Comparison of the Effects of e-cigarette Vapor with Cigarette Smoke on Lung Function and Inflammation in Mice

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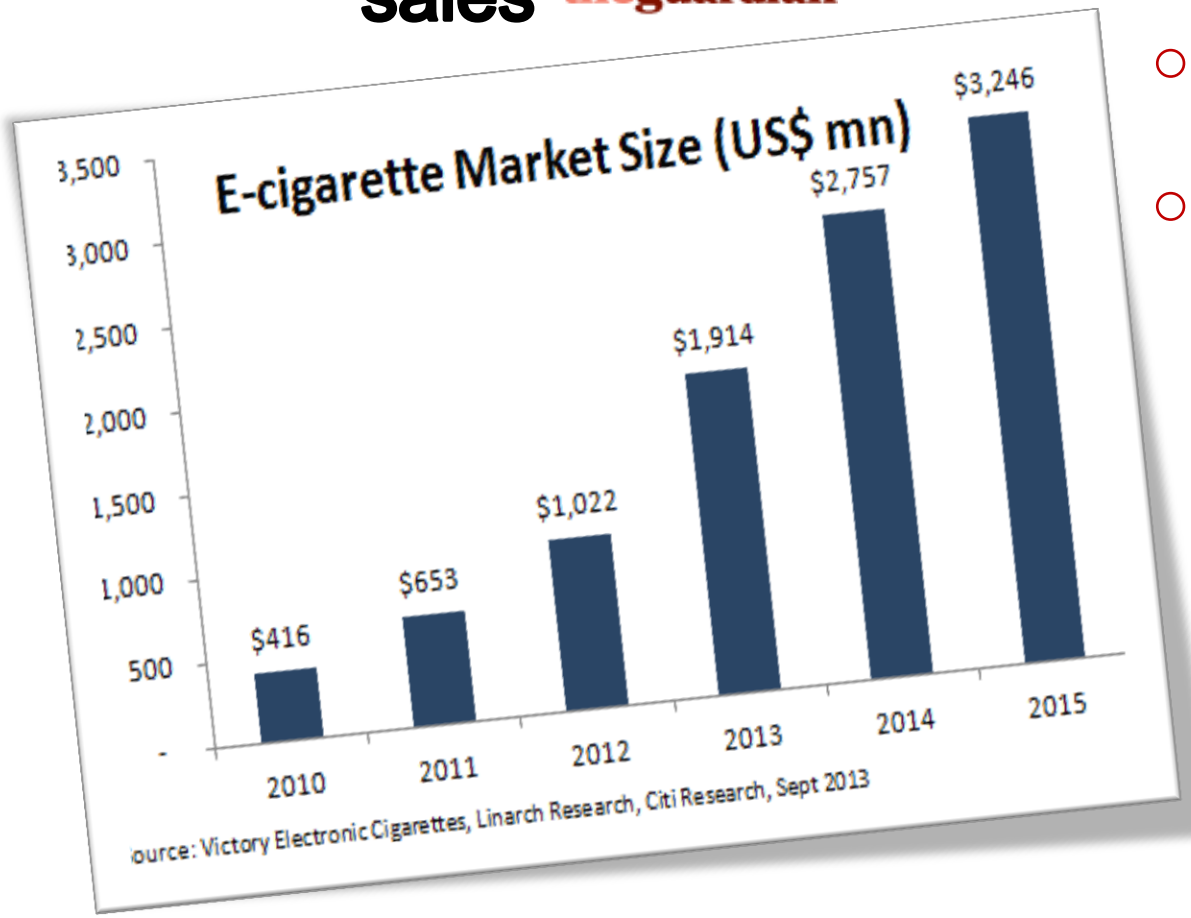
The Background

the ENDS: *Electronic Nicotine Delivery Systems*

Boom in e-cigarette sales theguardian



- 7,764 unique flavors
- 466 brands

What do we know about ENDS thus far?

