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Effects of e-cigarettes and vaping devices on cardiac and pulmonary physiology

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Abstract

E-cigarette aerosols are exceedingly different from conventional tobacco smoke, containing dozens of chemicals not found in cigarette smoke. It is highly likely that chronic use of e-cigarettes will induce pathological changes in both the heart and lungs. Here we review human and animal studies published to date and summarize the cardiopulmonary physiological changes caused by vaping. In terms of cardiac physiology, acute exposure to e-cigarette aerosols in human subjects led to increased blood pressure and heart rate, similar to traditional cigarettes. Chronic exposure to e-cigarette aerosols using animal models caused increased arterial stiffness, vascular endothelial changes, increased angiogenesis, cardiorenal fibrosis and increased atherosclerotic plaque formation. Pulmonary physiology is also affected by e-cigarette aerosol inhalation, with increased airway reactivity, airway obstruction, inflammation and emphysema. Research thus far demonstrates that the heart and lung undergo numerous changes in response to e-cigarette use, and disease development will depend on how those changes combine with both environmental and genetic factors. E-cigarettes have been advertised as a healthy alternative to cigarette smoking, and users are under the impression that vaping of e-cigarettes is harmless, but these claims that e-cigarettes are safer and healthier are not based on evidence. Data from both humans and animal models are consistent in demonstrating that vaping of e-cigarettes causes health effects both similar to and disparate from those of cigarette smoking. Further work is needed to define the long-term cardiopulmonary effects of e-cigarette use in humans.

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MuChun Tsai and Min Kwang Byun contributed equally to this study.

Author contributions

M.C.T., M.K.B., J.S. and L.E.C.A. contributed to the design of the work; M.C.T., M.K.B. and J.S. reviewed references and drafted the initial article; M.C.T., M.K.B., J.S. and L.E.C.A. wrote, edited and revised the article. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. L.E.C.A. agreed to serve as corresponding author for the work.

Competing interests

The authors declare that they have no competing interests.

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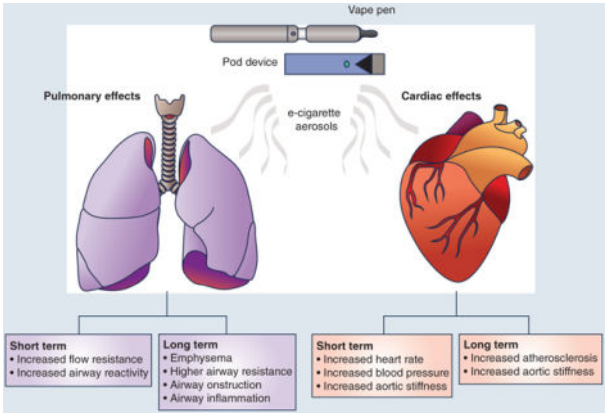
Graphical Abstract



Abstract figure legend Data across both human and animal studies demonstrate that daily inhalation of e-cigarette or vaping device aerosols on a chronic basis will cause significant cardiopulmonary disease. The data are stronger for short-term (months to years) effects, with the majority coming from human studies of acute (minutes to hours) and sub-acute (weeks to months) e-cigarette use. There are no data on long-term (years to decades) health effects in human subjects, because these devices have only been regularly used for the last few years; however, the data in animal models consistently demonstrate adverse effects on both cardiac and pulmonary physiology with long-term exposures. Because of the multitude of e-cigarette and vaping devices (cig-a-likes, vape pens, box Mods, pod devices, etc.) and the wide range of chemicals found within e-liquids, of which there are thousands on the market, data across studies is difficult to compare. Further detailed studies are needed to better define the long-term health effects of these popular devices, with an emphasis on defining the specific chemicals and devices associated with untoward physiological effects.

Keywords

animal models; cardiac function; cardiopulmonary physiology; clinical research; e-cigarette; inflammation; lung function; nicotine; vaping

Introduction

Conventional cigarette smoking is well-known to cause deleterious effects on both the cardiac and pulmonary systems, contributing to significant morbidity and mortality worldwide (Crotty Alexander *et al.* 2015*b*; Shin & Crotty Alexander, 2016). Electronic (e)-cigarettes, a relatively new product on the market, is an electronic device of variable design that heats up and aerosolizes e-liquids that most often contain nicotine, propylene glycol, glycerin and various flavorant additives. These devices are widely popular, with approximately 9% of the total U.S. population vaping and 16–28% of teenagers and young adults vaping (Miech *et al.* 2019; https://www.cdc.gov/tobacco/basic_information/e-cigarettes/about-e-cigarettes.html). Cigarette smoking has dramatically fallen in the US, with a total of 14% of the population smoking conventional tobacco, with some populations more heavily affected than others (23% of American Indians and Alaskan

Natives, 19% of non-Hispanic mixed race heritage, and 15% of both non-Hispanic Blacks and Whites) (https://www.cdc.gov/tobacco/data_statistics/fact_sheets/#fast-facts). Many cigarette smokers actively try to replace their use of conventional tobacco products with e-cigarettes because these vaping devices are marketed as a safer and healthier alternative to smoking, although these claims are not based on evidence.

The chemical profiles of e-cigarette aerosols are almost entirely different than cigarette smoke, with nicotine being one of the only chemicals consistently found in both inhalants. E-cigarette aerosols generally contain fewer toxic chemicals than traditional cigarette smoke (Eaton, 2018), but have been found to contain harmful substances including heavy metals, volatile organic compounds and cancer-causing chemicals (Kosmider *et al.* 2018; St Helen *et al.* 2020; Zhao *et al.* 2020). The short-term (weeks to months) effects of e-cigarette use, such as e-cigarette or vaping product associated lung injury (EVALI), are becoming more apparent as use of these devices with both nicotine and tetrahydrocannabinoids (THCs) escalates worldwide (WHO, 2019). However, the long-term (decades) effects of e-cigarette use are still unknown. There is hope that bench and translational research studies will identify health effects prior to recognition by epidemiological studies, and thus there is an urgent need to define what the acute and chronic effects of e-cigarette use will be on both pulmonary and cardiac systems.

Researchers have been racing to acquire data on e-cigarette effects on lung function and inflammation (Crotty Alexander *et al.* 2018), addiction (Alasmari *et al.* 2017), sleep (Boddu *et al.* 2019), renal disease (Crotty Alexander *et al.* 2018) and host defences (Hwang *et al.* 2016; Ghosh *et al.* 2019; Madison *et al.* 2019; Corriden *et al.* 2020), but unfortunately more work is needed before we will be able to draw definitive conclusions about the long-term effects on human health. Because e-devices have evolved rapidly and human use patterns have shifted every 12–24 months in response to the introduction of new devices, new flavours and aggressive marketing, research on vaping has been challenging (Kaisar *et al.* 2016; Hawk & Colbert Maresso, 2019). The first generation of e-cigarettes was designed to be disposable and mimicked the appearance of traditional cigarettes. However, as second and third-generation devices developed, e-cigarettes became rechargeable and contained refillable cartridges, where users could modify the contents of the e-liquid as well as alter the power or wattage of the device to modify the aerosol that was produced. The newest generation of e-cigarettes called pod-devices, are smaller, sleeker and utilize pod cartridges filled with e-liquid that utilize nicotine salts rather than free base nicotine used in the previous generations. The move to nicotine salts by e-liquid manufacturers allows for higher concentrations of nicotine to be inhaled with less irritation in comparison to free base nicotine, because this form of nicotine is paired with benzoic acid and has a lower pH (Harvanko *et al.* 2020).

The sheer number of chemicals utilized in vaping liquids is one of the greatest challenges for researchers, as none outside of nicotine have been studied for their health effects prior to the invention of the modern e-cigarette in 2003. There are over 1000 flavours available on the market, each of which has a different chemical profile. E-cigarette users (vapers) have also started incorporating other substances, such as THCs or CBD oil, into e-liquids. In December 2019, vitamin E acetate (VEA), an additive in THC-containing vaping products,

was identified as the likely causal agent for the EVALI outbreak. VEA was found in the majority of bronchoalveolar lavage fluids obtained from individuals with EVALI, and has been shown to induce lung injury in a mouse model (Bhat *et al.* 2020; Blount *et al.* 2020; Crotty Alexander *et al.* 2020). Due to the lack of regulation of e-cigarettes and e-liquids, these devices and their aerosols contain known and unknown harmful chemicals that can lead to deadly consequences (Perez & Crotty Alexander, 2020).

E-cigarettes only recently entered the international market in 2007 and thus epidemiological data on health outcomes from long-term vaping will take decades to acquire. Across research studies to date, >90% of e-cigarette users report vaping the devices for <4 years, with >80% of users vaping for <1 year (Lechner *et al.* 2015). Thus, to define the long-term health effects of vaping, we must rely on detailed and well controlled animal exposure models and extrapolation of unbiased acute and sub-acute exposure data from randomized, controlled human subject studies with appropriate controls. Herein, we review what is known thus far about the physiological effects of e-cigarettes on vital mediators of structure, function and inflammation and oxidative stress on the cardiopulmonary system (Fig. 1).

