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Systems toxicology analysis of cardiovascular and respiratory endpoints from Apoe$^{-/-}$ mice showed similar effects after switching to a candidate modified risk tobacco product, THS 2.2, or to smoking cessation

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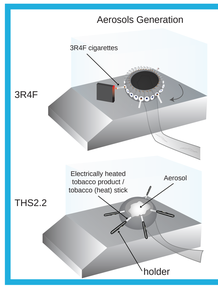
ABSTRACT

Cigarette smoking is a risk factor for chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD). Apoe-deficient mice are prone to developing premature atherosclerosis and emphysema making it an ideal model in which both pathologies can be assessed simultaneously. We evaluated the effects of cigarette smoke (CS) from a conventional cigarette (3R4F) and from THS 2.2, a modified risk tobacco product (MRTP). Apoe$^{-/-}$ mice were exposed for up to 8 months to an aerosol from 3R4F or from (THS 2.2) 3 hours/day, 5 days/week to a target nicotine concentration of 30 µg/l. After 2 months exposure to CS, cessation and switching groups were further exposed for up to 6 months to fresh air, or THS 2.2, respectively. A battery of markers of disease progression were investigated including atherosclerotic plaque formation, pulmonary inflammation (cell infiltration, cytokine levels), pulmonary function and lung emphysema. Exposure to CS induced time-dependent molecular, physiological and inflammatory responses in the lungs of Apoe$^{-/-}$ mice consistent with emphysematous changes. The size of atherosclerotic plaques measured in the aortic arches was higher in CS-exposed animals compared to both sham and pMRTP-exposed animals at all time-points examined. Micro computed tomographic (micro CT) analysis of aortic images after 8 months of exposure showed that larger plaque volumetric measurements in 3R4F-exposed animals than sham-exposed, cessation or MRTP-exposed mice. Significant changes in the lung transcriptome of Apoe$^{-/-}$ mice were observed in response to 3R4F-exposure compared to the gene expression levels observed in the transcriptome of sham-exposed mice. Smoking cessation and switching to THS2.2 resulted in lower activation levels compared to 3R4F. Both, smoking cessation and switching to the MRTP aerosol halted the rate of disease development as assessed by inflammatory, histopathological and molecular endpoints, typically within 1 to 3 months post exposure. Our work demonstrates the power of using the Apoe$^{-/-}$ mouse model to study comorbidities associated with cigarette smoking and investigate the mechanisms underlying the benefits of smoking cessation or switching to a MRTP.

CONCLUSIONS

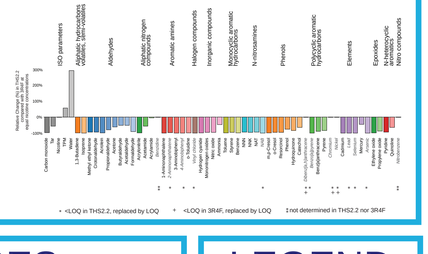
- The exposure to 3R4F cigarette smoke resulted in significant levels of pulmonary inflammation, decline in pulmonary function, and histopathological changes. These phenotypic changes were coherent with the molecular data.
- Chronic exposure to an aerosol from the THS2.2 resulted in very little difference in all measured parameters related to COPD and CVD when compared to the filtered air-exposed animals.
- The biological response to switching to a THS2.2 aerosol or filtered air following 2 months of 3R4F cigarette smoke exposure were very similar between the two conditions across the spectrum of endpoints assessed, and showed a generally positive effect.
- Differential 'omics' profiles associated with 3R4F exposure returned to nearly filtered air-like level following either switching to a THS2.2 aerosol or filtered air.
- Histopathological assessment also showed a marked effect of switching, in which a partial or complete (depending on the inflammatory cell type) reversal of pulmonary inflammation was observed.
- These data collectively indicate a halting or regression of the disease genesis following switching from conventional cigarette to THS2.2 aerosol in Apoe$^{-/-}$.

TEST ITEMS



3R4F reference cigarettes were purchased from the University of Kentucky (http://www.ca.uky.edu/refcig). The candidate MRTP, THS2.2, consisted of a stick containing a tobacco plug inserted into a holder that heats the tobacco in a controlled way to ensure combustion temperatures are not reached. The controlled heating of the tobacco generates an aerosol containing mainly water, glycerin, nicotine, and tobacco flavors.

Yields per cigarette of selected 3R4F mainstream smoke and THS aerosol constituents (HPHCs), normalized for nicotine levels.



