Vaping and Lung Inflammation and Injury

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Abstract

The use of electronic (e)-cigarettes was initially considered a beneficial solution to conventional cigarette smoking cessation. However, paradoxically, e-cigarette use is rapidly growing among nonsmokers, including youth and young adults. In 2019, this rapid growth resulted in an epidemic of hospitalizations and deaths of e-cigarette users (vapers) due to acute lung injury; this novel disease was termed e-cigarette or vaping use-associated lung injury (EVALI). Pathophysiologic mechanisms of EVALI likely involve cytotoxicity and neutrophilic inflammation caused by inhaled chemicals, but further details remain unknown. The undiscovered mechanisms of EVALI are a barrier to identifying biomarkers and developing therapeutics. Furthermore, adverse effects of e-cigarette use have been linked to chronic lung diseases and systemic effects on multiple organs. In this comprehensive review, we discuss the diverse spectrum of vaping exposures, epidemiological and clinical reports, and experimental findings to provide a better understanding of EVALI and the adverse health effects of chronic e-cigarette exposure.

Keywords

e-cigarette or vaping use-associated lung injury; EVALI; electronic nicotine delivery systems; ENDS; e-cigarette aerosols; acute lung injury; inflammatory lung disease; systemic inflammation

1. INTRODUCTION

Electronic (e)-cigarettes represent the newest tobacco product increasingly used by the public (1–4). However, despite their popularity, the effect of e-cigarette use (vaping) remains poorly understood, and chronic e-cigarette use will likely lead to serious health effects. A variety of e-cigarettes have been marketed to the public over the past decade in the context of a lack of regulatory action. E-cigarette or vaping use-associated lung injury, or EVALI, is a recently described entity at the forefront of current investigations and represents

an epidemiologic outbreak that focused attention of clinicians and researchers on vaping products. Though the cause of EVALI has been linked to vitamin E acetate (VEA), which is added to some vaping products, aerosols generated by e-cigarettes (termed electronic nicotine delivery systems or ENDS) have the potential to cause numerous pulmonary toxicities, both acute and chronic. The pathophysiology of EVALI and the host responses that correlate with chronic inflammation are described in this review, as well as the general pulmonary toxicity and pathophysiologic profile of vaping tetrahydrocannabinol (THC) products and ENDS.

2. THE SPECTRUM OF VAPING EXPOSURES

Modern e-cigarettes were invented in 2003 and entered the international market in 2007 (1–4). They have rapidly evolved from the first-generation "cig-a-like," designed to look like a conventional tobacco cigarette, to the second-generation vape pens, third-generation box Mods, and the currently popular fourth-generation pod-based devices (5, 6). With the rapid evolution of the hardware of these ENDS devices, four core components have remained the same: a liquid reservoir (called a tank, cartridge, or pod) to hold the e-liquid, a power source (most commonly a lithium rechargeable battery), a heating element (atomizer), and a mouthpiece (7). But the voltage, wattage, temperature, metals, plastics, and other factors differ across devices. Some of these factors have been found to play a major role in the formation of toxins, such as the production of high levels of formaldehyde with the application of high wattage or high temperature (8, 9) and production of carbonyls with combinations of wick length and coil design (10). Toxic metals and other substances have also been detected in e-liquids and e-cigarette aerosols, which may be due to the materials used to make the devices (11–13, 14–19) (Figure 1).

While e-devices have gone through four generations of evolution, the composition changes of e-liquids are innumerable. With hundreds of chemicals added to e-liquids to produce flavors appealing to every man, woman, and child from every country and culture on Earth, there is almost an infinite combination of chemicals being used to create the e-liquids available on the market. Some chemical additives have been approved for ingestion via the gastrointestinal tract, whereas others have never been approved for human consumption. Approval for gastrointestinal consumption does not confer safety for inhalation of the chemicals, as the gastrointestinal tract has evolved to protect the body from absorbing toxins and being harmed by them entering the body in this manner. The lungs, however, have evolved to allow passage of molecules entering the airways directly into the bloodstream, such that adding chemicals to e-liquids for aerosolization and inhalation into the airways is hijacking this evolutionary process to rapidly deliver chemicals contained in e-cigarette aerosols into the bloodstream (20).

Another aspect of vaping exposure is puff topography, which affects coil temperature and thus the composition of e-cigarette emissions. While the topography of smoking conventional tobacco cigarettes is similar between users—rapid (1–1.5 s) puffs, spaced by intervals of 20–30 s until the cigarette is finished, followed by either another cigarette or a break in between cigarettes—vaping of e-cigarettes involves a longer inhalation (2.3–4.3 s),with many different intervals between puffs (21–23) owing to the ability to take a puff at

any time throughout the day without needing to light a new one or commit to smoking a full cigarette. Although some e-cigarette users (vapers) are social vapers, only using e-cigarettes around friends or at parties, others vape continuously, with the first use before getting out of bed and the last use before bedtime. In addition, some people are exposed to secondhand e-cigarette aerosols, such that they are primarily inhaling aerosols that have entered someone else's lungs first. With the lack of sidestream vapor, because e-cigarettes only generate aerosols while the user is actively applying negative pressure to the mouthpiece, secondhand exposure to e-cigarette aerosols is likely to be less intense than that seen with cigarette smoke (which includes both sidestream and exhaled residual smoke).However, studies to date have confirmed that individuals standing close to e-cigarette users or within a confined space (a car or room without good ventilation) undergo significant exposure to e-cigarette aerosols (24–26).

