Endothelial stiffening: a new parameter of endothelial damage in athero-susceptible regions of aorta

Atherosclerotic plaques that clog major arteries and lead to heart attacks and strokes are known to develop in sites exposed to non-unidirectional disturbed flow, such as bends and branches, whereas arterial segments that are straight and exposed to uni-directional laminar flow are athero-resistant. Furthermore, while disturbed flow alone primes the region to plaque development, it is its combination with high cholesterol that leads to the development of atherosclerotic plaques. Numerous studies addressed the impact of both disturbed flow and dyslipidemia on endothelial cells, the inner lining of the blood vessels, focusing on multiple signaling cascades. Our work focuses on endothelial biomechanical properties.

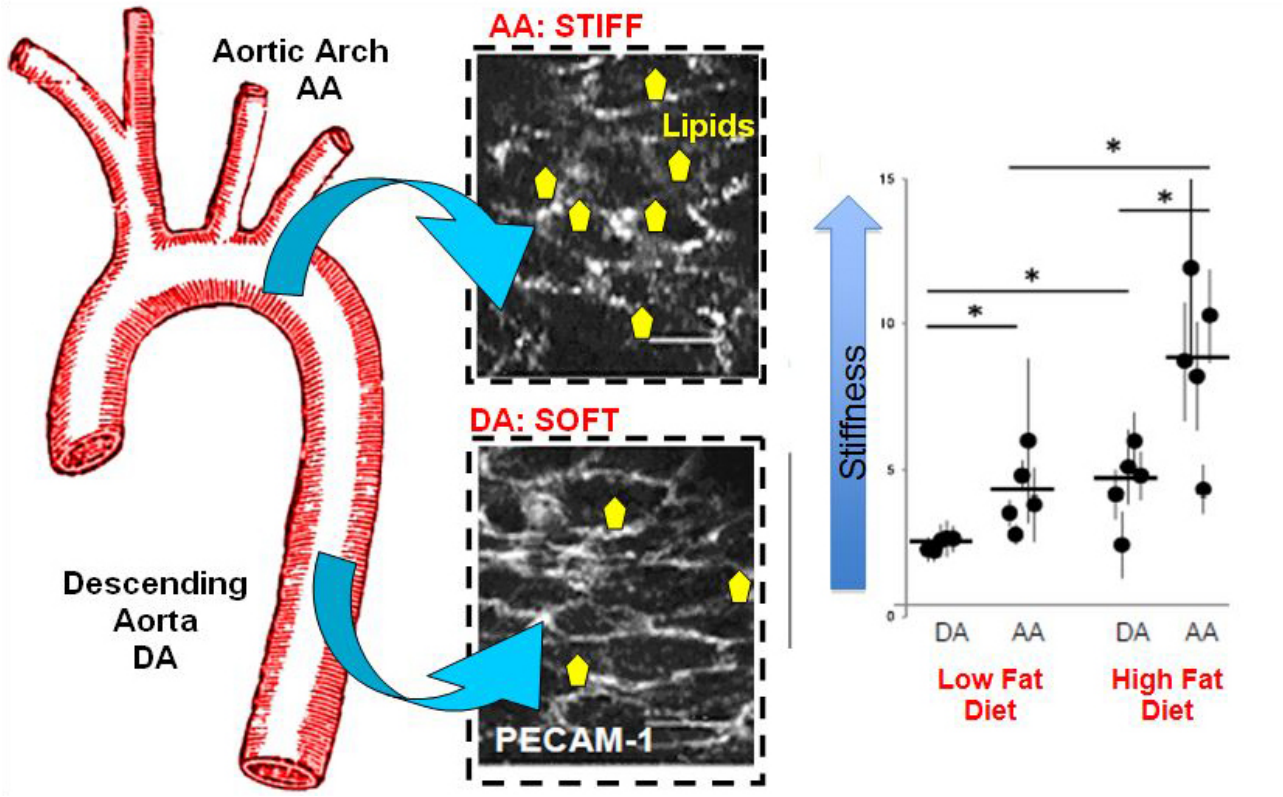

Fig. 1. Increased endothelial stiffening in athero-susceptible regions of the aorta
Left panel: an image of the aorta showing the location of the aortic arch and the descending aorta. Center panel: images of endothelial cells showing the difference in lipid accumulation (yellow pentagons) between the stiff aortic arch (top) and the soft descending aorta (bottom). Right panel: AFM data showing an increased endothelial stiffness between low fat diet and high fat diet mice's descending aorta and aortic arch regions.