+ <LOQ in THS2.2, replaced by LOQ + <LOQ in 3R4F, replaced by LOQ ‡ not determined in THS2.2 nor 3R4F

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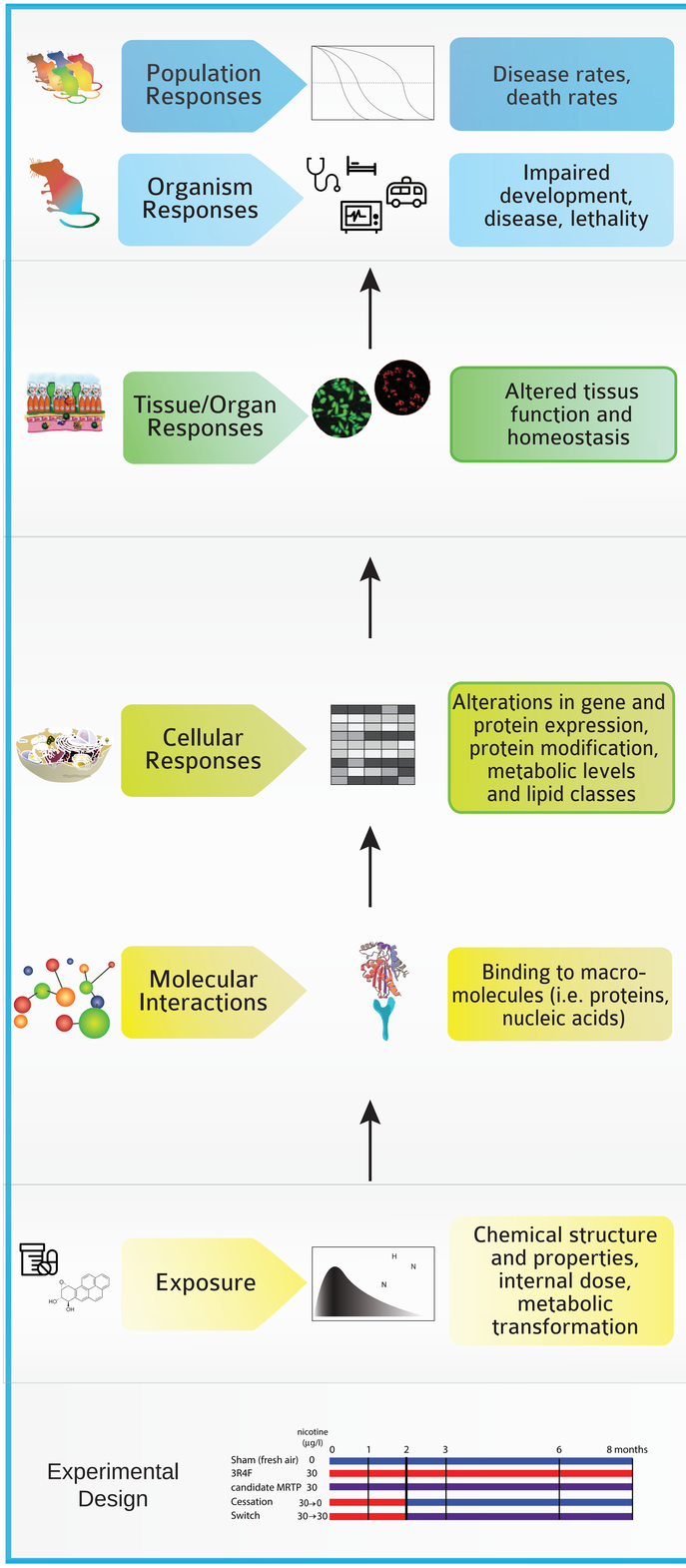
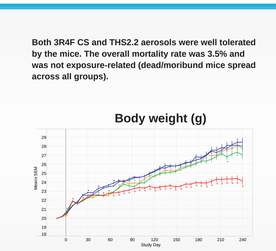
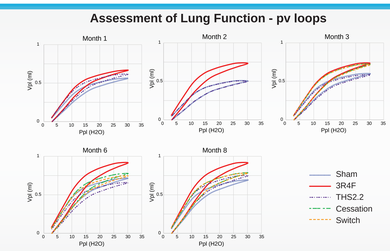
LEGEND

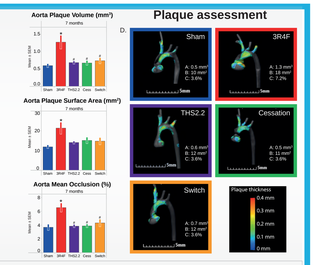
- Sham
- 3R4F
- THS2.2
- Cessation
- Switch

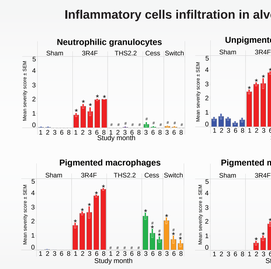
*: different from sham (p<0.05)
#: different from 3R4F (p<0.05)
&: different from Cessation (p<0.05)