Interestingly, many e-cigarette users are not committed to a single device or a single flavor. Thus, they expose themselves to chemicals produced by multiple e-devices, plus the multitude of chemicals within the flavored e-liquids they choose to use. This complexity of e-cigarette use makes it more challenging to track sources of lung injury and inflammation caused by any single device or chemical. Finally, many e-cigarette users are also conventional tobacco smokers, marijuana smokers, or vapers of THC. Each of these inhalants has its own range of host effects, known and unknown, and the consequences of combining the various inhalants are yet unknown.

3. EPIDEMIOLOGY OF ACUTE LUNG INJURY FROM ELECTRONICVAPING

In 2019, there were several outbreaks of acute respiratory failure of mysterious cause in persons who vape THC, nicotine, or both. Layden et al. (27) reported in the *New England Journal of Medicine* a cluster of cases from Illinois and Wisconsin in which patients presented with acute, severe respiratory distress after using e-cigarette products. Two letters published at the same time added further evidence of this new vaping-induced respiratory disease: a six-case cluster from Utah (28) and a report of imaging changes seen in a range of cases (29). The syndrome has been since termed EVALI by the US Centers for Disease Control and Prevention (CDC). As of January 9, 2020, the CDC reported a total of 2,602 hospitalized EVALI cases across all 50 states, the District of Columbia (DC), and two territories (Puerto Rico and US Virgin Islands). Fifty-seven deaths by then had been confirmed in 27 states and Washington, DC.

Inhalation of toxic environmental agents causes injury, both acute and subacute, to the airways and lung parenchyma (30–38). The pathologic outcomes of lung injuries depend on the dose of the inhaled toxic compound(s) and their physicochemical properties, including solubility and chemical composition (39–43). Much of our current knowledge about toxic inhalation syndromes derives from both occupational and community settings. In occupational settings, toxic compounds include acids, bases, metals, solvents, ozone, phosgene, or chlorine dioxide at high levels. In community settings, exposures occur during derailments of chemical-bearing train cars, factory explosions, and overexposure to household cleaning agents (30–38). Depending on levels and types of inhaled chemicals, patients may develop a wide range of symptoms, including minor respiratory tract

discomfort, acute airway injury and damage, parenchymal pneumonitis, alveolar edema, hypoxemic respiratory failure, and death (Figure 2). This constellation of damage and host responses is clinically called acute respiratory distress syndrome (ARDS) (44–46).

Prior to 2019, numerous vaping-associated lung diseases had been reported, with a heterogeneous collection of pneumonitis patterns, including acute eosinophilic pneumonia, organizing pneumonia, lipoid pneumonia, diffuse alveolar damage and ARDS, diffuse alveolar hemorrhage, hypersensitivity pneumonitis, peribronchiolar granulomatous pneumonitis, and the rare giant-cell interstitial pneumonitis (47–51). Though pathologic manifestations of respiratory injury caused by e-cigarette aerosol inhalation may be diverse, the recent EVALI epidemic was different in that pathologic patterns were consistent with a single common etiology. Most people diagnosed with EVALI reported having used products with THC or cannabidiol (CBD) that are formulated with other terpene oils (83%), while the remaining 17% reported using only nicotine-containing vaping products, which are not routinely mixed with terpenes, including VEA. In a report published in the *New England Journal of Medicine*, Blount and colleagues (52) found VEA in the bronchoalveolar lavage (BAL) fluid of 48 of 51 EVALI patients in a convenience sampling. Coconut oil and limonene were also found in a few patients. THC or its metabolites were found in the BAL of 94% of this group. In bulk samples seized by law enforcement, VEA was found in 20 of 20 seized samples in 2019 but in 0 of 10 seized samples in 2018. Hence, the most prevalent culprit appeared to be the additive VEA. It remains unknown whether VEA or its pyrolysis products is the causal agent of EVALI (52, 53). It is believed that the non-THC/CBD vapers who were diagnosed with EVALI were actually suffering from disparate vaping-associated lung diseases caused by different toxins within the aerosols or different host responses to the inhalants.

