



НОВЫЕ ЭКСПЕРИМЕНТАЛЬНЫЕ ПОДХОДЫ В ИССЛЕДОВАНИИ АЛЬТЕРНАТИВНЫХ СИСТЕМ ДОСТАВКИ НИКОТИНА (АСДН)

NEW EXPERIMENTAL APPROACHES TO STUDYING THE ALTERNATIVE NICOTINE DELIVERY SYSTEMS (ANDS)

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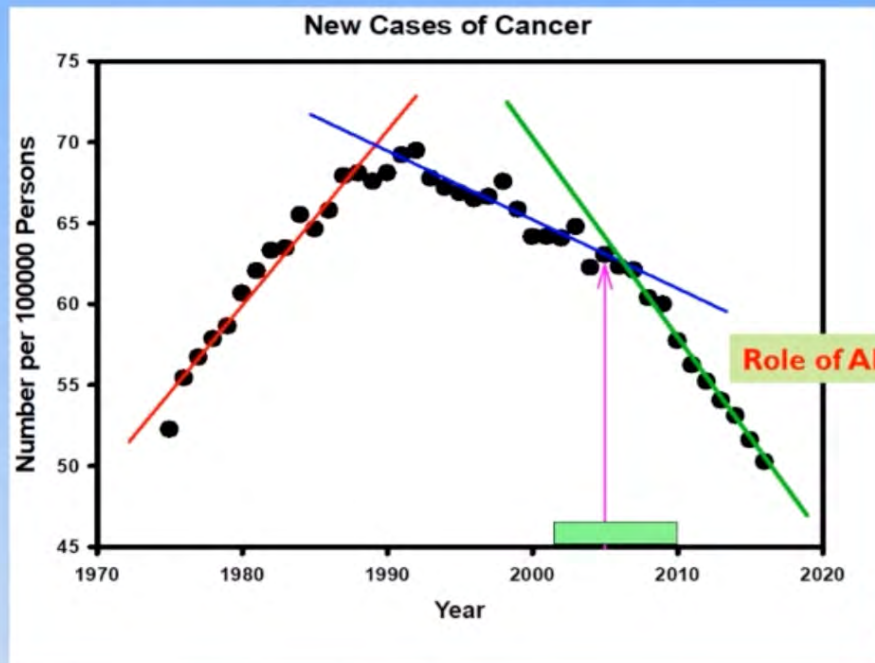
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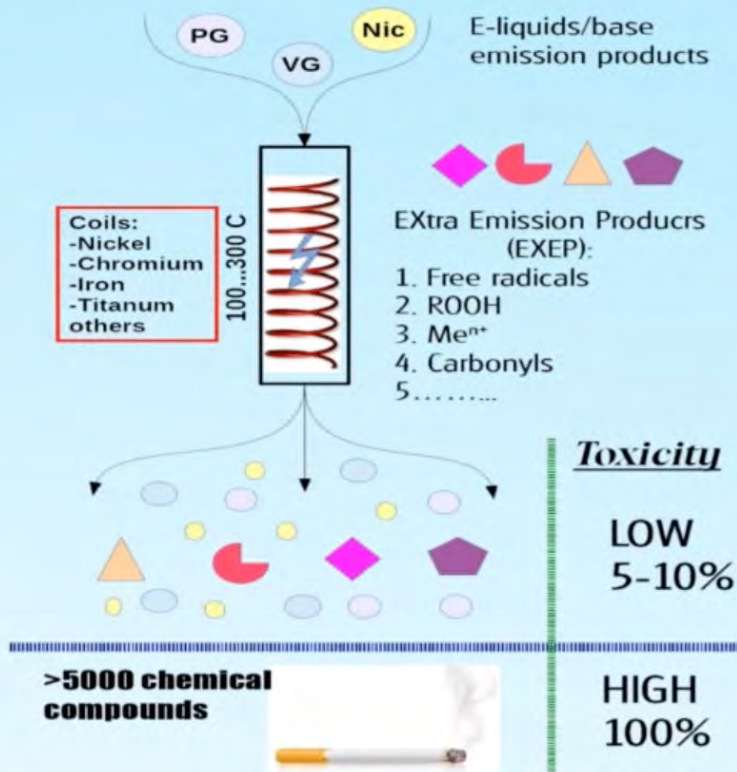
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Lung and Bronchial Cancer in the US (Reconstruction According to the National Cancer Institute, NCI)



