



# Proteins as biomarkers of *Shear stress (SS)* intensity: role of blood flow in atherosclerosis development

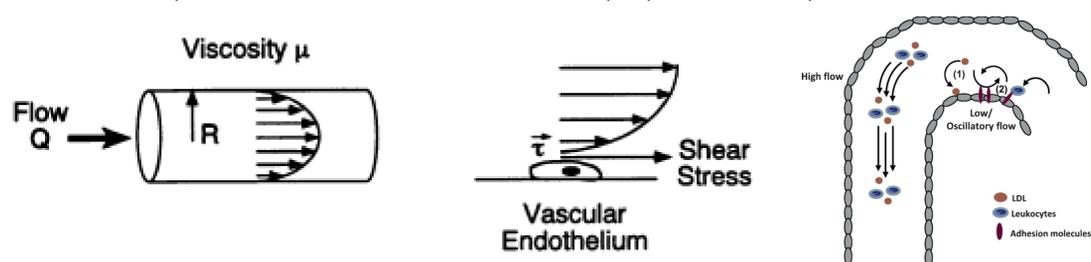
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## INTRODUCTION

Endothelial cells play an important role in the cardiovascular system as in a physical way, acting as a non-thrombogenic barrier and selective permeability, or biochemical way, acting as a signaling cell. These cells may be chemically modulated, or physically by hemodynamic forces of the blood flow as the shear stress (SS). The SS is a physical stimulus caused by the tensile force parallel to the endothelium of blood, being able to change proteins, secretory and signaling profile. Changes in SS are highly related to the formation and progression of atherosclerotic plaques. There are vascular regions with change in SS from laminar to oscillatory, as in bifurcations. In these regions, there is increased endothelial permeability, cytoskeletal changes and increased expression of adhesion molecules in the membranes of endothelial cells. Although very studied the SS and atherosclerotic plaques relationship, little is known about the signaling pathways of phosphorylation and changes involved in this process, and how this is associated with plaque vulnerability.



Modified of Chien, S. et al. *AJP Heart Cardiology Physiology* 292, H1209–24 (2007) and Warboys, C. M. et al. *F1000 medicine reports* 3, 5 (2011).

## AIM

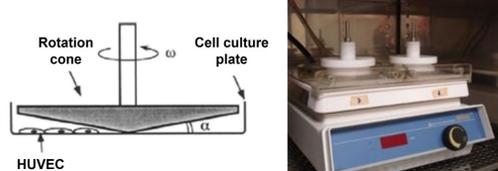
➤ This work aimed identifies and quantify proteins with differential expression between laminar and low SS, and connect these proteins to atherosclerosis development.

## METHODS

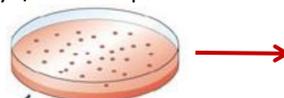
### Cell culture



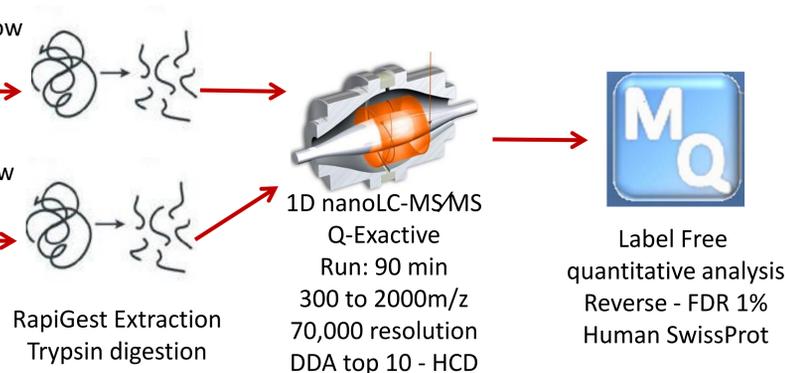
### In vitro shear stress cone plate system



15 dyn/cm<sup>2</sup> atheroprotective flow



5 dyn/cm<sup>2</sup> proatherogenic flow



Total cell protein and Media secreted protein

RapiGest Extraction  
 Trypsin digestion

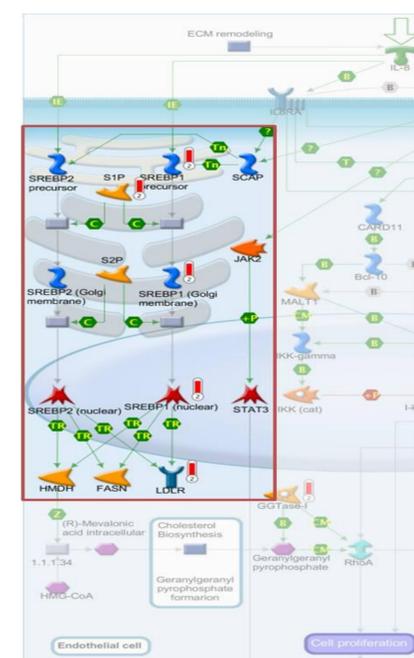
## RESULTS

- In total, 2728 proteins were found in cell proteome analysis. Proteins increased in 5 dyn/cm<sup>2</sup> SS are related to blood clotting, vascular development and cholesterol metabolism, whereas proteins upregulated in 15 dyn/cm<sup>2</sup> are related to inflammatory response, oxidative stress and tissue remodeling, according MetaCore Analysis.

- In secretome analysis, 1235 proteins were found and protein related to apoptosis, chemokine for cell adhesion and vesicles formation are up secreted in 5 dyn/cm<sup>2</sup> stimuli, whereas in 15 dyn/cm<sup>2</sup> stimuli there are the increase of vasodilator proteins and hypoxia response. These findings corroborated with endothelial changes during atherosclerosis development and NO measure in media culture.



Interestingly, we identified downregulation of every compounds participating in LDL receptor expression pathway in 5 dyn/cm<sup>2</sup> stimuli. These data suggest that laminar flow keep the expression of LDL receptor, activating LDL metabolism and avoiding the LDL inner in sub-endothelial space, in opposite of low SS. Subjects with mutation in LDL receptor show severe familial hypercholesterolemia with spontaneous plaque atherosclerosis formation. We are currently replicating these data in vessels with different levels of atherosclerosis.



## CONCLUSION

All together our data suggest that intensity of SS have an important role atherosclerosis development and proteins could be useful as biomarker of vulnerability plaque.