Methods

To identify original science peer-reviewed manuscripts pertaining to e-cigarette effects on human cardiac and lung physiology, the PubMed database was searched on July 1, 2020 using multiple searches with combinations of specific terms for each area of review. Because modern e-cigarettes were first invented in 2003, searches were limited to 2000–2020. For lung physiology, search terms included e-cigarettes, electronic cigarettes, lung physiology, respiratory function, lung function, spirometry, airway resistance, methacholine, airway hyperreactivity, lung mechanics, inflammation, emphysema and pulmonary fibrosis. For human cardiac physiology, the search terms used were electronic cigarettes, e-cigarettes, vaping, JUUL (the most popular pod-based vaping device 2017–2020), IQOS (a heat, not burn, tobacco device), heart, cardiac, cardiovascular, heart function, blood pressure, and ejection fraction. Searches were then limited to humans to separate out animal-based research. Tobacco company funded or influenced research (as identified in affiliations and conflicts of interest sections) was reviewed but excluded from discussion, because of the inherent bias of such studies, which has been shown to lead to results and conclusions skewed in the tobacco industries' favour (Bero *et al.* 2005; Capps, 2016; Velicer *et al.* 2018). However, these tobacco industry-influenced papers are included in the tables with methodological details and key findings, to allow readers to draw their own conclusions.

Effects of e-cigarette use on the pulmonary system

Chemicals within e-cigarette aerosols first come into contact with the airways, prior to absorption into the bloodstream where they come in contact with vascular endothelial cells while circulating to reach the other organ systems, including the heart. Thus, a great many of the research studies published to date have focused on the direct effects of e-cigarette aerosols on the pulmonary system. Because vaping devices are a recent phenomenon, the majority of data in humans is from acute (hours to days) or sub-acute (weeks to months) exposures, assessed via cross-sectional not longitudinal studies. Thus, we are highly reliant

on animal studies for understanding and defining the chronic effects of vaping on lung function. In addition, many e-cigarette users, especially adolescents and young adults, are never-smokers, such that many studies are not designed to directly compare the impact of vaping *versus* conventional tobacco smoking, since it is not relevant to this population of e-cigarette users who are never smokers. This is reasonable as e-cigarette use will have effects disparate from those of conventional tobacco, due to the fact that e-cigarette aerosol chemical profiles are unlike those of tobacco smoke except for nicotine, and restricting research studies to effects known to occur in the setting of smoking will lead to a limited understanding of health effects of vaping. For example, vaping led to the novel disease EVALI, which has never been seen with conventional tobacco use.

Human studies.

Fifteen studies assessing the effects of vaping on pulmonary physiology in human subjects were identified, six of which had ties to the tobacco industry (Table 1). Four of these studies utilized short (5 min) exposures to assess acute effects of e-cigarette vapour inhalation on lung physiology. These studies provide evidence that the inhalation of e-cigarette aerosols for even 5min can alter airway flow resistance, as measured by impulse oscillometry, a test that can be used to diagnose obstructive lung disease (Vardavas *et al.* 2012; Antoniewicz *et al.* 2019). Also, inhalation of e-cigarette aerosols for 30 min, both with and without nicotine, was found to increase the fraction of exhaled nitric oxide (FeNO) and decrease vital capacity (VC), suggesting immediate effects of non-nicotine chemicals on lung function and inflammation (Antoniewicz *et al.* 2019). In comparison to inhalation of tobacco cigarette smoke actively or passively by non-smokers for 30–60 min, which led to decreased FEV1/FVC (by 7.2%, $P < 0.001$ for active smoking and 3.4%, $P < 0.01$ for passive smoke inhalation) (Ferrari *et al.* 2015), acute inhalation of e-cigarette aerosols by non-smokers for 30–60 min did not change lung function as measured by spirometry (Vardavas *et al.* 2012; Flouris *et al.* 2013; Antoniewicz *et al.* 2019). However, in smokers, Ferrari *et al.* determined that exposure for 5 min to a nicotine-free e-cigarette led to reductions in FEV1 and FEF25 similar to that seen with conventional tobacco smoke (Ferrari *et al.* 2015). Chaumont *et al.* took a different approach, by assessing the effect of short-term e-cigarette cessation on regular e-cig vapers. They found that an acute vaping cessation for 5 days led to increased forced expiratory flow 25% (FEF25%), suggesting an improvement in lung function via decreased airway resistance due to cessation of vaping. Thus, these studies demonstrate that even short-term exposure to e-cigarette vapour leads to increased airway resistance and inflammation, which may be reversible with cessation of vaping (Chaumont *et al.* 2018).

On the molecular level, Staudt *et al.* assessed 10 healthy never-smokers before and after acute exposure to e-cigarette aerosols (Staudt *et al.* 2018) and found significantly higher levels of endothelial microparticles (EMPs) post-exposure to nicotine containing e-cigarette aerosols. Genome-wide expression profiles were assessed by mRNA-sequencing from small airway epithelium and alveolar macrophages, and it was found that e-cigarette users had altered activation of *p53*-dependent signalling, which is worrisome in that *p53* is one of the most important and well-characterized tumour suppressor genes (Stegh, 2012). Several genes affected by e-cigarette exposure are known to have significant roles in macrophage physiology and pulmonary health, including forkhead box M1 (*FOXM1*), coronin 1A

(*CORO1A*) and prostaglandin E receptor 3 (*PTGER3*). This study suggests that even limited, acute exposure to e-cigarette aerosols dysregulates the biology of the small airway epithelium, alveolar macrophages and lung capillary endothelium. These changes across pulmonary cells portend the development of lung pathology such as chronic obstructive pulmonary disease (COPD) and interstitial lung diseases (ILD).

Cigarette smoking is well-known to exacerbate airway reactivity and inflammation in asthma; however. this has not acted as a deterrent to asthmatics, who consistently have higher smoking rates than non-asthmatics across the US (https://www.cdc.gov/asthma/asthma_stats/people_who_smoke.htm). This holds true for e-cigarette vaping as well, with rates of e-cigarette use higher in the asthmatic population *versus* non-asthmatics (Reid, 2018). Thus, research into the effects of e-cigarette use in the setting of asthma is a priority (Bousquet *et al.* 2016). Lappas *et al.* (2018) investigated the immediate respiratory effects of e-cigarette use in combustible cigarette smokers with and without asthma and determined that a single 5 min session of e-cigarette use had effects on respiratory mechanics, as well as inflammatory effects, measured immediately after vaping (Table 1). These physiological and inflammatory effects were more prominent in asthmatics, suggesting that inhaling the 100+ chemicals within e-cigarette aerosols has greater detrimental effects on asthmatic airways relative to non-asthmatics (Garcia-Gomez *et al.* 2016).

The studies with the longest e-cigarette use or exposure published to date all assessed effects of e-cigarette exposure in the setting of previous or current combustible cigarette smoking. Cibella *et al.* (2016) assessed spirometry and respiratory symptoms in smokers invited to quit or reduce their cigarette consumption by switching to e-cigarettes over 1 year. They found improvement in FEF25–75% among those who completely gave up cigarette smoking, whether or not they continued using e-cigarettes. FEF25–75% is also known as maximum mid-expiratory flow and has not been found to be clinically useful in detection of small airways disease (Quanjer *et al.* 2014). Because the sole change in lung function detected was the FEF25–75% and because there was no control group of non-e-cigarette subjects, the clinical relevance of these data on the sub-acute effects of e-cigarette aerosol inhalation is low. In the longest study to date, Bowler *et al.* (2017) analysed e-cigarette use in two large observational cohorts of current and former smokers (SPIROMICS and COPDGene) including older current and former conventional cigarette smokers. SPIROMICS enrolled 2982 subjects aged 40–80 years who either had at least 20 pack-years of conventional cigarette smoking ($n = 2780$) or were never-smokers ($n = 202$). COPDGene enrolled 10,294 subjects aged 45–80 years who either had at least 10 pack-years of conventional cigarette smoking ($n = 10\ 192$) or were never-smokers (≤1 pack-year lifetime; $n = 102$). Both cohorts were asked about e-cigarette use beginning in 2014, such that this study includes data across 2 years of use (2014–2016). Bowler *et al.* determined that the use of e-cigarettes across cohorts was associated with greater loss of lung function, higher nicotine exposure, and higher risk of exacerbations (Table 1).

Overall these studies of acute and sub-acute e-cigarette exposure and use in human subjects suggest that vaping will worsen both asthma and COPD, induce pathological changes in the small airways, and dysregulate innate immunity in the lung (Table 1). These clinical and physiological effects induced by vaping may be less than those seen with conventional

tobacco, but because we as yet only have acute and sub-acute studies to draw conclusions from, it is clear that more studies, especially longitudinal studies, are needed to better understand the effects that e-cigarette use will have on pulmonary physiology. If e-cigarettes follow in the footsteps of conventional tobacco smoking, it may take years to decades before profound physiological changes occur with use. However, the fact that physiological changes are evident with acute and sub-acute exposures makes it clear that e-cigarettes will cause substantial physiological changes with chronic exposures.