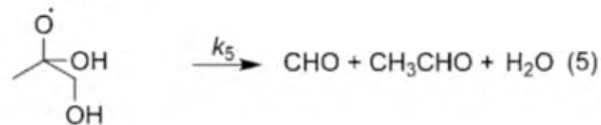
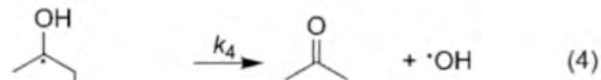
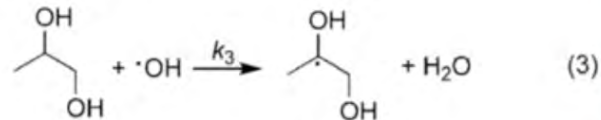
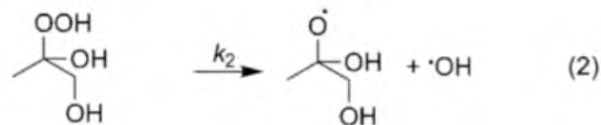
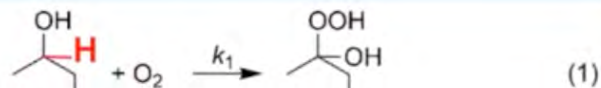
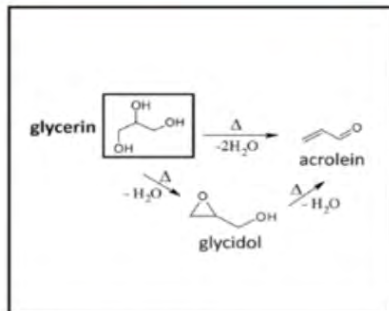
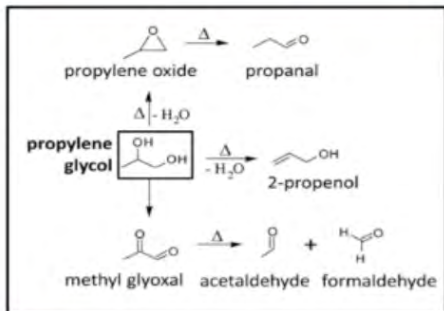
ELECTRONIC *versus* CONVENTIONAL CIGARETTES



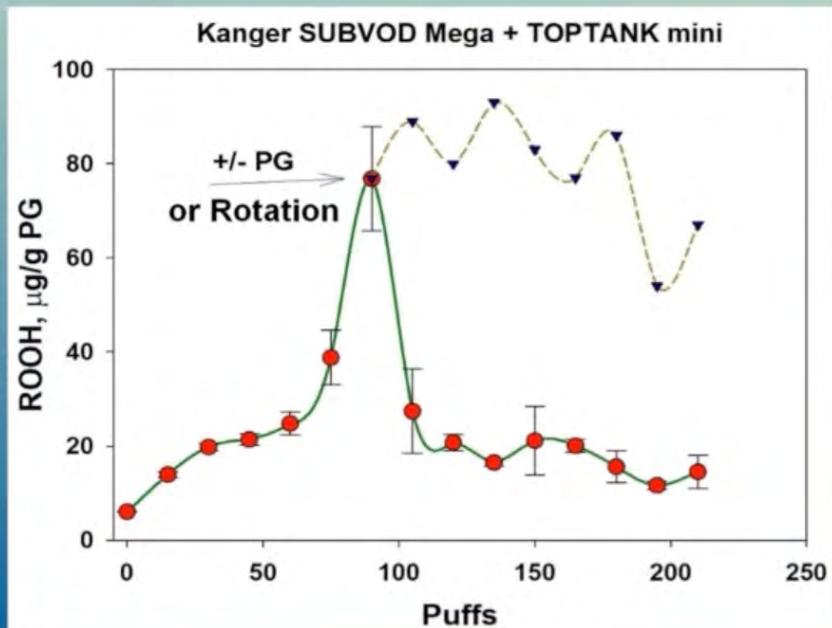
There exist also the problems. One of them refers to oxidation of e-liquids:

- High temperature evaporation?
- “Dry” puffing?
- Substandard thermocontrol?
- Specific e-liquids?
- Damaged coil or wick?

Exuberance of questions...



