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Evidence Review

EVIDENCE REVIEW

E-Cigarettes and Cardiopulmonary Health

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Abstract

E-cigarettes have surged in popularity over the last few years, particularly among youth and young adults. These battery-powered devices aerosolize e-liquids, comprised of propylene glycol and vegetable glycerin, typically with nicotine, flavors, and stabilizers/humectants. Although the use of combustible cigarettes is associated with several adverse health effects including multiple pulmonary and cardiovascular diseases, the effects of e-cigarettes on both short- and long-term health have only begun to be investigated. Given the recent increase in the popularity of e-cigarettes, there is an urgent need for studies to address their potential adverse health effects, particularly as many researchers have suggested that e-cigarettes may pose less of a health risk than traditional combustible cigarettes and should be used as nicotine replacements. This report is prepared for clinicians, researchers, and other health care providers to provide the current state of knowledge on how e-cigarette use might affect cardiopulmonary health, along with research gaps to be addressed in future studies.

Key words: e-cigarette; cardiovascular disease; pulmonary disease; policy; cessation

Introduction

Electronic cigarettes (e-cigarettes) are battery-powered devices that aerosolize e-liquids, which typically contain propylene glycol (PG) and vegetable glycerin (VG), nicotine, flavors, and stabilizers/humectants such as triacetin.[1] Although it is well known that combustible cigarettes cause multiple cardiovascular and pulmonary diseases, the effects of e-cigarettes on health have only begun to be studied. Alarmingly, there has been a rapid increase in e-cigarette use among adolescents and young adults, who could potentially be exposed to e-cigarette aerosols for decades if their use is lifelong.[1] Indeed, the US Surgeon General concluded that the use of e-cigarettes among youth and young adults has become a major public health concern.[1] A recent European Respiratory Society task force concluded that since the long-term effects of e-cigarettes are unknown, it is not clear whether they are in fact safer than tobacco and based on current knowledge, their negative health effects cannot be ruled out.[2]

In the USA, the Family Smoking Prevention and Tobacco Control Act of 2009 gave the Food and Drug Administration (FDA) the power to regulate tobacco products. While e-cigarettes were not covered in the original act, the FDA has clarified its position with its "deeming" rule, and since 2016, has begun to exert its regulatory authority over e-cigarettes and other non-combustible products. In 2020, in response to the growing popularity among youth, the FDA issued a policy to limit the sales of some flavored e-cigarette products.[3] As the FDA adheres to a public health impact standard, evidence on adverse health effects of e-cigarettes will be a consideration to impact on future sales of e-cigarettes and e-cigarette liquids. Such a regulation will likely be contingent upon their observed health effects, as well as effects on nicotine addiction. In addition, the recent emergence of acute and severe e-cigarette, or vaping, product use-associated lung injury (EVALI) across the US underscores the need, complexity, urgency, and importance of basic and clinical research on the health effects of e-cigarettes, particularly focused on cardiopulmonary systems.[4]

With regard to public health impact and cardiopulmonary health, availability and use of e-cigarettes might benefit those who switch from combustible cigarettes to e-cigarettes, that is, harm reduction. However, the potential benefits from the use of these products are uncertain: they deliver a poorly defined, highly variable, and potentially toxic aerosol that may have adverse effects, which may be dependent on patterns of age, reproductive status, health of the user, and use. In addition, there are major public health concerns surrounding the availability of e-cigarettes for children, adolescents, and young adults.[1] Nicotine addiction is of particular concern and the use of e-cigarettes is positively associated with increased risk of use of combustible cigarettes.[5] These issues complicate the question of how e-cigarettes might impact cardiopulmonary health and are explored further in the later sections of this review.

Recognizing the potential health impact of e-cigarettes when they first emerged, particularly impacting the heart and lung, the Division of Lung Diseases and the Division of Cardiovascular Sciences at the NIH's National Heart, Lung, and Blood Institute (NHLBI) conducted a workshop in the summer of 2015 entitled "Cardiovascular and Pulmonary Disease and the Emergence of E-cigarettes", to identify key areas of needed research as well as opportunities and challenges of such research. The workshop was organized around a framework recognizing that the public health impact of e-cigarettes would be influenced by a complex network of factors in addition to direct health and biological effects, including device characteristics, chemical constituents (including flavorings and nicotine), aerosol characteristics, and use patterns. In response to the significant gaps and research areas highlighted at the workshop, NHLBI subsequently directed research funding to projects aimed at understanding the cardiopulmonary health effects of e-cigarettes and inhaled nicotine. Funded investigators met in 2018, 2019, and 2020 to discuss their results, findings in the larger field, and remaining scientific questions. The focus of this review is the result of discussions recognizing a need for further understanding of cardiopulmonary health effects of

e-cigarettes that occurred at these NHLBI-supported workshops and investigator meetings.

This review takes a holistic view of e-cigarette use and cardiopulmonary health, with a major focus on the USA. A summary of the current understanding of the multitude of factors that ultimately affect health, including policies, behaviors, emissions, and biological effects associated with e-cigarette use, is provided herein, with the ultimate goal of identifying key research gaps that remain in the field. E-cigarettes inhabit a rapidly changing marketplace and an evolving pattern of use that typically precedes scientific exploration. Following a PubMed search for relevant literature using "e-cigarette" and/or "cardiopulmonary" and/or "pulmonary", and/or "cardiovascular disease" as search terms, we break down the pertinent fields to uncover critical research questions that will better enable an understanding of how e-cigarettes affect cardiopulmonary health at an individual and community level.

Device Characteristics

E-cigarettes are highly variable in design, and are comprised of a battery, a reservoir for holding the e-liquid, a heating element, an atomizer, and a mouthpiece. The first generation of e-cigarettes (called "cig-a-likes") were similar in size and shape to combustible cigarettes. First-generation devices typically used a prefilled nicotine solution cartridge that directly contacted the heating element. Many second-generation devices were pen-shaped; some included refillable cartridges, while others were closed systems that held only prefilled sealed cartridges. Third-generation devices were called "mods" since they are easily modified. They were more diverse, and featured customizable atomizers, resistance coils, and larger-sized batteries capable of heating made-to-order e-liquids to higher temperatures to create more aerosol and potentially deliver more nicotine.[6] Fourth-generation devices (eg, JUUL) were smaller and some resembled familiar items such as USB drives. Their sleek design and ease by which they can be concealed from parents and teachers have contributed to their growing popularity in school-age children. These e-cigarettes operate at lower wattages than third-generation devices.[7] Arguably the most significant change that came with the fourth-generation devices is the use of nicotine salts, such as benzoic acid, as opposed to free-base liquid nicotine. Freebase nicotine is alkaline, irritating, and harsh to inhale, which limits the concentration that can be inhaled. Addition of benzoic acid or other salts results in protonation of the tertiary amine on nicotine's pyrrolidine ring, forming nicotine salts. The reduced pH of the e-liquid allows for inhalation of much higher nicotine concentrations with less of the acute respiratory irritation ("throat hit") typical of alkaline e-liquids. Third and fourth generation devices achieve similar doses of blood nicotine as tobacco cigarettes[7] and in some cases are being used to deliver substances other than nicotine, including cannabinoids.[8]

User Profiles and Patterns of Use

The Centers for Disease Control (CDC) found that non-Hispanic white males are more likely to use e-cigarettes than Hispanic whites or African Americans.[9] Lower-income smokers are also less likely to use e-cigarettes.[10] There is concern that e-cigarettes could be a gateway to combustible cigarette use (among youth) or encourage continued or enhanced nicotine addiction (among older established smokers). Indeed, longitudinal studies have shown that young people who start using e-cigarettes are at risk of also using combustible cigarettes.[11,12] However, we know little about how e-cigarette use affects other tobacco-related behaviors, and since the marketplace and demographics are changing, whether this observation holds true for fourth-generation devices remains to be determined.

Obtaining reliable data on e-cigarette use will depend on the development and adoption of standardized/validated self-report measures of use behaviors, perceptions, and attitudes, which will allow researchers to compare across studies over time. While there are measures currently used in surveillance studies such as Population Assessment of Tobacco and Health (PATH) and CDC surveillance, the rapidly evolving landscape of e-cigarettes presents challenges including tracking the evolution of terminology and device design. Timeliness of data collection and availability for use are crucial given the rapidly evolving marketplace. The methods used for tobacco surveillance typically do not produce usable data until a year or more after data collection and rarely allow for more than annual data collection. While in-place surveillance (eg, the National Adult Tobacco Survey and National Youth Tobacco Survey) can remain the backbone for e-cigarette investigation, novel innovative methods are required, including real-time sentinels, such as using informatics to analyze online and big data resources including Google Trends or Twitter. Thus, despite the heterogeneity in tobacco and nicotine use behaviors and trajectories, the skyrocketing popularity of e-cigarettes suggests an urgent need to better understand the implications of e-cigarette patterns of use and uptake.

