Effects of Short- and Long-Term Exposure to E-Cigarette Vapour

DZL – Deutsches Zentrum für Lungenforschung
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General report:

The winners of the Balzan Prize 2019 – Erika von Mutius, Klaus Rabe, Werner Seeger, and Tobias Welte – proposed to dedicate the Balzan Prize money to the cross-disciplinary project *Effects of Short- and Long-Term Exposure to E-Cigarette Vapour*. Given the importance of the subject, the prize winners decided to equip the scientific project with further funding from the German Centre for Lung Research (DZL). A group of DZL early career scientists started three subprojects relating to potential side effects of vaping during adolescence for the next generation, namely for their own children; the potential damage to the airways conferred by vaping; and the health effects of vaping in a large German general population (the National Cohort)

A group of DZL early career scientists subsequently launched three subprojects:

1. Early responses to e-cigarettes and their components: airway structural changes, signalling pathways and survival in *Drosophila melanogaster* in the context of risk alleles (DZL site ARCN)
2. Effect of short- and long-term exposure to e-cigarette vapour on inflammation, endothelial barrier function, and structural alterations in murine lungs (DZL sites BREATH and UGMLC)
3. Association of e-cigarette use with respiratory status in lung-healthy subjects and pulmonary patients (DZL sites CPC-M and TLRC)

Scientific report:

1. **Early responses to e-cigarettes and their components: airway structural changes, signalling pathways and survival in *Drosophila melanogaster* in the context of risk alleles (Dr. Hanna Angstmann, Prof. Dr. Susanne Krauss-Etschmann, Research Center Borstel, DZL site ARCN)**

Background

a. *E-cigarette use by vulnerable populations*

Advanced age or pre-existing disease render individuals more vulnerable to environmental stressors. Lung organogenesis takes place during intrauterine life and therefore is another period of sensitivity to influences from the environment. Unlike age or morbidity, pregnancy represents a unique life stage in which harmful exposures can affect the respiratory health of the offspring for many decades to come. This has been firmly documented for maternal smoking in pregnancy, which increases the offspring's risk of developing asthma and COPD. In contrast, how use of ECs during pregnancy affects foetal lung development and consequently later respiratory health is largely unknown. Moreover, there is increasing evidence that even exposures in the preconception period may influence the respiratory health of offspring born many years later. In addition to flavours and base liquid, nicotine is a regular ingredient in ECs, in similar or even higher amounts than in combustion cigarettes, whose nicotine content is limited to 1 mg per manufactured cigarette by law (in the EU). Because nicotine readily crosses the placenta and accumulates in the foetal lung, it can adversely affect lung development.

In the present subproject we have used a simple model organism to investigate how preconception exposure to vaporized nicotine affects the respiratory health of offspring. Research on this subproject will beyond the scope of the Balzan funding as the data suggest that teenage vaping can have long-term consequences for future generations and therefore an enormous socio-economic impact for decades to come.

b. *The model organism* Drosophila melanogaster

To explain why the fruit fly was chosen as a model, we would like to briefly reiterate the usefulness of the *D. melanogaster* for respiratory research.

1) The fruit fly has a short life span of approximately 90 days which allows investigation of long-term consequences of parental vaping on offspring throughout the entire life cycle, from early developmental stages to the adult fly.

2) *D. melanogaster* develops extracorporeally so that all developmental stages are easy to visualize.

3) Similar to the human lung, the respiratory system of the fruit fly consists of hierarchically arranged branches that end in primitive alveolar-like structures (see **Fig. 1A** below).

4) As in humans, the airway epithelium consists of both a physical and an immunological barrier.

5) Like all inhaled pollutants, the vapour products first reach the surface epithelium of the lungs. The airways of fruit flies also consist exclusively of different – albeit less defined – subtypes of epithelial cells thus mimicking the human situation.

6) *D. melanogaster* has homologues to approximately 80% of all human disease-associated genes.

Results

I. Preconception maternal e-nicotine

To simulate teenage use of electronic cigarettes, we exposed young virgin female flies to vapoured nicotine (e-nicotine). As reported earlier, we observed marked growth deficits in the F1 generation which were more severe in males and beyond pupation. The latter is important, since the entire larval body disintegrates during the pupal stage except for precursor cells giving rise to the very different morphology of the adult fly. In other words, any difference seen beyond pupation must be rooted in stem cell-like precursor cells (El-Merhie *et al.*, 2021). Maternal preconception e-nicotine further reduced the length of the main trachea in female offspring as well as of the secondary branches in offspring (**Fig 1 B, C**). Surprisingly, we also discovered malformations in the F1 tracheae, with local narrowings that, in some places, led to almost complete stenosis (**Fig 1D, E**).