HOW ADEQUATE ARE THE MEASUREMENTS? I. CHEMISTRY: AEROSOL PEROXIDE (ROOH) GENERATION (NI200 HEATING COIL (0.15 Ω))



Level of propylene glycol (PG)



PG = 75 % V_{max}



PG = 20 % V_{max}

HOW ADEQUATE ARE THE MEASUREMENTS? II. BIOLOGY:

Phagocytosis and Inflammation: Exploring the effects of the components of E-cigarette vapor on macrophages

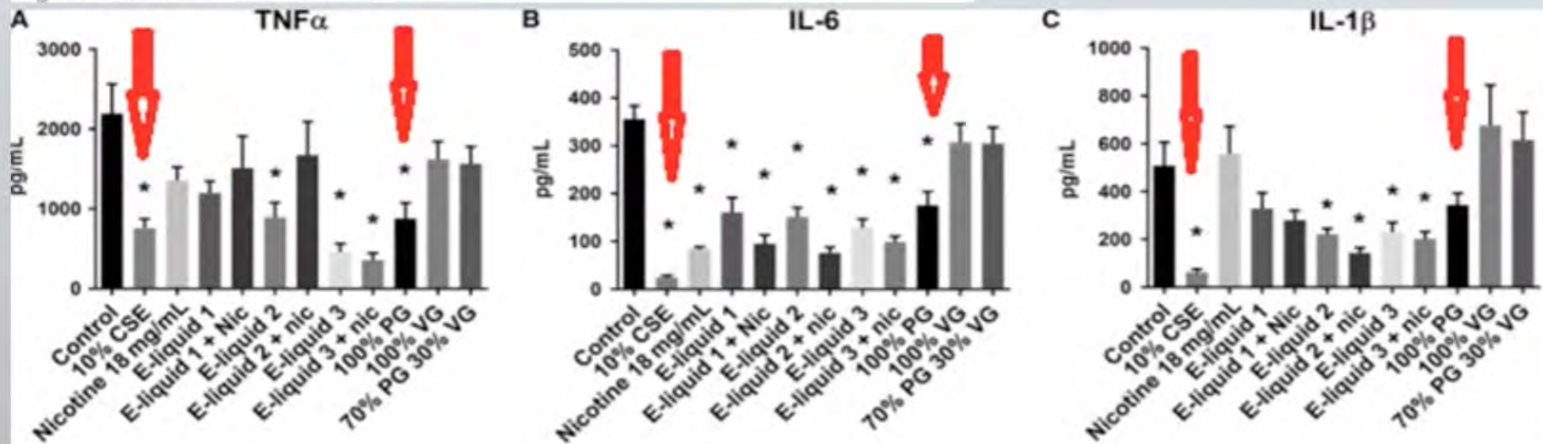
Miranda P. Ween,^{1, 2} Jonathan J. Whittall,^{1, 2} Rhys Hamon,^{1, 2} Paul N. Reynolds,^{1, 2} and Sandra J. Hodge²

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"...We conclude that E-cigarettes can cause phagocytic dysfunction of macrophages via alteration of bacterial recognition receptors and can alter cytokine secretion pathways. As such, E-cigarettes should be treated with caution by users, especially those who are nonsmokers, as this data adds to the growing literature showing E-cigarettes can potentially cause harm to a variety of cells, especially those in the airway where exposure is most direct..."

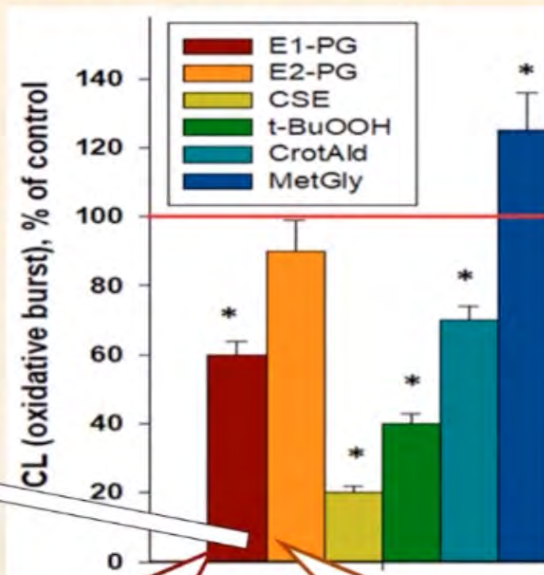


A PARTIAL RECONSTRUCTION OF WEEN'S EXPERIMENTS

- Same e-cigarette → Kanger EMOW (3.7 V) ✓
- Same coil → new dual coil heaters ✓
- Same e-liquids → PG ± nicotine ✓
- Same in vitro model → phagocytes ✓
- Same puffing topography → 60(?) / 3 / 5 ✓
- Healthy donors → COPD patients ✓

ROS GENERATION BY NEUTROPHILS

Conclusion: No immunosuppressive effect under physiologically relevant conditions for the aerosol generation!