Dosimetry

While cigarette smoke comes from combustion, e-cigarette vapor results from heating liquids to high temperatures, leading to a different type of exposure and dosimetry. Vaporized components of e-liquids cool and reach critical supersaturation conditions that result in a phase transition from vapor to aerosol (nucleation), followed by condensational growth.[13] Chemicals contained in the e-cigarette vapor-aerosol mixture are partitioned between the gas (vapor) and particulate phases and this partitioning affects the deposition pattern in the human respiratory tract.[14] E-cigarette aerosols have a wide particle size distribution (typically bimodal or even tri-modal) that ranges from nanometers to micrometers, making the prediction of delivery/deposition and dosimetry difficult.[15,16] The deposition of gas phase and nanoparticles (<100 nm) is mostly driven by Brownian diffusion, whereas larger particles (>1 μm in size) are deposited via inertial impaction, and submicron particles (0.1–1 μm) are deposited by both diffusional and gravitational forces.[17] E-cigarette aerosols consist of hygroscopic and relatively volatile compounds, which may either quickly grow or evaporate, depending on ambient conditions. This obviously adds additional complexity to dosimetric predictions. The development of computer algorithms to predict deposition of e-cigarette aerosol is an active, challenging area of investigation which includes models for multiple-path particle dosimetry, computational fluid-particle dynamics (considering hygroscopic growth of multicomponent droplets),[18] and thermodynamic interactions between the droplet and vapor phases.[19] Vapers of high power devices typically use low nicotine e-liquids, while users of low power devices use high nicotine e-liquids. However, the serum cotinine levels of these groups are the same, suggesting that the users of high power devices may be exposed to more aerosol that is generated at much higher temperature, which is likely much more harmful.

In addition to emission characteristics, vaping behavior is another critically important factor required to provide input parameters to the aerosol deposition models. Most studies have been conducted in the laboratory, and therefore reported puff profiles may differ from real-world scenarios.[20,21] Interestingly, properties of the e-cigarette aerosol seem to affect puffing topography. For example, St Helen et al. recently demonstrated that e-liquid flavor influenced puff duration: subjects took longer puffs on a strawberry flavored e-liquid (3.2 s) than on a tobacco flavored e-liquid (2.8 s).[22] The PG to VG ratio also affected puff topography. E-liquids containing more PG lead to shorter puff times, significantly greater nicotine delivery, but paradoxically, significantly less "pleasant" and/or "satisfying" feelings.[23] Additionally, users of powerful sub-ohmic devices and nicotine salt-based e-cigarettes may display different topography profiles compared to the users of earlier generation e-cigarettes. As devices change, more studies will be needed to determine the input parameters required to accurately determine lung deposition models.

Health Effects of E-liquid Constituents

E-cigarette aerosols are complex and contain multiple constituents of varying concentrations. Due to this reason, studies measuring health effects of aerosols have not always been consistent. In addition to studying the complete aerosol, there has been a parallel reductionist effort to understand the health effects of separate e-cigarette constituents. These studies have primarily focused on nicotine, flavors, PG, and VG, as well as thermal decomposition products generated during the course of e-liquid vaping. However, there is a growing appreciation that information is needed on the health effects of constituents not traditionally found in nicotine-based vaping products, including (-)-trans-Δ^9-tetrahydrocannabinol (THC) and cutting agents such as Vitamin E acetate (VEA).

Nicotine

Pharmacology

Nicotine is a water-soluble nitrogenous organic molecule that is typically extracted from tobacco leaves. It is composed of an aromatic pyridine ring bound to a pyrrolidine ring. Nicotine binds to nicotinic acetylcholine receptors (nAChRs), which are ligand-gated cation channels.[24] Binding of endogenous acetylcholine, or exogenous nicotine, opens the channel pore, allowing conduction of Na^+, K^+, and Ca^{2+}. The channel is subsequently closed and becomes temporarily unresponsive to further ligand stimuli (ie, desensitization).[24] nAChRs are broadly expressed throughout multiple organ systems, and in addition to the psychotropic effects on the brain, nicotine can also affect immune cells and resident, specialized cells of the heart and lungs (Figure 1). Despite the long history of nicotine research, understanding the role of nicotine in cardiopulmonary disease is extraordinarily difficult due to factors such as sensitization and desensitization responses; complicated dose-response relationships; the importance of exposure route; and differences in response dependent on species, age, sex, or disease status.[24]

Nicotine and Cardiovascular Disease

The known effects of nicotine on the cardiovascular system are numerous and complex. Nicotine affects angiogenesis, arrhythmogenesis, endothelial cells (ECs), hemodynamics, insulin resistance, and lipids.[25] Nicotine increases blood pressure, heart rate, myocardial contractility, and myocardial work. Nicotine also constricts coronary arteries, reduces coronary blood flow reserve and constricts blood vessels in the skin. The majority of nicotine-related clinical studies have involved combustible cigarettes, which expose smokers to nicotine as well as multiple combustion products. To isolate the specific effects of nicotine on human cardiovascular disease, researchers have studied populations who consume nicotine without combustion, including users of nicotine medications (ie, for smoking cessation), and smokeless tobacco users (ie, snus). Smokeless tobacco has been used by non-smokers in Sweden lifelong, with no evidence of accelerated atherosclerosis or most other cardiovascular harms.[26] Nicotine medication studies have provided evidence of tolerance to the cardiovascular effects of nicotine: Low doses of nicotine increase heart rate and systemic catecholamine release, while higher doses have little additional cardio-acceleratory and catecholamine effects. These factors are reassuring when considering whether there is a risk of treating smokers with nicotine replacement medications while they are still smoking. Limitations of nicotine medication studies include the fact that the subjects are all former smokers, nicotine medication use is generally of short duration (weeks or months), and the delivery of nicotine by gum or patch is sustained and does not simulate the spike in nicotine levels seen after smoking combustible cigarettes or e-cigarettes. In contrast, regular e-cigarette exposure likely takes place over years to decades, with vascular nicotine eliciting chronic hemodynamic changes.

Nicotine has complex dose-response patterns and may exert species- and cell/tissue-specific effects. Primary rat cardiomyocytes incubated with nicotine showed increased intracellular Ca^{2+} concentrations, which likely contributed to the observed hypertrophy.[27] Rats and mice that either consumed or were injected with nicotine showed weakening of the aortic walls and destruction of aortic elastin and collagen, all of which were likely a result of the upregulation of matrix metalloproteases. These findings indicate a potential risk for abdominal aortic aneurysms as a result of chronic nicotine consumption.[28,29] Nicotine also promotes trans-differentiation of vascular smooth muscle cells to a calcifying phenotype by inducing a pro-inflammatory state, impairing endothelial function, and causing oxidative stress.[30] The growing body of evidence in animal models has illustrated that nicotine can directly affect cardiovascular function without exposure to the byproducts of combustion-based delivery mechanisms.

The contribution of specific vapor constituents to the observed endothelial dysfunction has not been fully delineated. Some studies have suggested that nicotine may be partially responsible, as nicotine exposure can damage epithelial and ECs, induce epithelial-mesenchymal transition markers such as α-SMA and fibronectin, and release inflammatory mediators such as transforming growth factor-beta, all of which suggests that nicotine may promote tissue remodeling and fibrosis.[25] While some investigators have hypothesized that the adverse effects of e-cigarettes were largely due to nicotine delivery, others have proposed that e-cigarette aerosol without nicotine might also contribute to these effects. Of note, in the study by Schweitzer et al.,[31] adverse nicotine-independent effects of e-cigarette aerosol on endothelial barrier function were also observed, which the authors attributed to other toxic components of e-cigarettes such as acrolein.

Nicotine and Pulmonary Disease

The effects of nicotine on the lung have been established from studies in humans, animal models, and cultures of human cells. The use of smokeless tobacco permits the study of people who

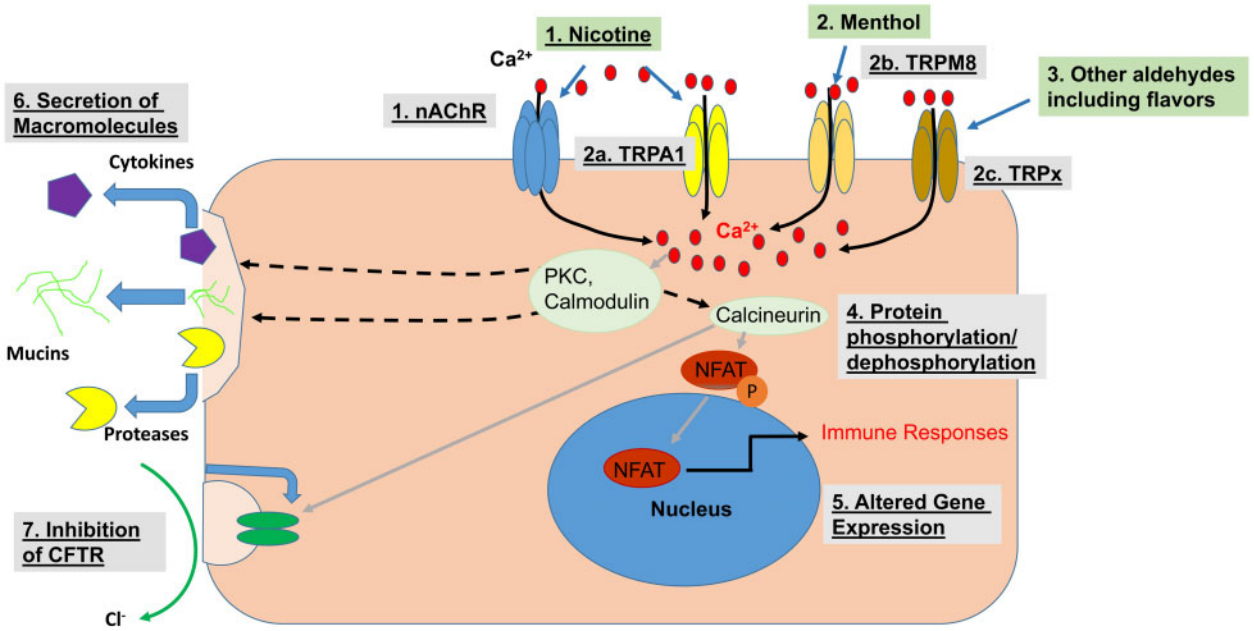

Figure 1. The Impact of E-Liquid Constituents on Ca^{2+} Homeostasis, and on Subsequent Cellular Functions. After exposure to e-liquid constituents, cytoplasmic Ca^{2+} can be elevated directly via nicotine binding to ligand-gated ion channels (ie, nAChR) (1); by menthol or nicotine activating TRP channel (2); by aldehyde flavors activating ion channels or other proteins (3); or indirectly via activation of PLC and subsequent IP_3 formation (3). These actions directly affect the lung since they can alter protein phosphorylation (4), gene expression (5), induce cytokine/protease secretion (6) and inhibit the CFTR anion channel (7).

