DEVELOPMENT OF BIOMARKERS OF EFFECT FROM CHRONIC TOBACCO USAGE: Part 2, Inflammation and Oxidative Stress

Research and Development, R.J. Reynolds Tobacco Company
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G. L. Prasad
Bobbette A. Jones
Peter Chen
Eckhardt Schmidt

Purpose

- To evaluate the long-term health effects of cigarette smoking and consumption of smokeless tobacco (ST), particularly moist snuff.

- To discover/develop potential biomarkers of effect (BioEff) that could predict long-term effects of cigarette smoking and consumption of ST, particularly moist snuff.

Outline of the Presentation

- Introduction
- Physiology, Pathways & Biomarkers
- Key Findings
- Comparison with Previous RJRT Work
- Summary & Conclusions
- Acknowledgements

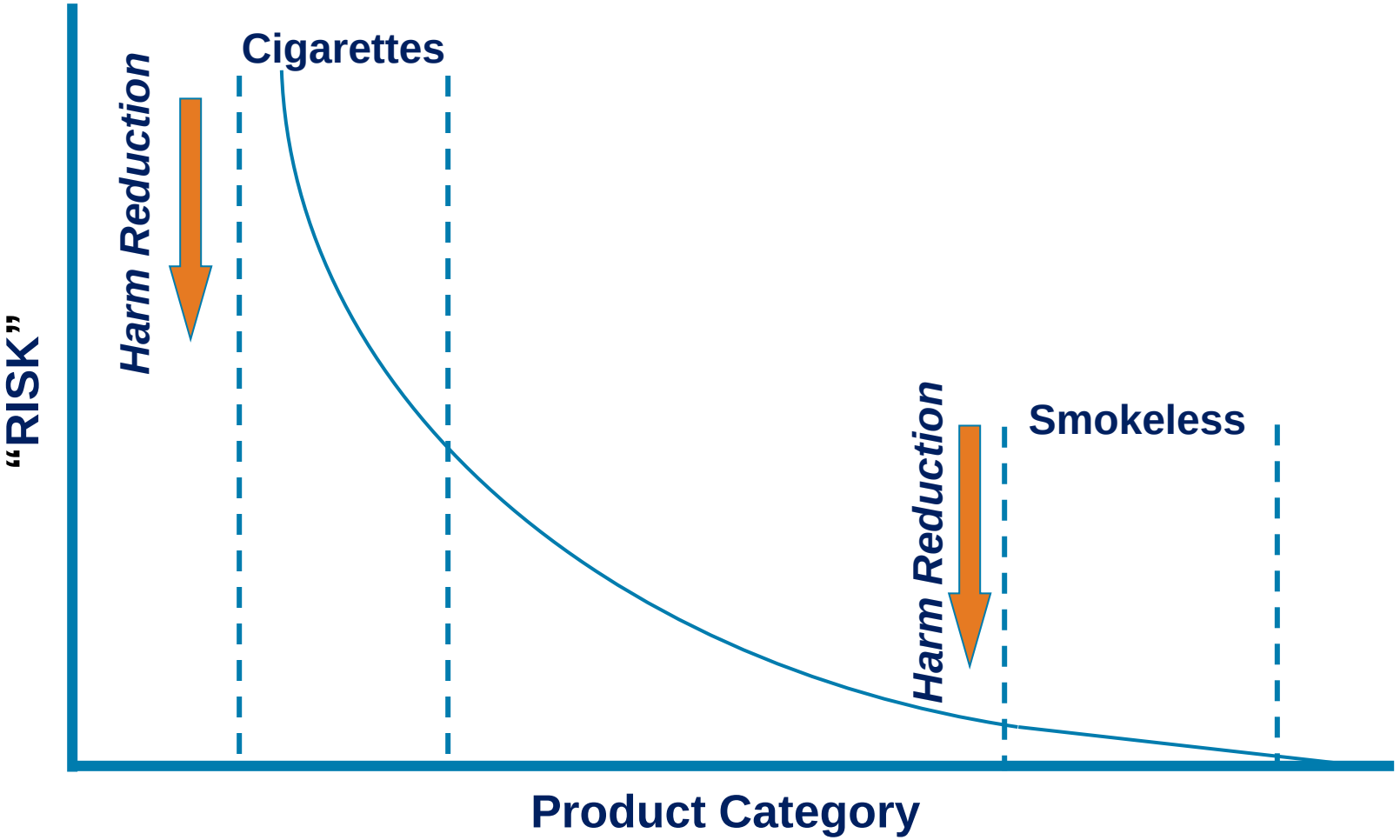
RJRT Beliefs & Risk Continuum

- **No tobacco product has been shown to be safe and without risks. The health risks associated with cigarettes are significantly greater than those associated with the use of smoke-free tobacco and nicotine products.**

- **The best course of action for tobacco users concerned about their health is to quit. Adults who continue to use tobacco products should consider the reductions of risks for serious diseases associated with moving from cigarettes to the use of smoke-free tobacco or nicotine products.**

RJRT, Our Guiding Principles and Beliefs (www.rjrt.com)

Relative Risk

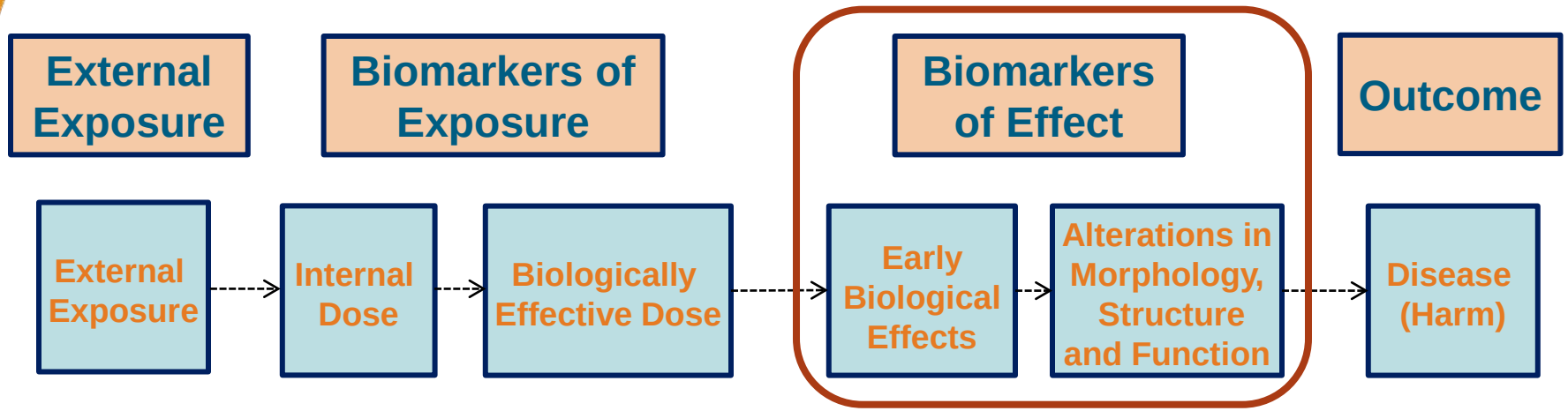


Biomarkers of Effect (BioEff)

- Defined as measure(s) of early biologic alterations with the potential to lead to harm due to exposure

- Important elements and tools in harm reduction strategies

Biomarkers of Exposure and Effect



- Biomarkers of effect measure early subclinical and biological effects.
  (Schematic was redrawn from LSRO Report)
- Relatively less information is available on tobacco related biomarkers of effect. This is an active area of investigation.