Type of studies	Research subject
Chemical studies	**Evaluation of ENDS liquids /aerosols**
Toxicological studies	**Evaluation the ENDS effect on cell or animals**
Clinical studies	**Studies on humans**

Bad news always makes better titles
Regulatory agencies have a right/duty to err on the side of caution

- ✓ **Small amounts of heavy metals and at least 20 known carcinogens** [1]
- ✓ **Glycerol forms acrolein, which causes respiratory tract irritation** [2]
- ✓ **Cyto-toxicity in embryonic cells** [2,] **oxidative stress** [2,3]
- ✓ **Lung tissue destruction and airway hyperreactivity in mice**[3]
- ✓ **Memory impairment** [7]
- ✓ **Cardiovascular toxicity** [7]
- ✓ **Increased dynamic airway resistance** [4]
- ✓ **Inhibition of pulmonary anti-viral/microbial defense mechanisms** [5]
- ✓ **Bronchitis, cough, and emphysema** [7]
- ✓ **COPD-emphysema pathogenesis** [6]
- ✓ **Contact dermatitis and burns** [7]

1. Grana R et al Circulation 2014
2. Bahl et al Reprod Toxicol. 2012
3. US EPA Acrolein: http://www.epa.gov 2013
4. Vardavas CI et al Chest. 2012
5. Sussan TE et al PLoS 2015
6. Kaisar et al Toxicology. 2016
7. Qasim et al J Am Heart Assoc. 2017

The status in a nutshell

Still no good consensus because of still poor experimental evidence

- Many cell-based tests and assays, in contrast to very few *in vivo* experimental studies

- There was a flurry of case reports but few substantial and conclusive studies

- Paucity of side-by-side comparison of e-cig inhaling with cigarette smoking

- Lack of data comparing directly individual components of e-cigs *in vivo*

Aims of our study

- Determine the effects on mouse lung inflammation and function of subacute (3d) and subchronic (4wk) exposure to e-cig vapor

- Tease apart the effects of the vehicle, the nicotine and the added flavor

- Compare these effects to those of conventional cigarette smoke

Comparison of the effects of e-cigarette vapor with cigarette smoke on lung function and inflammation in mice

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APPROACH / METHODOLOGY

Whole body exposure

Mice:

male C57/BL6
age: 8-12wks
n= 7-10 / group

Treatment :

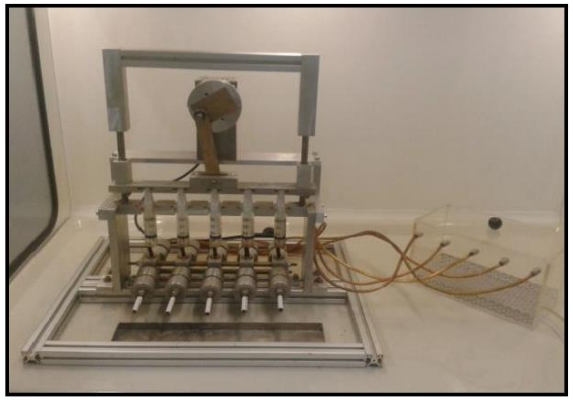
cigarette smoke or e-cigarette vapor

5 cigarettes /day

3 days (subacute)
or
4 weeks (subchronic)

A

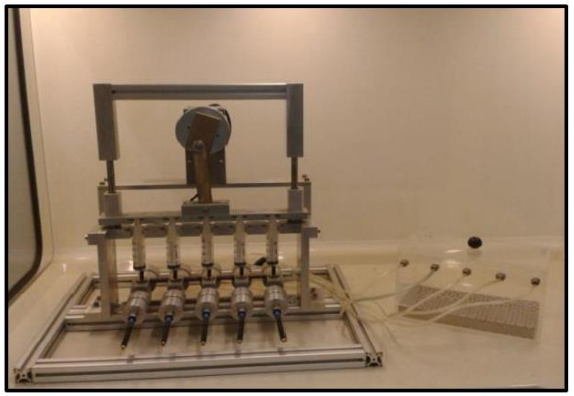
Modified cigarette smoking chamber



cigarette smoking

B

Modified e-cigarette smoking chamber

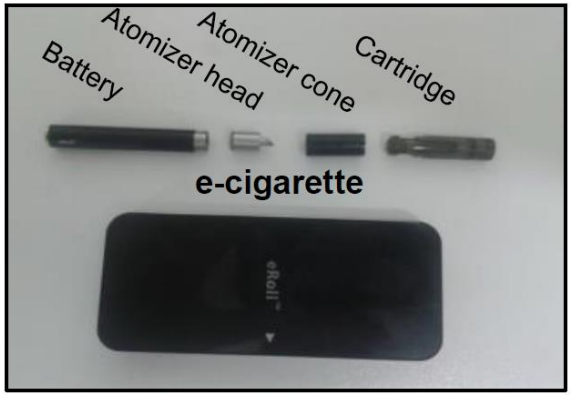


e-cigarette vaping

C

3R4F U of KY, USA

D



eRoll, Joye Technology

the e-cigarette machine

METHODOLOGY

Groups:

CTL
Ambient Air

CS
Cigarette smoking (3R4F U of KY, USA)

PV:VG
Propylene glycol/ vegetable glycerol 1:1

PG:VG-N
PG:VG plus nicotine 18mg/mL

PV:VG-N+F
PG:VG plus nicotine 18mg/mL and 4% “tobacco blend” flavor

Evaluations:

Lung Tissue / BAL fluid

Immune/biochemistry
- Cellularity
- Protein content
- Oxidative stress
- Cytokine determination

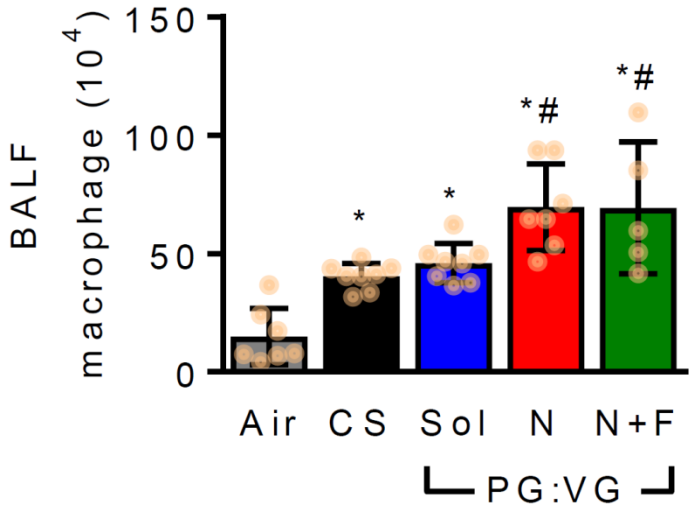
(Immuno)Histochemistry
- Muc5ac expression
- Lung injury score

***In vivo* Lung Mechanics**

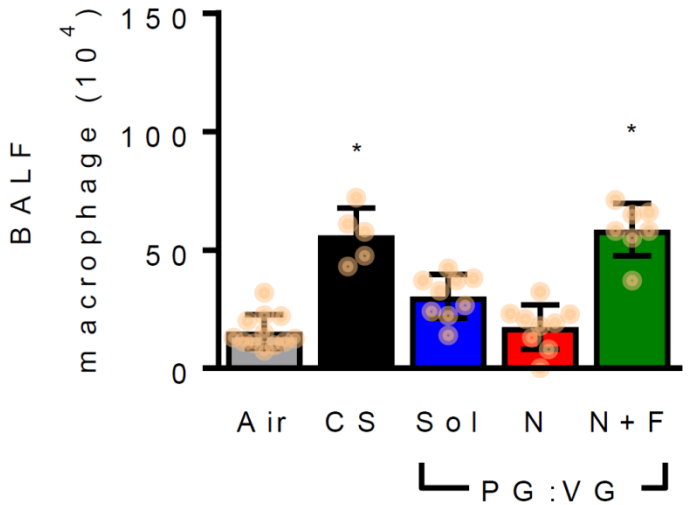
- Resistance
- Static compliance
- Elasticity
- Bronchial reactivity (3d only)

BALF CELLULARITY

3 days



4 wks



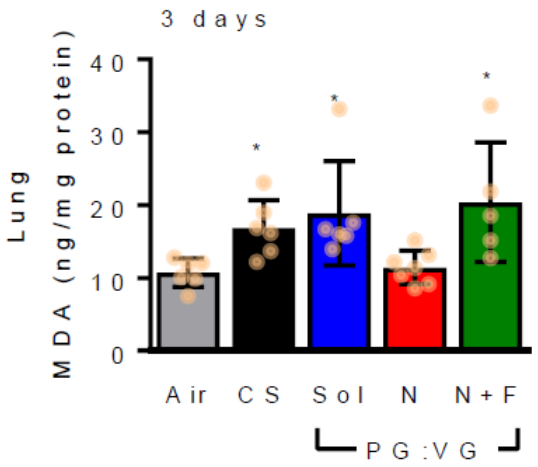
At 3 days, all treatments increase BALF macrophage content