were never smokers and have used nicotine for many years. For example, snus users have a lower mortality rate than tobacco smokers. As discussed before, nicotine levels are ~50-fold lower in plasma after use of e-cigarettes, snus, or tobacco than in the lung after tobacco or e-cigarette inhalation (~70 nM in plasma versus ~50 μM in sputum). This suggests that vaping and tobacco smoke inhalation lead to higher levels of pulmonary nicotine than snus due to their delivery route. Because of this, snus may not have as much effect on the lung as vaping due to different nicotine pharmacokinetics.[32–34] Incubating isolated alveolar epithelial cells with nicotine-induced oxidative stress via activation of NADPH oxidase 1, leading to cell apoptosis.[35] This observation was confirmed *in vivo* with nicotine injections to Wistar rats, which resulted in oxidative stress signaling in lung tissue.[36] In contrast to promoting oxidative stress, a study in mice found that nicotine can prevent inflammation by inhibiting the release of pro-inflammatory cytokines in the lungs.[37] However, a more recent study has challenged this observation by demonstrating that nicotine binding to α7 nAChR, lead to increased type I collagen deposition in lung fibroblasts: Fibroblasts from wild type (WT) and α7 nAChR knockout mice were exposed to nicotine, and U937 monocytes were co-cultured on matrices derived from these fibroblasts. Nicotine exposure in WT but not α7 nAChR knockout fibroblasts resulted in monocyte activation and release of IL-1ß.[38] Finally, nicotine has been shown to cause mucociliary dysfunction in human airway epithelial cells and animal models by reducing ion channel function via TRP channels.[39,40] However, the effects of nicotine may be both cell type- and concentration-specific, which may lead to the disparity of results (Figure 1).

Nicotine Exposure during Sensitive Windows of Development

Nicotine readily crosses the placenta and produces higher nicotine concentrations in the fetal circulation than in the maternal circulation.[41] Epidemiologic studies have shown that cigarette smoking during pregnancy is associated with an increased risk of cardiovascular disease in offspring, although this may or may not be due to direct effects of nicotine.[40,42] Moreover, nicotine may be a link between maternal smoking and the risk for sudden infant death syndrome.[43] It is also well-established that maternal smoking, mediated most likely through nicotine, results in impaired fetal lung development and function, and these effects, while small, persist into childhood and beyond.[44–46] Recent animal studies further indicate that pre-conception and prenatal e-cigarette exposure to nicotine and PG/VG impairs embryo implantation and offspring's metabolic health later in life.[47–49] This is of particular concern, given the current rate of e-cigarettes use among young adults of childbearing age.[50] Regardless of its source, fetal nicotine exposure during pregnancy has become a public concern and the impact of vaping nicotine-containing e-liquids on fetal development remains to be determined.[51]

Flavor Constituents

The Family Smoking Prevention and Tobacco Control Act of 2009 banned the use of all natural and artificial compounds characterized as flavors (except for menthol) in combustible cigarettes and any of their component parts to eliminate flavored tobacco products that held special appeal in the youth market. In 2020, the US FDA finalized their enforcement policy on flavored cartridge-based e-cigarette products, including fruit and mint flavors, but excluded menthol and tobacco flavors.[52] However, since this enforcement policy only applies to prefilled cartridges, flavored e-liquids in bottles and refillable empty cartridges are still commercially available. Flavored products can be legally marketed in the USA if they receive a tobacco product marketing order from the FDA, and this may become common in the coming years.

There is sufficient evidence to suggest that flavorings influence the perception, use, and safety of e-cigarettes.[53] Flavor compounds,

particularly sweet ones, have the potential to mask and/or ameliorate irritant and bitter sensations, such as the taste of nicotine.[54] The route of delivery has an impact on the toxicology of a compound. Indeed, many flavor compounds have only been tested for safety following oral delivery (ie, in the context of ingested human food products), and to date have not been tested for inhalation safety. The potential risk of flavor compounds when inhaled rather than ingested has been demonstrated by occupational and consumer inhalation of high doses of the buttery flavor diacetyl (2,3 butanedione), which leads to irreversible lung disease, namely bronchiolitis obliterans or "popcorn lung" in microwave popcorn factory workers.[55] At one time, diacetyl was found in ~70% of 159 sweet flavorings used in e-cigarettes,[56] while a more recent study found diacetyl in only a few flavorings.

Flavored e-liquids and individual flavors induce toxicity and/or exert biological effects: Sassano et al. found vanillin is the most common flavor.[57] The number of flavors used varies considerably across e-liquid products. Notably, in 20 e-liquids, Hua et al. found 99 different flavors.[58] Sassano et al. studied 148 e-liquids and found ~100 flavor constituents.[57] They also found the more flavors contained in an e-liquid, the more likely it was to be cytotoxic, while concentrations of vanillin and cinnamaldehyde in e-liquids significantly correlated with toxicity.[57] Cinnamaldehyde, was found to be >7.6 mM in multiple e-liquids, and in some cases, levels exceeded 1 M.[59] These concentrations were sufficient to induce cytotoxicity and ciliary dysfunction. However, flavor concentrations in most e-liquids have not yet been determined. Menthol is biologically active and can activate the transient receptor potential (TRP) channel in pulmonary neurons to suppress cough and irritation,[54] making it easier to tolerate cigarette smoking. Menthol flavors are significantly more popular among African Americans and tobacco companies have marketed them accordingly.[60] Flavorings including vanillin (4-Hydroxy-3-mehoxybenzaldehyde) and cinnamaldehyde are aldehydes that have the potential to form adducts with proteins and DNA. Vanillin can also activate TRP channels to exert biological effects (Figure 1).[61] They can also react with base constituents (eg, PG and glycerine) in e-liquids, leading to the formation of acetals, which can stimulate irritant receptors.[62]

Sweet and bitter taste receptors are G-protein-coupled receptors expressed in airway epithelia where they regulate innate immunity. The sweet (T1R) and bitter (T2R) taste receptors are expressed in the nasal passages/upper airways, while only bitter/T2R taste receptors are expressed in the lower airways.[63] This raises the possibility that inhalation of flavor compounds may stimulate airway taste receptors and affect immune function. Their activation may disrupt innate airway defense by suppressing the release of antimicrobial peptides that are capable of killing a variety of respiratory pathogens. In the lower airways, T2R activation leads to an increase in ciliary beating and may have other physiological functions via its effects on cytoplasmic Ca^{2+}, a universal second messenger. There is some evidence that toxicity is cell type-dependent, suggesting that mechanistic investigations must be cell-specific.[57,64] In summary, the adverse impact of flavor compounds in e-cigarettes include the potential for (1) increased appeal of these products, particularly to the youth market, (2) influence on patterns of use and smoking topography, (3) changes in cell signaling, and (4) increased cellular toxicity (Figure 1).

PG and VG

PG and VG are commercially available in different mixture ratios, and the ratio of PG to VG in the e-liquid can affect taste sensation, the amount of aerosol generated, the amount of nicotine delivered, and the overall user experience.[65] Predominantly PG-based e-liquids deliver more nicotine systemically, but taste less pleasant than VG-rich e-liquids.[23] PG is used to deliver pharmaceuticals intravenously and while it is generally considered safe, higher PG doses can lead to metabolic acidosis, acute renal injury, and sepsis-like syndrome.[66] In Sprague-Dawley rats, nasally inhaled PG of up to 2.2 mg/L for 90 days led to nasal hemorrhaging. Similar short-term exposures of mice to PG/VG resulted in changes to tissue elasticity, static compliance, and airway resistance, although these effects waned after 1 month of exposure, suggesting that there may be a long-term adaptive response.[67] In contrast, a recent study funded by Philip Morris International found that nasal exposure to PG/VG mixtures of up to 1.5 mg/L PG and 1.9 mg/L VG for 90 days had minimal effects on respiratory organs, gene transcription, proteomics, and lipid profiles in Sprague-Dawley rats.[68] Lipid-laden macrophages were recently observed in mice that were chronically-exposed to PG/VG.[69] While this exposure was not fatal, these mice had decreased macrophage function and were more vulnerable to influenza A infection.[69]

Mucin abnormalities correlate with a decline in forced expiratory volume in 1 s (FEV_1) in chronic obstructive pulmonary disease (COPD) patients,[70] indicating that mucins are important biomarkers of harm. Importantly, increased MUC5AC mucin levels were also detected in human e-cigarette users' bronchial epithelia obtained by bronchoscopy and in sputum,[71,72] and these increases in MUC5AC levels could be replicated in the laboratory by exposing both primary bronchial epithelial cultures and mice airways to PG/VG.[71] While the underlying mechanism(s) whereby PG/VG can exert their effects are unknown, Ghosh et al. found that PG/VG rapidly alters membrane rheology.[71] Altered membrane properties could affect a number of aspects of fundamental cell biology including endocytosis, exocytosis, and cell division. *In vitro*, higher levels of both PG and VG can prevent cell growth and/or induce cell death. However, more work will be required to fully appreciate the effects of PG/VG. The potential effects of PG/VG at the cellular level are summarized in Figure 2. The concentrations of PG and VG saw in the lung and systemically after vaping are poorly understood, and additional studies to determine PG/VG pharmacokinetics and pharmacodynamics after inhalation are required.