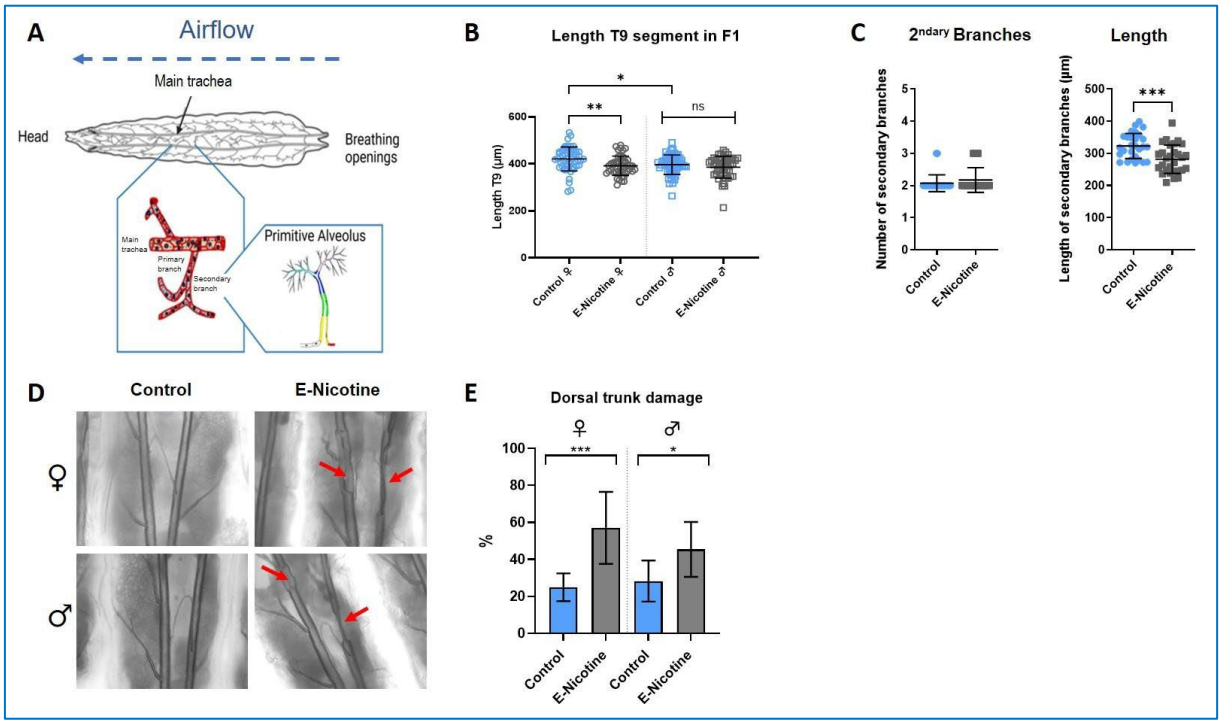

Figure 1: Tracheal malformations/*geometry* in offspring of preconceptionally exposed mothers. **(A)** Schematic drawing of larval airways with two main tracheae in the upper panel overview. The left insert shows one of in total ten metameric segments that are fused together in a row. The trachea gives rise to defined numbers of primary and secondary branches. The right insert shows one finger-shaped primitive alveolus rising from secondary branches **(B)** Length of the ninth tracheal segment (T9) in male and female offspring in L3 larval stage; 2way ANOVA (Šídák's). **(C)** Number of secondary airway branches (left panel) and their length (right panel) in the third tracheal segment (T3); unpaired t-test. **(D)** Representative images of the main tracheae. Red arrows mark tracheal narrowing. **(E)** Percentage of animals with tracheal narrowing; One-way ANOVA; mean ± SD; n = 3 independent experiments; ns ($p > 0.05$), *($p \leq 0.05$), **($p < 0.01$), ***($p < 0.001$), **** ($p < 0.0001$).

For deeper insight into the airway fine structure, we measured the diameter of the airway walls in F1, which was thickened in the trachea as well as primary and secondary branches following maternal preconception exposure to e-nicotine (**Fig 2A, B**). This finding was not explained by a higher number of epithelial cells as they were equal in offspring of both maternal exposure groups. However, the epithelial cellular size was significantly enlarged in offspring from mothers exposed to e-nicotine earlier (**Fig 2C, D**). In addition, visualization of the airway cell tight junction protein coracle revealed a massive epithelial cellular dysplasia with irregular cell shapes of varying sizes. This resulted in an overall distorted form of the entire airway after maternal preconception e-nicotine (**Fig 2C**). In contrast, the airways of offspring from control mothers were slim and even, and the individual epithelial cells had the physiological hexagonal circumference (**Fig 2C**).