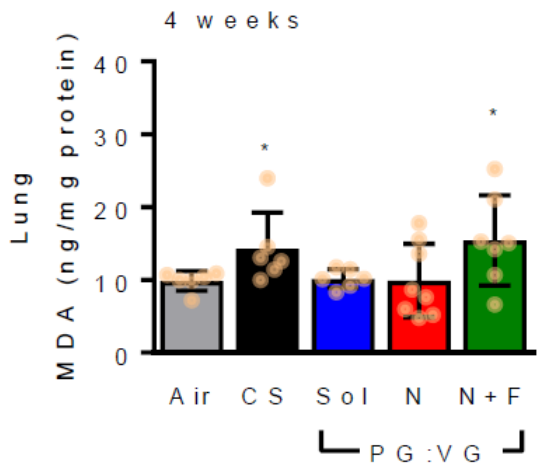
In contrast, after 4 wks of exposure, only BALF from mice exposed to CS and flavored vapor still show increased macrophage content

MARKERS OF OXIDATIVE STRESS IN LUNG TISSUE

MDA

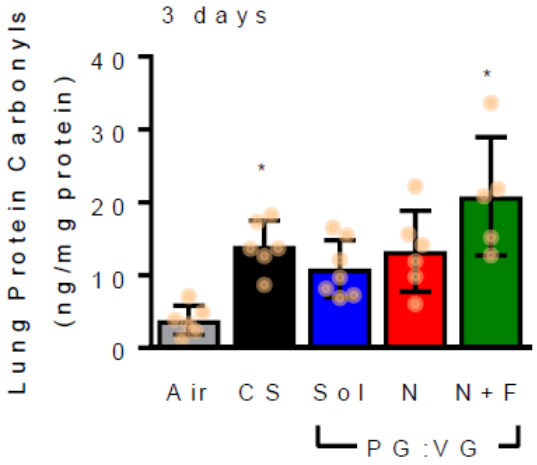
3 days

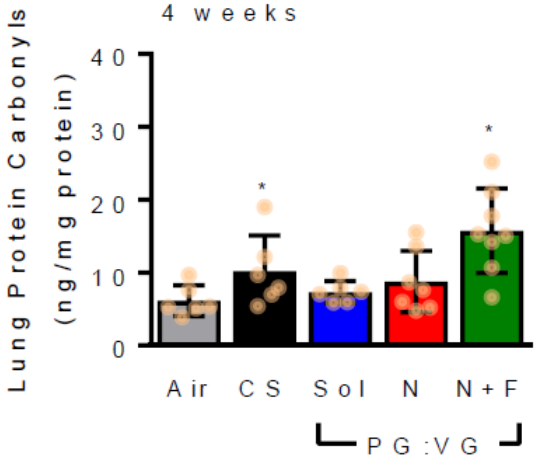




4 wks

carbonyls

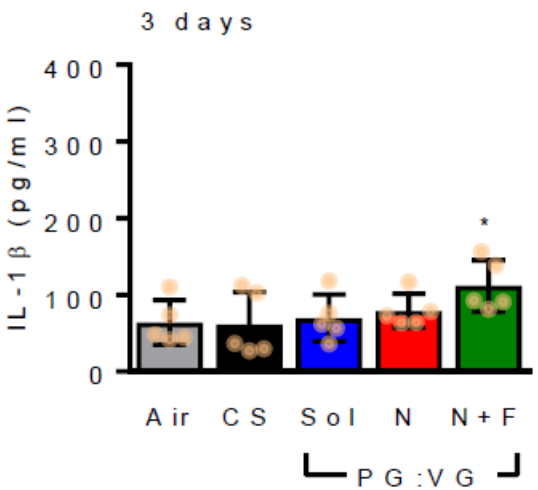


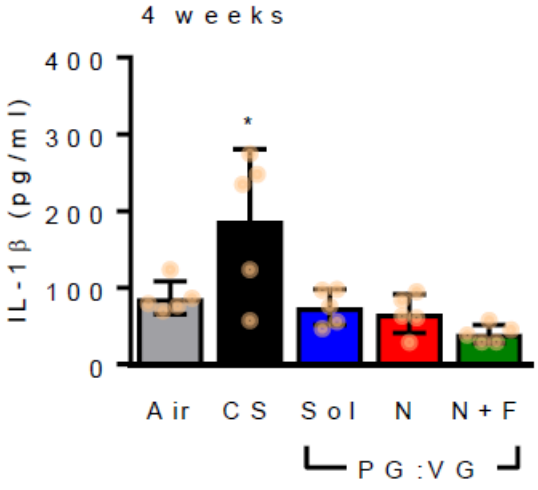


Exposure to cigarette smoke and to flavored vapor consistently induced oxidation markers in the lung at both 3d and 4wks