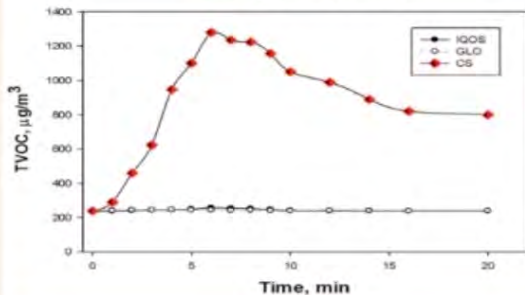


"Overheated" aerosol (Ween's conditions: 60мл-3с-5с)

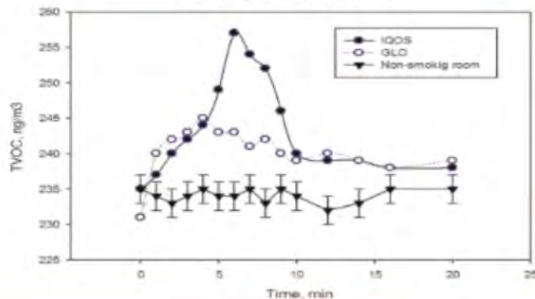
Physiologically relevant puffing conditions: 60ml-3s-30s

Air Quality (TVOC & PM_{2.5}) in the Smoking Room: ANDS versus Conventional Cigarettes

Smoking Room Air Quality



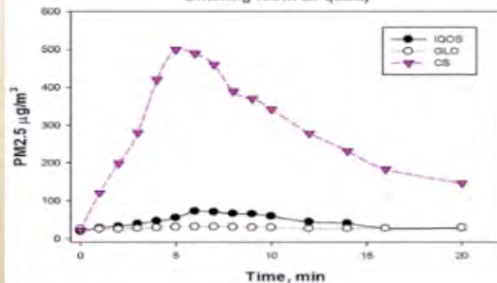
Smoking Room Air Quality



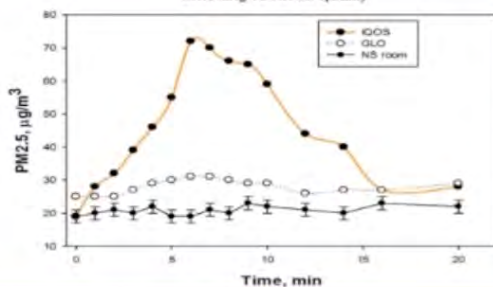
The TVOC gas analyzer measures the total concentration of substances with an ionization potential below 10.6 eV by the photoionization method.

Outcome: THP "vaping" results in 6-fold lower concentration of toxic organic compounds in the ambient air compared to cigarette smoking!

Smoking room air quality



Smoking room air quality



Persistent ambient concentrations of PM_{2.5} > 20 mg/m^3 are associated with increasing severity of respiratory symptoms related to airway obstruction, chronic bronchitis and asthma.

Outcome: Peak concentration of PM_{2.5} particles in the ambient air upon THP "vaping" is 10-fold lower compared to that upon smoking!

ELIAS METSCHNIKOFF



Elias Metschnikoff

*“Inflammation is a
phagocytic reaction of the
body”*


Elias Metschnikoff

In 2020, the Humanity celebrates the 175th anniversary of **Elias (Elie, Ilya) Metschnikoff**, the “father of the natural immunity” (the 1908 Nobel Prize in Physiology or Medicine). It was Metschnikoff who has discovered that in animals possessing the blood, WBC accumulate at the sites of inflammation and are directly related to the death of bacteria that cause such an inflammation.

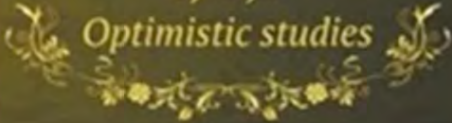
The
prolongation
of *smokers'* life
with *ANDS*:
Optimistic studies?

If Metschnikoff were our contemporary and would work on the immunity issues, then, perhaps, one of his books would be entitled somewhat differently...