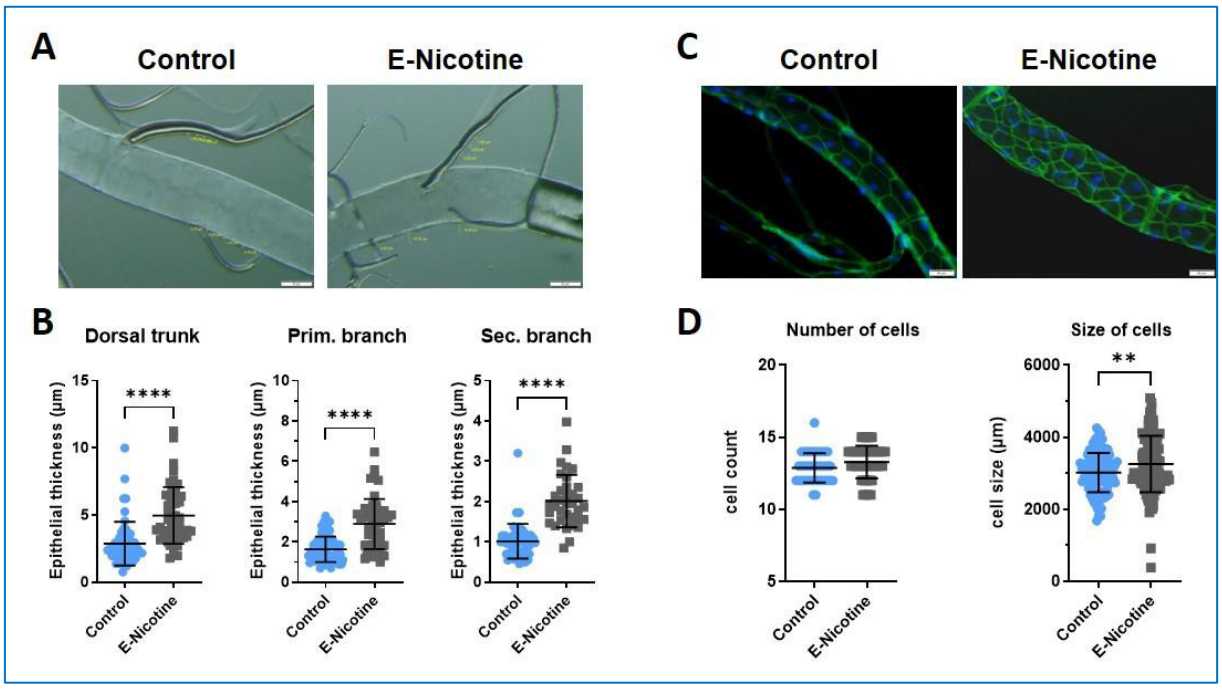

Figure 2: Airway epithelial remodelling in offspring from e-nicotine exposed virgins. (A) Representative image of epithelial thickness in offspring (L3 larvae) from e-water (left) and e-nicotine (right) preconceptionally exposed mothers. Bars indicate epithelial thickness. **(B)** Quantification of the epithelial thickness in the main trachea (left), primary (middle) and secondary (right) branches. In total 50 airways from 3 independent experiments were analysed per group. **(C)** Representative images of the tight junction protein coracle visualized by fluorescence histochemistry in the main trachea (9^{th} tracheal segment). **(D)** Number (left; 40 cells analysed per group) and size (right; 100 cells analysed per group) of airway epithelial cells in the ninth segment of the main trachea. Unpaired t-test; mean ± SD; n = 3 independent experiments; ns ($p > 0.05$), ** ($p < 0.01$), **** ($p < 0.0001$).

In the fruit fly, primitive correlates of mammalian alveoli, termed terminal cells, sprout from the secondary branches. They reach the surrounding tissue with long finger-shaped extensions (**Fig 1A)** to secure gas exchange. The effect of maternal e-nicotine on terminal cells was sexually dimorphic as only female F1 developed shorter terminal cells ($p = 0.005$), which also developed fewer branches ($p = 0.017$). Of note, the airway deformities described above presumably caused a reduced physical fitness of the animals, as evidenced by significantly lower larval locomotor activity of both sexes ($p < 0.0001$).

Airway precursor cells, termed tracheoblasts, are the main drivers of growth and repair in the larval airway epithelium. The stem cell niche is located at the secondary branches. To fulfil their tasks, the

stem cells need to proliferate, mirrored by the area they cover, followed by migration to the main trachea. Upon maternal preconception of e-nicotine, the area covered by tracheoblasts was significantly reduced in offspring (**Fig 3A-C).**