INFLAMMATORY CYTOKINE LEVELS IN LUNG TISSUE

IL-1β

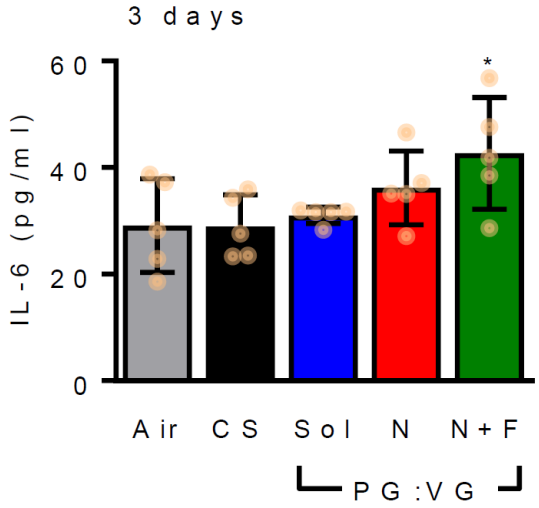


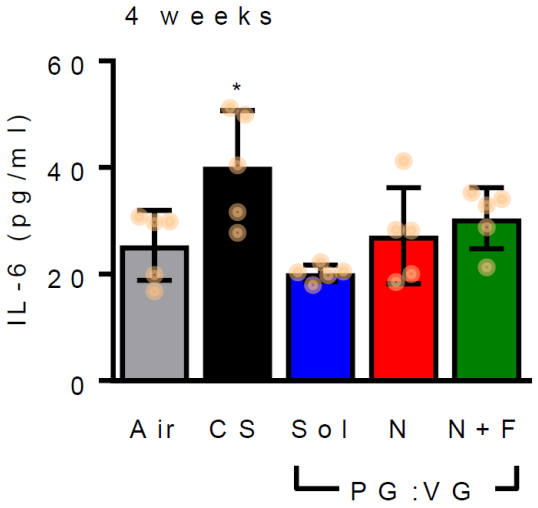


3 days

4 wks

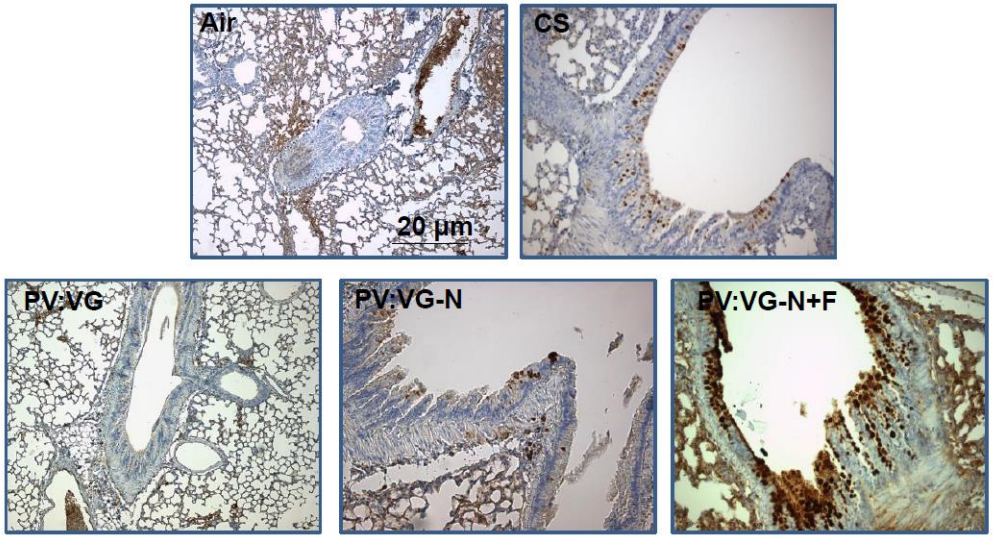
IL-6



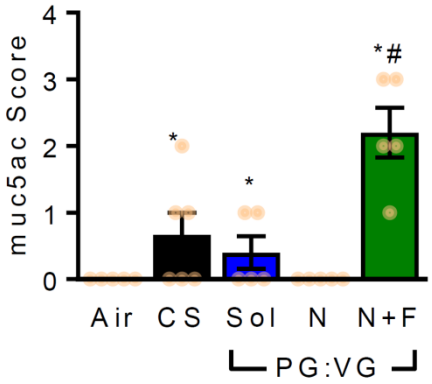


After a 3-day exposure, only exposure to flavored vapor elevated IL-1β and IL-6