It is important to study multiple components within e-cigarette aerosols, because while nicotine is a cholinergic drug and may have effects that directly mirror those seen in conventional tobacco use, THC activates different molecular pathways, and will have different effects on airway epithelium, smooth muscle cells and overall lung function. Furthermore, it is completely unknown what airway effects other chemicals in e-cigarette aerosols, including those used as flavorants and vehicles, will have. Because of their ability to model long-term, chronic inhalant exposures, animal studies will be the key to defining physiological effects of chronic inhalation of these chemicals and others on the lungs, before epidemiological studies are possible in humans over the course of decades of use.

Animal studies.

By utilizing animal models, researchers have the ability to more rapidly acquire data regarding the long-term health consequences of chronic e-cigarette use, relative to waiting decades for longitudinal human studies to be conducted. Murine models range from whole-body to nose-only exposure systems, where mice are exposed to and inhale e-cigarette aerosols. The majority of these models were originally designed for cigarette smoke exposures (10–12 mg nicotine per cigarette) and were adapted for e-cigarette aerosol exposures (typically 3–59 mg ml^{-1} nicotine within e-liquid). Mice are typically exposed to aerosols for 20–60 min per day to mimic human use (Table 2). To date, only four animal model studies on e-cigarette aerosol effects on lung physiology have been published, and all utilized whole-body exposure systems. These studies revealed an array of different pulmonary effects associated with what is considered acute (1–7 days), sub-acute (2–4 weeks) and chronic (2 months or more) e-cigarette aerosol exposures in humans (Table 2).

One sub-acute study by Glynos *et al.* (2018) found increased airway reactivity upon exposing male C57BL/6 mice to air, traditional cigarettes (10–12 mg ml^{-1}) or e-cigarettes with nicotine (18 mg ml^{-1}) plus flavour additives, nicotine alone, or no nicotine. The mice were exposed for 3 days (acute exposure) *versus* 4 weeks (sub-acute), during which they were exposed to inhalants 4 times a day for 7 min each, for a total of 28 min per day. Mice exposed to e-cigarette aerosols containing nicotine and flavouring had elevated protein, cellularity and lung oxidative stress markers, specifically malondialdehyde (MDA) and protein carbonyls, in bronchoalveolar lavage (BAL). The BAL cellularity increased primarily due to an influx of macrophages. The combined findings demonstrate increased inflammation and oxidative stress and indicate that pulmonary diseases such as asthma, COPD or acute interstitial pneumonia, will occur with chronic e-cigarette exposures. Interestingly, mice exposed to e-cigarette aerosols with nicotine and flavouring had similar increased airway reactivity with methacholine challenge compared to traditional cigarettes.

These data suggest that short-term e-cigarette exposure alone may increase airway reactivity to a similar degree as cigarette smoking, and that these effects are nicotine and flavour dependent. Because flavours are a core component of all e-liquids used in vaping devices, and nicotine is found in >95% of e-liquids, these data indicate that all e-cigarette users are at risk of developing airway reactivity (a key finding in asthma and COPD) with only short-term use of these drug delivery devices.

In a chronic exposure, Garcia-Arcos *et al.* (2016) exposed A/J mice to e-cigarette liquid with and without nicotine (18 mg ml^{-1}) via an aerosol nebulizer for 1 h per day, 5 days per week for 4 months. Mice whom inhaled the aerosolized (non-heated) e-liquid with nicotine had higher airway resistance by methacholine challenge, histological evidence of emphysema, increased BAL cellularity and elevated levels of IL-1β, MCP-1, Cxcl10, IL-6 and Cxcl2 in BAL. Thus, inhaling nicotine containing aerosols was associated with the development of airway obstruction and inflammation most similar to COPD. These results are comparable to the detrimental effects of traditional smoking (Crotty Alexander *et al.* 2015*a*).

The effects of traditional cigarette smoke exposure on infants are well-known, with proven associations with the development of asthma, wheeze, lower airways infections and sudden infant death syndrome (https://www.cdc.gov/tobacco/data_statistics/sgr/50th-anniversary/index.htm). It is also well-known that tobacco smoke consumption during pregnancy leads to poor birth outcomes and adversely impacts brain, lung and cardiovascular development (https://www.cdc.gov/tobacco/data_statistics/sgr/50th-anniversary/index.htm). However, many parents are vaping e-cigarettes without understanding the potential risk to their children. McGrath-Morrow *et al.* (2015) assessed effects of e-cigarettes on neonatal mice by exposing C57BL/6J mice to either nicotine (1.8%), no nicotine or air. The mice were exposed once a day for the first 2 days of life and then were exposed twice daily from days 3–9 of life. The mice exposed to e-cigarette aerosols both with and without nicotine gained significantly less weight over time relative to air control mice, suggesting that non-nicotine chemicals within these aerosols are impeding growth and weight gain by some pathological mechanism. Nicotine-exposed mice had larger air spaces (by mean linear intercept quantification) when compared to air control mice. These larger air spaces demonstrate alterations in lung structure, such as emphysema, which in this setting suggests that nicotine inhibits alveolar growth. By measuring KI67 levels through immunohistochemistry, nicotine-exposed mice were found to have significantly decreased levels, indicating impaired cell proliferation in the alveoli.

Water pipes used to smoke tobacco, such as hookah and shisha, have been in existence for over 400 years. Khan *et al.* (2019) evaluated the impact of both water pipe smoke (WPS) and e-cigarette aerosol exposures on male and female C57BL/6 mice over 3–10 days. Both inhalants altered expression of core clock and clock-controlled output genes, indicating disruption of the lung circadian rhythm. When comparing the two inhalant types, WPS exposure decreased the protein level of BMALI (by ELISA), and increased CLOCK and REV-ERBα (by western blot), while e-cigarette exposure increased protein levels of BMALI and PER2 (by western blot). Comparing gene expression, WPS exposure increased *Rev-erbα*, *Cry2* and *Rorα*, while e-cigarette exposure increased *Bmal1, Clock* and *Per2*. The alterations in gene expression and protein levels detected confirm that both WPS and

e-cigarette aerosols cause substantial effects on clock dependent lung pathophysiology, but that the effects of these disparate exposures are not identical and may be affected by post-translational modifications. The changes in both protein levels and gene expression predict pathological changes in both immune and inflammatory responses within the lungs in response to both inhalants.

These four murine studies provide evidence that lungs are negatively impacted by exposure to e-cigarette aerosols (Table 2). The largest signals were seen in the increase in oxidative stress markers, cytokines and airway resistance, suggesting that chronic e-cigarette use will lead to diseases such as COPD, asthma and acute interstitial pneumonia. More long-term (chronic) studies are needed as only one study performed to date was 4 months in duration, but chronic murine models of cigarette smoke effects are often run for 4–6 months before consistent detection of COPD and fibrosis is assured. There may be a need to increase the intensity of the e-cigarette exposures from 20–60 min a day up to 120–180 min, because mice have an excellent nose filtration system such that the amount of aerosol reaching the lower airways and lung parenchyma may be lower than expected. Researchers must assess the quantity of e-cigarette aerosol reaching the lower airways, or the amount absorbed into the bloodstream, to ensure that exposures are accurately modelling human use. Thus, as e-cigarette devices and e-liquids evolve over time, models used by researchers to study them will also need to evolve, to obtain the most relevant data as to their acute and chronic effects on pulmonary health. Thus, conducting animal research on e-cigarette aerosol effects on lung physiology is fraught with challenges, but remains the most likely path by which we will be able to define the long-term health effects of these vaping devices.

Effects of e-cigarette use on the cardiac system

Human studies.

Conventional cigarette smoking has various effects on the cardiovascular system, and there has been much work done to determine the potential effects of e-cigarettes on this system (Table 3). Multiple studies have been conducted on the acute effects of e-cigarette use on cardiac physiology, including heart rate (HR) and blood pressure (BP). Smokers who were given e-cigarettes with at least 18 mg ml^{-1} of nicotine were noted to have increased HR in four separate acute e-cigarette exposure studies (Vlachopoulos *et al.* 2016; Chaumont *et al.* 2018; Franzen *et al.* 2018; Kerr *et al.* 2019). Systolic blood pressure (SBP) was not significantly increased after e-cigarette use, although differences were found in diastolic blood pressure (DBP) across studies, and elevations in DBP are associated with increased cardiovascular disease risk and mortality, particularly in women (Wingfield *et al.* 2005). Vlachopoulos *et al.* (2016) determined that carotid-femoral pulse-wave velocity (PWV) increased after vaping an e-cigarette for 30 min to a similar degree as induced by smoking a traditional cigarette, but the vaping effect was more prolonged. One weakness of this study was the use of a 30 min vaping session relative to smoking one cigarette. The long vaping session may have led to exaggerated effects on PWV in the e-cigarette cohort.