Elie Metschnikoff

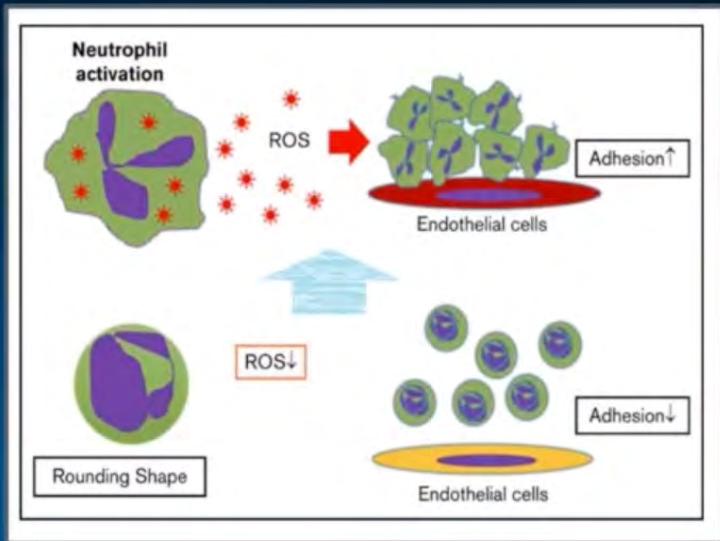


The
prolongation
of life



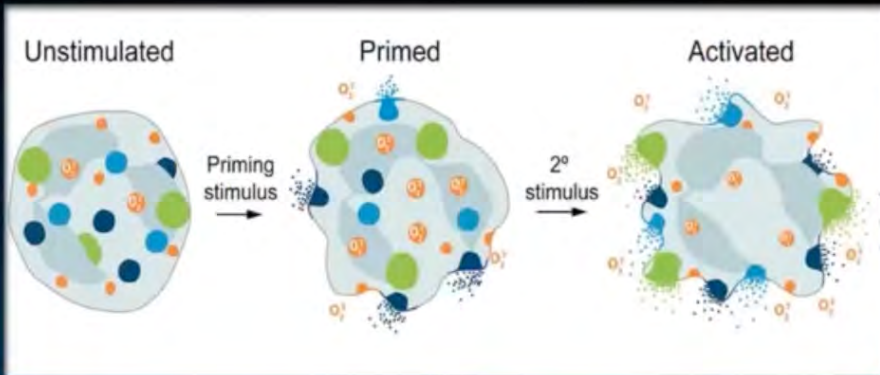
Optimistic studies

INDEED, NEUTROPHILS PLAY AN ESSENTIAL ROLE IN THE INNATE IMMUNE RESPONSE, BUT....

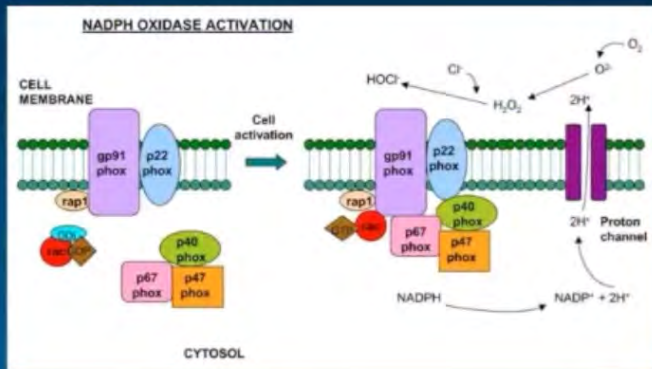


- *“...Uncontrolled or prolonged neutrophil activation uses antimicrobial responses to injure normal host cells, leading to pathologic changes to tissues and organs in autoimmune and inflammatory diseases...”*

(Nathan, 2006)



- ✓ There are clear associations between neutrophils and many chronic inflammatory diseases as diverse as COPD, myocardial infarction, arthritis and inflammatory bowel disease.
- ✓ Neutrophils exist in three states: quiescent, primed or active. Priming and activation appear distinct. In the primed state, there is no increase in oxidase activity; however, subsequent stimulation is able to provoke a response that is **10- to 20-fold larger** than in non-primed activated cells.



CIGARETTE SMOKE IS ABLE TO PRIME NEUTROPHILS FOR AN INCREASED BURST RESPONSE. AND HOW ABOUT E-CIGARETTES?