Thermal Decomposition Products

All e-liquids are heated and aerosolized prior to inhalation in a device-dependent fashion. This may subject them to chemical reactions that result in the formation of new compounds including reactive oxygen species (ROS). For example, the hydroxyl radical (OH) was produced from PG/VG at higher power settings.[73] Carbonyl compounds (eg, acetaldehyde, formaldehyde, and acrolein) were formed in e-liquid aerosols as a result of dehydration and oxidation, and was dependent on the PG/VG ratio, the wattage used to heat the e-liquid, as well as other factors including brand, and type of e-liquid used.[74,75] Exposing biological tissues to carbonyl compounds can deplete glutathione, induce DNA damage, alter ion channel function, and elicit cell death.[76,77] Thus, both reactive aldehyde production and subsequent reactive aldehyde metabolism in biological tissues need to be considered. This may be particularly important since nearly 8% of the world's population has an impaired capability to metabolize reactive aldehydes, and they may show altered responses to e-cigarette/reactive aldehyde exposure.[78] Importantly, aldehydes can form adducts with both proteins

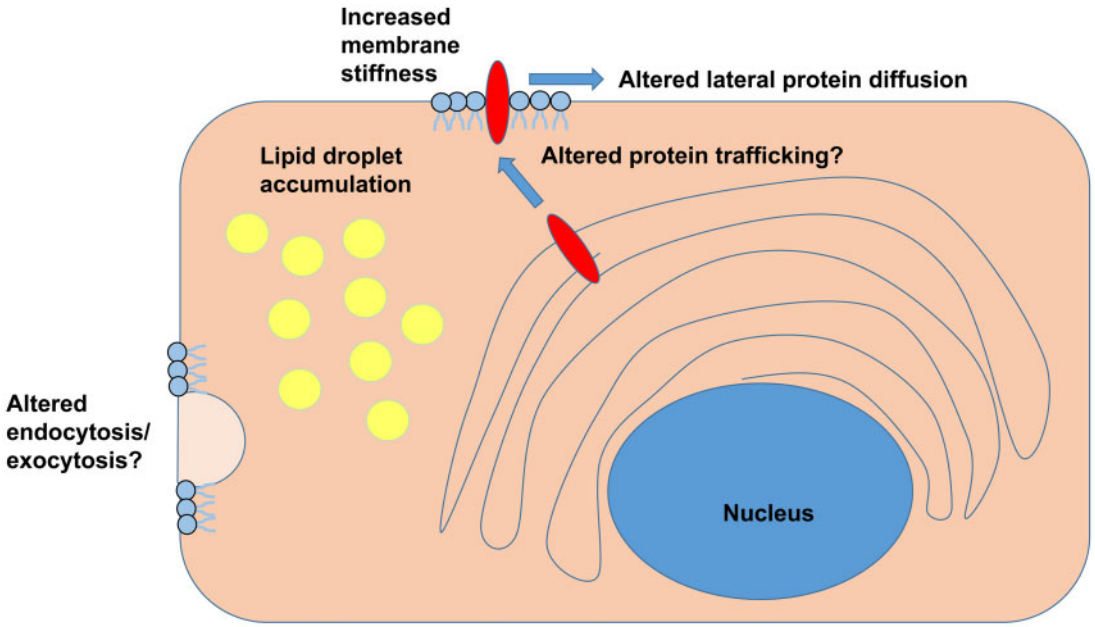

Figure 2. Do PG and VG Affect Cells? A combination of PG and VG have been shown to affect membrane rheology and alter protein diffusion.[71] Moreover, PG/VG-exposed mice develop macrophages with cytoplasmic inclusions and show altered lipid biochemistry.[69]

and DNA.[79] Adduct binding can impair protein function, as recently noted for the short palate and nasal epithelial clone 1 (SPLUNC1), which is an innate defense protein expressed in the lung. Here, crotonaldehyde bound to SPLUNC1, which prevented it from regulating lung hydration.[80] Similarly, acrolein can form adducts with surfactant protein A, which leads to impaired innate defense by decreasing antimicrobial activity and reducing phagocytosis by macrophages.[81] Aldehyde adducts can also bind to DNA, leading to frame shift and base-pair substitution mutations, which may contribute to cytotoxic and genotoxic effects.[82] These results indicate that as a result of reactive aldehyde inhalation from heat-coil aerosolization of PG/VG, the lung may be especially vulnerable to adduct formation and the associated macromolecule damage. In addition to decomposition products resulting from heating e-liquids, emissions may also contain contaminants from components of the e-cigarette device itself. These can include toxic metals such as chromium, nickel, and lead.[83] Therefore, understanding the potential health effects of thermal decomposition products remains key to delineating the overall e-cigarette health effects.

Cardiovascular Health Effects of E-Cigarette Aerosols

There is a common perception e-cigarettes may be safer than combustible cigarettes, since they deliver much lower levels of oxidants, volatile organic chemicals, and other noxious chemicals associated with tobacco cigarette smoke and cardiovascular risk.[25] However, both combustible tobacco products and e-cigarettes deliver oxidants, toxic metals, and potentially toxic carbonyls, which have been associated with cardiovascular disease.[84,85] Moreover, e-cigarette-derived particles are spread among a wider size range than those generated by standard cigarettes. Known toxicants in e-cigarettes (eg, acrolein, aldehydes, PG, and metals) may also contribute to cardiovascular damage in a different manner than toxicant-induced cardiovascular damage from combustible cigarettes. There is an urgent need to determine both the acute and the long-term effects of e-cigarettes on the hearts and blood vessels of healthy adults and children, as well as those with either risk factors for cardiovascular disease or outright cardiovascular disease[25] and to determine the comparative safety of e-cigarettes relative to combustible cigarettes.[86] A summary of the potential cardiovascular biomarkers of exposure/harm following vaping are shown in Table 1 and are discussed in more detail below.

Effects on Vascular Function and ECs

E-cigarette use has been consistently connected to reductions in vascular function and damage to ECs. For example, several studies have established that e-cigarette inhalation leads to increased arterial stiffness in humans and rodents, as evidenced by increases in augmentation index and pulse wave velocity.[87,100] One crossover design study compared e-cigarette vaping ± nicotine and found that pulse wave velocity, aortic pulse pressure, augmentation index corrected for heart rate, and subendocardial viability ratio were all significantly increased, but only when subjects vaped with nicotine.[87] However, another study found that inhalation of nicotine-free e-cigarette vapor caused an increase in aortic pulse wave velocity and resistivity index.[88] These results have made it unclear as to whether nicotine is required to elicit these adverse effects: Additional factors such as e-cigarette wattage, which affects toxicant production, and/or the presence of flavors may also affect arterial stiffness. In addition to arterial stiffness, e-cigarette use impaired endothelial nitric oxide synthase signaling,[101] which could be considered a biomarker of endothelial dysfunction in several vascular diseases including hypertension and atherosclerosis. Participants exposed to e-cigarettes showed significantly reduced flow-mediated dilation (FMD) of the brachial artery, demonstrating endothelial dysfunction compared to non-users.[95] However, in a separate study, smokers who switched to e-cigarettes for one month showed an improvement in FMD, suggesting that there may be a difference between acute and chronic effects of vaping on FMD.[102]

Table 1. Biomarkers of Cardiovascular Diseases by Vaping or E-Cigarette Exposure

Biomarker	Human/mouse	Biofluid	Post-exposure	References
Arterial stiffness indices	Human			
Pulse wave velocity	–	–	↑	87,88,89
Augmentation index corrected for heart rate	–	–	↑	87,89
Aortic pulse pressure	–	–	↑	87
Subendocardial viability ratio	–	–	↑	87
Blood pressure	–	–	↑	87
Abnormal pulse wave form	–	–	–	90,91
Oxidative stress	Human	–	–	
Myeloperoxidase	–	Plasma	↑	87
8-iso-PGF2α	–	Serum	↑	92,93
NO bioavailability	–	Serum	↓	92,93
s-NOX2-dp	–	Serum	↑	92,93
Vitamin E	–	Serum	↓	92,94
Thioredoxin	–	Sputum	↑	71
Endothelial dysfunction	Human	–		
FMD		–	↓	95
LDL-oxidizability/ ApoB		Plasma	↑	96
Platelet function	Human	–	–	
Activation	–	Plasma	↑	97,98
Aggregation	–	Plasma	↑	97,98
P-selectin	–	Plasma	↑↓	97,90,98
Platelet microparticles	–	Plasma	↑	90, 99

