Effects of Cigarette Smoke, Cessation and Switching to Aerosol from Two Heat-Not-Burn Tobacco Products on Lung Lipid Metabolism in Two Mouse Strains

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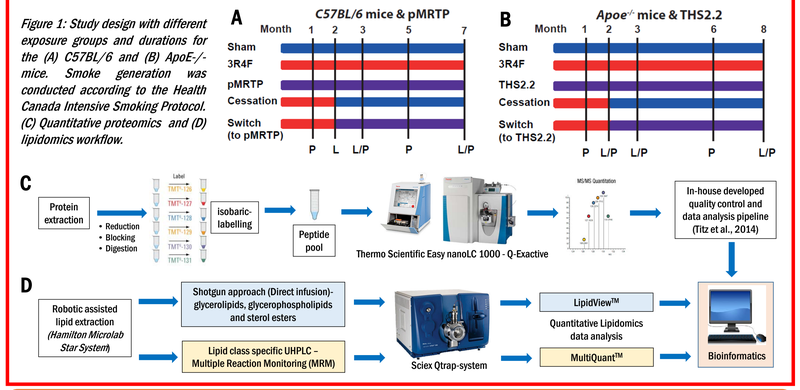
Introduction

Cigarette smoking causes cardiovascular disease (CVD) and chronic obstructive pulmonary diseases (COPD) such as chronic bronchitis and emphysema. Reliable system-wide measurements are key to a meaningful systems-toxicology approach by which impact of toxicants in cells are evaluated (Hoeng et al., 2012). We evaluated the advantages of using proteomics and lipidomics methodologies for the product assessment of a carbon heated prototype modified risk tobacco product (pMRTP) and of the tobacco heating system 2.2 (THS2.2), a candidate modified risk tobacco product (cMRTP). The objective was to integrate the proteomics and lipidomics with results from other endpoints such as transcriptomics and histology data within our systems toxicology approach. Two studies were conducted on 2 different mouse strains: 1) pMRTP was assessed using C57BL/6 mice which are prone to developing emphysema, a form of COPD (Phillips et al, 2015a), and 2) the cMRTP THS2.2 was assessed using ApoE-deficient mice which are prone to developing premature atherosclerosis and emphysema (Phillips et al., 2015b). We compared the effects of aerosols from the two different MRTPs with the effects of cigarette smoke (CS) from a reference cigarette (3R4F) on mice exposed for up to 7/8 months.

Methods

The C57BL/6 mice were whole body-exposed to diluted mainstream smoke from 3R4F (750 mg TPM/m^3, equivalent to 34.4 µg nicotine/l), pMRTP aerosol (nicotine-matched to 3R4F, 34.4 µg/l) or filtered air (Sham) for 4 h per day, 5 days per week (Fig 1A). The Apoe-/- mice were whole body-exposed to diluted mainstream smoke from 3R4F (600 mg TPM/m^3, equivalent to 29.9 µg nicotine / l), THS2.2 aerosol (nicotine-matched to 3R4F, 29.9 µg/ l) or filtered air for 3 h per day, 5 days per week (Fig. 1B), In order to evaluate the effects of cessation and switching, after 2 months of exposure to CS in both studies dedicated groups of animals were exposed to filtered air or pMRTP/THS2.2 aerosols.. L = lipidomics, and P = proteomics data were obtained at indicated dissection time points. Quantitative proteomics analysis was performed using isobaric-tag based labelling approaches (Fig 1C). Lipid concentrations were determined using shotgun and targeted mass-spectrometry based approaches (Fig 1D).



Results

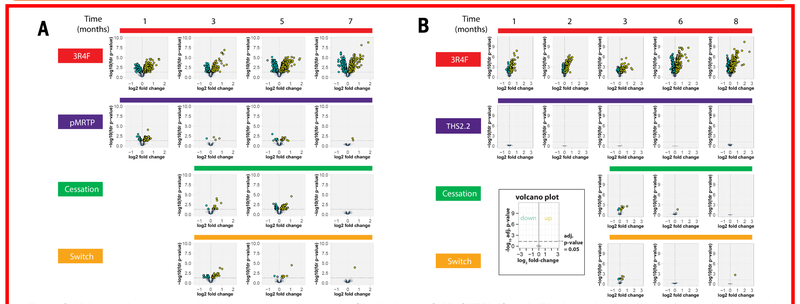


Figure 2: Volcano plots representing the proteome response profiles in lungs of (A) C57BL/6 and (B) Apoe-/- mice. For each protein, the protein expression change, calculated as the log2 fold change, is plotted on the x-axis and the statistical significance, proportional to the negative log10-adjusted P-value, is plotted on the y-axis. Yellow and blue dots highlight proteins that are statistically significantly up- or down-regulated, respectively, compared with the sham group at each respective time point (Benjamini-Hochberg adjusted p-value <0.05).