At 4wks, however, this was shown only in the cigarette smoke-exposed group

LUNG Muc-5ac EXPRESSION

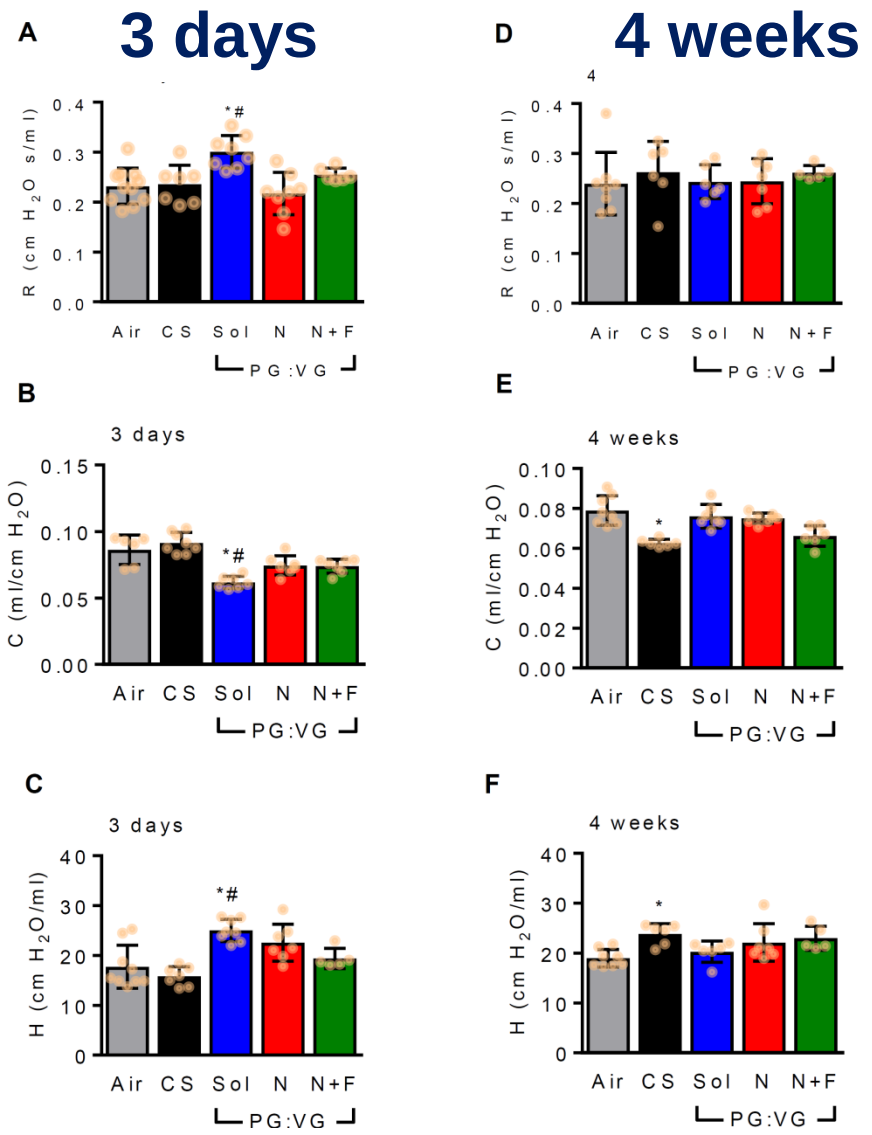


3 days



The levels of Mucin 5ac, an important effector in airway hyperreactivity, were increased by exposure to both cigarette smoke and e-cig vapor