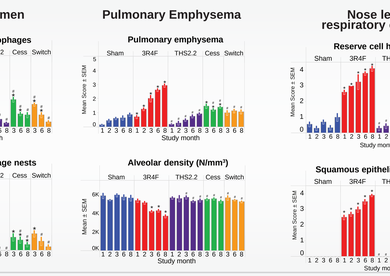


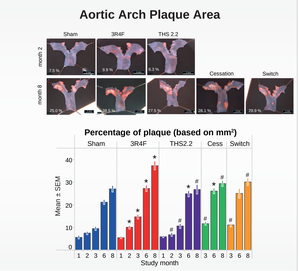


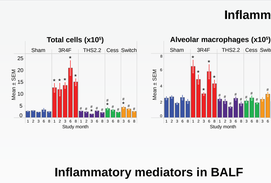


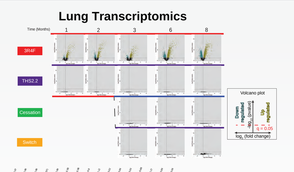


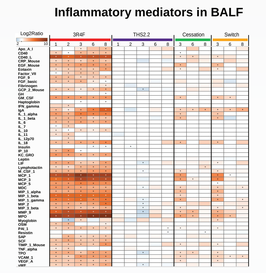


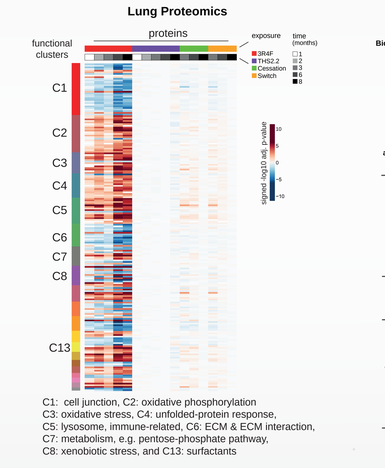




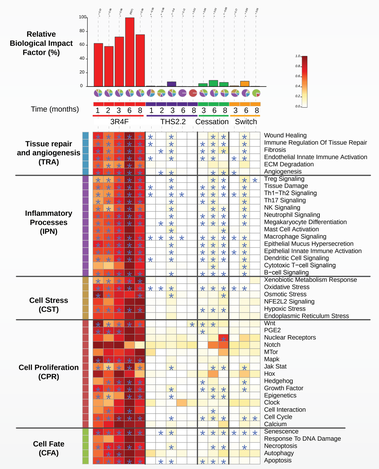


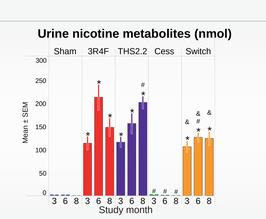


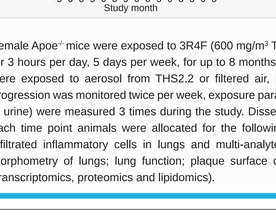


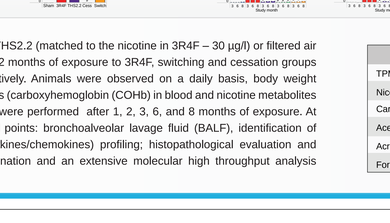


C1: cell junction, C2: oxidative phosphorylation
C3: oxidative stress, C4: unfolded-protein response,
C5: lysosome, immune-related, C6: ECM & ECM interaction,
C7: metabolism, e.g. pentose-phosphate pathway,
C8: xenobiotic stress, and C13: surfactants









Female Apoe$^{-/-}$ mice were exposed to 3R4F (600 mg/m^3 TPM), THS2.2 (matched to the nicotine in 3R4F – 30 µg/l) or filtered air for 3 hours per day, 5 days per week, for up to 8 months. After 2 months of exposure to 3R4F, switching and cessation groups were exposed to aerosol from THS2.2 or filtered air, respectively. Animals were observed on a daily basis, body weight progression was monitored twice per week, exposure parameters (carboxyhemoglobin (COHb) in blood and nicotine metabolites in urine) were measured 3 times during the study. Dissections were performed after 1, 2, 3, 6, and 8 months of exposure. At each time point animals were allocated for the following end points: bronchoalveolar lavage fluid (BALF), identification of infiltrated inflammatory cells in lungs and multi-analyte (cytokines/chemokines) profiling; histopathological evaluation and morphometry of lungs; lung function; plaque surface determination and an extensive molecular high throughput analysis (transcriptomics, proteomics and lipidomics).

Test Atmosphere Chemistry

	Sham	3R4F	THS2.2
TPM (mg/m³)	0 (250)	598.5 ± 27.1 (256)	368.9 ± 54.4 (250)
Nicotine (mg/m³)	0 (63)	29.4 ± 2.2 (256)	28.6 ± 3.2 (250)
Carbon monoxide (ppm)	0.1 ± 0.1 (250)	650.9 ± 34.2 (256)	14.5 ± 2.2 (250)
Acetaldehyde (mg/m³)	n.d.	32.9 ± 2.3 (50)	7.5 ± 0.7 (49)
Acrolein (mg/m³)	n.d.	3.1 ± 0.2 (50)	0.3 ± 0.0 (49)
Formaldehyde (mg/m³)	n.d.	0.6 ± 0.1 (50)	0.1 ± 0.0 (49)

Values given in the table are mean ± SD. Numbers in brackets are the number of measurements done during the study.

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