The mechanisms by which electronic cigarettes lead to these adverse vascular effects remain incompletely defined. However, a number of studies suggested that e-cigarettes might cause ROS-mediated damage, including damage to ECs.[103,104] Carnevale et al. performed a crossover study of 40 healthy subjects, half of whom were smokers. The subjects were asked to smoke combustible cigarettes for 1 week and were then crossed over to e-cigarettes.[92] Both combustible cigarettes and e-cigarettes increased markers of oxidative stress and worsened FMD after a single use. Lee et al. exposed human-induced pluripotent stem cell-derived ECs to various flavored e-cigarette liquids and assessed endothelial integrity. A cinnamon-flavored e-cigarette product was most potent in reducing cell viability and increasing ROS levels.[105] In multiple studies, subjects who used e-cigarettes either acutely or chronically were found to have altered blood and plasma biomarkers linked to oxidative stress and cardiovascular disease, including increased myeloperoxidase,[87] increased isoprostanes such as 8-iso-PGF2α,[94] reduced NO bioavailability, increased levels of the oxidant-generating enzyme nicotinamide adenine dinucleotide phosphate oxidase (s-NOX2-dp),[92] and reduced levels of the non-enzymatic antioxidant vitamin E.[92,94] Anderson et al.[104] showed that e-cigarette aerosol exposure induced ROS *in vitro*, which caused DNA damage and cell death. Of note, the antioxidants alpha-tocopherol and n-acetyl cysteine were effective in alleviating the damage. After e-cigarette exposure, activated ECs may have been the source of vascular ROS: Chatterjee et al. exposed serum to e-liquids and observed a NOX2-dependent increase in ROS, coupled with inhibition of NADPH oxidase 2 (NOX2) reduced ROS production by ~75%.[106] Kuntic et al. extended these observations and demonstrated that e-cigarette-induced ROS burden and endothelial dysfunction could be rescued by NOX2 inhibition or NOX2 gene knockout in mice.[107] These findings were nicotine-independent, and acrolein treatment alone was capable of causing NOX2-dependent ROS production in primary murine ECs. Together, these studies link e-cigarette use with NOX2 activation, endothelial oxidative stress, and subsequent endothelial damage/dysfunction, which may contribute to adverse vascular outcomes.

Effects on Platelets

Both mainstream and sidestream cigarette smoke have been shown to cause heightened platelet activation, adhesion aggregation, and inflammation.[97,108] Based on the possible clinical implications of altered platelet exposure, the potential effects of e-cigarette extracts on platelet function have also been investigated.[97] Platelet activation and aggregation were increased following exposure to e-liquid aerosol extracts. Platelet adhesion potential for fibrinogen and von Willebrand factor were also increased, indicating that e-cigarette extracts had a pro-thrombotic effect. In this study, the effects were caused by the non-nicotine constituents of e-cigarettes, while other studies have shown increased platelet aggregation and/or activation after exposure to e-cigarette vapor with nicotine.[94,109,110] One such study found shortened thrombosis occlusion and bleeding times in mice.[109] Increases in serum p-selectin were also detected, but these findings were contradicted by another study which reported decreased serum p-selectin levels. These conflicting data may be due to variability in devices and/or exposure conditions. Recently, endothelial and platelet-derived microparticles have been used as biomarkers of endothelial dysfunction and thrombosis, respectively. These particles, which are $<1\,\mu m$ in diameter, are formed as a consequence of cell blebbing during cellular stress and apoptosis. Microparticles play a role in arterial occlusion aided by p-selectin expression on activated platelets. Elevation of platelet microparticles in combustible smokers has been reported elsewhere,[111] and has recently been shown to occur following acute clinical exposure to e-cigarettes.[90,99] Combined, these data suggest one potential mechanism whereby e-cigarettes use could lead to myocardial infarction and stroke.

Effects on Hemodynamics and Sympathomimetic Activity

Consistent with the known sympathomimetic effects of nicotine, e-cigarette aerosol exposure in humans acutely increases heart rate and blood pressure.[112,113] Moheimani et al. measured

heart rate variability in participants who were habitual e-cigarettes users or healthy non-users. The high-frequency component of heart rate variability, an indicator of vagal activity, was decreased in e-cigarette users, while the low-frequency component and low-frequency to high-frequency ratio, reflecting cardiac sympathovagal balance, were increased by e-cigarettes use, consistent with an increase in sympathetic activity. In another study by the same group,[114] healthy subjects who were not current tobacco smokers or e-cigarette users were exposed to e-cigarette aerosols with nicotine and showed a shift in heart rate variability, suggesting an increase in sympathetic tone. Moreover, those exposed to only the non-nicotine components of e-cigarette aerosols did not show an acute sympathomimetic effect. The authors concluded that nicotine was needed to produce an increase in sympathetic activity. In contrast, D'Ruiz et al., found that 5 days of e-cigarettes use did not cause higher blood pressure or heart rate.[113] Other studies have found acute effects of e-cigarette use on heart rate, both with and without nicotine.[100] Hence, the effects of e-cigarettes on hemodynamics has been variable, and might be partially dependent on how much nicotine is delivered. Importantly, this could contribute to a sympathomimetic increase left ventricular hypertrophy, adverse LV remodeling, and potential rhythm disturbances.

Effects on Heart Tissue

While the effects of e-cigarette use on vascular pathology, sympathetic induction, and platelet abnormalities are relatively well established,[107] there is currently little to no evidence linking e-cigarette use to heart disease.[115] While there are no reports of cardiac dysfunction as a result of e-cigarette exposure in humans, chronic increases in arterial stiffness and blood pressure could potentially cause adverse cardiac remodeling. Unfortunately, these data are unavailable due to the novelty of e-cigarette devices and will likely emerge over time. However, animal models and *in vitro* studies can be used as a proxy, and these studies have found limited evidence of cardiac dysfunction as a result of e-cigarette exposure. One study in apolipoprotein-E knockout mice found that just 12 weeks of chronic e-cigarette exposure resulted in reduced ejection fraction and alterations in cardiomyocyte structure typical of cardiomyopathy.[116] However, this study included a small number of mice ($n = 5$), and this result could not be replicated by another group after as long as 6 months of exposure using the same mouse model and a larger sample size ($n = 10$–12).[117] While changes in ejection fraction have not been noted in exposed, otherwise healthy mice, one study noted significant collagen deposition in cardiac tissue as a result of e-cigarette exposure, with a 2.75-fold increase in cardiac collagen after 6 months of chronic e-cigarette exposure.[118] A study of cultured rat cardiomyoblast (H9c2) cells found that several e-liquids were cytotoxic at high concentrations, but that e-liquids were less cytotoxic than tobacco smoke extract.[119] Although the half-maximal inhibitory concentration (IC_{50}) of these extracts was 3-fold lower than cigarette smoke condensate, these observations indicated that e-cigarettes extracts were cytotoxic to cardiac myoblasts, and that the toxicity may have been related to the production process and/or the presence of different flavors.

Pulmonary Health Effects of E-Cigarette Aerosols

In chronic smokers, pulmonary disease generally occurs following decades of smoking. However, the adverse effects on lung function, coupled with earlier onset biochemical and/or structural changes in lung function, are present long before COPD or other chronic respiratory disease is detected. For example, adverse effects of conventional cigarette smoking on respiratory health including wheezing, coughing, and resultant decreased lung function have been found in adolescents.[44] Spirometry is typically used to determine the FEV_1. However, FEV_1 measurements can be noisy and FEV_1 over forced vital capacity (FEV_1/FVC) is also used for COPD diagnosis. FEV_1 has been measured immediately after vaping and some researchers reported airflow obstruction,[120,121] while others do not.[122,123] In healthy subjects who vaped regularly for at least 6 months, a decrease in FEV_1 was not apparent.[33,71] However, there were also no differences in FEV_1 in the healthy smokers' cohort (13 subjects) during this time period and longer studies are usually required to show accelerated loss of lung function.[124] In contrast, a reduced FEV_1 and FEV_1/FVC ratio was reported in vapers elsewhere.[125] Tobacco smokers who switched to e-cigarettes had little[126] or only moderate improvement in lung function.[127] In small scale studies of smokers and non-smokers without pre-existing lung disease, e-cigarette use was not associated with acute changes in FEV_1. While important diagnostically, spirometry does not always correlate well with early disease manifestations in COPD such as small airway/alveolar damage.[128] Thus, these measurements may not be a sensitive index of the early effects of vaping and should be interpreted with caution.