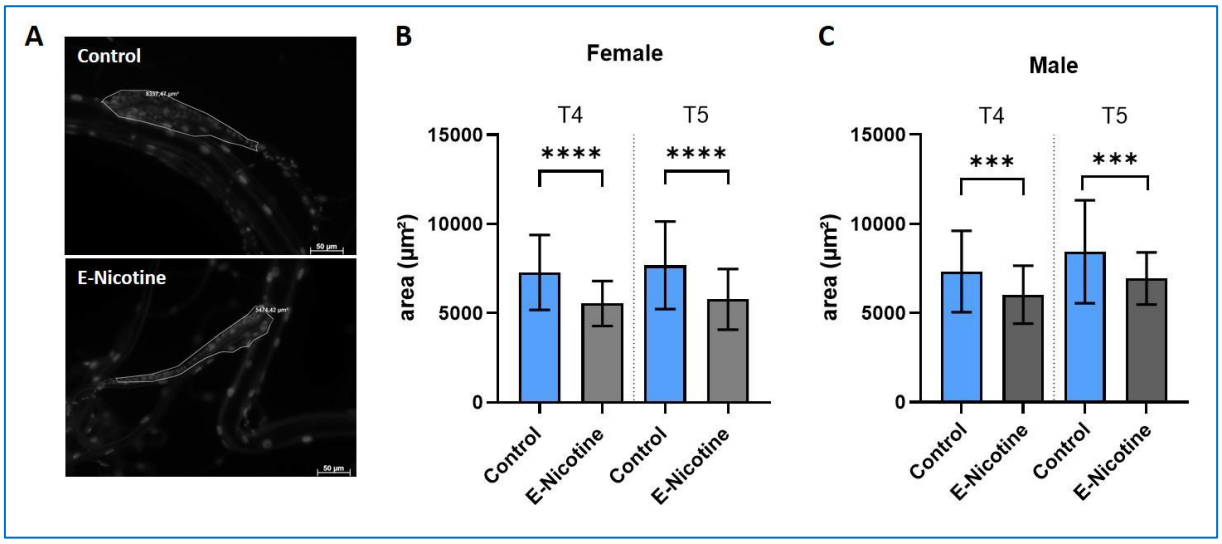

Figure 3: Airway stem cell niche in offspring from preconceptionally exposed mothers. (A) Representative image of stem cell niche in offspring in late L3 larval stage from e-water (top) and e-nicotine (bottom) preconceptionally exposed mothers; **(B)** and **(C)** Stem cell areas (µm²) measured in the thoracic segments T4 and T5 in female **(B)** and male **(C)** late L3 stage larvae. Unpaired t-test; mean ± SD; n = 3 independent experiments; *** ($p < 0.001$), **** ($p < 0.0001$).

Ongoing Research

To obtain clearer insight into the mechanisms underlying the prominent developmental deficits in offspring from preconceptionally exposed mothers, we isolated the airways from male and female offspring and subjected them to RNA sequencing. The results are currently analysed with focus on genes involved in airway development to be included in a current manuscript draft.

In summary, the airway development of offspring was significantly disturbed upon maternal e-nicotine exposure exclusively during her own unfertilized life period. The shorter and locally constricted F1 airways presumably led to perturbations of the gas flow and lower tissue oxygenation, although formal proof of the latter is lacking.

A limitation of our study is that the e-nicotine exposure cannot be directly translated to human exposures. A strength is that infant stem cells could be studied, which would be impossible in humans. Our findings however advocate for future epidemiological and mechanistic investigations to clarify the impact of e-cigarette use in adolescents on the health of their future children, to protect them from lasting harm.

II. Airborne pollutants can enter the entire respiratory tract of the fruit fly

The tracheae of fly larvae have two pairs of openings termed spiracles that form the first physical barrier to the airways. The spiracular lumen is filled with hair-like extensions building a dense *filzkörper* which, together with four hairs that can bend over each opening, form an efficient filter against foreign material. In addition, fly larvae – being very tolerant to hypoxia – can actively close these tracheal openings in an adverse environment for a prolonged time, thus conveying further protection.

Although we had seen prominent effects of maternal preconception e-nicotine exposure on the offspring (see Chapter above), we were not sure if e-nicotine is predominantly resorbed via the future mothers' body surface or large eyes instead of being inhaled. If this would be the case, it would lessen the transferability to the human situation.

Since vaping products are hard to visualize, we went a step backward by exposing larvae to conventional combustion cigarettes (Sirocko *et al.*, 2022). To clarify if cigarette smoke passes the breathing openings, Cyp18A1 (homologue to human Cyp1a1) expression was analysed in isolated and transversally bisected tracheae from male and female larvae. Cyp18a1/Cp1a1 metabolize smoke-derived polycyclic aromatic hydrocarbons. Indeed, Cyp18a1 was markedly increased in all parts of the trachea, proving that smoke enters the airways in their entirety, whereas the remaining carcasses gave a low signal. This finding was further confirmed by using airway specific HSP70 reporter lines, which gave a signal along the entire airway tract. Of note, from the beginning, male larvae showed an increase in mortality that extended across all developmental stages including adulthood.

To better understand the consequences of smoke exposure on the airway transcriptional response, transcriptomic analyses (RNAseq) of dissected larval airways yielded genes involved in immune response, oxidative stress, and airway development in a partly sex-specific manner.

Summary: Cigarette smoke induces an oxidative stress response in *D. melanogaste*r larval airways and sex-specifically deregulates airway developmental and immune pathways. This is connected to higher mortality until adulthood in male flies only.