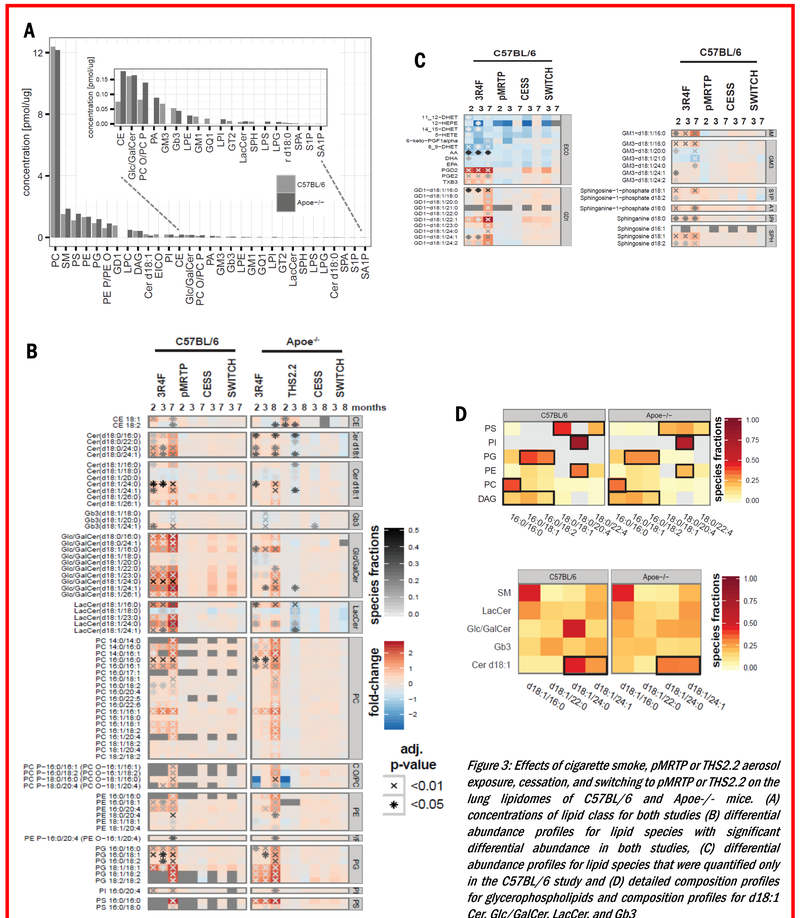


Figure 3: Effects of cigarette smoke, pMRTP or THS2.2 aerosol exposure, cessation, and switching to pMRTP or THS2.2 on the lung lipidomes of C57BL/6 and Apoe-/- mice. (A) concentrations of lipid class for both studies (B) differential abundance profiles for lipid species with significant differential abundance in both studies, (C) differential abundance profiles for lipid species that were quantified only in the C57BL/6 study and (D) detailed composition profiles for glycerophospholipids and composition profiles for d18:1 Cer, Glc/GalCer, LacCer, and Gb3

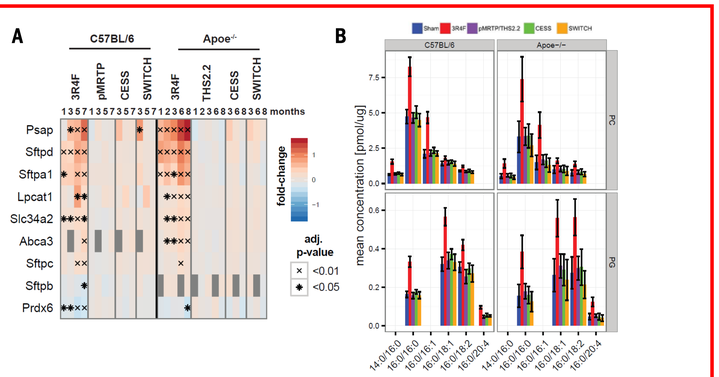


Figure 4: Surfactant protein (A) and surfactant lipid (B) responses in C57BL/6 and Apoe-/- mice. CS exposure strongly affected both protein and lipid components of surfactant, while pMRTP and THS2.2 exposure did not induce such changes and the cessation and switching groups rapidly returned to sham levels of these proteins and lipids. Cess = cessation.