Potential Applications of BioEff

Product Understanding

- Evaluation of existing/ new products as potential Modified Risk Tobacco Products (MRTPs)
- Placement of products on the tobacco product risk continuum

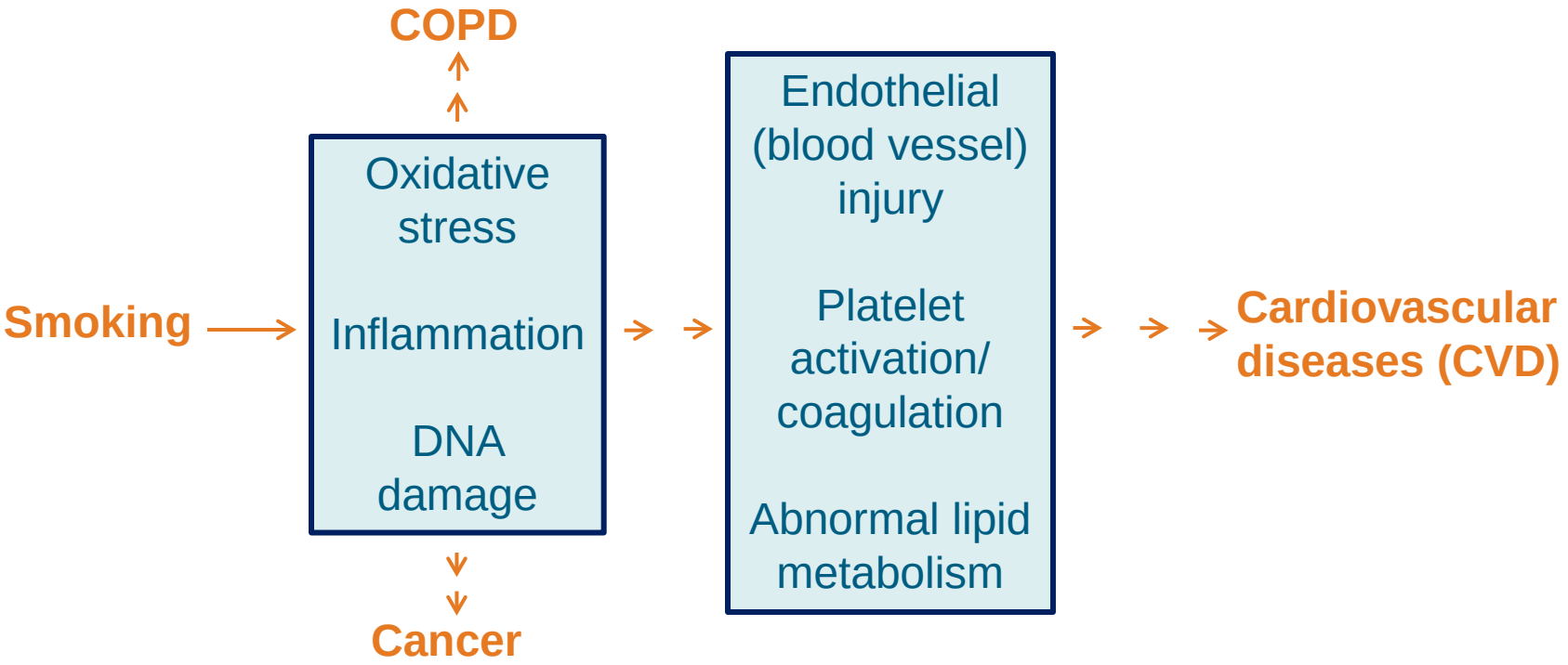
Product Regulation

- Potential use in New Tobacco Product (NTP), Substantial Equivalence and MRTP applications
- Use in MRTP post-marketing surveillance

Mechanisms of Tobacco-related Disease

- Understanding of pathophysiological effects of smoking
- Prediction of the onset of smoking-related illnesses
- Identification of at risk individuals

Mechanisms of Smoking-Related Diseases: A Simplified View



A simplified view of a few complex pathophysiological mechanisms implicated in smoking-related diseases.

BioEff: Examples & Approach

Smoking impacts multiple organs

- Physiological processes

  Examples:
  - Hemostasis (blood coagulation, platelet function)
  - Lipid metabolism

- Mechanisms include oxidative stress and inflammation

  Examples of biomarkers:
  - Isoprostanes
  - C-reactive protein

Two approaches

- Candidate/ targeted approach (this presentation)
- Global profiling/ untargeted methods (Part 3 and Part 4)

Clinical Study & Samples

Biomarker Discovery Study

- Clinical conduct and biomarkers of exposure were described in the preceding presentation (Part 1)

24h urine and fasting blood from healthy males enrolled into 3 study cohorts

- Long-term smokers (SMK, n=40)
- Long-term moist snuff consumers (MSC, n=40)
- Non-tobacco consumers (NTC, n=40)

A number of BioEff, including those indicating inflammation and oxidative stress, were investigated.

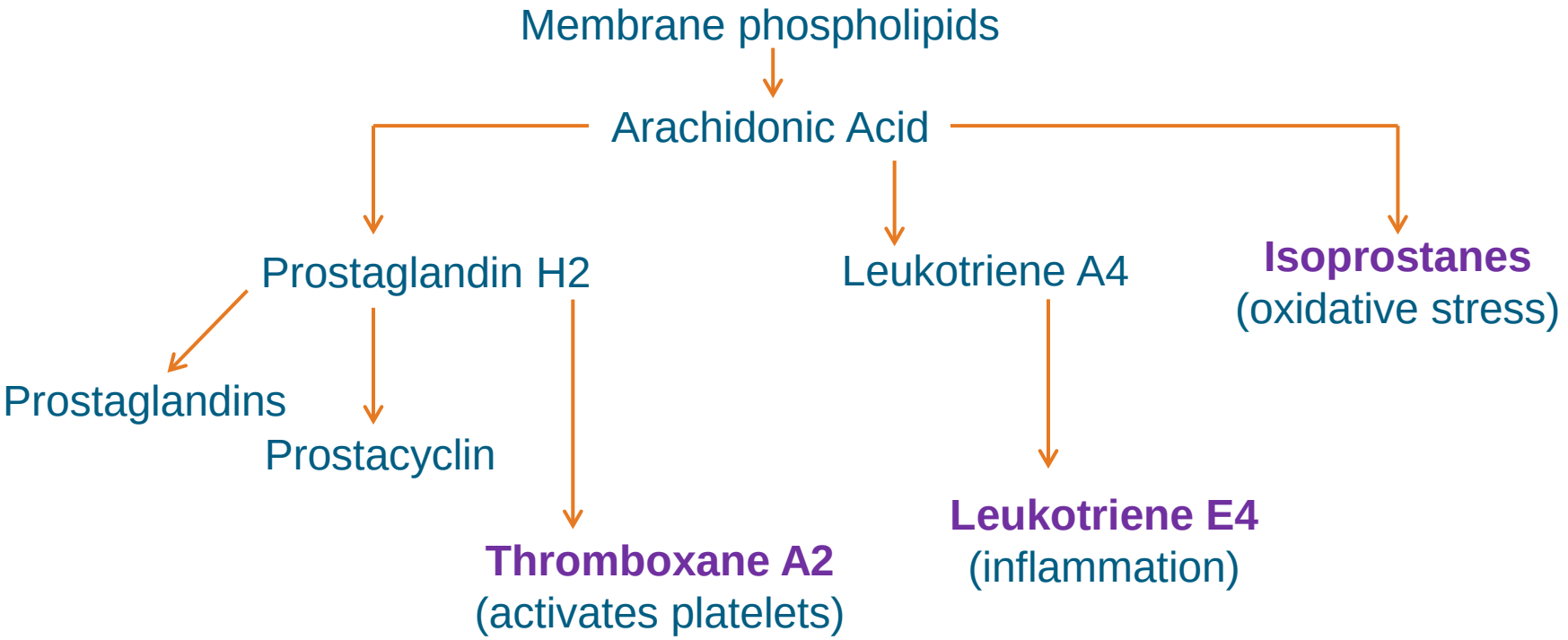
Biomarkers Evaluated

Physiological Process/ Pathway	Matrix	Biomarkers (examples)
Coagulation	Blood	Fibrinogen, vWF, complement C3
	Urine	Thromboxanes
Oxidative stress	Blood	Glutathione
	Urine	Isoprostanes
Inflammation	Blood	White blood cells
	Urine	Leukotriene-E4
Lipid metabolism	Blood	Cholesterol, triglycerides, LDL, lipoproteins
Nitric oxide pathway	Blood	Arginine, citrulline
DNA damage	Urine	DNA adducts

Data on select biomarkers will be presented.
Statistical significance is defined as $p \leq 0.05$.

