# PROTEIN DISULFIDE ISOMERASE MODULATES NITRIC OXIDE OUTPUT DURING LAMINAR SHEAR STRESS IN ENDOTHELIAL CELLS

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Laminar shear (LS) is atheroprotective due mainly to nitric oxide(NO) release from eNOS. Caveolae lipid microdomains, which increase after LS, regulate eNOS and contain NADPH oxidase isoform Nox1. Nox1 was recently shown to be regulated by thioredoxin superfamily protein disulfide isomerase (PDI), which in turn can promote transnitrosation-dependent NO cell uptake. Considering this dual effect, we hypothesized that PDI controls NO output during sustained LS. Rabbit aortic endothelial cells (RAEC) were submitted *in vitro* to LS (15dyne/cm$^2$) with cone-plate system. After 18h (vs. static controls), RAEC exhibited 50% decrease in ROS production (2-hydroxiethidium/HPLC), associated with decreases of 20% in membrane fraction NADPH oxidase activity and of 50% in Nox4 mRNA (QRT-PCR). To investigate the role of PDI in NO output, RAEC were transiently transfected with sense or antisense PDI cDNA. With PDI overexpression, LS promoted greater increase in nitrite levels (15.5±1.8 *vs* 7.8±4.4 µM, empty vector), while PDI knockdown decreased nitrite output. Transient incubation of smooth muscle cells (which lack eNOS) with NO donors promoted strong sustained NADPH oxidase down-activity. Fractionation studies showed that PDI is present in caveolin1-enriched fractions in static cultures but migrates to higher-density fractions during sustained LS. Preliminary confocal microscopy experiments suggest increased PDI-Nox1 co-localization after LS. Thus, PDI may exert a key role in NO bioavailability in LS, through compartment-dependent NADPH oxidase down-regulation. (FAPESP, CNPq-Milênio *Redoxoma*)