Although our current knowledge comes from studies with small sample sizes, evidence is growing that asthma and other respiratory disorders can be induced and/or exacerbated by e-cigarette use, albeit less so than seen with combustible cigarettes.[129] In a study on e-cigarette use and chronic bronchitis, the risk of disease symptoms (odds ratio) was increased approximately by 2-fold in current and past e-cigarette users compared with non-vapers/non-smokers, and the risk was also increased with the frequency of vaping.[130] Moreover, studies in adolescents found a positive association between e-cigarette use and asthma risk that was independent of smoking tobacco or marijuana.[131,132] Decreased fractional exhaled nitric oxide concentration (FeNO) is a biomarker of airway inflammation that has been used to study vapers. In a study with 30 participants, as little as 5 min of vaping caused a significant reduction in FeNO.[120] In contrast, another study showed a significant increase in FeNO levels in subjects who vaped for 2 h.[133] In a longitudinal study, vaping was found to be an independent risk factor for respiratory disease.[134] Similarly, in a retrospective analysis of two cross-sectional cohorts (COPDGene, $n = 3536$; SPIROMICS, $n = 1060$), switching from combustible cigarettes to e-cigarettes did not improve lung function, and was associated with an increased incidence of COPD.[135] These discrepancies suggest that depending on the length or the frequency of e-cigarette consumption, NO levels can change in either direction, indicating a need for more research. A summary of the potential pulmonary biomarkers of exposure/harm following vaping are shown in Table 2 and are discussed in more detail below.

Biochemical Effects on the Conducting Airways

In a series of cross-sectional studies, samples were obtained from different regions of vapers' lungs and subjected to both "omics"-style and targeted approaches. These studies may show changes at the molecular/biochemical levels before gross structural and/or physiological changes can be detected. Nasal biopsies from e-cigarette users showed greater reductions in immune-related gene expression than those from tobacco

Table 2. Biomarkers of Pulmonary Diseases by Vaping or E-Cigarette Exposure

Biomarker	Human/animal	Biofluid/tissue	Δ	References
Spirometry	Human			
Forced expiratory volume			↑	122,125
FEV/FVC			↑	122,125
Fractional exhaled nitric oxide			↑↓	120,133
Mucus properties	Human			
MUC5AC/ MUC5B		Sputum	↑	
MUC5AC	Bronchial brushings		↑	71
MUC4		Sputum	↑	71
DMBT1		Sputum	↓	72
LYSC		Sputum	↓	72
Mucus concentration	Sheep	Tracheal aspirate	↑	39
Inflammasome	–			
Caspase 1	–	BALF	↑	136
ASC	–	BALF	↑	136
Respiratory disease risk	Human			
Bronchitis OR = 2			↑	130
BAL proteases levels (neutrophil elastase, MMP-2, and MMP-9)	Human	BALF	↑	33
Inflammatory mediators	Mouse			
MCP-1	–	BALF	↑	137
IL-6	–	BALF	↑↓	137–139
Edema (wet:dry)	–	Lung	↑	139
KC	–	BALF	↑	140
TREM-1	–	BALF	↑	140
Angiopoietic-1	–	Serum	↑	118
LIX	–	Serum	↑	118
MMP3	–	Serum	↓	118
Neutrophil extracellular trap marker proteins (S100A8, S100A9, cornonin-1, and protein-arginine deiminase4)	Human	Sputum	↑	72
Immunosuppression				
Bacterial growth	Mouse	BALF	↑	138
EGR1, ZBTB16, PIGR, PTGS2, and FKBP5	Human	Nasal scrape	↓	141
CSF-1	Human	Nasal scrape	↓	141
CCL26	Human	Nasal scrape	↓	141
Eotaxin 3	Human	Nasal scrape	↓	141
Epithelial dysfunction				
CC10/CC16	Human	Serum	↑	142
DNA damage/adducts/detoxifying enzymes				
O^6-methyldeoxyguanosines	Mouse	Lung	↑	143
and γ-hydroxy-1,N^2-propano-deoxygeuanosines	Mouse	Lung	↑	143
XPC	Mouse	Lung	↑	143
OGG1/2	Mouse	Lung	↑	143
CYP1A1/2	Mouse	Lung	↑	144
CYP2B1/2	Mouse	Lung	↑	144
CYP3A	Mouse	Lung	↑	144
8-hydroxy-2′-guanine	Mouse	Lung	↑	144
Aldehyde dehydrogenase 3A1	Human	Sputum	↑	72
Glutathione S-transferase	Human	Sputum	↑	72

smokers, indicating e-cigarette induced immunosuppression.[141] Another study found increased platelet-activating factor receptor (PAFR) expression in e-cigarette users' nasal epithelia.[132] Importantly, *Streptococcus pneumoniae* adhere to PAFR, and e-cigarette vapor increased pneumococcal adhesion to airway cells *in vitro*, independently of nicotine, suggesting increased virulence.[145] Together, these studies are suggestive of dampened immunity in the upper airways from those who vape.

Reidel et al. induced sputum from healthy non-smokers, vapers, and cigarette smokers and performed proteomics. Interestingly, they found more changes in sputum protein in vapers compared to that of smokers, relative to non-smokers.[72] Ghosh et al. performed bronchial brush biopsies on non-smokers, smokers, and vapers and also found a significant number of unique proteins were independently elevated in vapers airways.[71] Elevated levels of aldehyde-detoxifying enzymes, including aldehyde dehydrogenase 3A1, metabolic isozyme, glutathione S-transferase, and an antioxidant, thioredoxin were found.[71] These enzymes play an important role in detoxifying e-cigarette toxicants as well as by maintaining the redox homeostasis in cells. Tsai et al. found that inflammasome complex proteins, caspase-1 and apoptosis-associated speck-

like protein containing caspase activation and recruitment domain, which promotes cellular pyroptosis, were elevated in the BAL fluid of e-cigarette users.[136] The anti-inflammatory club cell protein 16 (CC16) was significantly elevated in serum of vapers after 25 puffs of the aerosol, suggesting an acute response to epithelial dysfunction/injury in the lungs.[142] These data suggest that the lung responds to the increased toxic burden from vaping by upregulating metabolic processes.

Increased proteolysis in the lung airspaces is associated with emphysema and bronchiectasis.[146] Using proteomics, Reidel et al. found evidence of increased proteases (neutrophil elastase and matrix metalloproteinase-9) in vapers' sputum. These data were suggestive of increased neutrophil lysis. Ghosh et al. subsequently measured protease levels and activity in vaper's bronchoalveolar lavage (BAL) fluid and found protease levels (neutrophil elastase, matrix metalloproteinase-2, and matrix metalloproteinase-9) were equally upregulated at the protein level and by activity in both vapers and smokers.[33] Furthermore, a subset of vaper/never-smokers also had increased lung protease levels. Consistent with these observations, both neutrophils and alveolar macrophages were stimulated by nicotine to secrete these proteases. In contrast, PG/VG did not stimulate protease secretion. Taken together, these data indicate that a number of molecular markers that are associated with pulmonary disease are upregulated in vapers' airways.

Despite not controlling for either e-cigarette device type or e-liquid brand, the human omics studies and targeted studies found remarkably consistent results.[71,72,141] These data suggest that many of the changes in gene/protein expression were driven by exposure to nicotine, PG/VG, and/or their metabolites. However, with control for e-cigarette type and flavor type, more changes may have been found. Importantly, the human and murine findings reinforce the need for standardized protocols to fully understand the lung effects of e-cigarette constituents and aerosols. Use of different e-cigarette solutions of varying humectant (PG/VG) composition, nicotine concentrations, flavorings, and temperature settings for the generation of aerosols complicates the difficulty in interpretation of findings.

Lessons Learned from Animal Studies

Mucus clearance is a key component of the lung's innate defense system, and reductions in mucus clearance lead to airway obstruction, inflammation, and chronic infection, as seen in cystic fibrosis and COPD.[147] E-cigarette vapor containing nicotine led to significant mucociliary dysfunction in sheep.[39] Given the impact that mucus clearance has on lung function, these findings are important and need to be addressed further in humans. The ability of e-cigarette aerosols to induce inflammatory responses in animals has been varied. Lerner et al. demonstrated significantly increased MCP-1 and IL-6 cytokine levels in BAL fluid (BALF) after exposure of C57BL/6 mice for 3 days to side-stream aerosols of Blu e-cigarettes.[137] However, another group showed reduced IL-6 levels after a 2-week exposure of mice to NJOY aerosols.[138] Additional studies, using different exposures, e-cigarettes, and mouse strains, have added to the uncertainty. Many mouse exposure studies have supported the hypothesis that exposure to e-cigarette aerosols induces an inflammatory response in the lung,[137,139] while others found that exposure to e-cigarette aerosols induced little or no inflammation or oxidative stress.[113,138,140] These outcomes suggest that pulmonary inflammation may depend on the time of exposure, the e-cigarette brand used, the operation of the device, and possibly the strain of mice tested.

Several murine studies suggest that innate defense is impaired in e-cigarette-exposed mice. Reduced clearance of *S. pneumonia* and influenza virus A suggests that e-cigarettes may have the potential to dampen immune responses to infection.[138] BALF from the mice exposed to NJOY e-cigarette aerosol for 2-weeks prior to challenge with *S. pneumonia* had significantly greater bacterial growth compared to the unexposed counterparts.[138] These data suggest exposure to e-cigarette aerosols may cause both inflammation and immunosuppression.