Key Findings

Arachidonic Acid Metabolism is a Key Pathway



Arachidonic acid serves as a precursor for many signaling molecules which regulate several physiological processes.

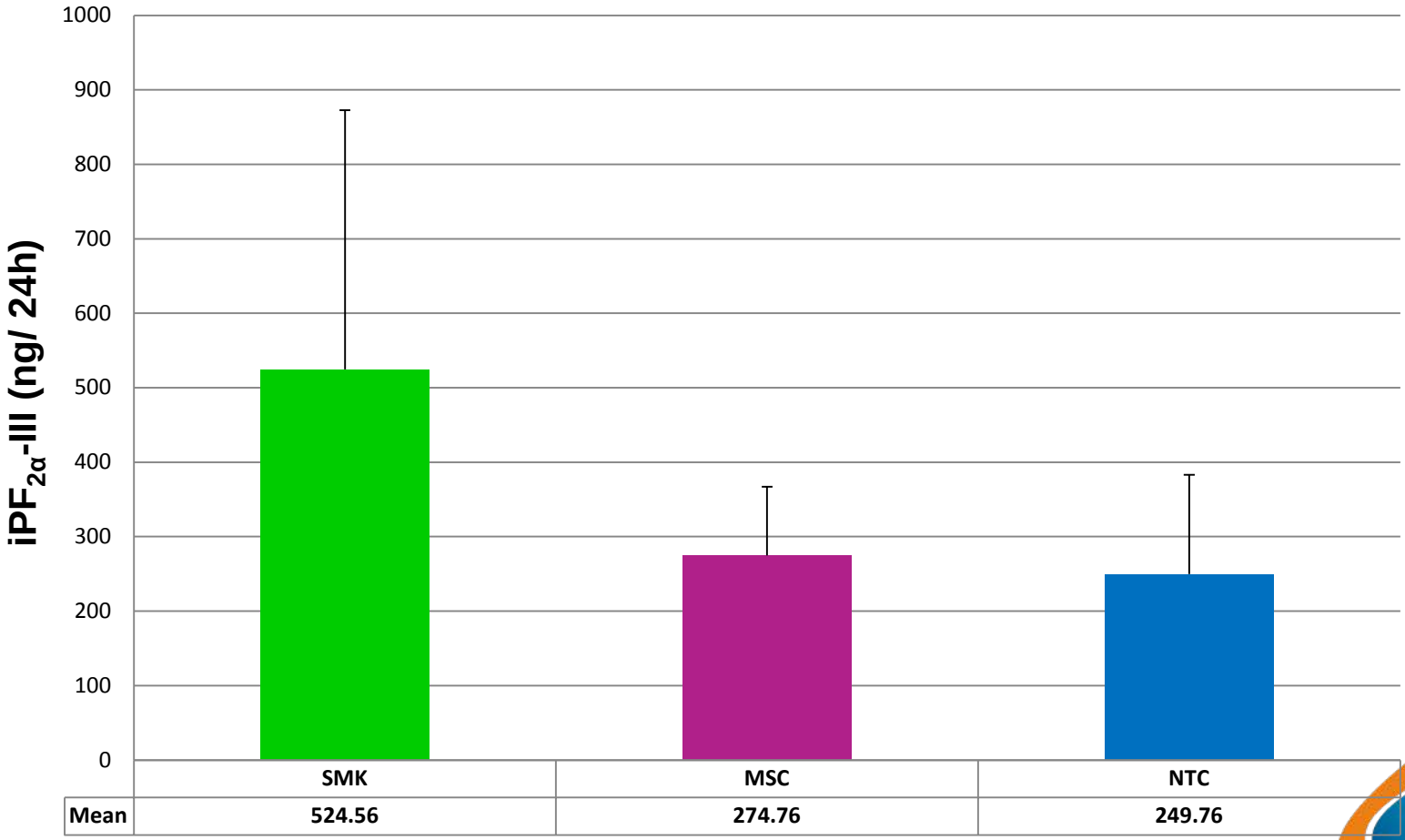
Oxidative Stress: Isoprostanes

- **Isoprostanes are:**
  - Non-enzymatic, free radical catalyzed peroxidation products of arachidonic acid in membrane phospholipids. Several isomers exist.
  - Markers of oxidative stress.
    Chronic smoking elevates several isoprostanes.
- **Isoprostane $iPF_{2\alpha}III$ (also known as 8-epi $PGF_{2\alpha}$ and 8-iso Prostaglandin $F_{2\alpha}$) is widely used as a marker to assess oxidative stress in Smokers.**

Oxidative Stress: $IPF_{2\alpha}$-III (8-epi $PGF_{2\alpha}$)