In one cross-over study, active cigarette smokers underwent three acute exposures: e-cigarette with nicotine, nicotine-free e-cigarette, and conventional cigarette. In this study, inhalation of nicotine (via e-cigarette or conventional cigarette) led to increased SBP of

more than 3% compared to baseline, while no changes were detected in the nicotine-free e-cigarette group (Franzen *et al.* 2018), which supports the previous findings by Vlachopoulos *et al.* (2016). A cross-sectional study of non-smokers, cigarette users, e-cigarette users and dual cigarette and e-cigarette users by Fetterman *et al.* (2020) revealed that the augmentation index – another measure of vascular stiffness – was similar between cigarette smokers, e-cigarette users and dual users. They also noted that endothelial cells obtained from cigarette smokers and sole e-cigarette users produced less nitric oxide when stimulated compared to non-smokers, suggesting impaired endothelial nitric oxide synthase signalling by both inhalants. In a separate cross-over study looking at the effects of e-cigarette use on endovascular changes, occasional cigarette smokers (maximum of 10 cigarettes per month) were exposed to e-cigarette aerosol or not. Endothelial progenitor cells were noted to be significantly increased after e-cigarette exposure, and these changes were persistent at 4 h (Antoniewicz *et al.* 2016). Endothelial progenitor cells originate from the bone marrow and possess the ability to repair endothelial dysfunction and are often used as a biomarker of endothelial function to assess cardiovascular risks in human subjects, such as acute myocardial infarction, revascularization or death from a cardiovascular cause (Werner *et al.* 2005). The data thus far demonstrate that short-term (acute) inhalation of e-cigarette aerosol induces acute cardiovascular changes including increased HR, DBP, PWV and endothelial progenitor cells in blood. Some of these are identical to changes induced by smoking conventional tobacco. These changes are consistent with the development of significant downstream cardiovascular disease, whichmay be secondary to e-cigarette use.

Longer studies in humans have also been conducted. George *et al.* (2019) conducted a prospective, randomized control trial on cigarette smokers without cardiovascular disease. Subjects were randomly assigned to 2 groups: e-cigarette with nicotine and e-cigarette without nicotine with a parallel arm for individuals who were unwilling to stop cigarette smoking for a month. The flow-mediated dilatation, which is used to assess endothelial function, showed some improvement in the e-cigarette groups compared to cigarette smokers. However, there was no difference noted among the 3 arms in regard to HR, PWV and platelet reactivity, except in certain subgroups. They found that smokers with a <20 pack-year history had an improvement in vascular stiffness and increased HR in the e-cigarette groups compared to the cigarette smokers group. In comparison, the smokers with a >20 pack-year history did not have any change in vascular stiffness and there was a noted decrease in HR in the e-cigarette users compared to cigarette smokers. These findings suggest that a switch to e-cigarette use does not improve vascular stiffness in long-term smokers. In another study, 40 smokers without any cardiovascular disease were randomized to smoke either conventional cigarettes or e-cigarettes for 4 months. PWV was found to decrease in smokers who switched to e-cigarettes in comparison to an increased PWV in the subjects who continued to smoke cigarettes (Ikonomidis *et al.* 2020). They also did not observe any changes in platelet function markers compared to baseline in the e-cigarette group, whereas the cigarette group had further impairment noted. Together these sub-acute e-cigarette exposure studies demonstrate that in some subsets of cigarette smokers, a switch to e-cigarettes may lead to improvements in cardiovascular health (decreased vascular stiffness and HR), while other smokers will not benefit from switching. Further work is

needed to determine the mechanisms underlying different responses to switching between tobacco products.

Because the nicotine within e-cigarette aerosols is the main tie to cigarette smoke, multiple research groups have assessed the effects of inhaled nicotine in non-smokers. Multiple groups detected cardiovascular differences when e-cigarette aerosols containing nicotine were inhaled for an acute period, but not when nicotine-free aerosols were inhaled (Fogt *et al.* 2016; Moheimani *et al.* 2017; Chaumont *et al.* 2018; Antoniewicz *et al.* 2019). In particular, HR, SBP, DBP and PWV were altered in subjects who acutely inhaled nicotine-containing e-cigarette aerosols. The one study that assessed HR variability found a sympathetic predominance in those individuals exposed to e-cigarettes containing nicotine, a pattern associated with increased cardiac risk in populations with and without known cardiovascular disease (Moheimani *et al.* 2017). A similar cross-over study by Mobarrez *et al.* (2020) observed increased platelet and endothelial derived extracellular vesicles 4 h following exposure to inhalation of e-cigarettes with nicotine, which is consistent with activation of platelets by acute e-cigarette exposure. In another crossover study (Chaumont *et al.* 2018), occasional smokers were randomized to short-term (acute) exposure to high-wattage nicotine-free e-cigarette use, e-cigarette use with nicotine and sham use. High-wattage e-cigarettes are often used by individuals to increase the flavour and generate a larger, denser aerosol, but the higher wattage also increases the heat generated by the coil and utilizes more e-liquid. The data revealed that an hour after vaping with nicotine, acetylcholine-mediated vasodilatation was decreased compared to sham and NF e-cigarette use. This suggests that nicotine alters vasodilatation and decreases prostaglandin production in the vascular endothelium via an increase in oxidative stress.

The data from human subjects thus far demonstrate that e-cigarettes are not less harmful than conventional tobacco since >99% of e-liquids contain nicotine, and nicotine itself has a significant impact on cardiovascular physiology (Table 3). Both acute and sub-acute exposures to e-cigarette aerosols containing nicotine were found to alter HR, BP and PWV in patterns that are consistent with increased risk of developing cardiovascular disease with chronic use. This is highly worrisome for the long-term health of the thousands of adolescents and young adults vaping this addictive chemical.

Animal studies.

With the lack of long-term studies to define the chronic cardiovascular effects of e-cigarettes in humans, we are reliant on the eight animal studies published to date (Table 4). Shi *et al.* (2019) performed echocardiographic measurements in C57BL6 mice after sub-acute exposures to e-cigarette aerosols (14 days) and found no significant change in ejection fraction (EF), left ventricular (LV) size or aorta dimension. They did detect an increase in CD31, an endothelial and angiogenesis marker, in cardiac and kidney tissue of female and male mice after e-cigarette exposure, suggesting that sub-acute exposures cause tissue damage leading to recruitment of leukocytes into these tissues. The authors used a high concentration of nicotine, 24 mg ml^{-1}, which is higher than that most commonly used in third-generation customizable e-cigarette devices, such as box Mod devices (3–6 mg ml^{-1}), but lower than that used in the newest generation of e-cigarette pod devices (59 mg ml^{-1}).

The length of exposure was short (2 weeks lies in the acute range for mice) in comparison to other studies, which may explain the lack of statistically significant changes in cardiac function.

Olfert *et al.* (2018) conducted a chronic exposure of C57BL6 mice to e-cigarette aerosols, cigarette smoke, or filtered air for 1 h, 4–5 times a week for 8 months and found an increase in aortic stiffness in e-cigarette- and cigarette-exposed mice. Crotty Alexander *et al.* (2018) also conducted a chronic e-cigarette exposure study with both inbred C57BL/6 and outbred CD-1 mice for 3–6 months. E-cigarette-exposed mice developed increased SBP, decreased HR and cardiac fibrosis. Cardiac fibrosis was also detected by Mayyas *et al.* (2020) in 8–9 week old Wistar rats that were exposed to e-cigarette aerosol, cigarette smoke and waterpipe smoke for 4 weeks. In another study (Espinoza-Derout *et al.* 2019), male apolipoprotein E knockout (ApoE−/−) mice were exposed for 12 weeks to saline, nicotine-free e-cigarette aerosols, and e-cigarettes with 2.4% nicotine aerosol to assess for the development of atherosclerosis as well as changes in cardiac function. The nicotine dose used, 2.4%, is high for vape pen and box Mod e-devices, but low-normal for pod-based devices (which typically have 3–5% nicotine). Echocardiograms of these mice noted a decrease in LV shortening and ejection fraction (EF). The authors found evidence of ultrastructural abnormalities in cardiomyocytes, including shrunken nuclei with chromatin condensation and fragmentation (apoptosis), nuclear malformation with extensively convoluted nuclear membranes, myofibrillar derangement, thinning and destruction, intramyocardial lipid accumulation and mitophagy, which are associated with myocardial dysfunction. Mice exposed to e-cigarette aerosols also had an increase in atherosclerotic lesions compared to saline-treated mice.

In a study assessing vascular endothelial function in response to acute exposure (Nabavizadeh *et al.* 2018), anaesthetized rats were exposed via a nose cone to IQOS aerosol, smoke from tobacco cigarettes and clean air. Arterial flow-mediated dilatation (FMD), which is used as a measure of vascular endothelial function, was obtained before and after exposure, and the impairments caused by IQOS aerosol and tobacco smoke were found to be comparable. Rao *et al.* (2020) also detected impairment of FMD in 10-week old male and female Sprague-Dawley rats that were exposed to either Virginia Tobacco flavoured JUUL or tobacco cigarettes for 5 min compared to clear air. These studies utilizing animal models demonstrate that chronic e-cigarette exposure affects aortic stiffness, EF, atherosclerosis and vascular endothelial function, and thus confirm that long-term exposures to e-cigarettes are detrimental to cardiovascular health (Table 4).

