

IMPAIRMENT IN NOS PATHWAY CAN SERVE AS AN EARLY MOLECULAR MARKER FOR RED BLOOD CELL DYSFUNCTIONALITY



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Consequences of Smoking During Pregnancy



Pregnancy + Additional Intake of Toxins = Acute Oxidative Stress

Tobacco smoke:

~4700 chemicals:

- Tar phase
- Gas phase

Strong pro-oxidants:

- Metals/Heavy metals, epoxides, peroxides, NO, ONOO⁻, Q/QH₂, O₂^{•-}, OH[•], etc.



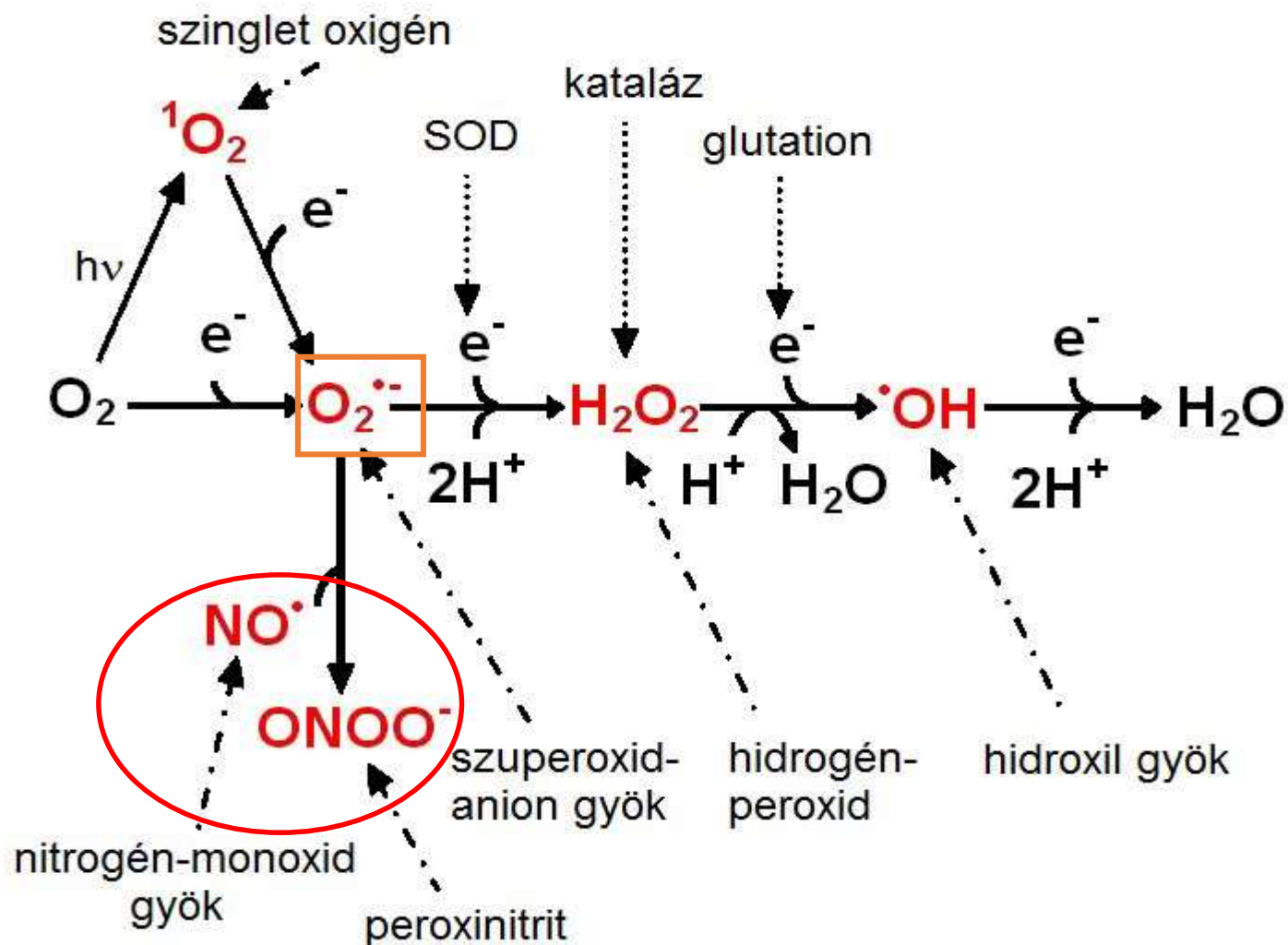
Macromolecular
damage

Oxidative Stress

Cell and tissue
damage



Free Radicals and Antioxidant System





SMOKING INDUCED RISK FACTORS

IN ADULTS

- Artherosclerosis
- Hypertension
- Dyslipidaemia
- Diabetes Mellitus
- Cancer

IN PREGNANCY

- Still Birth
- Preeclampsia
- IUGR
- Low Birth Weight
- Miscarriage

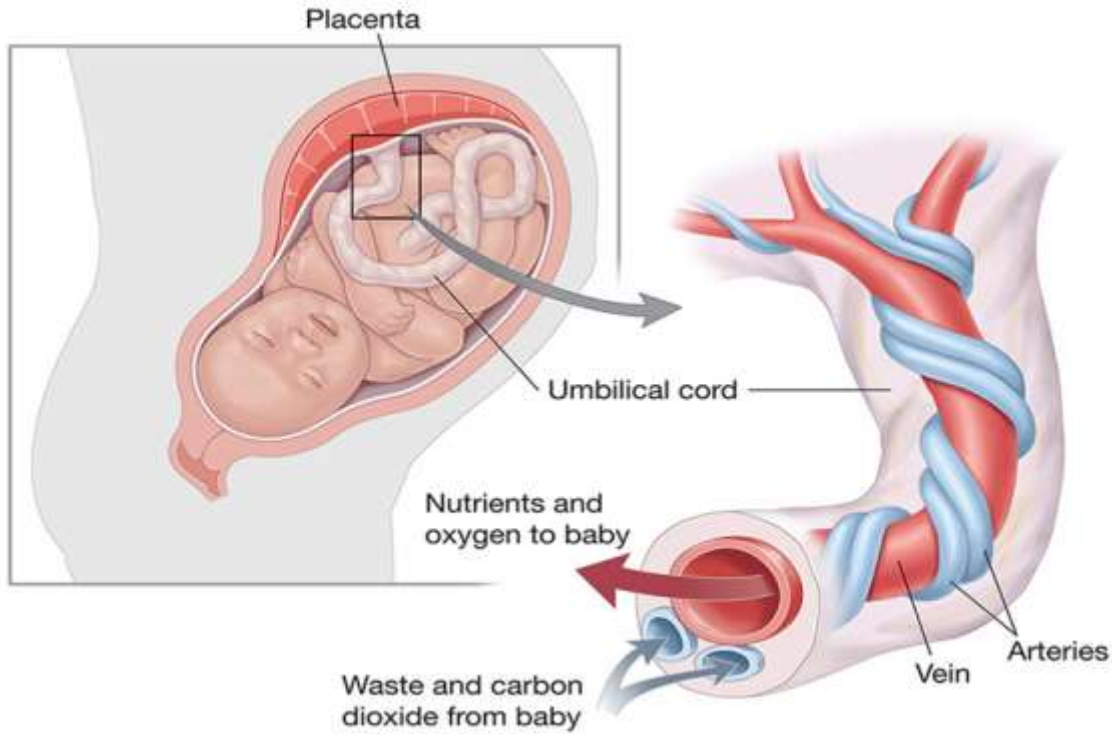
IN DEVELOPING FETUS



- Mechanisms are poorly understood !!!