SMK > MSC & NTC

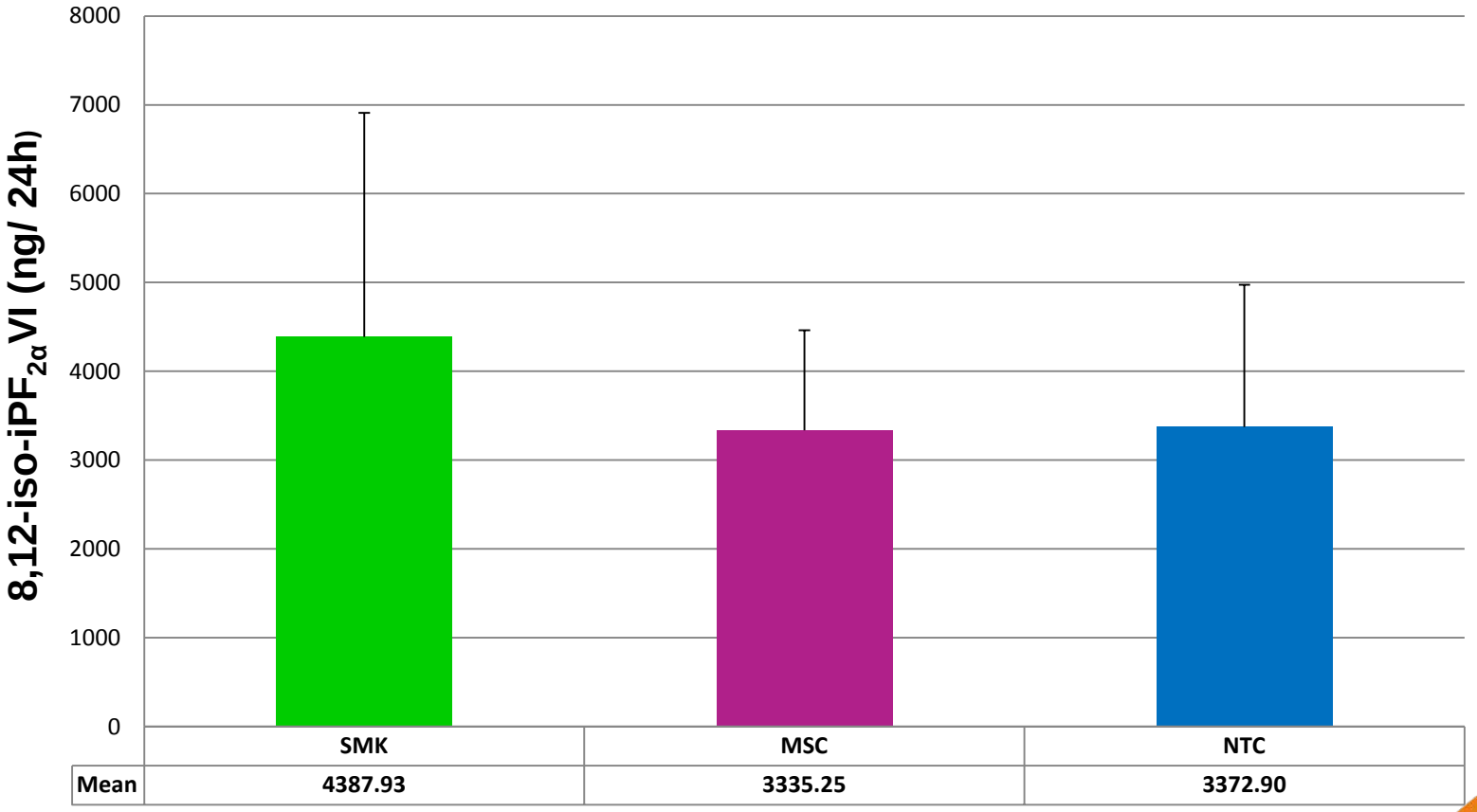
No difference between MSC & NTC



Oxidative Stress: 8,12-iso-IPF$_{2\alpha}$VI

SMK > NTC

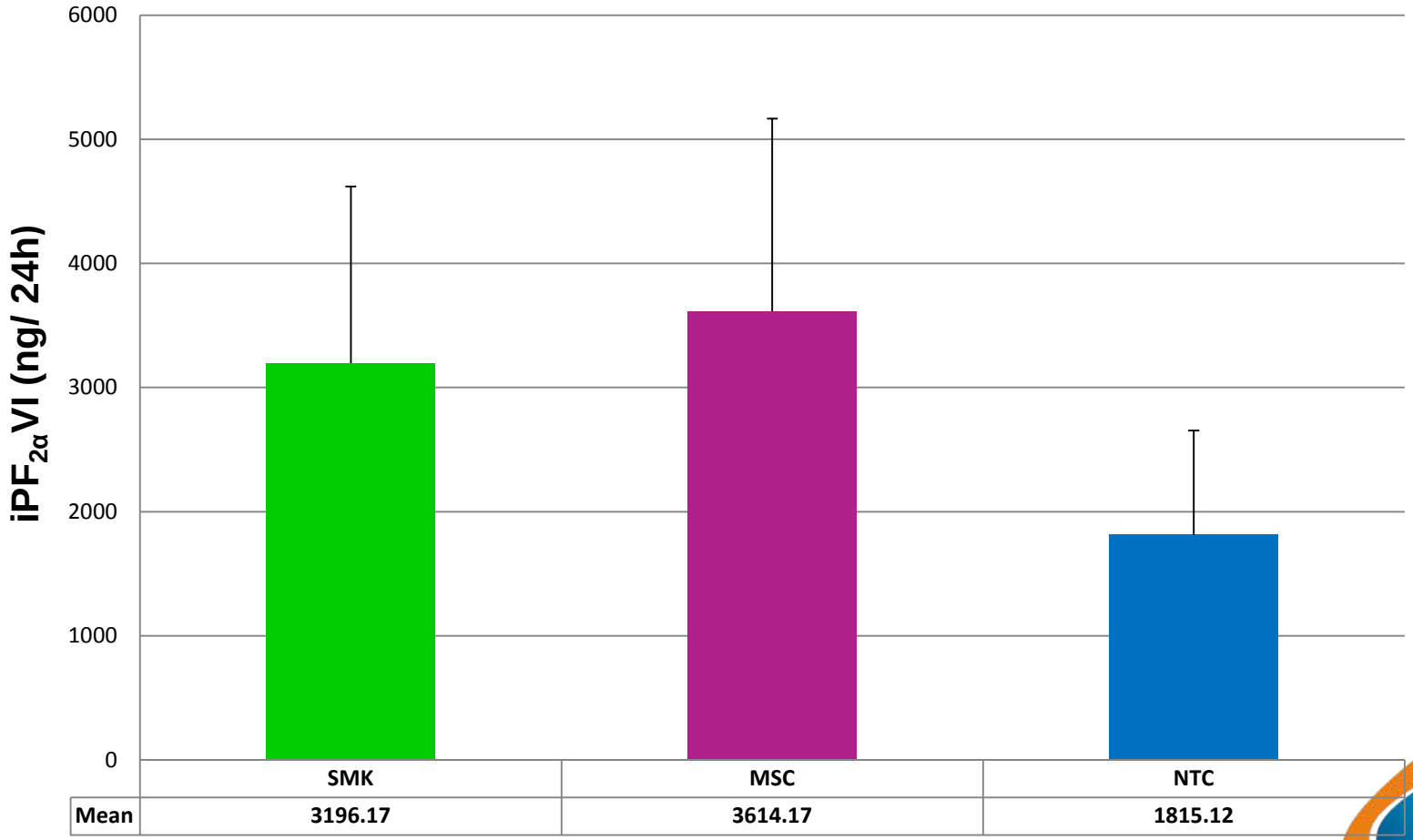
No difference between SMK & MSC*, or MSC & NTC



*p values were derived from ANCOVA model with adjustments for physical factors (systolic blood pressure and pulse): SMK vs MSC p=0.056; MSC vs NTC p=0.954; SMK vs NTC p=0.046.

Oxidative Stress: $IPF_{2\alpha}VI$

SMK & MSC > NTC
No difference between SMK & MSC



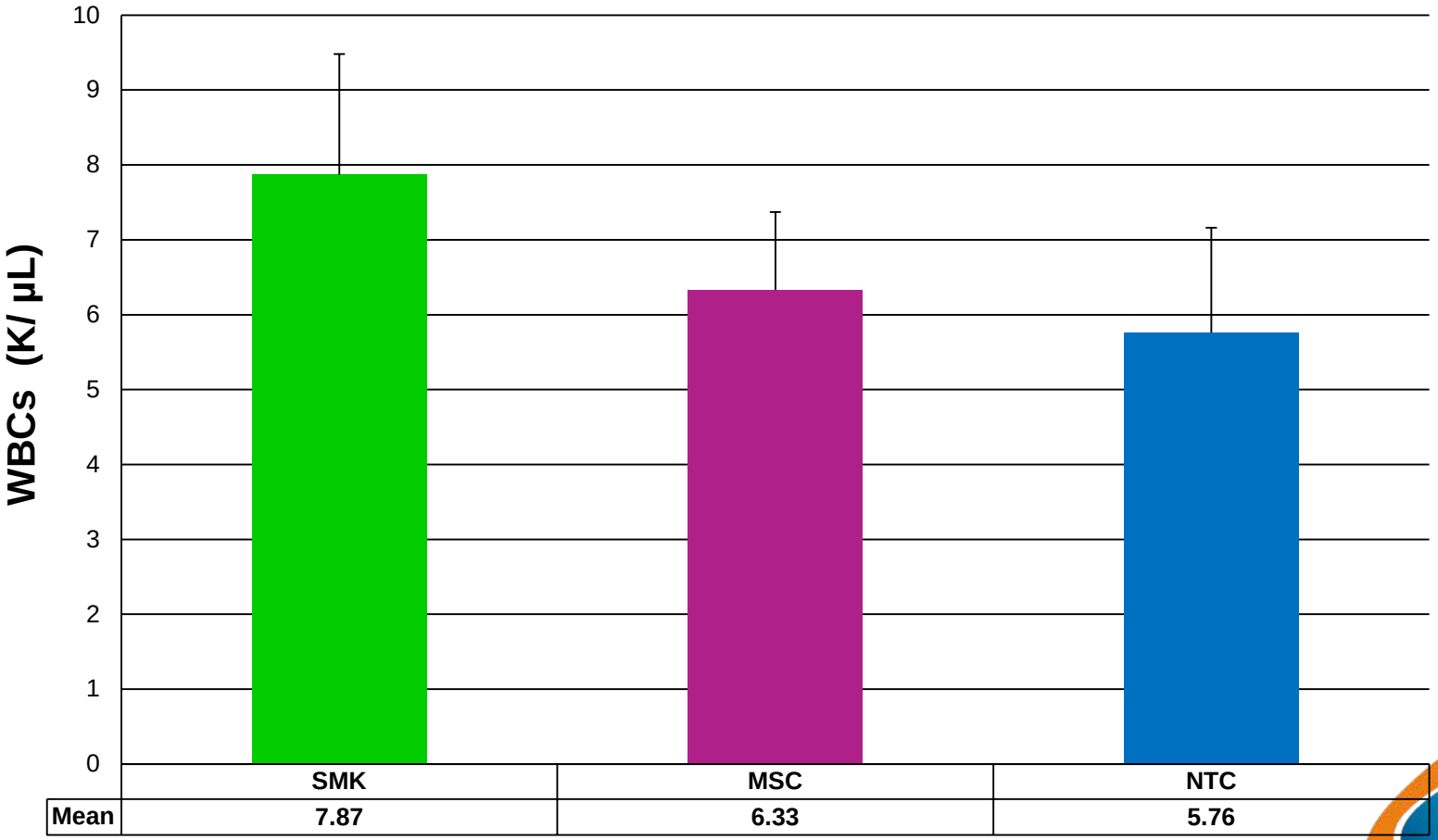
Inflammation: White Blood Cells (WBCs)