Discussion

E-cigarette use is known to cause acute changes in cardiopulmonary physiology and is predicted to cause chronic changes in cardiopulmonary physiology based on animal models, and thus both short-term and long-term studies are vital. Multiple components of e-cigarette aerosols have been linked to airway hyperreactivity, such that it is decidedly unlikely for the inhalation of any of the chemicals contained in this aerosol to be safe for the airways. In particular, those who already have airway reactivity (sufferers of asthma, bronchiectasis and COPD) may have increased bronchospasm and poorer control of their disease in the setting

of e-cigarette use, due to irritant effects of multiple chemicals found within e-cigarette aerosols, including flavourings (Kosmider *et al.* 2016; Tierney *et al.* 2016). Data are mixed on whether e-cigarette use by adults will consistently cause parenchymal disease leading to alterations in lung compliance, such as emphysema or fibrosis. But it is highly possible that some chemicals within e-cigarette aerosols will induce pathological changes leading to emphysema, while other chemicals will trigger fibrotic changes. Exposure to e-cigarette aerosols *in utero* adversely affects alveolar development, which further supports the belief that e-cigarette use will alter lung function in adolescents and young adults. The lung undergoes a vast number of changes in response to e-cigarette use, and the development of lung disease will depend on how those changes interact with both environmental and genetic factors.

Studies of acute effects involving human subjects have most notably shown that e-cigarette exposures cause increased cardiac sympathetic responses, increased HR and BP. Measurements of arterial stiffness in human subjects have also increased even after a short exposure to e-cigarettes. These effects on cardiac physiology may be primarily caused by nicotine. This is not unexpected considering that nicotine is one of the major active constituents in e-liquids and is known to have significant effects on the cardiovascular system, including activation of the sympathetic system and inducing atherosclerosis (Lee & Cooke, 2011; Middlekauff *et al.* 2014). Data from chronic e-cigarette exposure models in animals suggest that long-term e-cigarette use will also increase arterial stiffness and induce angiogenesis. These effects may also be attributable to nicotine, which is known to induce vascular dysfunction Markers of vascular endothelial function impairment have also been observed, which may suggest increased risk of heart failure, although evidence of decreased heart function has been mixed in the animal studies. Furthermore, cardiac fibrosis has been discovered in animals with long-term e-cigarette exposure, and although the aetiology is still unclear, it can be implicated in various forms of cardiovascular disease, such as arrhythmias, atherosclerosis, hypertension and heart failure (Neunteufl *et al.* 2002). Overall, the chronic inhalation of the multitude of chemicals contained in e-cigarette aerosols will lead to many adverse effects on the cardiovascular system, with an overall increased risk of myocardial infarct and cerebrovascular accidents. These health effects of e-cigarettes may potentially be more diverse and detrimental than those caused by conventional tobacco, given the numerous chemicals within vaping aerosols and the interactions that are occurring amongst them, and the current lack of methods for capturing the broad range of variables at play (device type, flavorants, wattage, puff duration, etc.) compared to the relatively simple process of assessing conventional tobacco use via number of cigarettes per day, frequency of smoking sessions and menthol flavour *versus* none.

The aerosols produced by e-cigarettes, which may contain nicotine, marijuana or THC, is not water vapour and thus is destined to put stress on the cardiopulmonary system of e-cigarette users. This stress is new to the human body, which, apart from nicotine, has never been exposed to the chemicals found in e-cigarette aerosols in the millenia since homo sapiens came into existence. Beyond primary e-cigarette users, exposure to e-cigarette aerosols is also likely to affect bystanders through secondary exposure. While we wait for more adverse health effects of these drug delivery devices to become apparent in human users, we must continue conducting the best research possible to give the public, policy

makers and clinicians guidance as to what the future holds health-wise for vapers. It is imperative thatwe regulate these drug delivery devices to minimize the health effects as far as possible. Regulation of both the concentration of nicotine and the flavorants allowed within e-liquids would be very likely to lead to a decrease in the number of e-cigarette users, as the nicotine concentration is the driver of addictive potential while the flavorants are what appeal most to adolescents and young adults.

A major caveat to drawing firm conclusions from the currently available research studies is the difficulty in standardizing the products across studies. Each study used different e-devices, e-liquids containing different concentrations of nicotine, propylene glycol, glycerin and types of flavorants, and various e-cigarette exposures. The studies using mice included a range of breeds, ages and sexes, and methods of exposure to e-cigarette aerosols also varied (whole body *vs.* nose only), which may have lead to different results. Amongst the human population, men are more likely to report using e-cigarettes, but the different animal studies reviewed here used various combinations of genders – male only, female only or both sexes. In addition, research funded by tobacco companies or by authors heavily involved in pro-e-cigarette advocacy movements may produce biased findings (Pisinger *et al* .2019).For this review, we highlighted studies where findings were likely to be biased by funding and authors had significant conflicts of interest (Tables 1–4). Overall, more research is needed to define how each e-device and each e-cigarette aerosol will affect cardiopulmonary physiology. To determine how chronic vaping will affect health, detailed, long-term studies are needed using animal models, assessing each of the many vaping devices, and comparing them to air controls and to each other. These studies need to be 4–6 months in duration, use animals from multiple genetic backgrounds and the most common e-liquid components. Human research studies need larger cohorts, longitudinal assessments, inclusion of at-risk subjects (adolescents, young adults, pregnant and non-pregnant women and minorities) and evaluation of pulmonary and cardiac function. It is not possible to study all of the thousands of e-liquids, combined with the hundreds of e-devices, to determine the absolute impact first-hand use of these drug delivery devices will have on human health in the long term. However, by studying the most common components of e-liquids and the most popular e-devices, we may begin to unravel the pathophysiological effects of e-cigarettes and better inform users, physicians and policy-makers about their impact on human health.

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Biography

Dr MuChun 'Joanna' Tsai obtained her BSc in electrical engineering at University of Maryland, College Park and her MD at Ohio State University. She completed her internal medicine residency training and pulmonary/critical care fellowship training at the Ohio State University. She joined the faculty of the Ohio State University in 2019. When she is not seeing patients, her research involves the effects of electronic cigarette use on lung immunity and host defense when infected with respiratory pathogens.

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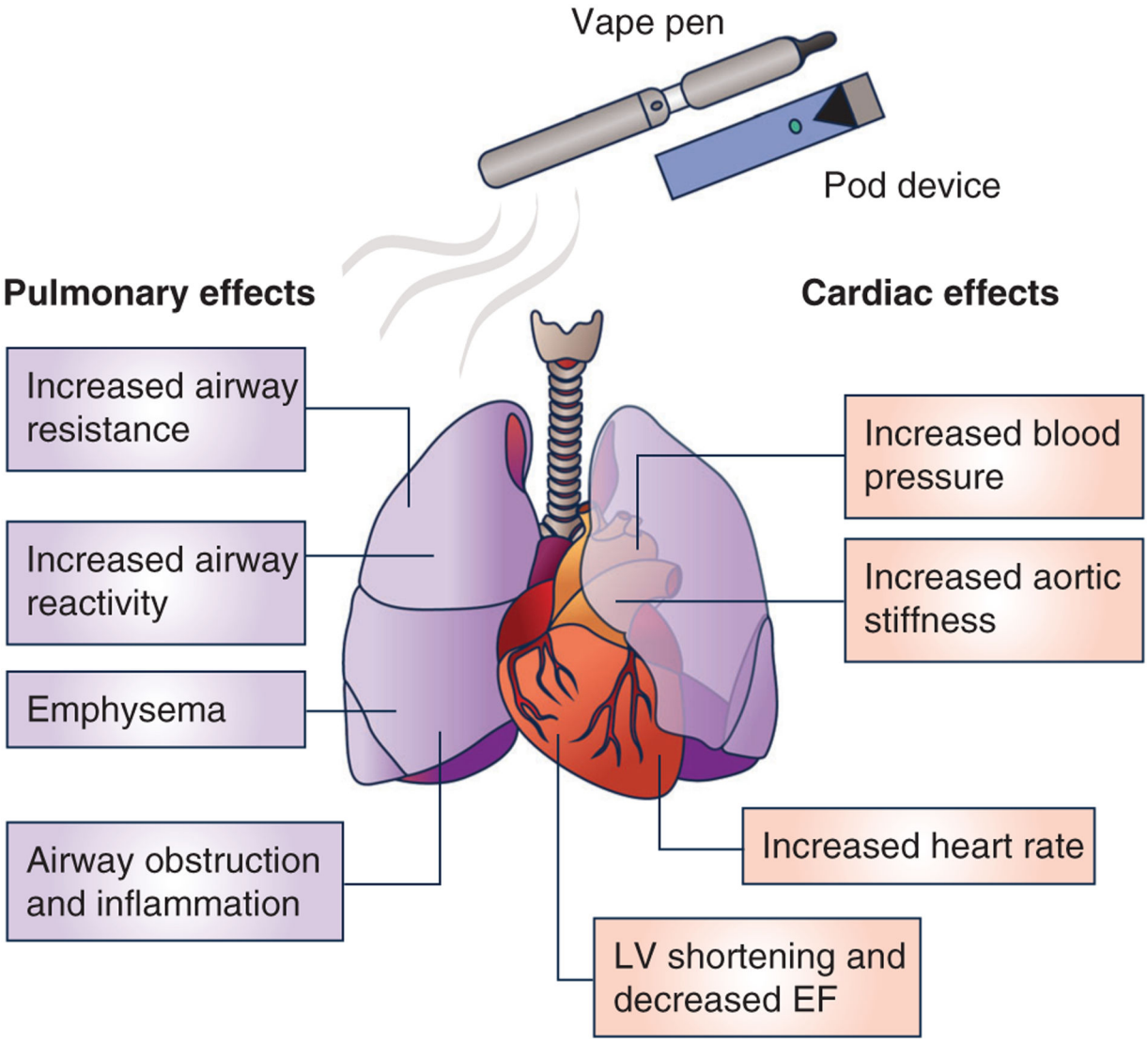

Figure 1.
E-cigarette research conducted to date, utilizing both humans and animal models, has defined a multitude of changes in cardiopulmonary physiology induced by the inhalation of e-cigarette aerosols

Table 1.