Ongoing Research: We currently investigate the epigenetic regulation of three selected pathways central to airway development. The information generated in this project is therefore not specific to cigarette smoke, but to any inhaled toxicant affecting airway development.

c. Airway structural changes in the context of asthma risk alleles

Proteases are key elements of the airway epithelial host defence and are tightly regulated by anti-proteases to prevent lung damage from an overshooting proteolytic activity. In line, an enhanced proteolytic activity has been noted for chronic lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). Among clade B serpins, Serpin B3, also termed *Scca1,* and *Spink5*, have been described for their role in airway diseases, but their function beyond antiproteolytic activity is insufficiently understood. To close this gap, we had generated fly lines with overexpression or knockdown for each gene in the airways. Overexpression of both fly homologues of *Scca1* and *Spink5* induced the growth of additional airway branches, with more variable results for the respective knockdowns. These morphological changes in the airways were associated with lower tolerance to hypoxia. At the level of physical fitness, dysregulation of *Scca1* resulted in a general delay in fruit fly development, and an increase in larval and pupal mortality following overexpression of this gene.

d. Paternal preconception exposure to e-nicotine

For decades, it has been assumed that a father's contribution to the health of his offspring is exclusively anchored in his DNA sequence (e.g., by uniparental disomy) or rooted in his social behaviour. More recently, it has been proposed that the intergenerational transfer of paternal information involves epigenetic changes in sperm cells. The differentiation of spermatogonia into spermatocytes, including changes in RNA types takes place within the testis. The immature spermatocytes then progressively mature in the epididymis, which is a highly segmented organ with specific microenvironments critical for sperm maturation. During puberty, primordial germ cells develop into spermatogonia, which in turn develop into mature spermatozoa. Therefore, environmental insults during prepubertal development may have long-lasting effects on the epigenetic make-up of mature sperm (reviewed in Svanes, Holloway, Krauss-Etschmann, *J Internal Med,* 2024).

To mimic this situation, freshly hatched male flies were exposed to vapored nicotine for three days followed by mating to non-exposed female flies. The F1 generation showed sex-specific differences since male F1 larvae – but not females – from e-nicotine exposed fathers were significantly smaller than F1 from fathers exposed to vapoured water. Similarly, the length of terminal cells, which are primitive alveoli, was reduced only in males, while the number of branches was lower both in males and females. The airway epithelial thickness was increased in males only. Of note, similar to maternal e-nicotine, the stem cell niche located at the secondary branches was smaller in offspring from exposed fathers.

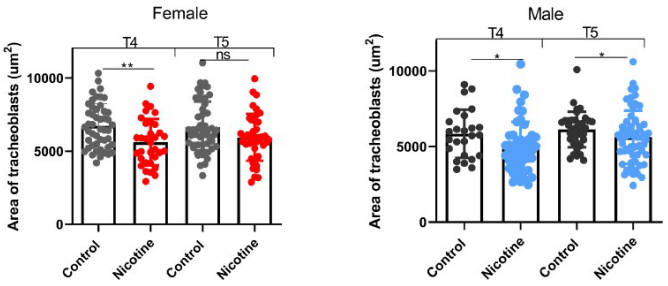

Figure 4: Airway stem cell niche in offspring from preconceptionally exposed fathers. (Left) Female offspring **(Right)** Male offspring. Representative image of stem cell niche in offspring in late L3 larval stage from e-water (top) and e-nicotine (bottom) preconceptionally exposed mothers; **(B)** and **(C)** Stem cell areas (µm²) measured in the thoracic segments T4 and T5 in female **(B)** and male **(C)** late L3 stage larvae. ANOVA 2-way; mean ± SD; n = 3 independent experiments.

Summary: Paternal exposure to e-nicotine also had an impact on the airway structure as well as on the airway stem cell niche. The latter indicates a reduced capacity for airway development and repair in F1.

Ongoing Research: Currently we perform a deeper analysis of the airway morphology and investigate how paternal e-nicotine exposure influences the longevity of their offspring.

2. **Effect of short- and long-term exposure to e-cigarette vapour on inflammation, endothelial barrier function, and structural alterations in murine lungs – preliminary results (Prof. Dr. Sascha David & PD Dr. Benjamin Seeliger, Hannover Medical School, DZL site BREATH; Prof. Dr. Natascha Sommer, Justus-Liebig University, Giessen, DZL site UGMLC)**

BREATH subproject: Effect of exposure to nicotine containing e-cigarette vapour on endothelial barrier integrity in human-umbilical cord endothelial cells (HUVECs)

The subproject at BREATH investigated the impact of e-cigarette vapour extract (ECVE) on endothelial barrier integrity in human umbilical cord endothelial cells (HUVECs). Previous findings had demonstrated a dose-dependent increase in supernatant angiopoietin-2 (ANGPT-2), a marker of endothelial damage, when HUVECs were exposed to ECVE with or without nicotine.