- **Inflammation is a general term used to describe the body's protective (immune) response to harmful stimuli.**
  - Chronic, deregulated inflammation is associated with many diseases, such as cancer and atherosclerosis.
- **Smoking causes inflammation, and yet suppresses immune responses.**
- **Increase in WBCs, which are key mediators of inflammation, are elevated in SMK.**
  - This suggests a chronic inflammatory state in SMK.

Inflammation: WBCs

SMK > MSC & NTC

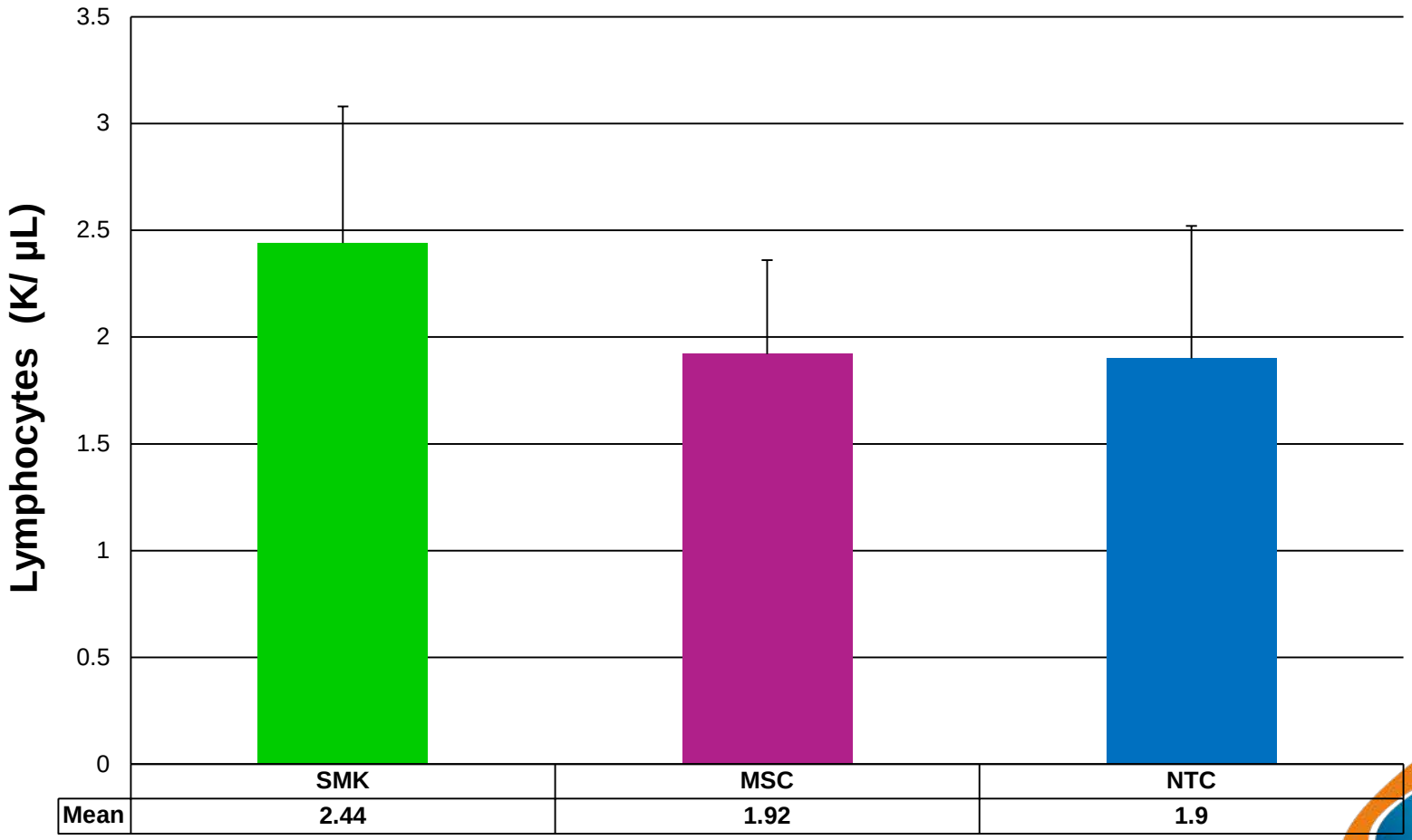
No difference between MSC & NTC



Inflammation: Lymphocytes

SMK > MSC & NTC