Studies assessing the effects of e-cigarette aerosol inhalation on human lung physiology to date

Author, year	Subjects	Study design and e-cig exposure	Key findings	Limitations
Antoniewicz, 2019	15 e-cig users (9 females. 6 males) Mean age 26 ± 3 years	Randomized, double-blinded, crossover design Inhaled 30 puffs of e-cig aerosol with or without nicotine during a 30-min period on two separate occasions. The wash out period was a minimum of 1 week. Lung function assessments – dynamic spirometry, impulse oscillometry (IOS), and fractional exhaled nitric oxide (FeNO) – were conducted 30 min after exposures.	• E-cig with nicotine led to increased resistance to air flow at 30 min post-exposure (at 5, 11, 13, 17 and 19 Hz), indicating obstruction of the conducting airways. • Resonance frequency decreased following inhalation of e-cig without nicotine. • FeNO increased at 2 h and vital capacity (VC) remained decreased after 2 h, following both e-cig aerosol exposures. • FEV1 did not change, indicating that the changes in airway resistance and air flow induced by e-cig aerosol exposures were not great enough to impact this measurement of pulmonary function.	Small sample size Acute e-cig exposure (30 min)
Chaumont, 2019	30 regular and exclusive e-cig users (average use of 38 ± 3 months) Mean age 38 ± 2 years Average past conventional cigarette use of 18 ± 2 pack-years	Randomized, investigator-blinded, three-period crossover study: **1** Nicotine session: regular vaping of e-cigs containing nicotine for 5 days (nicotine-session) **2** No nicotine session: nicotine-free-vaping for 5 days **3** Stop session: complete cessation of vaping for 5 days	• Forced expiratory flow-25% (FEF-25%) was higher in the stop-session compared with the nicotine-free-session. All other parameters of lung function and diffusing capacities were not modified by any of the three experimental sessions. • Short-term e-cig cessation in regular users increases CC16 (an anti-inflammatory protein) and FEF-25%, suggesting a slight improvement in lung health.	Only male participants enrolled Sub-acute exposure (5 days)
Lappas, 2018	54 smokers: • 27 healthy (healthy smoker group) • 27 with intermittent asthma (mild asthma group)	Smokers underwent a control session (no e-liquid, no resistor coil inside e-cig cartridge) and an experimental session of e-cig vaping using standardized puffing settings, each for 5 min. Measurements were obtained immediately after each inhalant session.	• Immediately post e-cig vaping, both asthmatics and non-asthmatics exhibited a significant increase in respiratory mechanics by IOS and inflammatory effects, which were more prominent in smokers with asthma. Specific changes detected by IOS: – increased respiratory mechanics total impedance at 5 Hz (Z5) – increased respiratory system resistance at 5 Hz (R5), 10 Hz (R10), and 20 Hz (R20)	Acute e-cig exposure (5 min)

Author, year	Subjects	Study design and e-cig exposure	Key findings	Limitations
			– increased resonant frequency and reactance area	
Polosa, 2018	48 COPD patients	22 e-cig users and 22 age- and sex-matched regularly smoking patients were followed for 36 months.	• Although there was no change in lung function, significant improvements were observed consistently in the e-cig user group over the 3-year period in: – COPD exacerbation rates – COPD assessment test (CAT) scores – 6-min walk distance (6MWD)	*Ricardo Polosa has direct and indirect relationships with the Tobacco Industry
Staudt, 2018	10 healthy never-smokers with no history of exposure to any tobacco products or e-cigs	Subjects inhaled 10 puffs of 'Blu' brand e-cig (EC), waited 30 min, then inhaled another 10 puffs $n = 7$ randomized to EC with nicotine $n = 3$ randomized to EC without nicotine.	• Exposure to EC with nicotine resulted in significantly higher levels of total endothelial microparticles. • Acute aerosol inhalation of EC with or without nicotine led to global changes in small airway epithelium transcriptome profiles. • Acute aerosol inhalation of EC with nicotine led to global changes in alveolar macrophage transcriptome profiles. • There were no consistent changes in vital signs, lung function tests.	Small number of subjects ($n = 10$) Acute e-cig exposure (20 puffs total)
Walele, 2018	209 healthy smokers, age 21–65 years	147 used e-cigs in the previous study and 62 used conventional cigarettes. Subjects were asked to use e-cigs containing 16 mg ml^{-1} nicotine and either tobacco or menthol flavour for 2 years.	• Lung function showed small, statistically significant decreases from baseline to month 24 in FVC, FEV1, FEF25–75 and peak expiratory flow (PEF) were observed. These were also observed in the previous 12-week study, with decreases being of greater amplitude in subjects who had continued smoking conventional cigarettes. Therefore, these decreases were not judged to be clinically significant. • From month 2, nicotine withdrawal symptoms decreased. • Smoking desire and conventional smoking consumption steadily decreased over time in all subjects.	Funded by tobacco company
Bowler, 2017	COPDGene (N = 3536) SPIROMICS (N = 1060) Current/former smokers, age 45–80 years	Observational study using surveys to determine whether e-cig use was associated with longitudinal changes in COPD progression or smoking habits	• No evidence of harm reduction with use of e-cigs. • E-cig use was associated with higher nicotine exposure, higher risk of COPD	Mixed inhalant exposures Observational design

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Author, year	Subjects	Study design and e-cig exposure	Key findings	Limitations
			exacerbations, and greater loss of lung function.	
D'Ruis, 2017	105 healthy adult smokers, age 21–65 years	Smokers were randomized to one of six groups (n = 15 each) each using blu e-cigs containing 24 mg ml^{-1} nicotine, and either tobacco or cherry flavouring. Three groups used e-cigs only, and three groups used e-cigs with conventional tobacco. Subjects used e-cigs for 5 days.	• No significant changes in pulmonary function test results.	Funded by tobacco company Sub-acute e-cig exposure (5 days)
Polosa, 2017	16 e-cig users of ≥3 months who had never smoked and 15 non-smoking controls	Six e-cig users were consuming nicotine-containing e-liquid. Three reportedly consumed zero-nicotine strength e-liquid throughout the 3.5 years follow-up.	• No decrements in spirometric indices, development of respiratory symptoms, changes in markers of lung inflammation in exhaled air or findings of early lung damage on HRCT, when compared with a carefully matched group of never-smoking non-e-cig users.	Small number of subjects *Ricardo Polosa has direct and indirect relationships with the Tobacco Industry
Cibella, 2016	300 smokers not intending to quit were invited to switch to e-cigs	12 weeks of e-cig use E-liquids contained 2.4%, 1.8% or 0% nicotine	• Smoking phenotype classification (Quitters, Reducers, Failures) had no significant effect on spirometric indices (FEV-$_1$, FVC and FEV_1/FVC) with the exception of $FEF_{25–75\%}$, which significantly ($P = 0.034$) increased over time among Quitters, suggesting slight improvements in small airway obstruction.	Sub-acute e-cig use (3 months)
Ferrari, 2015	20 healthy adult smokers ($n = 10$) and non-smokers ($n = 10$)	Nicotine free e-cig and standard cigarette *ad libitum* for 5 min in two different sessions with a cross-over design. FeNO, fractional concentration of CO (FeCO) and PFT were obtained immediately after the inhalant session ended.	• The use of the specific brand of nicotine free e-cig for 5 min assessed in this study did not change FeNO or FeCO or PFT measurements immediately after the inhalant session in non-smokers. • Nicotine free e-cig use for 5 min by smokers led to decreased FEVi and FEF25. • Use of a conventional cigarette by non-smokers led to decreased FEF75. • Use of a conventional cigarette by smokers led to decreased FEF25, FEV1, and PEF.	Small number of subjects – all of whom were pulmonary fellows or attendings Acute e-cig exposure (5 min)
Flouris, 2013	30 healthy adults, 15 smokers and 15 never-smokers 18–57 years of age	Smokers underwent a control session, an active tobacco cigarette smoking session and an active e-cig vaping session, each lasting 30 min. Never-smokers underwent a control session, a passive tobacco cigarette smoking session and a passive e-cig vaping session, each lasting 60 min. PFTs were conducted before, immediately after, and 1 h after.	• Neither 30 min of active e-cig vaping nor 60 min of passive e-cig vaping significantly affected FEV1/FVC. • Active (30 min) but not passive (60 min) tobacco cigarette smoking decreased FEV1/FVC.	Acute e-cig exposure (30–60 min)