TOPICALITY OF THE ISSUES ADDRESSED IN OUR WORK

- Over the past 10 years, 3 or 4 generations of alternative nicotine delivery systems (ANDS) have been changed. The market offers the smoker thousands of different modifications of e-devices and e-liquids, promising to maintain his/her health for many years but showing very limited reliable evidence for risk reduction.
- Science and medicine simply do not have time to test properly this stream of new devices and their modifications, since there are no reliable and effective biomarkers for assessing long-term risks.

PURPOSE AND OBJECTIVES OF THE STUDY

- Search for effective biomarkers of the biological response towards traditional and alternative nicotine delivery systems for a long-term risk assessment.
- Studying the mechanism of priming of neutrophils in smokers and users of nicotine-free e-cigarettes in smoking cessation programs and immune cells reactivity modulation.

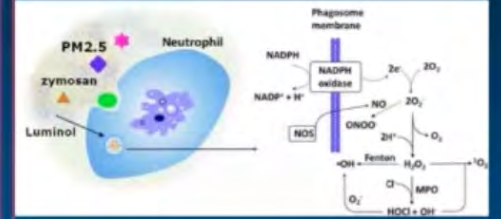
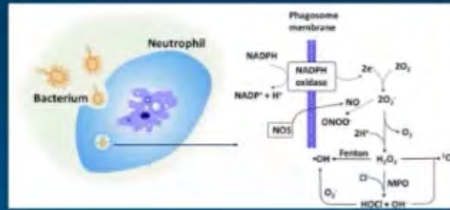
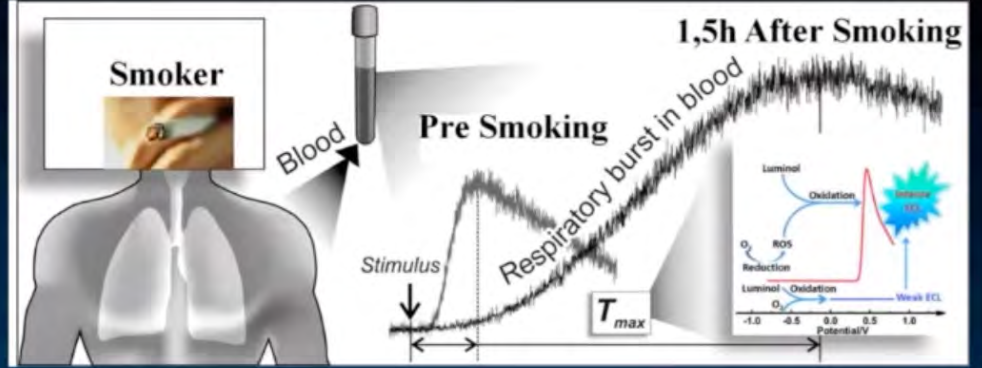
DESIGN OF THE STUDY:

METHODS AND
COMPOSITION OF
VOLUNTEERS



STUDIES ON THE **ROS** GENERATION BY LEUCOCYTE CELLS AFTER ACUTE SMOKING USING NON-OPSONIZED ZYMOSEAN-INDUCED AND LUMINOL-ENHANCED CHEMILUMINESCENCE IN A CONTINGENTLY WHOLE BLOOD

■ Particulate matters of cigarette smoke or bacterial cells stimulate phagocytosis and ROS production, whose the rate correlates with the number of primed white blood cells and the composition of the plasma.



AGE COMPOSITION & SMOKING STATUS OF VOLUNTEERS

| Age group | Age, years | Smoking stage, packs | Healthy Smokers | | | Healthy Non-Smokers/ Ex-Smokers | | |
|-----------|------------|----------------------|-----------------|-----------------|-----------------------|------------------------------------|----------------------|-----------------------|
| | | | N (m/f) | exCO, ppm | WBS, $\times 10^{-9}$ | N/Ex-Sm (m+f) | exCO, ppm (*p<0,001) | WBS, $\times 10^{-9}$ |
| I | < 30 | < 10 | 12 (6 + 6) | 18.9 \pm 7.6 | 7.4 \pm 1.8 | 16/2 (7 + 9) | 3.3 \pm 2.1* | 5.9 \pm 1.9* |
| II | 31-50 | 10-30 | 26 (17+9) | 20.2 \pm 5.9 | 6.2 \pm 1.7 | 31/4 (21+10) | 3.6 \pm 2.6* | 6.8 \pm 2.3 |
| III | > 50 | > 30 (30-60) | 8 (8+0) | 27.4 \pm 8.8* | 5.9 \pm 2.1 | 17/3 (11 + 6) | 2.8 \pm 1.7* | 6.4 \pm 1.6 |