Feto-Placental Circulation

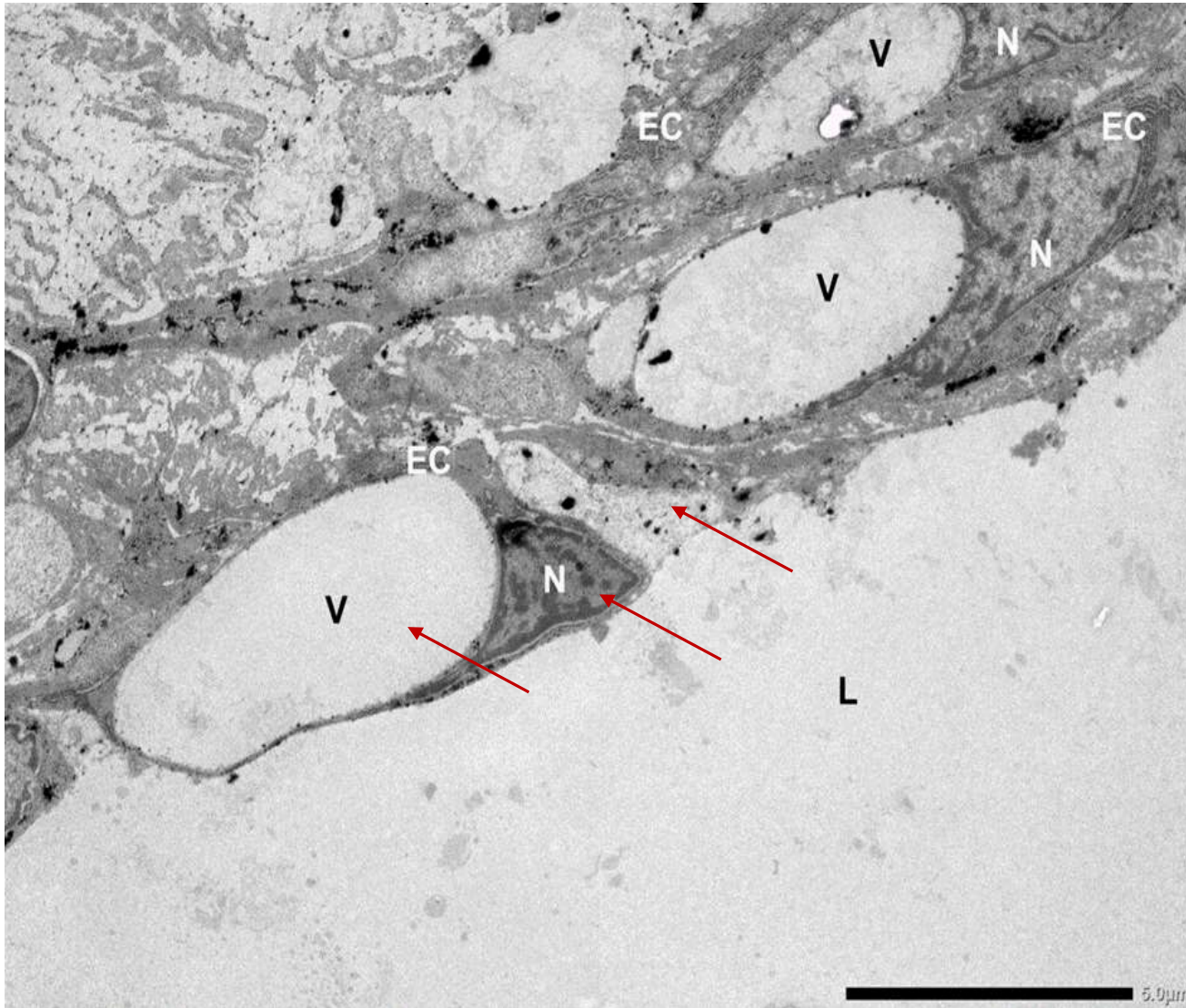


- Umbilical cord lacks Innervations
- The nitric oxide (NO) derived from NOS3 or Nitrate/Nitrite sources regulates the vascular tone and integrity

Important !

The harmful toxins from cigarette smoke easily infiltrate through the placenta and pass down to fetal circulation.

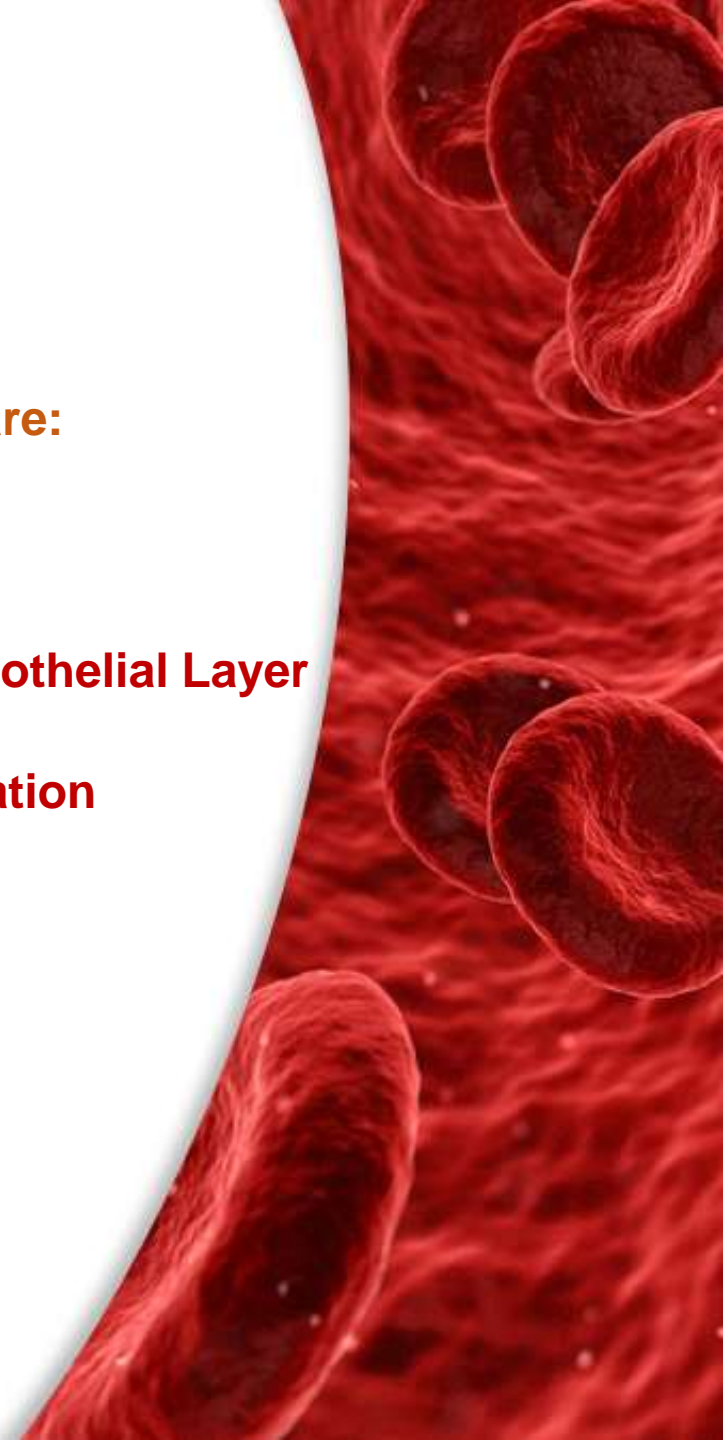
Representation of Cord Endothelial Cell – Damage