We started the project with experiments on the interplay between ECVE-induced endothelial damage and inflammation (here: stimulation by lipopolysaccharide (LPS)). We analysed the effect of pre-incubation with ECVE followed by lipopolysaccharide (LPS) stimulation on the Angiopoietin/TIE2 system as well as continuous trans-endothelial resistance (TER) measurements using an electrical cell impedance sensing (ECIS) device. Pre-incubation with ECVE medium increased the susceptibility of HUVECs to subsequent LPS stimulation by 20% with regard to ANGPT-2 production. The ANGPT2/TIE2 mRNA ratio increased significantly with nicotine exposure comparable to effects of LPS alone and was even higher with combined nicotine and LPS stimulation. On the functional level, pre-treatment with nicotine-containing ECVE resulted in an increased susceptibility to LPS-induced reduction in endothelial barrier integrity. To evaluate potential treatment options within the Angpt2/Tie2 pathway and ECVE-induced damage, we induced phosphorylation of the protein kinase Akt by a monoclonal TIE2 agonist (PMC-403).

In a trans-well model with human PBMC and HUVECs, we did not see an alteration of the absolute transmigration of PBMC. However, ECVE selectively down-regulated CD4 T Cell transmigration. In our most recent experiments, we found that trans-endothelial migration induced by LPS could be normalized by using a TIE-2 agonist (PMC-403). Interestingly, PMC-403 did not, however, inhibit ANGPT-2 production upon ECVE or LPS-stimulation, implying additional effects outside of ANGPT-2 induced capillary leakage.

In summary, these results suggest that ECVE induces endothelial activation and that nicotine-containing ECVE increases vulnerability to LPS-induced barrier breakdown, potentially increasing susceptibility to sepsis-induced capillary leakage. Moreover, nicotine-containing ECVE selectively down-regulates CD4 T Cell trans-endothelial migration. Using a TIE-2 agonist, we were able to reverse this effect.

UGMLC subproject: Effect of short- and long-term exposure to e-cigarette vapour on inflammation, endothelial barrier function, and structural alterations in murine lungs

In the UGMLC subproject, we showed in isolated ventilated and perfused mouse lungs that inhalation of e-cigarette vapour (ECV) with nicotine or cherry, but not without any additive, induced endothelial dysfunction characterized by increased capillary filtration coefficient and oedema development. Endothelial dysfunction was not enhanced by an additional inflammatory bacterial (lipopolysaccharide, LPS) or viral co-stimulus. Furthermore, endothelial dysfunction was completely inhibited by application of a selective TIE2 receptor agonist (PCM-403), which was provided in cooperation with Sascha David & PD Dr. Benjamin Seeliger. Similar to the experiments on isolated lungs, presence of nicotine in ECV promoted pulmonary inflammation and structural alterations after 8

months of *in vivo* exposure of mice. ECV without nicotine also caused slight alterations albeit to a lesser degree. The data on ECV with and without nicotine were published in the *European Respiratory Journal* (Roxlau *et al.*, 2023). In the last year of funding, we focused on the effect of flavours in ECV, finalized experiments on isolated lungs for publication and published a review on the effect of flavours in ECV in *Deutsche Medizinische Wochenschrift* (Sommer *et al.*, 2025).

3. **Association of e-cigarette use with respiratory status in lung-healthy subjects and pulmonary patients (Dr. Stefan Karrasch, LMU Munich, DZL site CPC-M, and Dr. Claudius Melzig, Heidelberg University Hospital, TLRC)**

CPC-M subproject: This subproject focused on the collection and analysis of non-invasive data on respiratory status including lung function and smoking behaviour regarding e-cigarette use in the general population, taking into account the use of conventional cigarettes and tobacco products as well as possible influencing factors. In particular, the investigations focused on the ongoing comprehensive assessments in the German National Cohort (NAKO) with 18 study centres throughout Germany. Despite significant challenges during the project period due to both the pandemic emergency and severe technical limitations following a cyberattack, the targeted investigations were successfully carried out with appropriately adapted precautionary measures as well as substantial further development and optimization of data preparation and provision and an extension of the project period.

Overall, information on e-cigarette use was obtained in the NAKO baseline survey for more than 64,000 adult participants aged 20 to 75 years who either currently smoked conventional cigarettes or had never smoked these at the time of the survey. Of these participants, around 10% reported that they had ever used e-cigarettes or were currently using them, and the vast majority of these were dual users, i.e. using e-cigarettes and conventional cigarettes in parallel. Furthermore, the percentage of e-cigarette users was higher in men than in women, and the use of e-cigarettes decreased with increasing age from around 18% in the youngest age group to less than 5% in participants over 65 years of age, with significant overall heterogeneity between the different NAKO study centres. Consequently, possible health effects of e-cigarette use may particularly affect young adults in view of these usage proportions.

Regarding the relationship with conventional smoking, there was a slight trend towards a positive association between e-cigarette use and conventional smoking, driven by current or former regular e-cigarette users. In addition, a significant difference in daily consumption of e-liquids was observed between dual users and individuals who only used e-cigarettes, and a positive association was found between the number of conventional cigarettes smoked per day and the volume of e-liquids consumed daily in individuals who currently consumed both e-cigarettes and conventional cigarettes. These results indicate that, overall, e-cigarette use among current users shows similar patterns to conventional smoking.