Animal studies also have shown e-cigarette aerosol exposure induces changes in lung (airways or alveoli) structure.[118] Crotty Alexander et al. exposed C57BL-1 and CD-1 mice to e-cigarette aerosol for 3 and 6 months, respectively, and observed a differential response when evaluating secreted inflammatory proteins in mouse sera. In both strains, angiopoietin-1 was increased, suggesting remodeling of the lung tissue.[118] Similarly, LIX (CXCL5) was also elevated in the two strains, suggesting an influx of immune cells to the site of inflammation in the lung. Conversely, matrix metalloprotease-3 levels were highly and significantly attenuated in the aerosol exposed groups compared to the unexposed group, suggesting the involvement of the tissue-remodeling pathway.[118] Two studies indicated that exposure of mice to aerosolized nicotine-containing e-liquids led to significant emphysema,[40,148] while two studies did not.[69,118] Little is known regarding the impact of chronic vaping on the pulmonary vasculature. However, exposure of 6-week-old mice to Blu e-cigarettes for 5 weeks resulted in a significant reduction in pulmonary capillaries number, that was equal to the decrease seen after conventional tobacco exposure.[148] Due to the importance of this issue, more work is required to fully understand the impact of vaping on the pulmonary vasculature in humans.

Vaping may also result in DNA damage in the lungs: Lee et al. showed significantly elevated levels of O^6-methyldeoxyguanosines and γ-hydroxy-1,N^2-propano-deoxygeuanosines adducts in the lung tissues of FVB mice exposed e-cigarette aerosols for 12 weeks.[143] They also reported significantly reduced nucleotide excision repair and base excision repair along with a reduction in repair proteins, XPC and OGG1/2.[143] Sprague Dawley rats exposed to e-cigarette aerosols for 4 weeks showed induction of carcinogen-metabolizing enzymes, including cytochrome p450 family members CYP1A1/2, CYP2B1.2, and CYP3A, as well as increased 8-hydroxy-2′-guanine oxidative DNA lesions in the lung tissues, compared to the unexposed counterparts.[144] These studies show the potential for DNA damage, adduct formation, and genotoxicity/carcinogenicity of e-cigarette aerosols. Consistent with these results, it was recently shown that e-cigarette aerosols can induce lung adenocarcinomas along with bladder urothelial hyperplasia in a mouse model.[149] Thus, the majority of animal model studies are consistent with the human data and typically demonstrate that e-cigarette aerosol exposure affects the lung. However, due to a variety of animal models mouse strains (including strain differences), non-standardized exposure paradigms, and a lack of a standard/reference e-liquid, the results are mixed.

THC and Vaping

In addition to the growing use of e-cigarettes for nicotine delivery, aerosolization of cannabis and cannabis extracts has become increasingly popular over recent years. Strikingly, a June 2019 study in North Carolina found that ~10% of high schoolers

had used an e-cigarette to aerosolize cannabis, THC concentrates, butane hash oil, or THC waxes.[150] Aerosolization of THC-containing substances for both medicinal and recreational use is marketed as an alternative to traditional marijuana combustion. E-cigarettes and aerosolizers are capable of aerosolizing dry cannabis herbs, oil concentrates, and cannabis-based e-liquids.[151] Oil concentrates containing THC and other cannabinoids are commonly extracted using either butane or supercritical CO_2 in its liquid form at high temperature and pressure.[151] These oil extracts can be aerosolized directly or mixed with PG to make e-liquids. Other solvents are also used to dilute THC, including lipophilic substances like VEA, medium-chain-triglycerides, and coconut oil. Butane-based oil extracts, termed butane hash oil or butane honey oil, may also be concentrated into a waxy substance that is aerosolized using wax-globe atomizers, commonly known as "dab rigs".[151] The composition of butane hash oil aerosols with and without PG shows volatile organic compound concentrations similar to those of nicotine-based e-liquids. However, devices used to aerosolize concentrated oils and waxes have not been studied extensively.[152] A recent animal study showed pulmonary damage caused by VEA inhalation.[153] However, potential therapeutic potential for cannabinoids must also be considered.[154] Therefore, there is legitimate public health concern for the growing recreational use of unregulated cannabis, especially for youth of high school age in areas where accessibility is prevalent.

The growing concerns for aerosolization of cannabis-based products need to be addressed in the laboratory. Most animal exposures to date have used PG as a solvent to deliver THC via e-cigarettes to rats. These studies have validated their method by demonstrating the presence of THC-related effects, including increased tolerance to pain and hypothermia, in the exposed rats.[155] Major gaps in the body of literature include testing oil and wax-based products and the cardiopulmonary consequences of all cannabis-based aerosols, and these urgently need to be addressed experimentally.

Update on the Recent E-Cigarette, or Vaping, EVALI Epidemic

Nearly every state in the USA has found e-cigarette users to develop a lung condition that has been termed EVALI, leading to ~2800 reported cases, the hospitalization of >2500 patients, and >60 deaths.[4] The cause or causes for EVALI are not well understood. The US CDC found THC and VEA, were present in the lungs of many EVALI patients. A murine model also suggested inhaled VEA may cause EVALI-like lung injury,[153] but the underlying mechanism remains to be determined. The age range of cases and deaths is broad, and the e-cigarette use patterns are diverse, although ~75% of EVALI patients were young Caucasian males and an overwhelming majority (~80%) admitted to THC vaping. While VEA from THC vaping has been most commonly and consistently linked to EVALI cases, the spectrum of usage patterns and clinical manifestations suggest a possible role of multiple toxicants from unregulated products. Chemical analysis of counterfeit cartridges obtained from EVALI patients demonstrated the presence of several toxicants including volatile organic compounds, semi-volatile hydrocarbons, silicon conjugated compounds, terpenes, pesticides, and metals, which were not found in medical-grade THC cartridges.[156]

The typical symptoms of EVALI include dyspnea, chest pain, cough, fever, and fatigue. Additionally, many of the EVALI patients also presented with nausea and vomiting and other gastrointestinal symptoms. Chest radiography of most cases was abnormal; images typically showed ground-glass opacities in both lungs.[4] Four radiographic patterns were identified in EVALI patients including acute eosinophilic pneumonia, diffuse alveolar damage, organizing pneumonia, and lipoid pneumonia.[157] Arterial hypoxemia and elevated neutrophil counts may be present. Histological analysis of lung biopsies showed patterns of acute fibrinous pneumonitis, diffuse alveolar damage, or organizing pneumonia.[157] EVALI patients may have slightly different phenotypes and have been diagnosed with (1) acute respiratory distress syndrome, (2) lipoid pneumonia, and (3) pneumonitis. Patients have been treated with antibiotics and glucocorticoids,[158] and the steroidal treatment has been shown to improve symptoms and lung function.[159,160]

Recent Epidemiological Studies

Although this is a new field, where initial cross-sectional epidemiological studies have demonstrated several limitations, adolescents who vaped have been found to be more likely to try cigarettes than non-smoking non-vaping youth.[7] For example, a cross-sectional analysis of PATH study data indicated an association between e-cigarette use and self-reported wheeze,[161] and an analysis of data from 402 822 never-smoking participants in the behavioral risk factor surveillance system indicated an association between self-reported asthma and e-cigarette use intensity.[162] It is important to recognize that the above studies were observational in nature, and the chronology of e-cigarette use and disease development are often not clear, so more evidence is needed that will further clarify the cause-effect relationship between e-cigarette use, cardiopulmonary disease, and cerebrovascular events. Regardless, these publications serve as an impetus for future research into the causative and mechanistic relationships between e-cigarette use and cardiopulmonary disease risk.

Smoking Cessation Trials

E-cigarettes have been proposed as an effective strategy to quit conventional cigarette smoking, but they have not been approved for this purpose in the USA or elsewhere. To date, the clinical trials that have been carried out do not address the question of effectiveness in the "real world", that is, does the availability of e-cigarettes in the marketplace decrease smoking at the population level. Instead, clinical trials have compared the delivery of nicotine by an e-cigarette to other modalities of nicotine delivery. The most recent review on this topic concluded: "The evidence is inadequate to infer that e-cigarettes, in general, increase smoking cessation. However, the evidence is suggestive but not sufficient to infer that the use of e-cigarettes containing nicotine is associated with increased smoking cessation compared to the use of e-cigarettes not containing nicotine, and the evidence is suggestive but not sufficient to infer that more frequent use of e-cigarettes is associated with increased smoking cessation compared with less frequent use of e-cigarettes".[44]

Second- and Third-Hand E-cigarette Exposure Risk

To predict the relative dangers of second- and third-hand e-cigarette exposures, an understanding of the degree to which e-cigarette use might lead to an increase in ambient nicotine and

particulate matter, and the degree to which nicotine and other e-cigarette constituents deposit on surfaces, will be critical. Since there are no side stream aerosols from e-cigarettes, unlike combustible cigarettes, secondhand e-cigarette exposure is almost exclusively from user exhalation. Thus, it remains unclear and somewhat controversial as to what level of additional particulate matter, vapor phase, and nicotine emissions are released into the environment from e-cigarettes. Some of this uncertainty may relate to variability in device design and liquid composition. However, several studies have demonstrated e-cigarette use by individuals can contribute to worse indoor air quality, including release of toxicants and particulate matter.[163] For example, indoor e-cigarette use can generate fine particulate matter in high concentrations during natural use conditions in indoor environments, as well as an increase in particle numbers and concentrations of 1,2-propanediol, glycerin, and nicotine.[133] Increased levels of 1,2-propanediol, diacetin, and nicotine were also measured by gas chromatography from one exhaled e-cigarette puff. E-cigarettes containing nicotine-free solutions may have higher particulate levels than those containing nicotine. However, these particles dissipate much more quickly than cigarette smoke particles and further studies will be needed to fully understand the risk of second and third-hand exposures.