Indicated Findings are:

- Huge Vacuoles
- Discontinuous Endothelial Layer
- Nucleus Fragmentation

Courtesy: Electron Microscopy



GROUND-BREAKING FUNCTIONAL FEATURES OF RED BLOOD CELLS



Redox Biology

journal homepage: www.elsevier.com/locate/redox



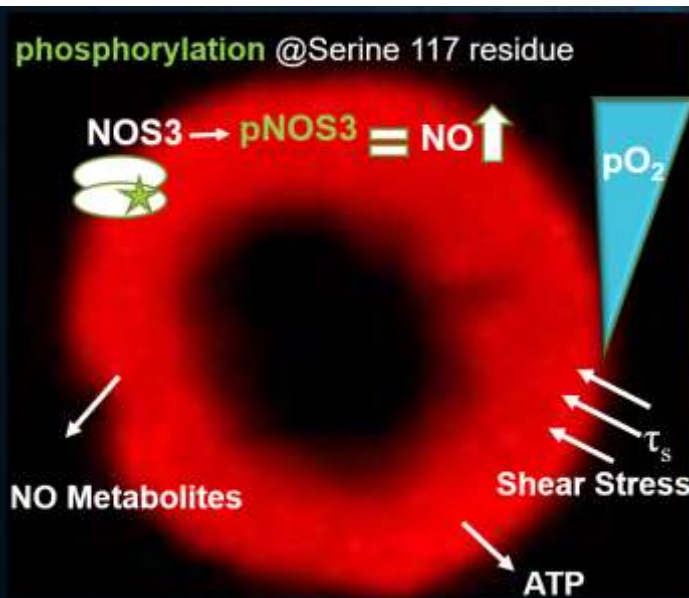
Review Article

Endothelial nitric oxide synthase in red blood cells: Key to a new erythrocrine function? ☆



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Cardiovascular Research Laboratory, Department of Cardiology, Pneumology and Angiology, Medical Faculty, Heinrich Heine University of Düsseldorf, Düsseldorf, Germany

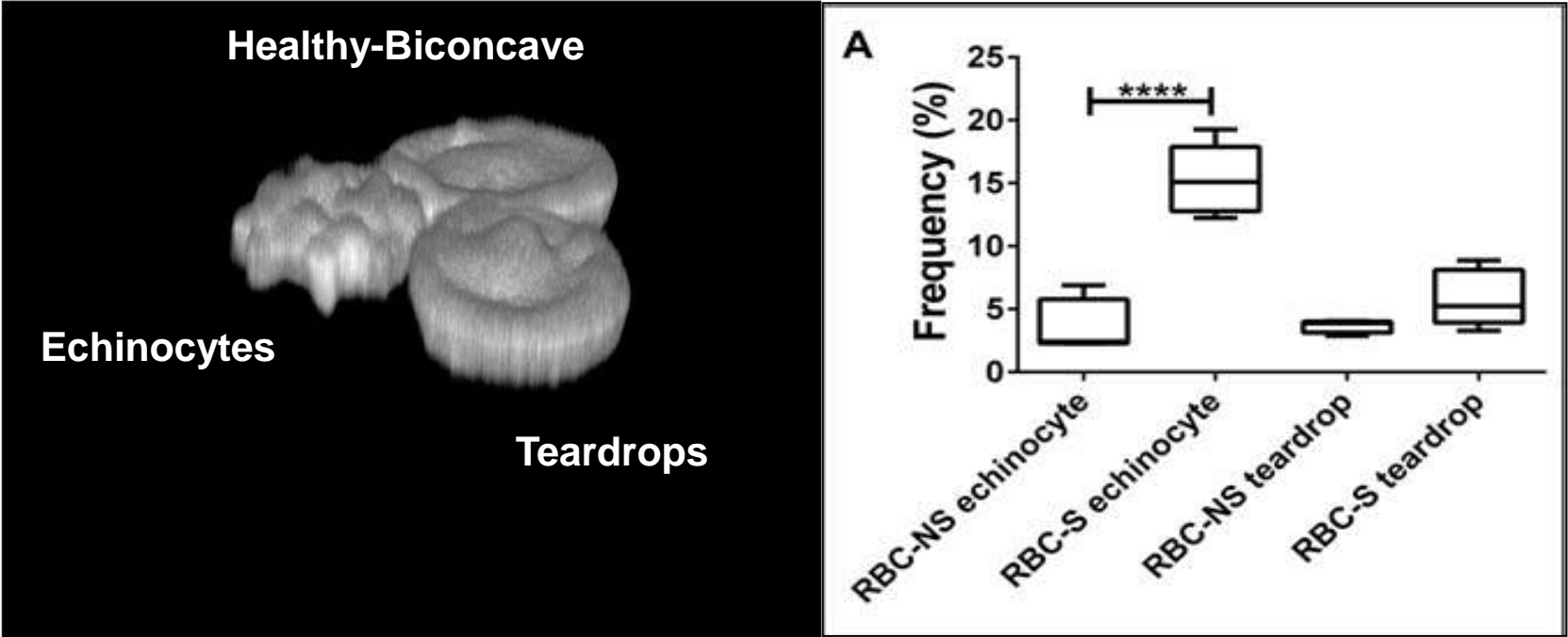


- ❖ Previously RBC was considered as only nitric oxide(NO) “Sink”
- ❖ **Erythrocrine Function-** The RBC-Nitric oxide synthase (NOS) pathway can synthesize and release NO, thus “Sink” to “Source”
- ❖ RBCs can perform “**Rescue Mechanism**” under oxidative stress.
- ❖ Intimate Crosstalk between RBCs & Vascular Endothelium
- ❖ RBCs - Protagonist player under Endothelial Dysfunctions

In smoking background do RBCs still retain its “Rescue Mechanism”?

(RBC-S)-Fetal-RBCS from heavy smoker > 10 cigarettes/day and **(RBC-NS)**-from non-smoker origin