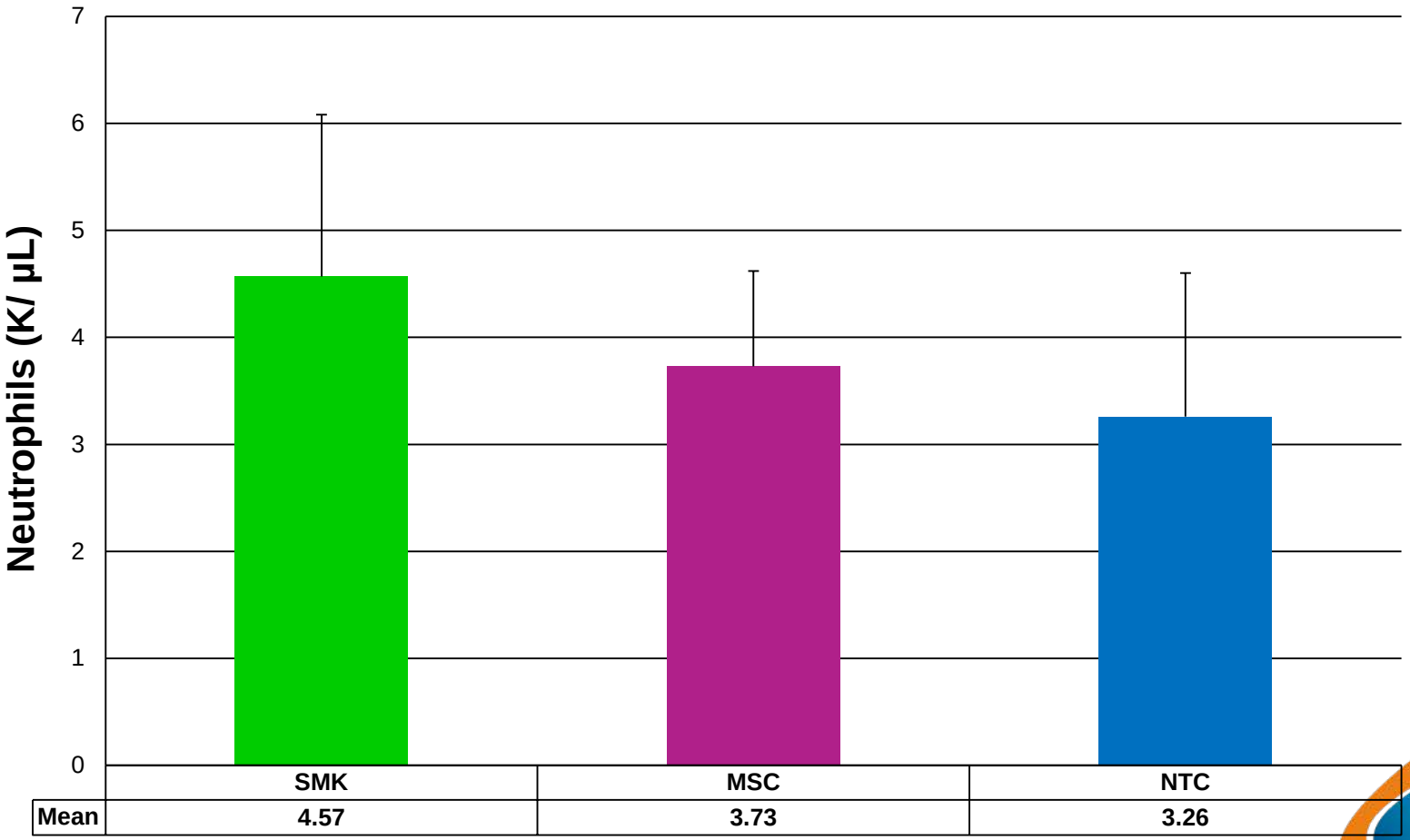
No difference between MSC & NTC



Inflammation: Neutrophils

SMK > MSC & NTC

No difference between MSC & NTC



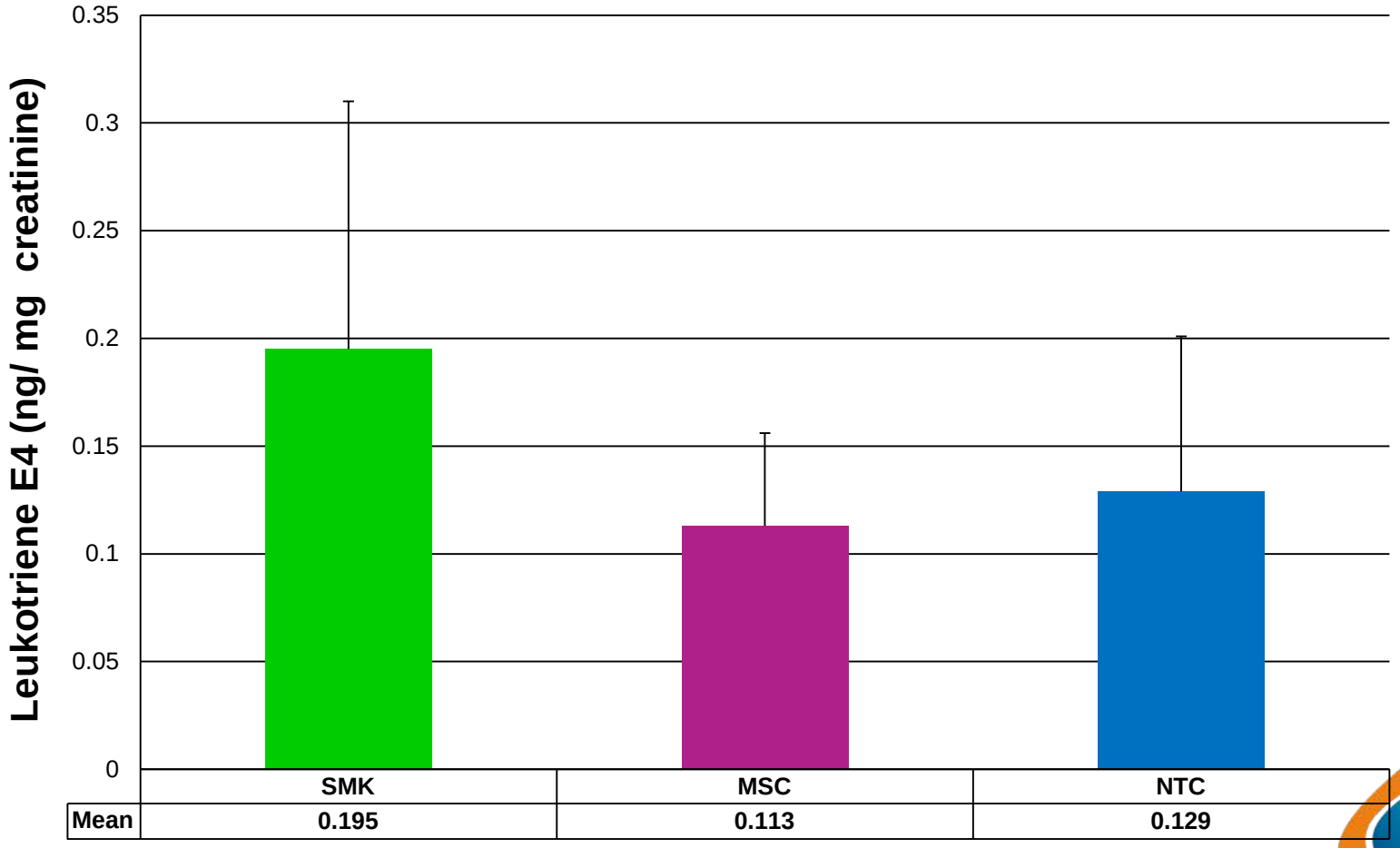
	SMK	MSC	NTC
Mean	4.57	3.73	3.26

Inflammation: Leukotrienes

- **Leukotrienes (LTEs) are:**
  - Produced from arachidonic acid by 5-lipoxygenase in WBCs.
    Several different leukotrienes exist.
  - Markers of inflammation.
    Smoking elevates urinary leukotrienes.
    Increased in COPD, CVD and inflammatory diseases.
- **Leukotriene E4 is measured as a marker of cytsteinyl LTE4.**