RESPIRATORY SYSTEM MECHANICS



R = airway resistance
dynamic lung resistance, bronchoconstriction

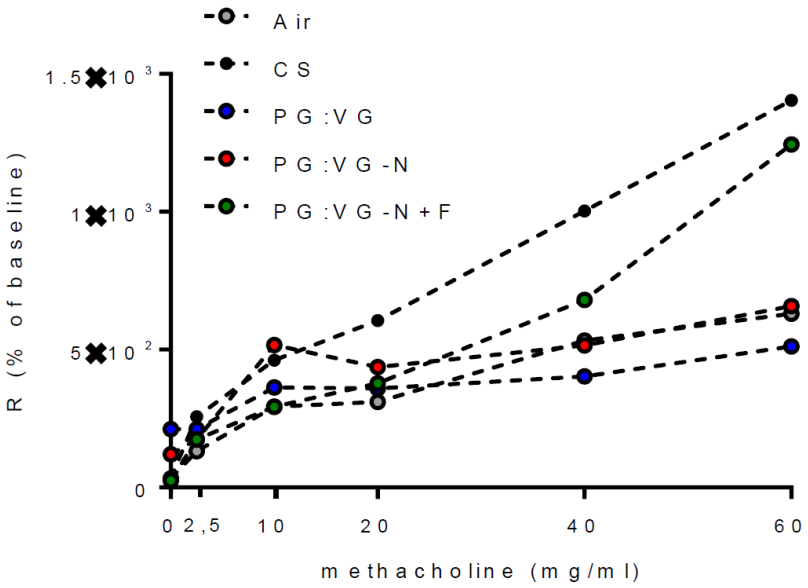
C = static compliance
ease with which lungs can be extended

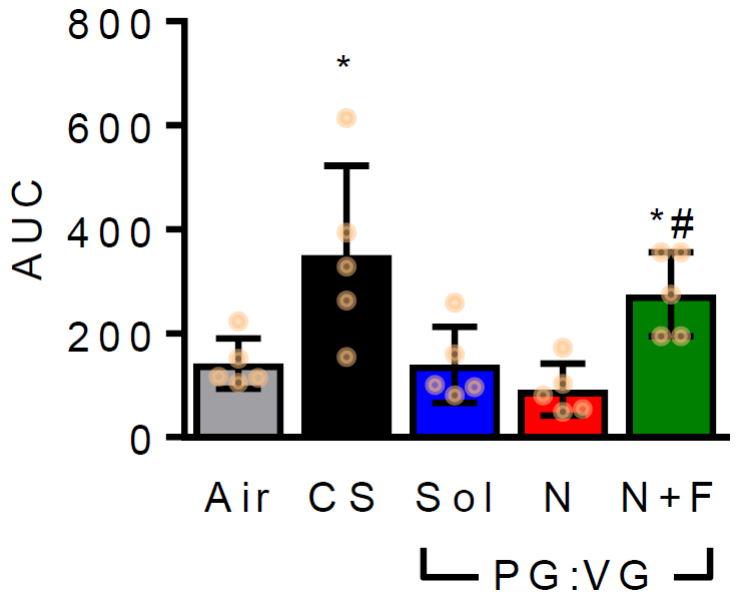
H = tissue elasticity
elastic rigidity of the lungs

Lung mechanics parameters are affected only by PG:VG at 3d of exposure
In contrast, only cigarette smoke exposure affects them at 4wks

AIRWAY HYPERRESPONSIVENESS

3 days





Subacute exposure to cigarette smoke or to flavored e-cig vapor induced increased pulmonary reactivity to methacholine

CONCLUSIONS

- The length of the exposure influences the respiratory responses of mice to cigarette smoke and e-cig vapor
- E-cig vapors, especially when containing flavor, can affect BALF cellularity, induce oxidative stress and perturb lung mechanics
- These effects are more prominent after subacute (3d) exposure to e-cig vapors
- The PG:VG solvent by itself is able to elicit markers of oxidative stress, affect Mucin-5ac production and alter lung mechanics in experimental animals
- The flavor contained in e-cig fluids may adversely affect responses of the lungs to the e-cig vapor and should be more closely monitored

THANK YOU
FOR YOUR ATTENTION!!!!