Author, year	Subjects	Study design and e-cig exposure	Key findings	Limitations
Vardavas, 2012	30 healthy smokers, age 19–56 years	Use of an e-cig for 5 min with the cartridge included (experimental group, $n = 30$) or removed from the device (control group, $n = 10$). For the control group, e-cig aerosol was not created nor inhaled.	• E-cig use was found to cause an increase in impedance, peripheral airway flow resistance by impulse oscillometry system, and oxidative stress by FeNO among healthy smokers. • Pulmonary function assessed via spirometry did not change in either group.	Acute e-cig exposure (5 min)
Smoking cessation studies				
Cravo, 2016	387 smokers, age 21–65 years	Smokers of conventional cigarettes switched to e-cig products for 12 weeks.	• No significant changes were found in lung function parameters (FVC, FEV1, FEF25–75, and PEF) at baseline *versus* weeks 2, 4, 8 and 12.	Funded by tobacco company Sub-acute e-cig exposure (3 months)
Polosa, 2014	18 asthmatic e-cig users	Retrospective review of changes in spirometry data, airway hyper-responsiveness, asthma exacerbations and subjective asthma control in smoking asthmatics who switched to e-cigs.	• Compared to baseline, at 6 months there were significant improvements in $FEF_{25–75\%}$ and asthma control questionnaire (ACQ) scores in asthmatic smokers who switched to e-cigs. • At 12 months significant improvements were observed on all asthma outcomes measures, including methacholine PC20, after smokers switched to e-cigs.	Small retrospective study *Ricardo Polosa has direct and indirect relationships with the Tobacco Industry

Papers are sorted by year and alphabetically by first author. Abbreviations: e-cig, e-cigarette; CC16, club cell protein 16; IOS, impulse oscillometry; FeNO, fractional exhaled nitric oxide; VC, vital capacity; FEF-25%, forced expiratory flow-25%; COPD, chronic obstructive pulmonary disease; CAT, COPD assessment test; 6MWD, 6-min walk distance; EC, Ecig; PEF, peak expiratory flow; HRCT, high resolution computed tomography; FeCO, fractional concentration of CO; ACQ, asthma control questionnaire.

Table 2.

E-cigarette aerosol effects on lung physiology utilizing animal models

Author, year	Subjects	Exposure duration	Exposure	Key findings	Pathways studied/ affected	Limitations
Khan, 2019	C57BL/6 14–20 weeks old male and female	3 days or 10 days	Whole body waterpipe smoke (WPS) exposures for 30–60 min twice a day for 10 days. Whole body e-cig exposure 2 h a day to PG and nicotine (25 mg ml^{-1}), PG only or air, for 3 days.	Acute WPS exposure increased CLOCK and REV-ERBα protein levels (by western blot), while decreasing BMAL1 (by ELISA only). Acute e-cig exposure increased BMAL1 and PER2 protein levels (by western blot). Acute WPS exposure increased gene expression of *Rev-erbα, Rora* and *Cry2,* while e-cig exposure increased *Bmall, Clock* and *Per2.* E-cig changes were only found with aerosols containing PG + nicotine, not PG alone.	CLOCK REV-ERBα-	Short (sub-acute) exposure Exposure was different for WPS *vs.* e-cig
Glynos, 2018	C57BL/6 8–12 weeks old male	3 days or 4 weeks	Whole body exposures to ambient air, cigarette smoke (CS), or an e-cig aerosol containing: **1** propylene glycol/ vegetable glycerol (PG:VG) **2** PG:VG with nicotine (PG:VG-N) **3** 3) PG:VG with nicotine and flavour (PG:VG-N+F)	E-cig aerosols with nicotine and flavours (PG/VG/Nic+F) increased bronchoalveolar lavage (BAL) cellularity, Muc5ac production, BAL and lung oxidative stress markers, and elevated BAL protein content. Non-nicotine containing aerosols (PG/VG) was the only inhalant exposure to alter lung function after 3 days (acute exposure), causing decreased lung compliance and increased airway resistance. After 4 weeks (chronic exposure), only CS induced lung function changes, with a decrement in lung compliance. Both e-cigarette aerosols with nicotine and flavours (PG/VG/Nic+F) and CS increased airway reactivity, as demonstrated by methacholine challenge.	Muc5ac	Funded by a tobacco company Only male mice were studied
Garcia-Arcos, 2016	A/J 12 weeks old	4 months	Whole body exposed to 0.4 mL of phosphate-buffered saline (PBS) or e-cigarette vehicle (PG:VG 50:50) containing 0 or 18 mg ml^{-1} nicotine 5 days/week	Inhalation of nicotine-containing e-cigarette aerosols increased airway hyper-reactivity, distal airspace enlargement, mucin production, cytokine and protease expression. NHBE cells exposed to nicotine-containing e-cigarette aerosols showed impaired ciliary beat frequency, airway surface liquid volume, cystic fibrosis transmembrane regulator and ATP-stimulated K+ ion conductance and decreased expression of FOXJ1 and KCNMA1.	mucin protease FOXJ1 KCNMA1 IL-6 IL-8	

Author, year	Subjects	Exposure duration	Exposure	Key findings	Pathways studied/ affected	Limitations
				Exposure of NHBE cells to nicotine for 5 days increased interleukin (IL)-6 and IL-8 secretion.		
McGrath-Morrow, 2015	C57BL/6J neonatal	9 days	Vape Pen whole body exposure once a day for first 2 days of life (began at 24 hours of life). For days 3–9, pups were exposed twice daily. Three exposure types: **1** Room air **2** 1.8% nicotine in propylene glycol (PG) **3** 3) 100% PG (0% nicotine)	Nicotine e-cigarette aerosol exposed mice had larger MLI (mean linear intercepts – indicate impact on postnatal alveolar growth) *vs.* air (adjusted for sex and weight), as well as diminished levels of KI67, demonstrating inhibition of alveolar growth and decreased cell proliferation, consistent with impaired lung growth during early postnatal life.	KI67	Short (sub-acute) exposure

Papers are sorted by year and alphabetically by first author. Abbreviations: WPS, waterpipe smoke; E-cig, e-cigarette; PG, propylene glycol; CS, cigarette smoke; VG, vegetable glycerol; N, nicotine; F, flavour; BAL, bronchoalveolar lavage; PBS, phosphate-buffered saline; NHBE, normal human bronchial epithelial; IL, interleukin; MLI, mean linear intercept.

Table 3.

Studies assessing the effects of e-cig aerosol inhalation on human cardiac physiology to date

Author, year	Subjects	Exposure	Key findings	Limitations
Fetterman, 2020	467 volunteers without cardiovascular disease (CVD) risk factors age 21–45 years	Cross-sectional study of non-smokers, cigarette users, e-cig users and dual cigarette and e-cig users	Augmentation index – measure of vascular stiffness – were higher in cigarette smokers compared to non-smokers Augmentation index was similar between cigarette smokers and sole e-cig users and dual users Endothelial cells from cigarette smokers and sole e-cig users produced less nitric oxide after stimulation compared to non-smokers – suggesting impaired endothelial nitric oxide synthase signalling	Cross-sectional study
Ikonomidis, 2020	40 smokers 8 males 32 females mean age 45 ± 11 years	Randomized to smoke either conventional cigarettes or e-cig (nicotine 12 mg/ml) for 4 months	Decrease in pulse wave variation (PWV) in the e-cig group but increase in PWV in the cigarette group compared to baseline No significant change in platelet function markers in the e-cig group but further impairment noted in cigarette group compared to baseline	Subacute exposure (4 months)
Mobarrez, 2020	17 healthy occasional sm 6 males 9 females mean age 26 ± 3 years	Crossover study with 2 visits where subjects vaped e-cigs with or without nicotine for 30 minutes	Platelet and endothelial derived extracellular vesicles were increased with peak levels at 4 h following exposure to inhalation of e-cig with nicotine Platelet activation marker, P-selectin, and inflammation marker, CD40 ligand, were both significantly increased following inhalation of e-cig with nicotine	Small number of subjects Acute exposure (30 min ×2)
Antoniewicz, 2019	17 healthy occasional tobacco smokers 6 males 9 females mean age 26 ± 3 years	Crossover study with 2 vaping visits: **1** E-cig with nicotine **2** E-cig without nicotine **3** 30 puffs over 30 min	Systolic blood pressure (SBP) and diastolic blood pressure (DBP) increased with e-cig use with and without nicotine Heart rate (HR) and PWV – measurement of arterial stiffness - increased after e-cig with nicotine	Small number of subjects Acute exposure (30 min ×2)
Chaumont, 2019	30 regular and exclusive e-cig users (average use of 38 ± 3 months) mean age 38 ± 2 years Average past tobacco use 18 ± 2 pack-years	Randomized, investigator-blinded. three-period crossover study: **1** Nicotine session: regular vaping of e-cigs containing nicotine for 5 days (nicotine-session) **2** No nicotine session: nicotine-free-vaping for 5 days **3** Stop session: complete cessation of vaping for 5 days	Acute nicotine vaping increased heart rate and blood pressure, while sham (no nicotine) vaping did not, demonstrating nicotine specific effects on the cardiovascular system. Short-term e-cig cessation in regular users decreased baseline heart rate, suggesting a slight improvement of cardiac function.	Only male participants Acute exposure (5 days)