Inflammation: Leukotriene E4

SMK > MSC & NTC
No difference between MSC & NTC



Leukotriene E4 was measured in the first void urine

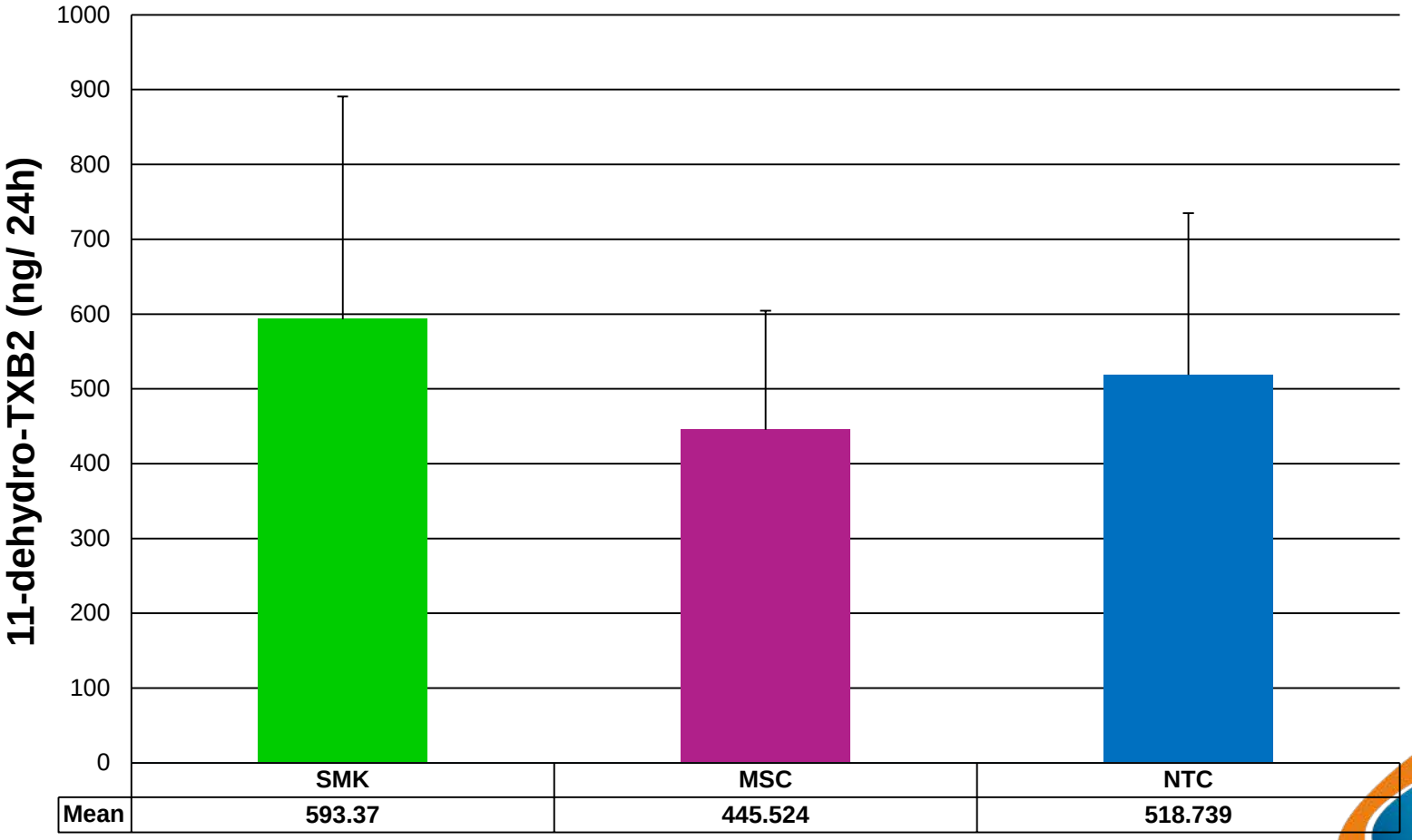
Coagulation: Platelet Function

Thromboxanes

- Thromboxane (Tx) A2 is a labile eicosanoid derived from arachidonic acid in platelets. Biologically active TxA2 is converted to stable 11-dehydro TxB2, which is the measured analyte.
- TxA2 is a powerful vasoconstrictor, platelet aggregator and a mitogen.
- Smoking increases pro-inflammatory arachidonic acid and 11-dehydro TxB2 levels, and contributes to thrombotic risk and CVD.

Coagulation Factors: 11-dehydro TxB2

SMK > MSC & NTC
No difference between MSC & NTC



Coagulation Factors

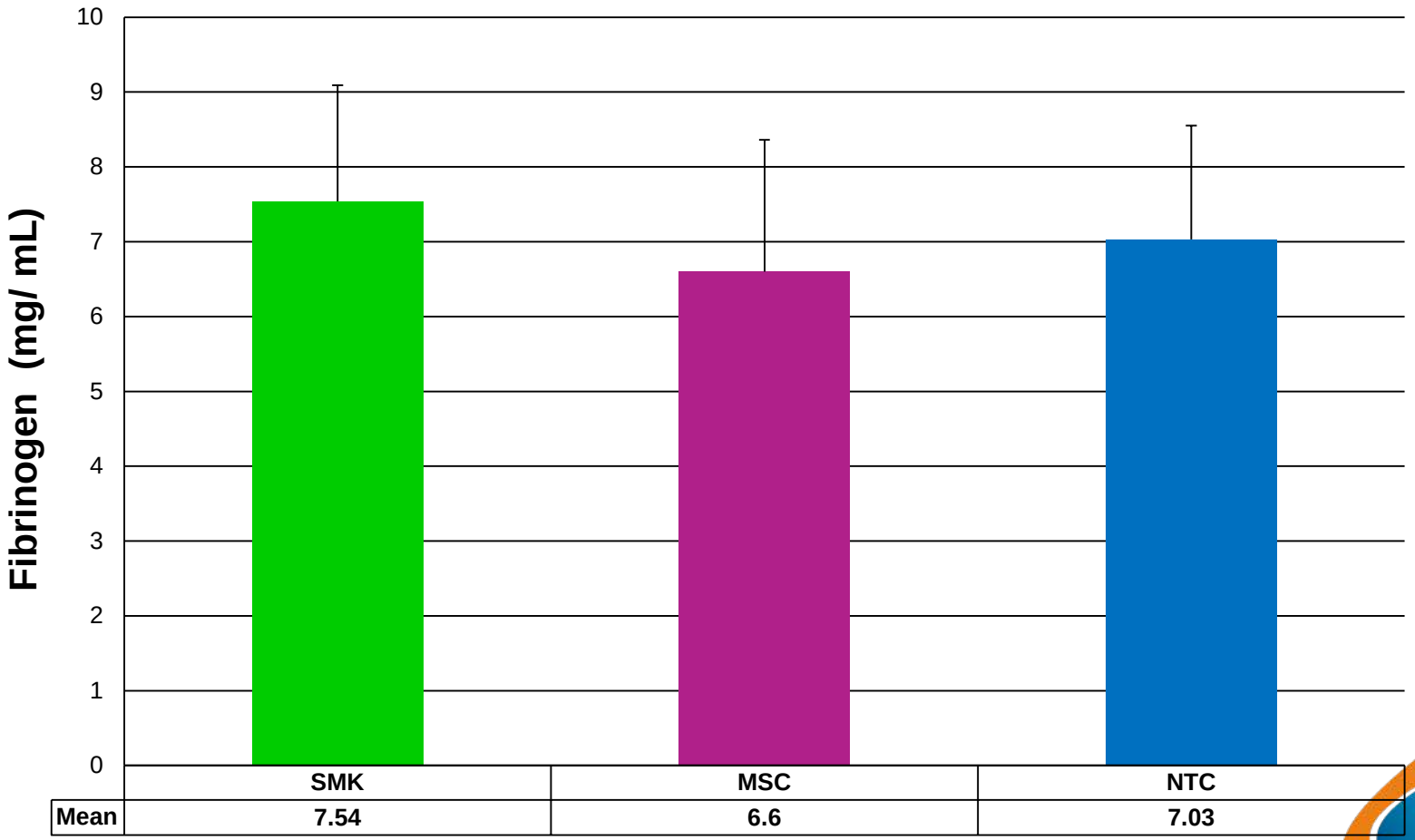
Fibrinogen

- A protein involved in blood clot formation.
- Chronic smoking elevates fibrinogen levels.
- High levels of fibrinogen is a thrombotic risk factor. Thrombosis occurs when fibrinogen is converted to fibrin (clot forming form) by thrombin.