Selection Criteria:

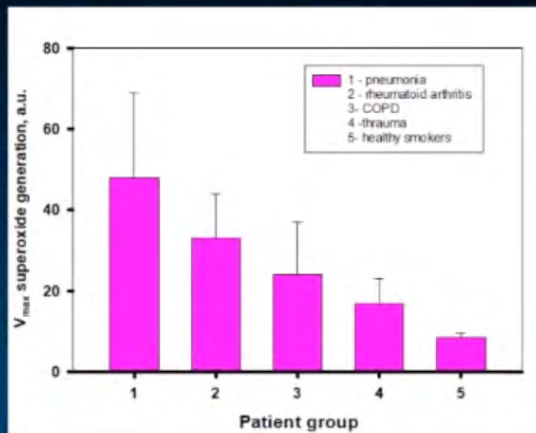
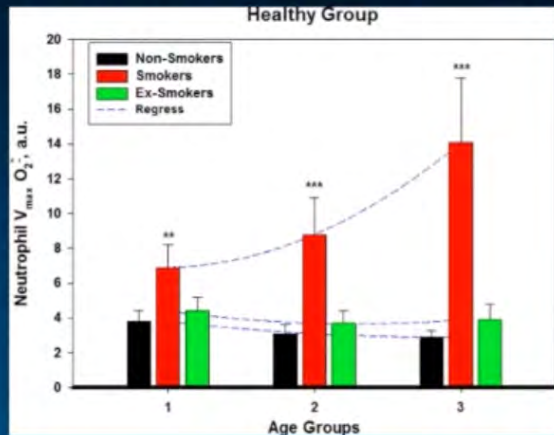
- **Body Mass Index – [18-30];**
- **Body Temperature (Ear) – [36.3-36.8];**
- **Blood Pressure, FEV₁/FVC, Complete blood count in accordance to the age norm. No drug administration during last month;**
- **At least 3 years of abstinence from smoking for ex-smokers.**



MAIN RESULTS

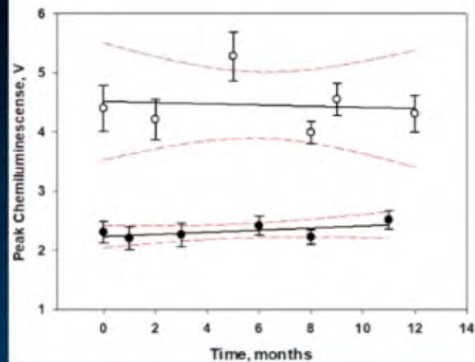
PART № 1. PHYSIOLOGY

MAXIMAL RATE OF THE ROS GENERATION BY ZYMOZAN-STIMULATED NEUTROPHILS IN THE CONTINGENTLY WHOLE BLOOD IN SMOKING AND NON-SMOKING HEALTHY VOLUNTEERS (LEFT) AND PATIENTS (RIGHT)

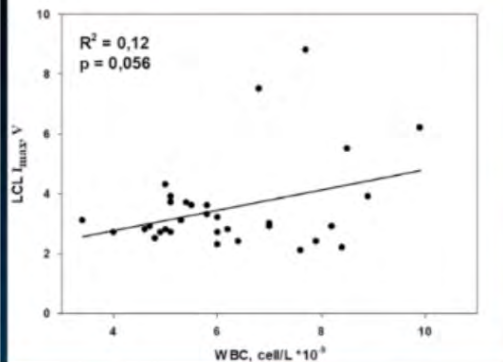


Blood sampling and analysis of neutrophils' activity in smokers was performed 8-10 hours after the last puff. At the left, the differences in neutrophils' activity in the groups of never smokers and former smokers are not statistically significant.

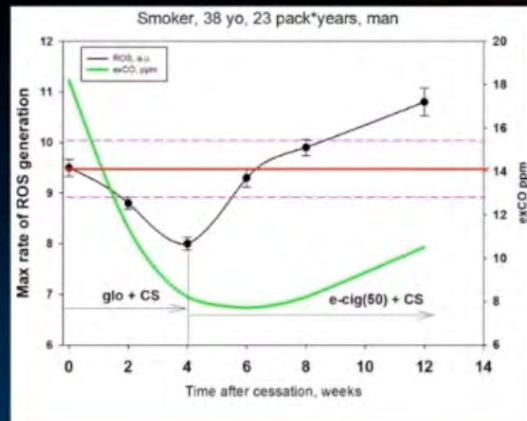
The membrane expression of CD11b, CD15 and CD63 was significantly higher on neutrophils from smokers, indicating upregulated neutrophil functions. Stimulated neutrophils from subjects with various inflammatory tissue-destructive conditions, such as trauma, periodontal, pulmonary, gastrointestinal and cardiovascular diseases, generate more oxygen radicals and proteases, involved in tissue destruction, than neutrophils from healthy persons. Cigarette smoking aggravates these diseases.