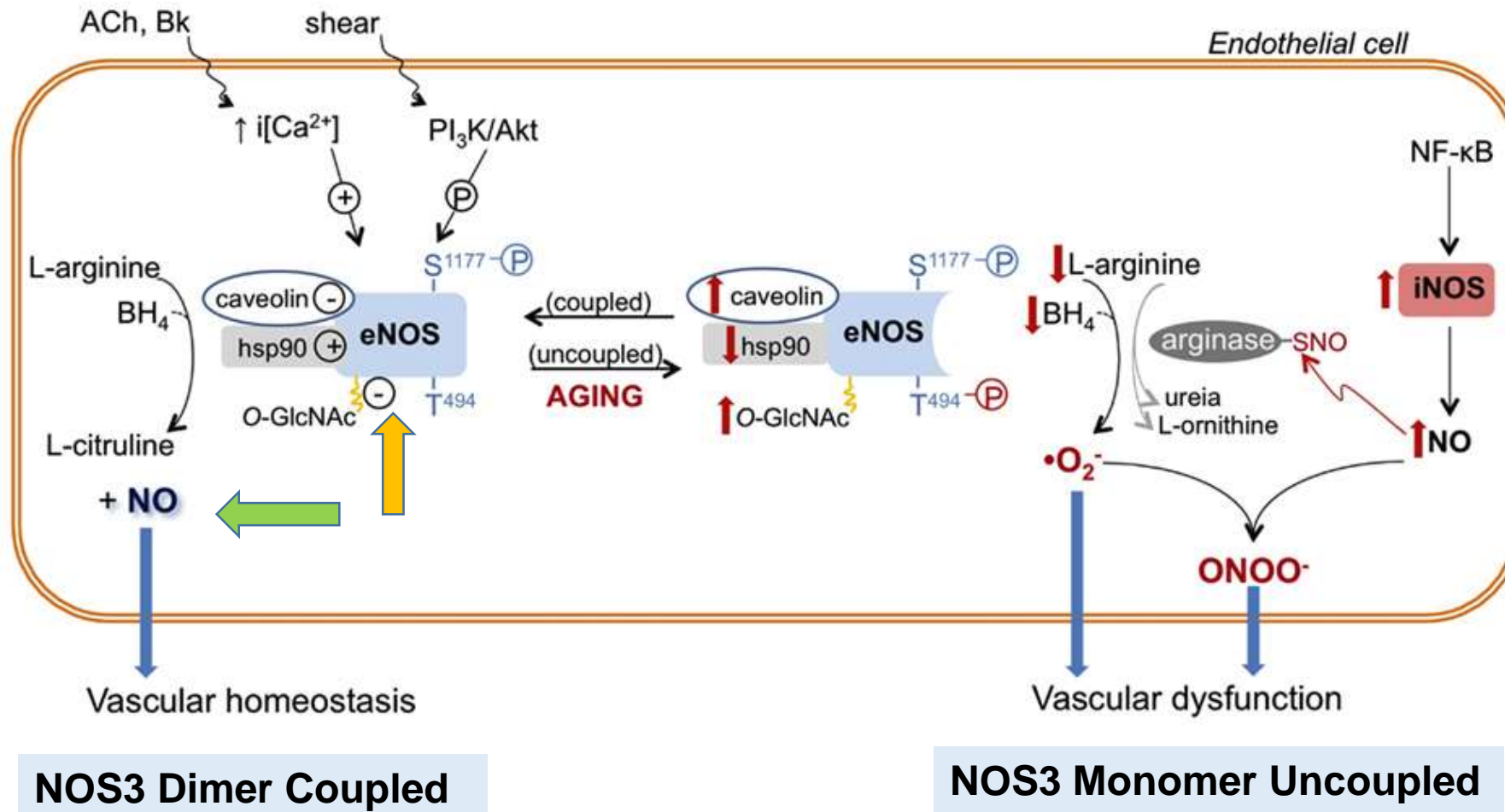
1. RBC – Morphological Variants



Software: Advanced Cell Classifier™

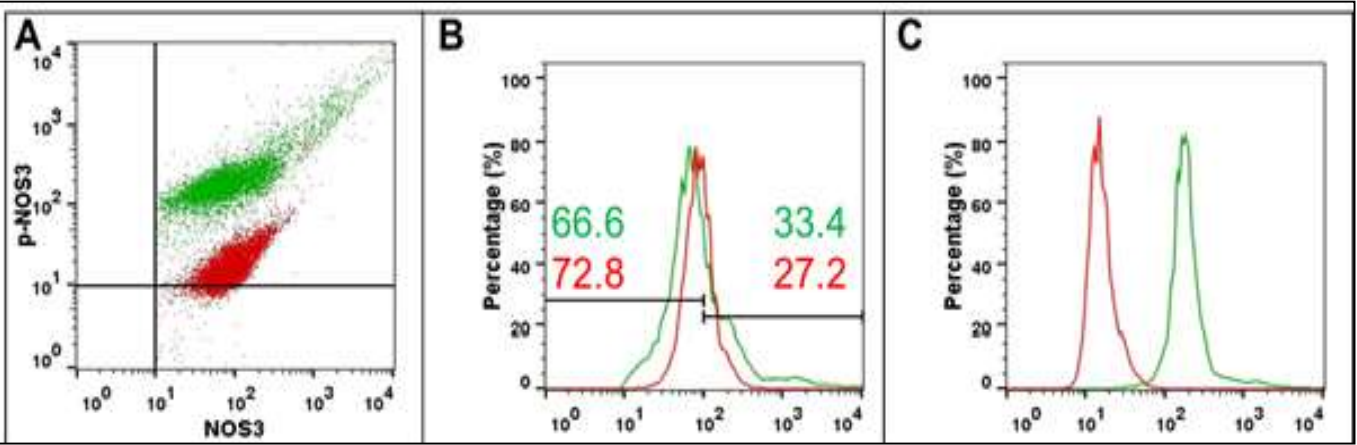
Mechanism of NOS3 Pathway

2. Functionality



Analysis of NOS3 –Expression and Activation

CONFOCAL MICROSCOPY MEASUREMENTS

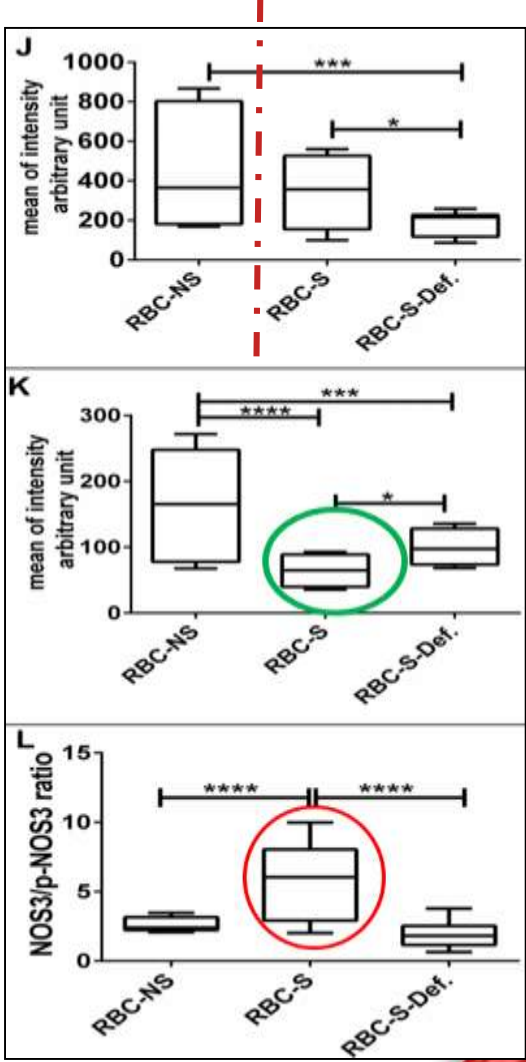


Flow Activated Cell Sorting - (FACS)