Coagulation Factors: Fibrinogen

SMK > MSC

No difference between SMK & NTC, or MSC & NTC



Lipid Metabolism

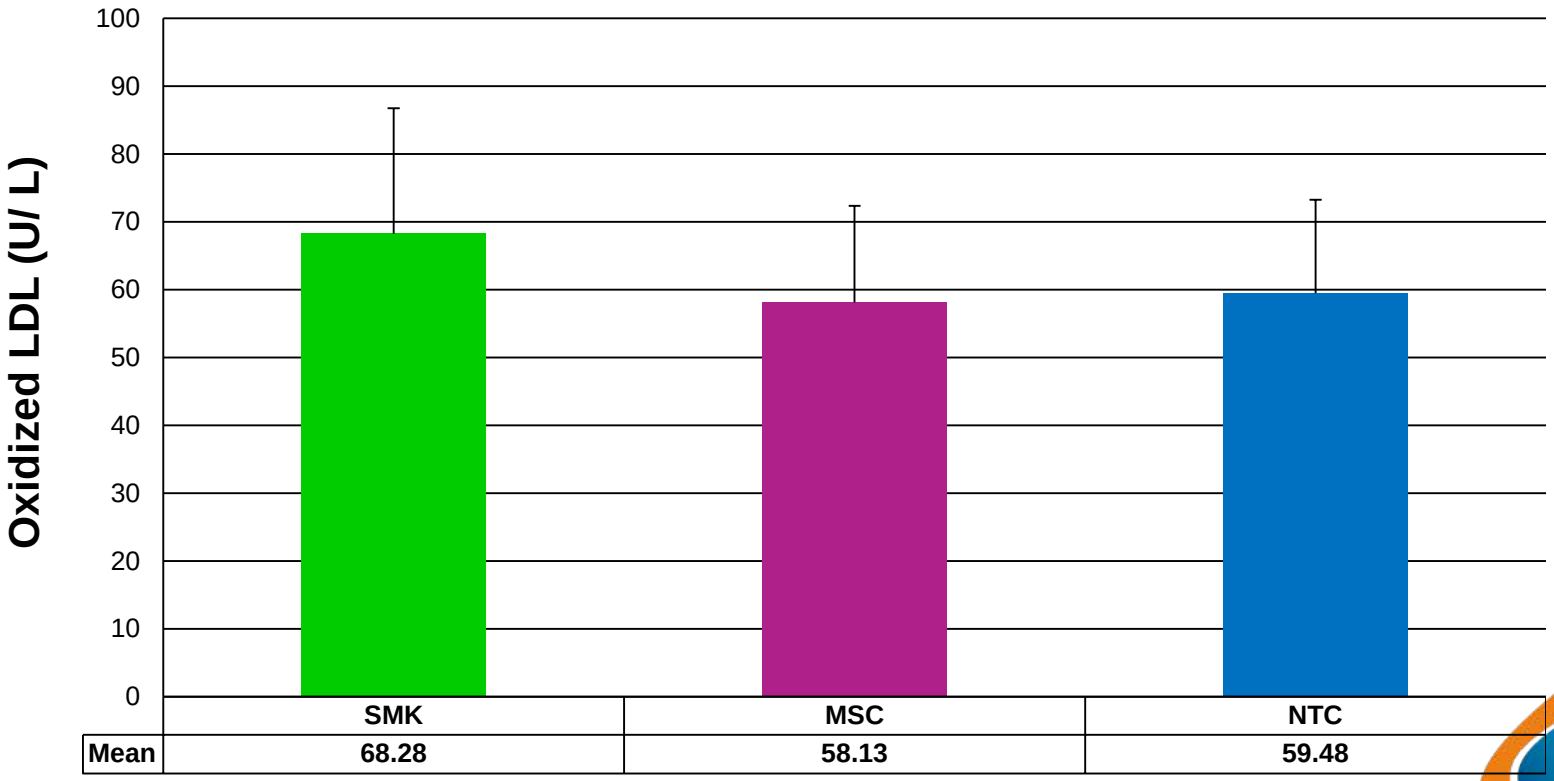
- **Deregulated lipid profiles and cigarette smoking are known risk factors for developing CVD.**
- **Altered low density lipoprotein (LDL), high density lipoprotein (HDL), lipoprotein ApoB 100 and other lipids were observed in SMK.**
  - Elevated oxidized LDL and Apo B100 are CVD risk factors.

Lipid Metabolism: Oxidized LDL

- LDL particles transport cholesterol from liver to tissues. LDL is oxidized by free radicals.

SMK > MSC & NTC

No difference between MSC & NTC

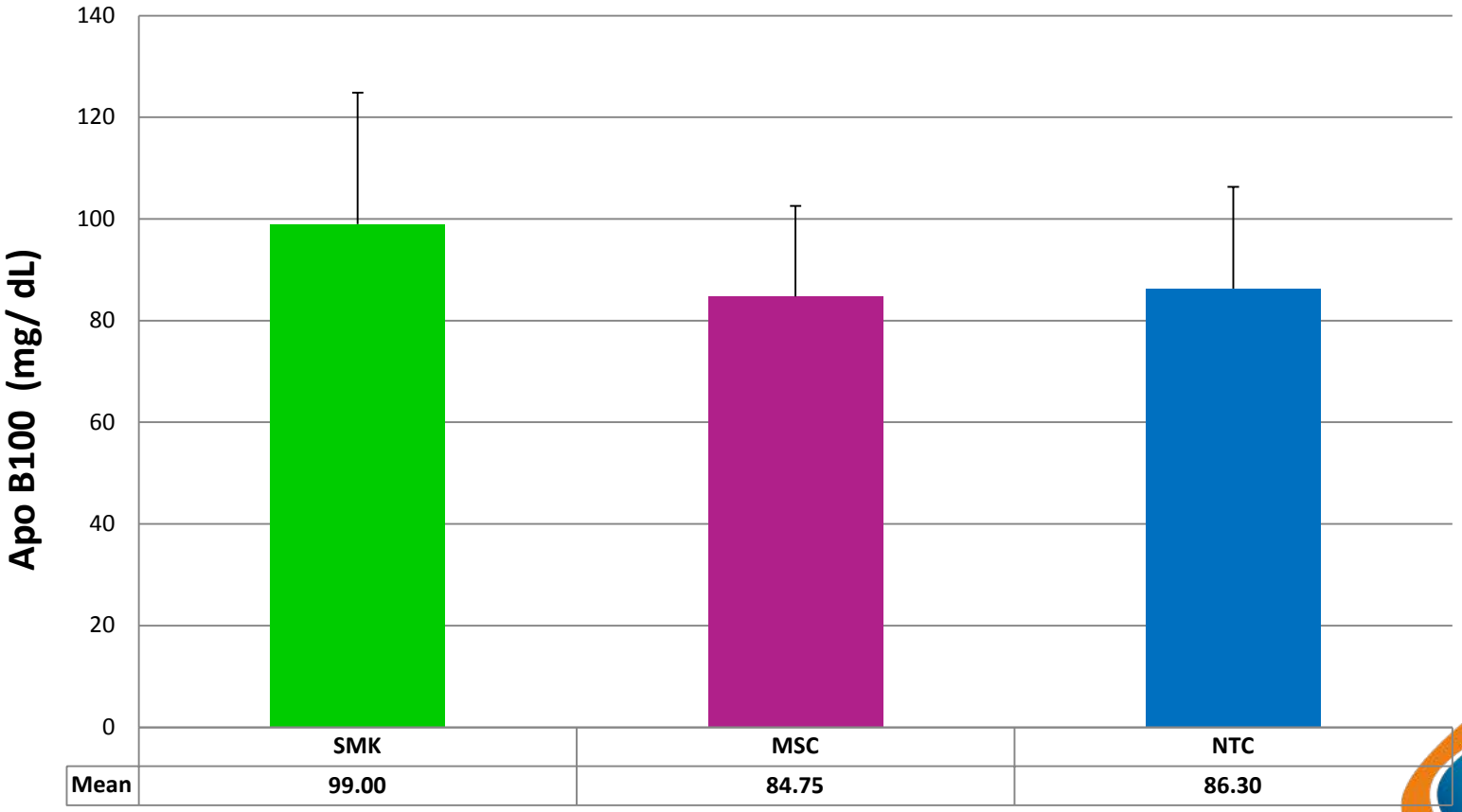


Lipid Metabolism: Apo B100

- Apo B100 is a large constituent protein of the shell of LDL particles.

SMK > MSC & NTC

No difference between MSC & NTC



Comparison with a Previous RJRT Study (Repetition Phase)

Among the biomarkers investigated in both studies, several biomarkers reproducibly distinguished SMK from the non-smoking cohorts (MSC and NTC).

Examples:

- WBC
- Fibrinogen

- $IPF_{2\alpha}$ III
- 11 dehydro TxB2

Increased inflammation in SMK, relative to MSC and NTC, is suggested.

Summary and Conclusions

- Several biomarkers of effect that distinguish long-term Smokers and MSC have been identified.
- Smokers exhibit elevated oxidative stress, inflammation, platelet activation, and altered lipid metabolism, relative to MSC and NTC.
- Collectively, Smokers exhibit perturbations in pathways potentially leading to smoking-related diseases, particularly CVD, compared to the non-smoking cohorts.
- The biomarkers of effect described herein will be useful in assessing health effects of NTPs.

Acknowledgements

Study Partners

- **Clinical Study Site**
  - High Point Clinical Trials Center, High Point NC (formerly Mendenhall Clinical Research Center)
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  - Pacific Biomarkers, Inc., Seattle WA
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