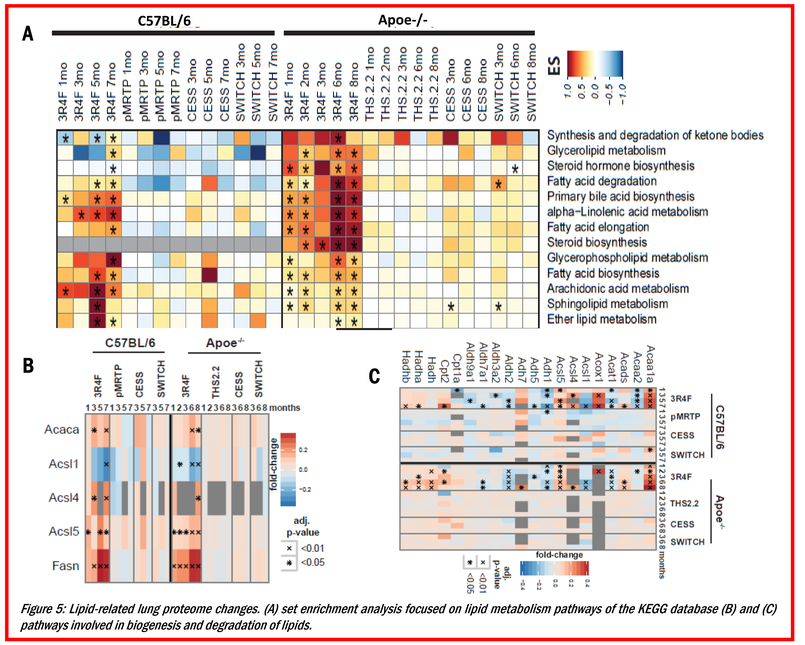


Figure 5: Lipid-related lung proteome changes. (A) set enrichment analysis focused on lipid metabolism pathways of the KEGG database (B) and (C) pathways involved in biogenesis and degradation of lipids.

Conclusions

- We successfully implemented proteomics and lipidomics workflows and we were able to capture the main known smoke effects in the lung tissue.
- Exposure to 3R4F smoke induced a strong effect in a time-dependent manner with significant changes in protein and lipid profiles in both mouse strains.
- Continuous exposure to the aerosol from either the pMRTP or cMRTP resulted in very limited changes in protein and lipid profiles in both mouse strains.
- Switching to pMRTP or cMRTP resulted in a response decrease in protein and lipid profiles reaching similar levels as cessation in both mouse strains.
- Biological functions affected in the proteome as a result of 3R4F smoke exposure included xenobiotic response, ROS response, immune-response, metabolism, surfactants, cell adhesion and unfolded protein response (for details see Titz et al., 2015).
- Very close similarities in surfactant protein and surfactant lipids responses for all the groups in both studies were observed. Inflammatory eicosanoids, their metabolic enzymes and several ceramide classes were elevated.
- The integration of proteomics and lipidomics with transcriptomics and histopathology proved to be valuable in the systems toxicology approach to perform product assessment of MRTPs.

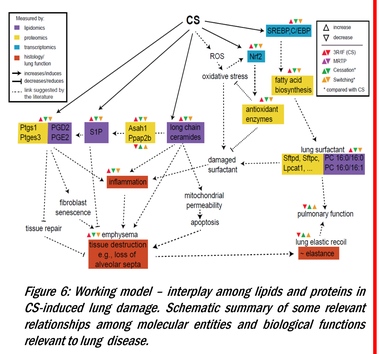


Figure 6: Working model – interplay among lipids and proteins in CS-induced lung damage. Schematic summary of some relevant relationships among molecular entities and biological functions relevant to lung disease.

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Competing Financial Interest

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