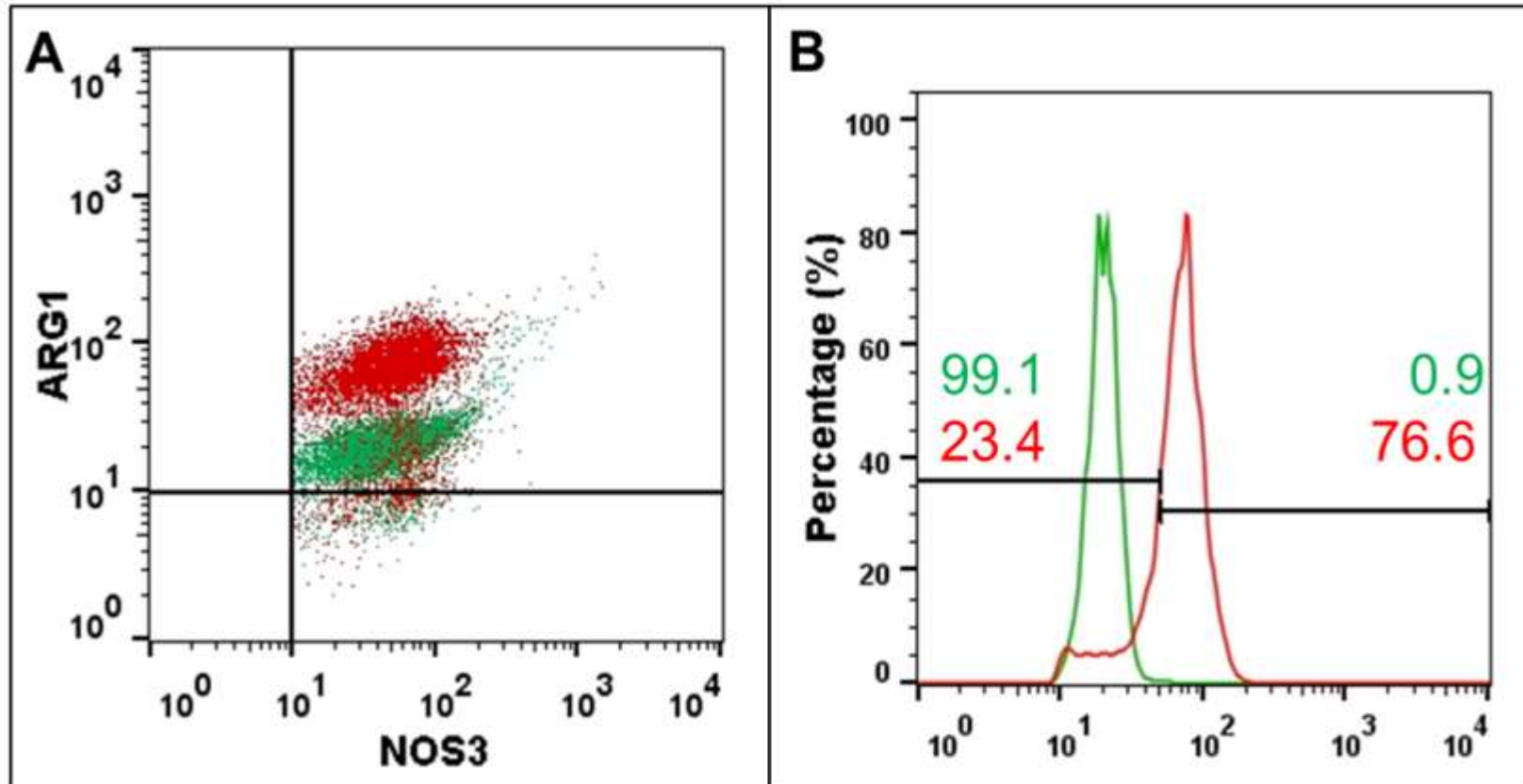
Software:



Software:



Arginase Expression



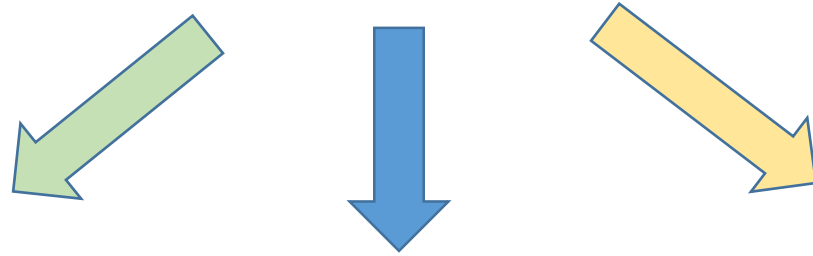
Flow Activated Cell Sorting - (FACS)

Software:



ASPECTS OF HARMFUL CONSEQUENCES ON **FETAL RBCS** WITH SMOKER ORIGIN

RBC-NOS PATHWAY



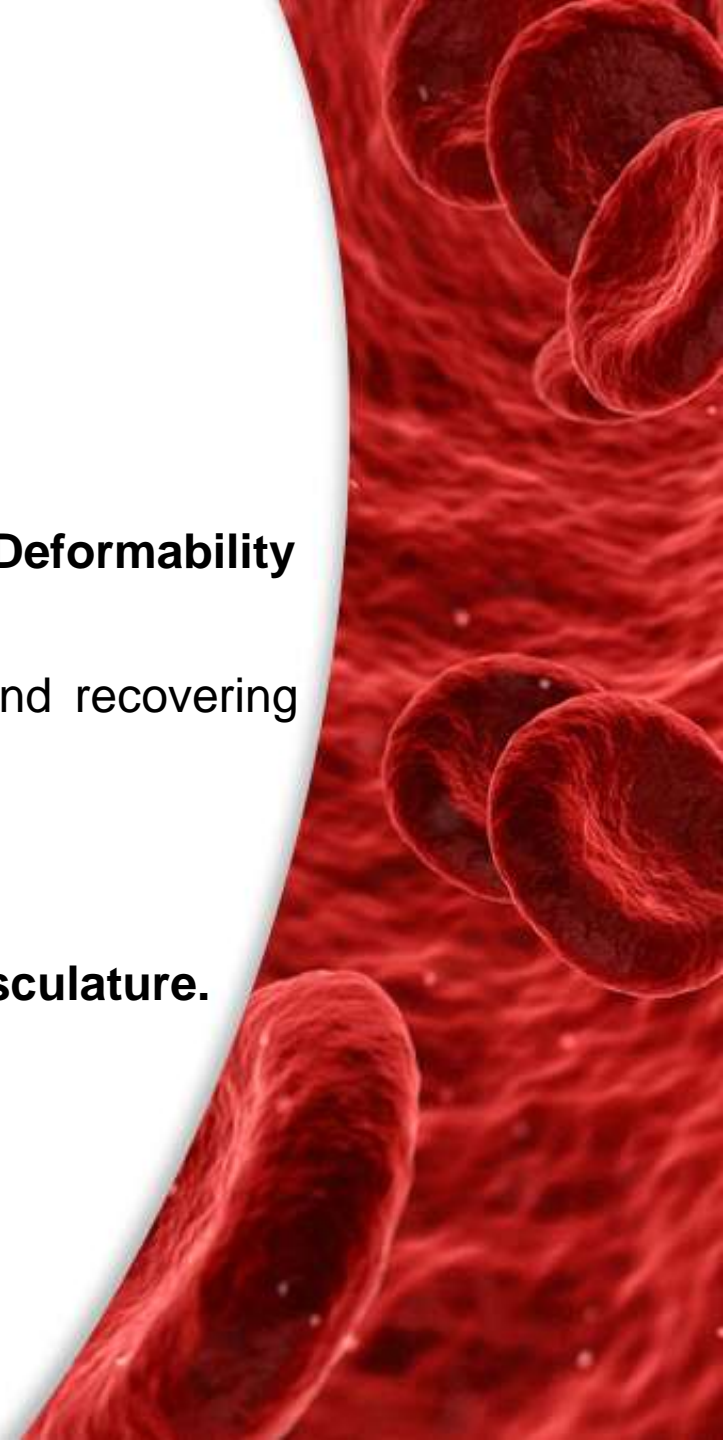
Deformability Property

Macromolecular Damages

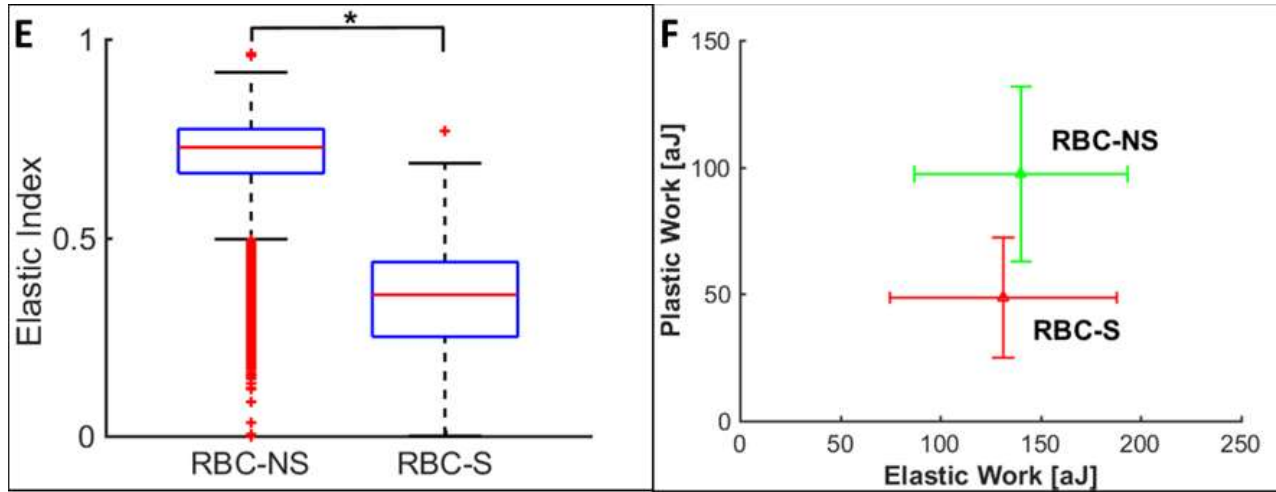
Membrane Lipidomic
Alterations

RBC-DEFORMABILITY PROPERTY

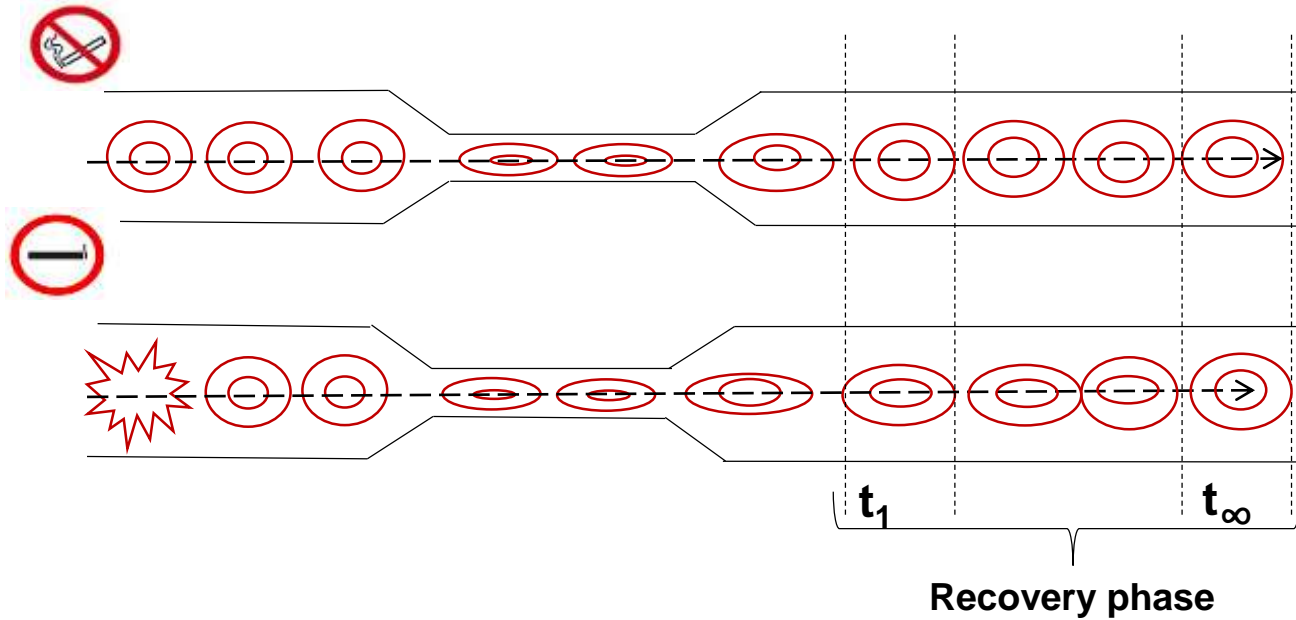
- Atomic Force Microscopy (AFM) study elucidated the RBC Deformability Index
- The new “**Erythrocrine Function**” in some way connected to the maximum **RBC Deformability**
- **Deformability** are defined by the applied shear stress as the **Elastic Work** and recovering ability of the cell after indentation as **the Plastic Work**.
- Hemorheological properties highly regulate the blood flow characteristics in the **Vasculature**.