In terms of smoking history, the total duration of e-cigarette use was well below 10 years in the vast majority of cases, whereas the duration of smoking conventional cigarettes ranged up to more than 50 years. However, in this respect the differences in availability over time for these different types of cigarettes have also to be taken into account. Compared to e-cigarette only users, dual users more often used nicotine-containing e-cigarette liquids than nicotine-free liquids, and the self-reported amount of e-cigarette liquids consumed per day was higher among those who used nicotine-containing liquids. These findings suggest that e-cigarettes may primarily be used by these participants as an alternative way to consume nicotine alongside conventional smoking.

Across all participants with e-cigarette use, an increase in respiratory symptoms such as coughing and whistling or wheezing was observed compared to participants without e-cigarette use, even when conventional cigarette smoking was taken into account, and this was especially observed in subjects without diagnosed respiratory disease. Overall, these effects were slightly more pronounced in women than in men. In addition, there was a trend towards stronger effects of e-cigarette use on respiratory symptoms in subjects with a BMI below 25 kg/m2 compared to overweight or obese subjects. This association was mainly observed for coughing and sputum production; the difference between the BMI groups was smaller for wheezing or whistling. These observations suggest that e-cigarette use may exert additional adverse effects on respiratory health beyond the well-established effects of conventional smoking, even in the absence of pre-existing respiratory disease, and that these effects depend on defined characteristics associated with higher individual susceptibility to adverse respiratory impact of e-cigarette use.

An analysis of possible effects of smoking behaviour on exhaled nitric oxide (FeNO) showed significantly reduced values associated with conventional cigarette smoking as well as typical changes in this marker due to further influencing factors such as the presence of respiratory allergy, recent respiratory tract infection, sex, and age. Taking these factors into account, however, no significant additional effects of e-cigarette use were observed, and this was also the case when only subjects with or without respiratory allergies or bronchial asthma were considered. This indicates that potential effects of e-cigarette use on FeNO may play only a minor role in the spectrum of other everyday influences.

In a further study of a possible correlation between e-cigarette use and medical treatment for respiratory diseases, a higher proportion of patients tended to be treated for chronic bronchitis or COPD among e-cigarette users than among non-users. However, this was not observed for bronchial asthma. These findings are consistent with the previously described observation of a lack of correlation with FeNO in terms of asthma and respiratory allergies. Regarding the tendency towards a positive association with current medical treatment for COPD, which on the one hand might be interpreted as an indication of respiratory deterioration due to e-cigarette use, it must also be considered that this may partly be due to the fact that the affected patients are trying to reduce their conventional cigarette smoking by partially switching to e-cigarettes, as observed in other studies.

An investigation of the possible effects of e-cigarette use on spirometric indices taking into account the status of conventional cigarette smoking showed no significant effects on the z-scores for FEV1 and FVC, but a negative trend for the obstruction marker FEV1/FVC. For expiratory flow rates, stronger negative effects were observed, and this was most pronounced for FEF75. Quantitatively, the additional adverse effect of e-cigarette use was about ten times lower than the effects of conventional cigarette smoking, and these effects were driven by associations in participants without diagnosed respiratory disease. These observations suggest that e-cigarettes have detectable adverse effects on

pulmonary mechanical indices beyond the impact of conventional smoking, which can most probably be interpreted as mild alterations of peripheral (i.e. small) airways.

Whether these effects are reversible when e-cigarette use is discontinued and whether they show progression over a longer period of use, also taking into account parallel use of conventional cigarettes, will be investigated on the basis of longitudinal data from the follow-up examinations of the participants that have recently been obtained.

TLRC subproject: The aim of this subproject was to identify potential, automatically derived imaging-based biomarkers on whole-body magnetic resonance imaging (MRI) for the effects of e-cigarette use. This required establishing a deep learning-based method for fully automated 3-dimensional delineation of the lungs on thoracic and whole-body MRI examinations from two large nationwide cohort studies: the COSYCONET cohort of patients with chronic obstructive pulmonary disease (COPD) and the population-based German National Cohort (NAKO). The project was carried out in collaboration with the Department of Diagnostic and Interventional Radiology of the Medical Center at the University of Freiburg and the Division of Medical Image Computing at the German Cancer Research Center.

MRI is a non-invasive imaging method that is increasingly being used in population-based cohort studies around the world and that can serve as a radiation-free alternative or provide additional information compared with computed tomography for thoracic imaging in certain scenarios. We utilised available thoracic MRI data of ~500 participants of the COSYCONET study and ~11.000 participants of the NAKO study in a cross-validation approach for our project. To enable the analysis of structural alterations of the lungs based on this imaging data, a deep learning-based algorithm using the nnU-NET architecture in an ensemble configuration of five networks was trained to automatically separate the lung parenchyma from surrounding tissue on axially acquired T1-weighted MRI sequences in inspiratory breath-hold on a voxel-by-voxel basis (lung segmentation). To achieve this, voxel-accurate manual annotation of right and left lungs was first carried out in a small number of ~15 datasets per study by two medical experts and used as initial training data for the algorithm. Resulting automatic segmentations were then iteratively improved based on computationally derived measures of segmentation uncertainty, manual review, and subsequent revision of segmentation masks in select cases and re-training of the algorithm.