Seasonal fluctuations in the peak ROS generation rate in the contingently whole blood obtained from the two donors during the year (seasonal factor of variability). On the graphs: white symbols and the regression line with the 95 % confidence interval refer to the male smoker (37 y.o.), while black symbols and the regression line with the 95% confidence interval pertain to the non-smoking female volunteer (42 y.o.).



Correlation between the maximum rate of the ROS generation (manifested by the maximum luminal chemiluminescence intensity, LCL I_{max}) by the cells of the contingently whole blood and the concentration of leucocytes in the peripheral blood. The variability of the peak LCL I_{max} value is determined only up to 12 % by the variability of the concentration of leucocytes.



Dynamics of the reactivity of the blood phagocytes and the CO level in the exhalation of the volunteer upon the prolonged use of ANDS according to the smoking cessation program.

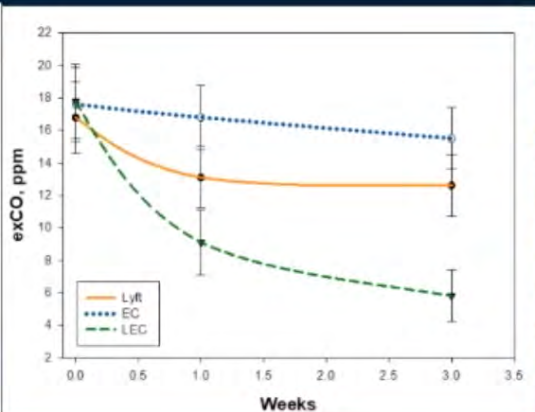
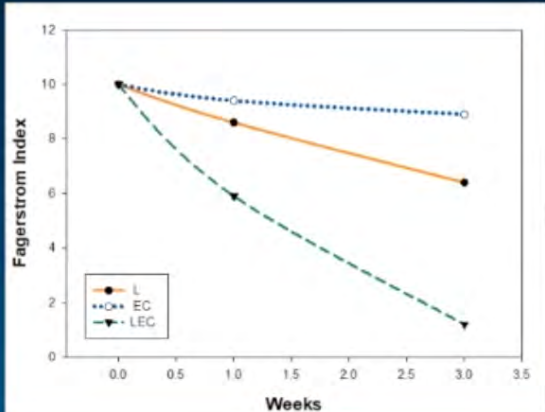
MAIN RESULTS.

PART № 2. PSYCHOLOGY

EXAMINING THE UTILITY OF NICOTINE-FREE E-CIGARETTES IN THE PROGRAM ON SMOKING CESSATION: PILOT STUDIES

- **Experimental Design:** Three groups of middle-aged smokers of 4 people each (> 20 cigs / day) with the Fagerström index IF = 10 switched to different types of ANDS, including white snus LYFT (group 1), nicotine-free e-cigs (group 2) or their combination (group 3). Volunteers passed the Fagerström test for the tobacco dependence with the determination of the CO content in the exhale after 1 week and 3 weeks from the start of the experiment.
- **Aim of the Experiment:** To achieve the complete cessation of cigarette smoking by volunteers within 3 weeks.

COMMENDATION TO A NICOTINE-FREE E-CIGARETTE



- It was the nicotine-free e-cigarette that played a decisive role in quitting smoking using the tobacco-free oral nicotine product as ANDS!
- Only in group No. 3, 3 out of 4 volunteers (75 %) completely gave up using cigarettes during the of the experiment.

SUMMARY & CONCLUSIONS

- Adequate experimental design is the key prerequisite in the ANDS studies!
- The search for adequate biomarkers is a major problem in such kind of research.
- Modulation of neutrophil oxidative activity is a valuable biomarker of the biological response towards using traditional cigarettes and ANDS.
- Switching to nicotinic packs reduces harm due to the decrease in cigarettes consumption.
- Nicotine-free cigarettes are the effective tools to assist quitting smoking along with the appropriate ANDS choice!