Analysis of RBC-Deformability Property

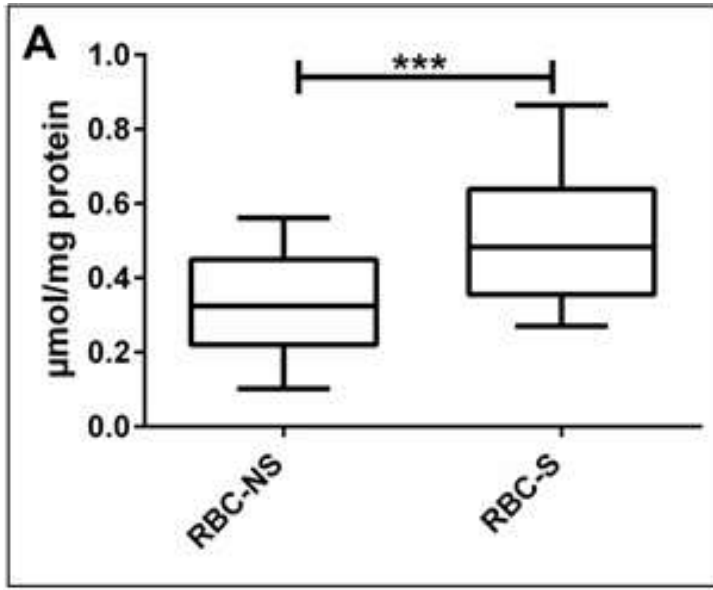


Elastic Index = Ratio of Elastic Work/Plastic Work

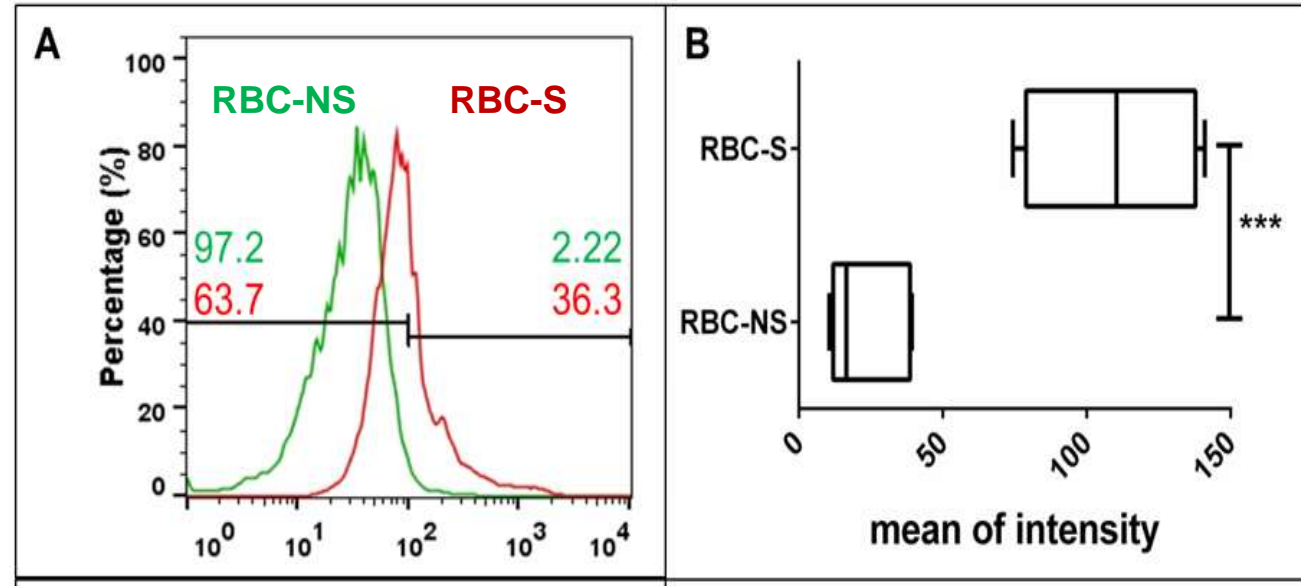


MACROMOLECULAR DAMAGES

Spectrophotometric measurements showed a higher per-oxynitrate (**ONOO⁻**) in the RBC-S population



Measurement of ONOO⁻ Level

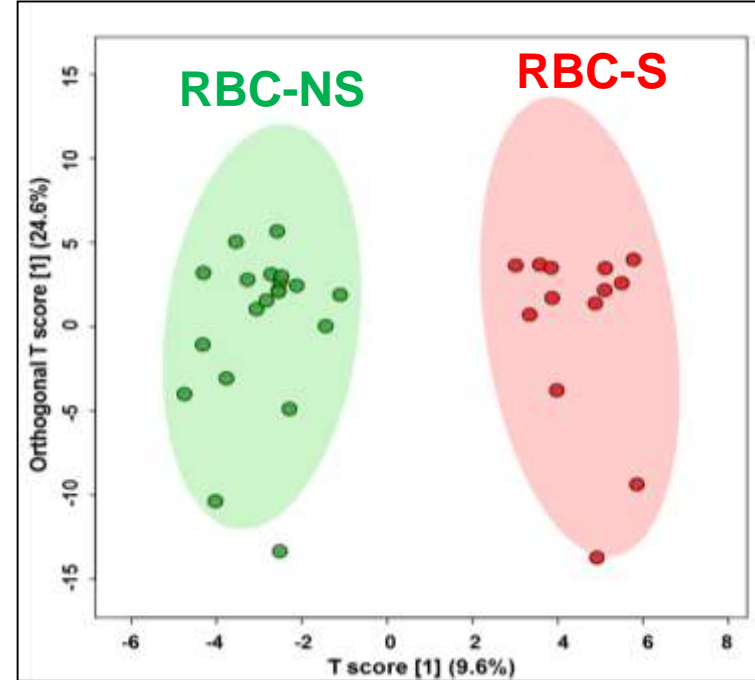
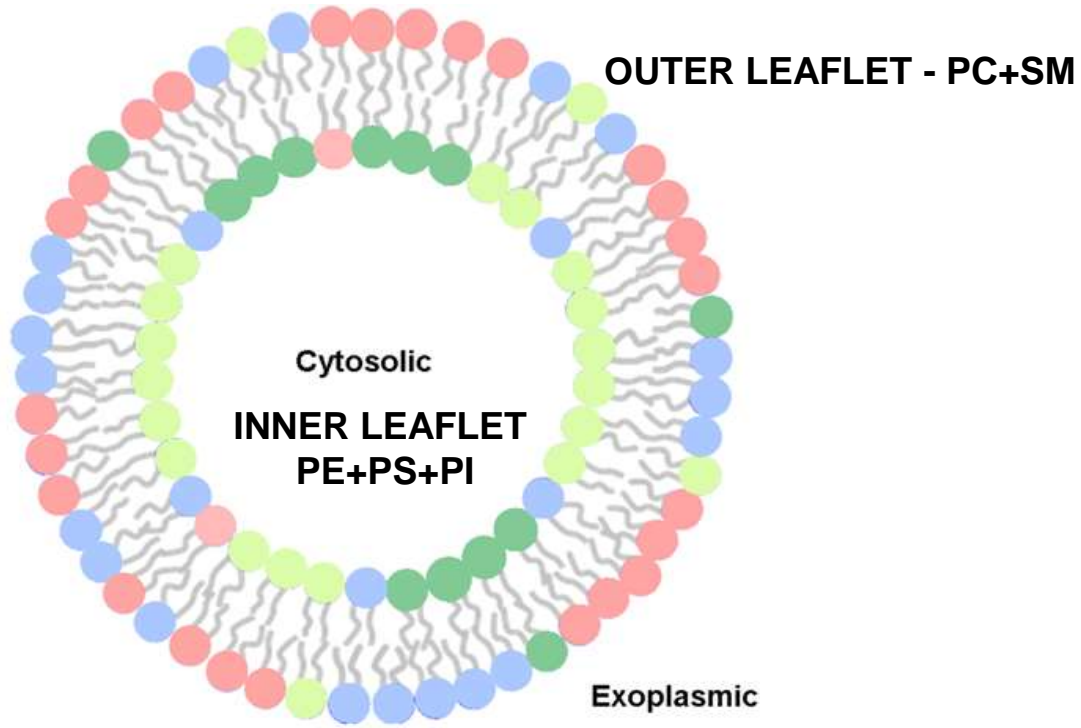


Measurement of 4-hydroxy-2-nonenal (HNE)

High Lipid peroxidation rate was also followed by the level of HNE immunostaining, a product of lipid peroxidation by Flow Activated Cell Sorting (FACS) in the RBC-S origin.