Author, year	Subjects	Exposure	Key findings	Limitations
George, 2019	114 smokers ages ≥18	Prospective, randomized controlled trial of smokers with two groups: **1** e-cig with nicotine (n = 37) **2** e-cig without nicotine (n = 37) Smokers were asked to switch to e-cigs for 1 month. The study had a third arm for individuals unwilling to quit cigarettes for 1 month (n = 40).	Flow-mediated dilatation – assessment of endothelial function – noted to be improved in e-cig users, both with and without nicotine, compared to cigarette smokers There was no difference among the 3 arms in regards to PWV, HR and platelet reactivity except in subgroups noted below: • Smokers with < 20 pack-years had improvement in vascular stiffness when switched to e-cigs, but smokers with >20 pack year history had no difference • Smokers with <20 pack-years had increased HR with e-cig use compared to cigarette smokers, but smokers with >20 pack-years had decreased HR with e-cig use compared to cigarette smokers	Sub-acute exposure (1 month)
Kerr, 2019	20 healthy male smokers mean age 32 ± 11 years	A cross-over study with 2 visits at which the subjects: Smoke one regular tobacco cigarette Vape e-cig with 18 mg ml^{-1} nicotine (15 puffs)	HR significantly increased after the use of both an e-cig (8±5) and tobacco cigarette (23±12). An acute increase in augmentation index (a measure of arterial stiffness) occurred following e-cig use.	Only male participants Small number of subjects Acute exposure (15 puffs)
Chaumont, 2018	25 healthy occasional tobacco smokers 18 males 7 females mean age 23 ± 0.4 years	3-period crossover design: **1** vaping without nicotine **2** vaping with nicotine **3** sham-vaping 25 puffs of PG:VG (50:50)	Vaping with nicotine: • impaired acetylcholine mediated vasodilatation • increased indices of arterial stiffness • increased SBP/DBP and HR • raised plasma myeloperoxidase	Small number of subjects Acute exposure (25 puffs)
Franzen, 2018	15 young, active, traditional cigarette smokers 5 males 10 females mean age 23 ± 4 years	Crossover study of: **1** e-cig with nicotine **2** e-cig without nicotine **3** conventional cigarettes 10 puffs. E-liquid formula: 55:35 PG:VG with 0 or 24 mg ml^{-1} nicotine and tobacco flavorant.	SBP and HR increased with acute cigarette and e-cig with nicotine exposures. DBP increased >5% by cigarettes and decreased > 4% by e-cig without nicotine Elevation of PWV changed significantly in cigarette and e-cig with nicotine group.	Small number of subjects Acute exposure (10 puffs)
Moheimani, 2017	33 non-smoking healthy volunteers, age 21–45 years	Three exposure sessions: **1** e-cig with nicotine **2** e-cig without nicotine **3** sham control 60 puffs over 30 min	Following exposure to e-cigarettes with nicotine, the sympathovagal balance was significantly shifted to sympathetic predominance, a pattern of heart rate variability that is associated with increased cardiac risk in multiple populations with and without known CVD.	Two different e-cigs used Acute exposure (30 min, 60 puffs)
Antoniewicz, 2016	16 healthy volunteers seldom smokers 11 males	Two groups either exposed or not to 10 puffs of e-cig aerosols for 10 min, in a crossover design, with blood draws at 1, 4 and 24 h.	Endothelial progenitor cell levels in blood were significantly increased 1 hour following exposure to e-cig aerosols and returned to baseline values after 24 h.	Small number of subjects

Author, year	Subjects	Exposure	Key findings	Limitations
	5 females mean age 27 ± 5 years		FeNO, a clinical measurement of degree of respiratory tract inflammation, was unaffected by exposure to e-cig aerosols.	Acute exposure (10 puffs)
Fogt, 2016	20 non-smoking, female healthy volunteers mean age 23 ± 3 years	Two groups: **1** placebo (0 mg nicotine) e-cig aerosol **2** nicotine (18 mg nicotine) e-cig aerosol inhaled every 30 s for 10 min (20 puffs)	Resting DBP was 3 mmHg higher following nicotine inhalation. Resting and exercise DBP was higher following nicotine use.	Changes in DBP may not be clinically relevant Acute exposure (20 puffs)
Vlachopoulos, 2016	24 smokers mean age 30 ± 8 years	Crossover study with 4 separate visits: **1** cigarette smoking 5 min **2** e-cig vaping 5 min **3** e-cig vaping 30 min **4** sham 60 min	HR increased in both the cigarette smoking and e-cig 30 min vaping sessions. Vaping for 30 min also resulted in a PWV increase similar to that of cigarette smoking.	Acute exposures (max 60 min)

Papers are sorted by year and alphabetically by first author. Abbreviations: CVD, cardiovascular disease; E-cig, E-cigarette; DBP, diastolic blood pressure; HR, heart rate; PWV, pulse wave velocity; SBP: systolic blood pressure.

Table 4.

Studies assessing the effects of e-cig aerosol inhalation on animal cardiac physiology to date

Author, year	Subjects	Exposure	Key findings	Limitations
Mayyas, 2020	Male Wistar rats 8–9 weeks old	4 groups: **1** fresh air **2** e-cig aerosol **3** conventional cigarette smoke **4** waterpipe smoke exposed 1 h/day, 6 days/week for 4 weeks	Cardiac endothelin-1 and myeloperoxidase increased in rats exposed to e-cig and tobacco smoke Increase in cardiac TGF-beta in all inhalant groups (tobacco smoke, waterpipe, and e-cig) Cardiac fibrosis observed in all inhalant groups relative to control	Only male rats were used
Rao, 2020	Male and female Sprague-Dawley rats 10 weeks old	4 groups: **1** Virginia Tobacco flavour **2** JUUL **3** E-cig tank system with unflavoured freebase nicotine **4** tobacco cigarettes **5** clean air 10 cycles of 2 second puffs over 5 min	Flow mediated dilatation (FMD) was measured pre and post-exposure and was impaired by JUUL and e-cig tank aerosols, and cigarette smoke Serum nicotine was highest in the JUUL exposed rats	Brief (acute) exposures (10 cycles of 2 second puffs over 5 min)
Szostak, 2020	Female apoE−/− mice 12–14 weeks old	5 groups: **1** fresh air **2** cigarette smoke **3** PG/VG **4** PG/VG/nicotine **5** PG/VG/nicotine/flavouring exposed for 3 h/day, 5 days/week for 3 and 6 months	E-cig aerosol exposures had smaller effects on systolic and diastolic heart function relative to cigarette smoke, but significant differences were found compared to controls Significant increase in pulse wave velocity (PWV) was noted in the cigarette smoke and groups with nicotine-containing aerosols compared to the control group	Funded by tobacco companies Only female mice used
Espinoza-Derout, 2019	Male apolipoprotein-E knockout (apoE−/−) mice 8 weeks old	2 groups: **1** e-cig with 2.4% nicotine **2** e-cig without nicotine exposed for 12 h/day for 12 weeks	E-cig aerosols with nicotine decreased both left ventricular fractional shortening and ejection fraction. Cardiomyocytes of mice treated with e-cig with nicotine exhibited ultrastructural abnormalities indicative of cardiomyopathy. E-cig with nicotine treated mice had increased oxidative stress and mitochondrial DNA mutations within cardiac tissue.	Only male mice used
Shi, 2019	Male and female C57BL/6 mice 2–3	2 groups: **1** room air	E-cig vaping had no effect on cardiac contractility as measured by ejection fraction.	Short (sub-acute) e-cig exposure (2 weeks)

Author, year	Subjects	Exposure	Key findings	Limitations
	months old	**2** e-cig vaping exposed for 3 h/day for 2 weeks	E-cig vaping significantly increased CD31 – biomarker of angiogenesis – in mouse heart and kidney tissue	
Crotty Alexander, 2018	Female C57BL/6 and CD-1 mice 8–10 weeks old	2 groups: **1** room air **2** e-cig vaping exposed for 1 h/day, 5 days/week for 3–6 months	E-cig exposed mice developed increased SBP and diminished heart rate. Chronic e-cig aerosol inhalation induced cardiac fibrosis in both genetic backgrounds with induction of Col3a1 expression.	Only female mice used
Nabavizadeh, 2018	Male Sprague Dawley rats 8–10 weeks old	3 groups, exposed for 1.5–5 min under anaesthesia via nose cone to: **1** IQOS aerosol **2** Cigarette smoke (Marlboro) **3** Clean air	FMD - measurement of vascular endothelial function impairment – was impaired to IQOS and cigarette smoke exposure but not clean air. Post-exposure nicotine levels were 4.5-fold higher in IQOS relative to cigarette.	Brief (acute) e-cig exposure (1.5–5 min)
Olfert, 2018	Female C57BL6 mice 10 weeks old	3 groups: **1** E-cig aerosol **2** cigarette smoke **3** filtered air exposed for 4 h/day, 5 days/week for 8 months	Aortic arterial stiffness, measured as PWV, increased in chronically exposed e-cig aerosol and cigarette smoke exposed mice. The maximal aortic relaxation to methacholine was lower in both e-cig aerosol and cigarette smoke mice.	Only female mice used

Papers are sorted by year and alphabetically by first author. Abbreviations: E-cig, E-cigarette; FMD, flow-mediated dilatation; PWV, pulse wave velocity; SBP, systolic blood pressure.