To avoid distortion or falsification of downstream analyses based on potentially undetected erroneous automatic lung segmentations, a novel approach to visual quality control of lung segmentations had to be established. Review of voxel-labelling on every single slice of cross-sectional imaging of the lungs is a time-consuming process. To minimise the amount of time required for human expert review, a single coronal and axial projection image of the segmentation masks was created per dataset. This way, only two images instead of a complete cross-sectional image stack had to be inspected. Different projection methods and the use of colour encoding for improved detection of segmentation errors were explored. The method allowed for rapid quality control within a few seconds per case with high diagnostic accuracy, underlining its usefulness for application in large-scale cohort studies. A manuscript describing the methods and respective diagnostic accuracies compared with the gold standard slice-based review is currently under peer review in an international scientific journal.

Finally, automatic segmentation of right and left lungs on MRI data of ~500 participants of the COSYCONET study and ~11.000 participants of the NAKO study was successfully carried out and validated. To derive quantitative imaging biomarkers, primarily based on lung volume and shape,

statistical shape models of the lungs were created. These enable quantitative description and comparison of lung shapes in relation to smoking status, parameters of lung function and anthropometric parameters. The respective analysis of promising imaging-based surrogate parameters is still being finalised. However, the derived lung segmentations and shape models enable further MRI-based morphological and functional analyses of the lungs beyond the initially planned investigations within the Balzan Prize project, and corresponding follow-up projects are already being planned.

Résumé

The Balzan Prize Project *Effects of Short- and Long-Term Exposure to E-Cigarette Vapour* aimed to improve our understanding of how e-cigarettes affect human health. To achieve this, scientists from all five DZL sites employed complementary scientific models and resources.

The specific goals were:

- To identify the early morphological and transcriptional changes in the airways of the fruit fly (*Drosophila melanogaster*), potentially leading to reduced survival in the context of known risk alleles for airway disease.
- To elucidate the mechanisms by which short-term e-cigarette exposure causes acute respiratory failure, and to characterize the long-term effects on pulmonary integrity.
- a) To investigate associations between e-cigarette use and respiratory status within the population-based NAKO cohort (Nationale Gesundheitsstudie) and b) to assess structural alterations of the cardiorespiratory unit associated with e-cigarette use based on whole-body magnetic resonance imaging (MRI) scans within the NAKO cohort, in relation to respiratory function parameters.

Our experiments in the **fruit fly** model showed that maternal exposure to e-nicotine prior to conception significantly disrupted airway development in offspring, resulting in structural deformities, epithelial remodelling, and reduced stem cell activity. Although these findings cannot be directly extrapolated to humans, they highlight potential transgenerational risks associated with adolescent e-cigarette use and underscore the need for further epidemiological and mechanistic research on its long-term impact on offspring health.

In **murine lungs**, we found that e-cigarette vapour extract—particularly when containing nicotine—compromised endothelial barrier function and increased susceptibility to inflammation-induced damage. It also selectively affected immune cell trafficking by reducing $CD4^+$ T-cell migration. Activation of the TIE2 pathway mitigated some of these adverse effects, suggesting a potential therapeutic strategy to protect vascular integrity under inflammatory conditions linked to e-cigarette exposure. Moreover, nicotine and specific flavouring components contributed to endothelial dysfunction, lung inflammation, and structural damage, whereas TIE2 pathway activation appeared protective against these effects.

Our **epidemiological analyses** within the German NAKO cohort indicated that e-cigarette use exerts measurable, though relatively modest, adverse effects on lung mechanics and respiratory symptoms – effects that extend beyond those observed with conventional cigarette smoking. Future longitudinal follow-up studies will determine whether these effects are reversible or progressive with continued e-cigarette use and concurrent tobacco consumption.

The **imaging subproject** successfully achieved automatic segmentation and validation of MRI data for all COSYCONET and NAKO participants. Statistical lung shape models were generated to quantify differences in lung volume and geometry in relation to smoking behaviour, lung function, and body characteristics. Analysis of these potential imaging biomarkers is ongoing. The resulting lung segmentations and shape models also provide a valuable foundation for future MRI-based morphological and functional studies of the lungs beyond the scope of the current project.

As members and leaders of the German Center for Lung Research we are convinced from our own and other data that e-cigarette smoking poses a significant public health concern. While tobacco manufacturers promote e-cigarettes as a healthier alternative to tobacco smoking, our results demonstrate that components of e-cigarette vapour have profound physiological effects. We therefore intend to pursue our research on e-cigarettes beyond the scope of the Balzan Prize project.

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