MC, Lee RT. Distribution of circumferential stress in ruptured and stable atherosclerotic lesions: a structural analysis with histopathological correlation. *Circulation*. 1993; 87:1179–1187.
4. Ohayon J, Finet G, Gharib AM, Herzka DA, Tracqui P, Heroux J, Rioufol G, Kotys MS, Elagha A, Pettigrew RI. Necrotic core thickness and positive arterial remodelling index: emergent biomechanical factors for evaluating the risk of plaque rupture. *Am J Physiol Heart Circ Physiol.* 2008;295:H717–H727.
5. Ohayon J, Dubreuil O, Tracqui P, Le Floc'h S, Rioufol G, Chalabreysse L, Thivolet F, Pettigrew RI, Finet G. Influence of residual stress/strain on the biomechanical stability of vulnerable coronary plaques: potential impact for evaluating the risk of plaque rupture. *Am J Physiol Heart Circ Physiol*. 2007;293:H1987–H1996.
6. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb*. 1994;14:133–140.
7. Johnson JL, Jackson CL. Atherosclerotic plaque rupture in the apolipoprotein E knockout mouse. *Atherosclerosis*. 2001;154:399–406.
8. Gregersen H, Zhao J, Lu X, Zhou J, Falk E. Remodelling of the zero-stress state and residual strains in apoE-deficient mouse aorta. *Biorheology*. 2007;44:75–89.
9. Lee RT, Grodzinsky AJ, Frank EH, Kamm RD, Schoen FJ. Structure-dependent dynamic mechanical behavior of fibrous caps from human atherosclerotic plaques. *Circulation*. 1991;83:1764–1770.
10. Coleman R, Hayek T, Keidar S, Aviram M. A mouse model for human atherosclerosis: long-term histopathological study of lesion development in the aortic arch of apolipoprotein E-deficient (E0) mice. *Acta Histochem*. 2006;108:415–424.