Measurable nicotine levels have been detected in samples from hard surfaces and cotton surfaces exposed to e-cigarette emissions.[164,165] Recent developments in detection strategies by use of autofluorescence have further elucidated e-liquid deposition topography. One study found that for each 70-mL aerosol puff, 0.019% (vol/vol) of the aerosolized e-liquid was deposited on hard surfaces.[166] These studies may also be an overestimate when compared to real-life scenarios because aerosol puffs were directly administered to the observed surfaces and were not inhaled and exhaled prior to surface deposition. However, in an attempt to provide a better model of surface deposition, deposition as a result of inhaled and exhaled e-cigarette aerosol was performed.[167] This study found no significant increase in surface nicotine levels following 80 puffs per participant. The authors noted these results may not indicate a lack of risk for third-hand exposure, since they did not account for gradual accumulation on surfaces over time. Together, these results indicate that potentially hazardous e-cigarette emissions, including PG/VG, nicotine, and heavy metals may be deposited on household surfaces as a result of typical vaping behavior. Furthermore, they suggest a potential risk for third-hand exposure which could serve as a public health concern. However, more studies are needed to better understand the risk of vaping for second and third-hand exposures.

E-Cigarette Public Health Risk Assessment

In assessing the public health impact of e-cigarette use, there is an implicit comparison to alternative or counterfactual scenarios; in the case of e-cigarettes, the comparison is to the hypothetical situation of a world lacking e-cigarettes. There are established methods for quantitative risk assessment that are widely used for public health decision-making, such as the four-element paradigm set out in the 1983 National Research Council Report generally referred to as “The Red Book”. The elements include: (1) hazard identification, that is, is there a risk?; (2) exposure assessment, that is, what is the pattern of exposure?; (3) dose-response assessment, that is, how does risk vary with dose?; and (4) risk characterization, that is, what is the burden of disease and who is affected? These four elements have general applicability to characterizing the impact of e-cigarettes in terms of the prevalence of nicotine addiction and its profile across groups in the population and the associated additional (or reduced) burden of disease. Population impact is quantitatively assessed using conceptual models that capture an understanding of the relationships between independent and modifying factors and their outcomes. Models are implemented using statistical approaches and evidence-based estimates of the values of parameters at key steps in the model, for example, the rate of initiation of use of tobacco products with e-cigarettes present (versus not present). This approach was used by the FDA’s Tobacco Products Scientific Advisory Committee to estimate the impact of menthol-containing tobacco products. The overall approach was to formulate a conceptual framework, conduct systematic reviews around the framework, and implement an evidence-based statistical model for making estimates related to public health impact. The systematic reviews highlighted those gaps in scientific evidence, pointing to the most critical research needs for strengthening the evidence foundation for potential regulation of menthol. For e-cigarettes, the research priorities identified in this article relate to key evidence gaps that need to be addressed to achieve a greater and more certain understanding of the population impact of e-cigarettes.

Research Gaps

There are many unknowns regarding the impact of e-cigarettes on cardiopulmonary health, especially in the face of emerging acute lung injuries associated with e-cigarette use. Some of the most important gaps in this area can be addressed in the following manner.

- Studies to determine aerosolized constituents of e-cigarette liquids produced by the latest generation of devices, as a function of different power, coil composition, coil age, and nature of wick.
- Observational and mechanistic studies, using human and animal models, to determine the acute and chronic pulmonary and cardiovascular effects of heated and aerosolized e-cigarette liquid constituents, including nicotine, solvents, flavorings, and toxicants.
- Studies on the impact of dual tobacco and e-cigarette use.
- Studies of the impact of inhaled THC and associated constituents (eg, VEA) on cardiopulmonary health, both alone and in parallel with nicotine vaping.
- Studies to determine lung and nasal deposition patterns of particles and constituents that are emitted during e-cigarette use and encountered through second-hand exposures.
- Development of valid and standard self-report measures of e-cigarette use.
- Studies to assess second- and third-hand exposures to e-cigarette emissions by measuring indoor air and human biomarkers of exposure.
- Studies, using human and animal models, of the effects of long-term exposure to e-cigarettes in those with pre-existing pulmonary or cardiovascular disease, as well as age-dependent effects of long-term use.
- Assessment of patterns for uptake of e-cigarettes among youth; motivating factors (including psychosocial) for e-cigarette uptake, and the implications of e-cigarette uptake at a young age for subsequent nicotine addiction and cardiopulmonary health.
- Assessment of the efficacy of e-cigarettes in promoting tobacco product cessation and decreasing cardiopulmonary harm due to smoking combustible cigarettes.

- Identification of susceptibility states that increase the cardiopulmonary risk of e-cigarette use.
- Evaluation of the association of e-cigarette use with established pulmonary and cardiovascular outcomes and subclinical pulmonary and cardiovascular disease in well-defined longitudinal cohorts.
- Population health risk assessment: continued surveillance of e-cigarette use, frequency and duration of illnesses, hospital visits and admissions, and deaths related to e-cigarette use.
- Studies on how policies, such as emerging restrictions on flavors, age of purchase, and taxes influence both combustible and e-cigarette product use, uptake, and cessation.
- Recommendation that e-liquid type, device type/wattage, and a list of constituents should be published along with every study in order to facilitate reproducibility by other researchers.

Limitations and Caveats

The use of e-cigarettes has dramatically increased, with the majority of new users being in the 18–44 age range. While traditionally designed as a means to promote traditional smoking cessation, a large number of vapers are former or current smokers. Additionally, a number of vaper/never smokers also exist. Therefore, understanding the acute and chronic health effects of e-cigarette use alone is important and not well understood. The majority of research in e-cigarette health effects has focused on pulmonary implications. However, a growing body of knowledge is emerging suggesting direct and distinct cardiovascular consequences of e-cigarette use. A major limitation of the literature is understanding the pulmonary and cardiovascular effects of e-cigarette use in smokers attempting to quit, in never smokers using e-cigarettes and in people with pre-existing cardiopulmonary conditions. These important studies must be done in order to design effective policies to target cessation at the user level, and to inform key stakeholders.

First-generation e-cigarettes delivered very little nicotine, while subsequent generations delivered similar amounts of nicotine as compared to conventional cigarettes. Thus, the vapor and aerosol output from e-cigarettes are highly variable and device-dependent and the cardiopulmonary effects of e-cigarettes will also be device-dependent. Ideally, all cardiovascular studies should also measure blood nicotine levels and all pulmonary studies should measure BAL or sputum nicotine levels. Detailed information on e-cigarette type, e-liquid type, and use patterns should be provided. However, few studies have provided this information or measured nicotine levels. Additional cardiopulmonary toxicants including acrolein, formaldehyde, and metals will also vary according to device type, e-liquid composition wattage, and user puff profile.

Laboratory-based cell culture and animal e-cigarette studies aerosols use a range of exposures to both neat e-liquids, e-liquid condensate, and aerosol and vapor. Determining the relevant dose that replicates real world vaping is challenging and no standards exist for device or e-liquid type. Aerosol deposition patterns in animals, especially rodents may differ greatly to humans. Acute transient changes in biomarkers of harm may not be useful predictors of impending cardiopulmonary disease. Nicotine causes acute vasoconstriction and transient increases in aortic pulse wave velocity, which is not equivalent to stiff vessels seen in chronic vascular disease. Similarly, altered heart rate variability is a predictor of future cardiovascular events, that reflects sympathetic tone.[168] Nicotine increases sympathetic tone alters heart rate variability,[169] but is not equivalent to increased sympathetic tone that is a consequence of underlying disease. Epidemiological studies are difficult to conduct and understand because many e-cigarette users are current or former combustible tobacco smokers. However, smokers who switched e-cigarettes can be monitored for future cardiopulmonary disease by using current and former smokers as comparators. Thus, any conclusions regarding e-cigarette exposure must be interpreted with caution. However, as the field matures and more people vape for longer periods, certainty regarding the impact of e-cigarettes on cardiopulmonary health may increase. Another concern is reproducibility. As stated above, inclusion of the constituents of each e-liquids studied would help to alleviate this. However, since manufacturers do not usually give out e-liquid recipes, this information would need to be generated by the research lab and results may differ depending on the mass spectrometer used. Moreover, any identified effect of a given e-liquid should be verified with other e-liquids to determine whether it is reproducible or not. For example, Rowell et al. found that some e-liquids elicited a cellular Ca^{2+} signaling response. Using high throughput screening, they then found that 42/100 e-liquids elicited a similar response,[170] suggesting that this approach is feasible.

Conclusions

It is clear the cardiopulmonary effects of e-cigarette use are not completely understood. Many unknowns remain and the field has been hampered by inconsistent methods for assessment and exposure. Moving forward, it is paramount that researchers find a way to perform investigations that use consistent methodologies, including characterization of the e-cigarette device used and its operation, in order to compare results and provide updates to decision makers. While electronic means of nicotine inhalation may help some people quit smoking traditional cigarettes, the number of unknowns related to e-cigarette design, chemicals generated by the heating process, and potential health effects make it difficult to assess the potential benefits of switching from combustible to e-cigarettes and/or the impact of chronic vaping on the cardiopulmonary health of never-smoking youth. Until the major health effects of e-cigarettes are known, it is recommended to proceed with caution in approaches to address the implications of e-cigarette use at both the individual and population levels.

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Conflict of Interest Statement

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