In this study, we explore the impact of disturbed flow and hypercholesterolemia on the stiffness of the endothelial monolayer of mouse aortas using atomic force microscopy (AFM). Endothelial stiffness is measured in intact freshly-isolated aortas opened longitudinally to allow AFM access the inner lining of the vessel. We demonstrate that endothelial monolayer is significantly stiffer at athero-susceptible region of the inner aortic arch as compared to athero-resistant region of descending aorta. Notably, this effect is strongly exacerbated in mice fed high cholesterol diet even just for one month. These observations provide the link between endothelial stiffening and two major pro-atherosclerotic risk factors, disturbed flow and hypercholesterolemia. Furthermore, our data indicate that the two factors act synergistically and that the stiffening effect of disturbed flow is mediated by increased lipid uptake. The two dimensional correlation between endothelial stiffening and pro-atherosclerotic environment provided the first evidence that endothelial stiffening might play an important role in the development of atherosclerosis. The causality between endothelial stiffening and atherosclerosis is further supported by our finding that genetic deletion a scavenger receptor CD36, which is known to confer athero-protective effect, also abrogates endothelial stiffening.

Our earlier studies discovered that uptake of oxidized lipids, such as oxidized modifications of low-density lipoproteins (ox-LDL), as well as oxysterols and oxidized phospholipids result in endothelial stiffening via a signaling cascade that involves endothelial contractile response. Here we show that the uptake of oxidized lipids is also responsible for endothelial stiffening in the pro-atherogenic environment in vivo. Specifically, several lines of evidence indicate that endothelial stiffening in mouse aortas is mediated by the endothelial uptake of oxidized lipids. First, using lipid mass spectrometry, we found significant accumulation of oxidized lipids in the aortic arch compared to the descending aorta, which was exacerbated by high fat diet, both correlated with endothelial stiffening. Next, we found that these results could be replicated in vitro using human aortic endothelial cells (HAECs). We created flow environments that reproduced conditions seen in the aortic arch and the descending aorta and discovered that disturbed flow increases oxLDL uptake in HAECs and that this increased oxLDL uptake in disturbed flow regions resulted in endothelial stiffening. The fact that an increase in oxLDL uptake under disturbed flow resulted in endothelial stiffening in a controlled in vitro experiment led us to conclude that this mechanism plays a role in endothelial stiffening in aortic arch in vivo. Finally, we show that an increased oxLDL uptake under disturbed flow results from a focal increase in CD36 expression, whereas genetic deletion of CD36 abrogates both the accumulation of oxLDL and the stiffening effect, both in vitro and in vivo.

Taken together, these studies suggest that endothelial stiffening in the aortic arch is a result of increased CD36-mediated uptake of oxidized lipids. We propose that this is one of the critical components of endothelial dysfunction in pre-atherosclerotic environment.

Maha Qureshi, Elizabeth Le Master, Irena Levitan
University of Illinois at Chicago, USA

Publication

Proatherogenic Flow Increases Endothelial Stiffness via Enhanced CD36-Mediated Uptake of Oxidized Low-Density Lipoproteins.

Le Master E, Huang RT, Zhang C, Bogachkov Y, Coles C, Shentu TP, Sheng Y, Fancher IS, Ng C, Christoforidis T, Subbaiah PV, Berdyshev E, Qain Z, Eddington DT, Lee J, Cho M, Fang Y, Minshall RD, Levitan I

Arterioscler Thromb Vasc Biol. 2018 Jan