MEMBRANE LIPIDOME ANALYSIS

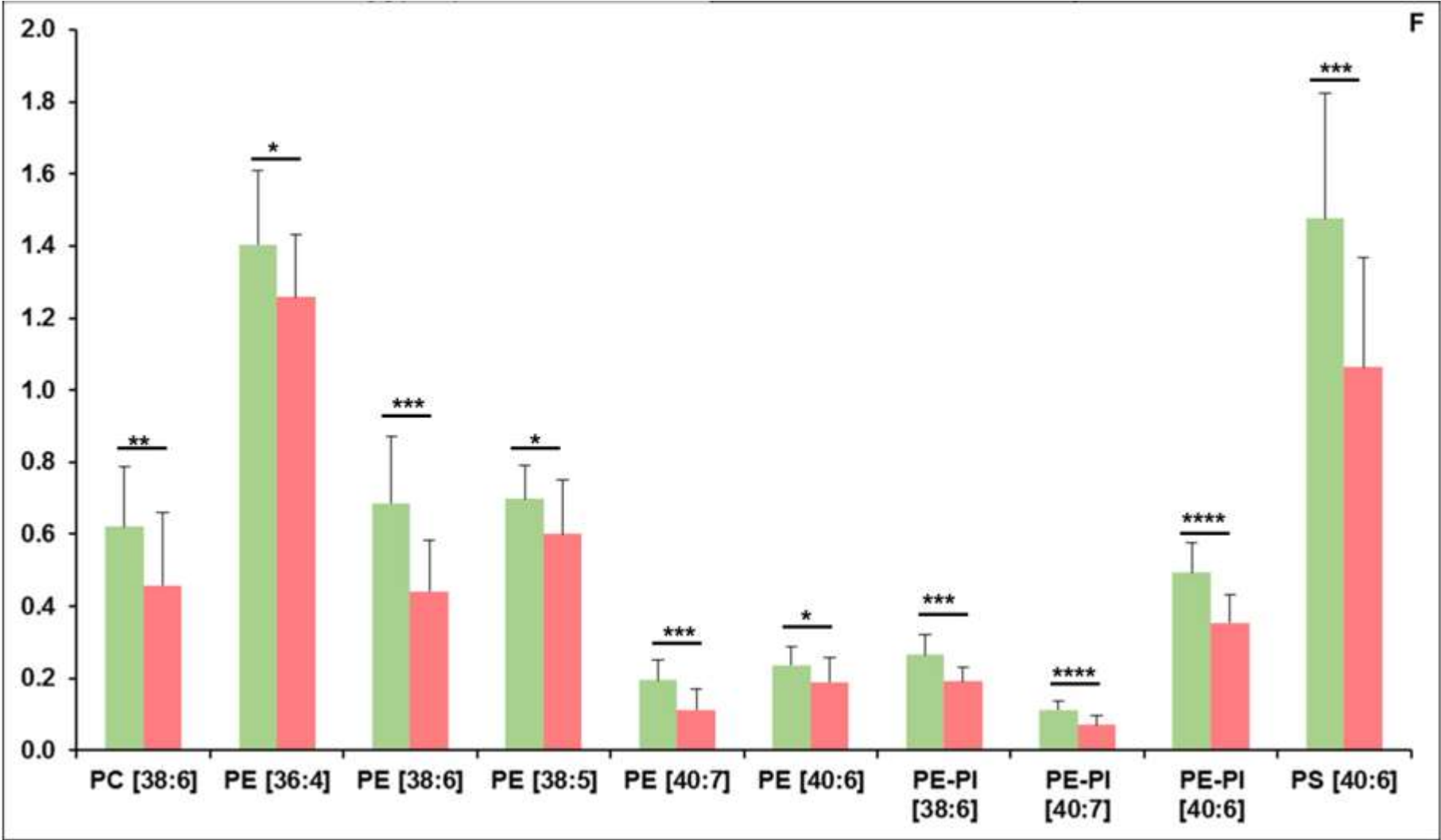
❖ Multi-dimensional mass spectrometry-based shotgun lipidomics (MDMS-SL) was used



- ❖ RBC membrane forms an **unique Elastic network** with Lipid Bilayer Cytoskeletal Protein and Transmembrane protein responsible for “**Membrane Stiffness**” and **Deformability property**
- ❖ **Membrane Asymmetry** promotes diverse antigenic, transport and mechanical characteristics
- ❖ Using MetaboAnalyst™ software we analyzed all aspects of the lipidome data and projected in the **Orthogonal T-score**

Increased level of **ONOO⁻** causes decrease in **Polyunsaturated fatty acids (PUFA)** in **RBC-S**

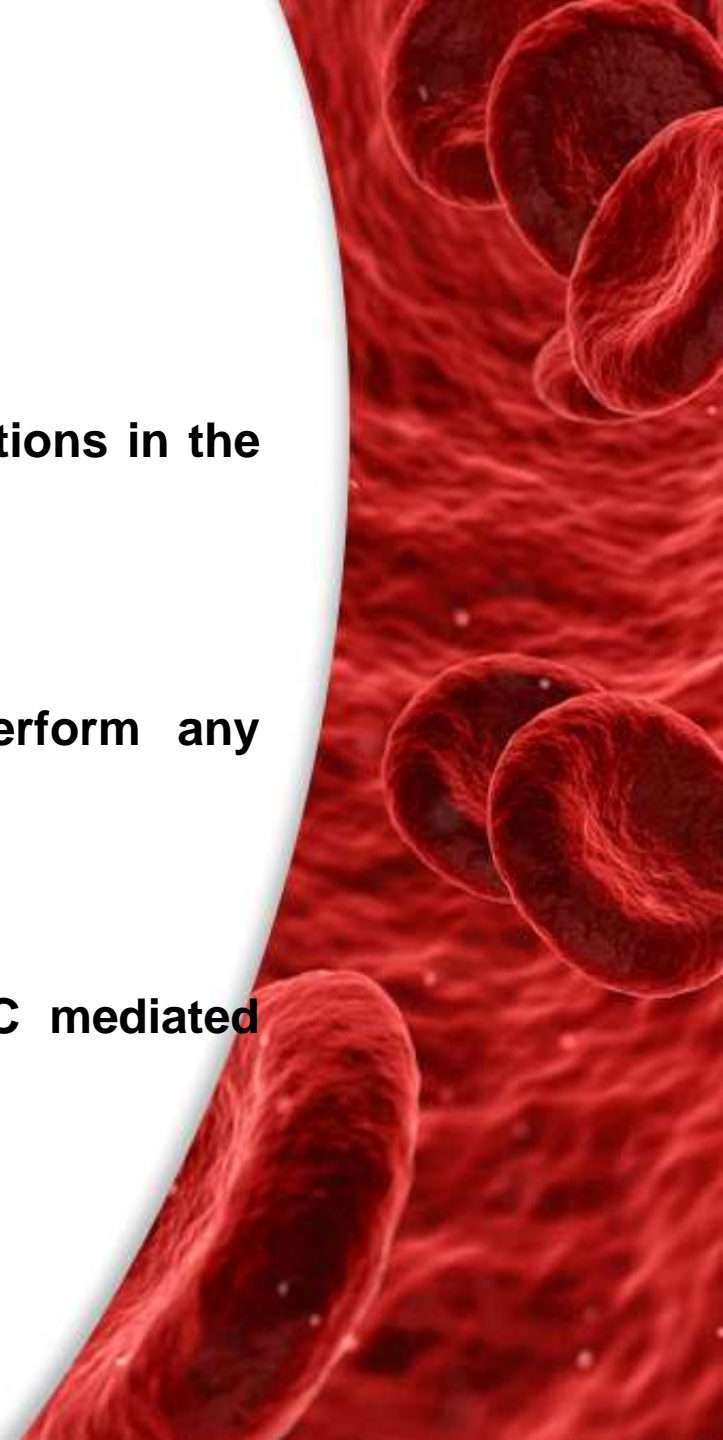
RBC-NS RBC-S



Alterations in the membrane Lipid Components

SUMMARY AND CONCLUSION

- ❑ Functional Impairment are far away preceded by the RBCs' structural variations in the smoker origin**
- ❑ The RBC–NOS pathway in the Smoker origin most likely fails to perform any “Rescue Mechanism”**
- ❑ The phosphorylated NOS level might be the prognostic marker for RBC mediated anomalies along with vascular comorbidities.**



ACKNOWLEDGEMENTS



Szabolcs Zahoran
(Team Member)

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Biochemistry and
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OUR COLLABORATORS

- I. **Attila Gergely Végh** - Institute of Biophysics Biological Research Centre
of Hungarian Academy of Sciences
- II. **Reka Hollandi** Institute of Biochemistry Biological Research Centre of
Hungarian Academy of Sciences
- III. **László Vigh and team members** - Laboratory of Molecular Stress Biology,
Institute of Biochemistry Biological Research Centre of Hungarian
Academy of Sciences
- IV. **Dr. Hajnalka Orvos**, Department of Gynecology and Obstetrics at the
University of Szeged





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