Common histopathologic features in EVALI include lipid-laden alveolar macrophages that frequently coincide with vacuolization and vacuolated pneumocytes (27, 52). These findings are typically observed in patients with chemical-induced pneumonitis. Although VEA itself may be the key common exposure culprit for EVALI, underlying mechanisms of toxicity may be more complicated. Specifically, severe inflammatory responses and pulmonary edema may be caused by pyrolysis products of vitamin E oil rather than the parent compound itself. However, examining the toxicity of the pyrolysis products is difficult because some are gases, such as ketene, which are not easily measured in biological samples. Controlled studies using animal models have provided early insight into whether exposure to VEA alone can directly cause acute lung injury (54). In mice exposed to VEA, the level of albumin in BAL fluid (a surrogate marker of lung epithelial damage) and the total number of leukocytes in the lungs were increased to a greater extent than those in mice exposed to air or propylene glycol (PG) and vegetable glycerin (VG). Moreover, cells isolated from the BAL fluid of mice exposed to VEA contained numerous lipid-laden alveolar macrophages, a finding consistent with clinical observations in patients with EVALI (27, 52).

An autopsy series of 23 suspected cases showed that 21 met the EVALI definition and had histological evidence of acute to subacute lung injury, including diffuse alveolar damage or organizing pneumonia (55). Transbronchial and surgical lung biopsies from eight men

aged 19–61 years with respiratory symptoms following e-cigarette use showed acute lung injury, including organizing pneumonia and/or diffuse alveolar damage (56). This is the predominant pattern in the acute process we call EVALI. Additional common features seen in the lung biopsies were fibroblast plugs, hyaline membranes, fibrinous exudates, type 2 pneumocyte hyperplasia, and interstitial organization. Some cases featured a sparse interstitial chronic inflammatory infiltrate. Although macrophages were present within the airspaces in all cases, this feature was not prominent, and findings typical of exogenous lipoid pneumonia were not present (56). While acute features of vaping-induced lung injury are becoming clearer, pathological aspects of chronic lung disease due to vaping are still unclarified.

The epidemiologic profile of EVALI patients who were hospitalized was published in a large series ($n = 2,558$) in 2020 by Werner and colleagues (57). Most EVALI patients were male [32 of 60 (53%) in fatal and 1,666 of 2,498 (67%) in nonfatal cases, respectively]. The proportion of patients was higher among those who were non-Hispanic white [39 of 49 (80%) in fatal and 1,104 of 1,818 (61%) in nonfatal cases, respectively] than among those in other race or ethnic groups.In fatal cases, the proportion was higher among those 35 years of age or older [44 of 60 (73%)] than among those younger than 35 years. Among the patients who had an available medical history, a higher proportion of those with fatal cases than those with nonfatal cases had a history of asthma [13 of 57 (23%) versus 102 of 1,297 (8%)], cardiac disease [26 of 55 (47%) versus 115 of 1,169 (10%)], or a mental health condition [32 of 49 (65%) versus 575 of 1,398 (41%)]. A total of 26 of 50 patients (52%) with fatal cases were obese. The study highlighted that premorbid chronic conditions, including cardiac and respiratory diseases, as well as mental health conditions, were common among hospitalized patients with EVALI.

Although the numbers of EVALI cases dropped dramatically during 2020, cases are still occurring. Unfortunately, the onset of the coronavirus disease 2019 (COVID-19) pandemic and its progress in the United States have presented challenges to both clinicians and epidemiologists in diagnosing, treating, and accounting for disease incidence (58, 59). Moreover, a recent study in youth demonstrated an increased risk of COVID-19 among vapers,most of whom use only ENDS and not THC/VEA solutions. However, it remains unknown whether smoking of cigarettes and vaping e-cigarettes in youth increase risk of COVID-19. To address this question, in May 2020, Gaiha et al. (60) conducted a national online survey among adolescents and young adults aged 13–24 years ($n = 4,351$). Multivariable logistic regression was performed to determine the relationships between COVID-19-related symptoms, testing, and diagnosis with multiple variables, including use of e-cigarettes only,dual use (e-cigarettes and cigarettes),sociodemographic factors, obesity, and complying with shelter-in-place. COVID-19 diagnosis was five times higher among ever-users of e-cigarettes only, seven times higher among ever-dual-users, and seven times higher among past 30-day dual-users. Frequency of positive COVID-19 testing was nine times higher among past 30-day dual-users and 2.6 times higher among past 30-day e-cigarette only users. Symptoms of COVID-19 were 4.7 times higher among past 30-day dual-users. This study revealed that while COVID-19 is less common in youth, use of e-cigarettes only or the dual use of e-cigarettes and cigarettes increases the risk of COVID-19 in this demographic.

4. EPIDEMIOLOGY OF CHRONIC RESPIRATORY ILLNESS IN VAPERS

The focus of the outbreak in 2019 turned to THC vaping products, but it is important not to lose sight of the larger health issues around vaping. Global usage of ENDS has increased in the last decade, especially among youth and young adults (61). In 2019, the prevalence of ENDS among middle and high school students in the United States was 10.5 and 27.5%, respectively (62). ENDS are noncombustible tobacco products that heat and aerosolize a liquid containing humectants and solvents (5–7, 63). The liquid contained in the tanks, cartridges, and pods used in the e-devices is commonly referred to as e-liquid, and commercial labels list the primary ingredients as PG and VG (also known as glycerol), plus flavorings and nicotine (15, 16). The e-devices heat the liquid via activation of the battery and conduction of the energy through a heating coil within the liquid. Application of negative pressure via the mouthpiece is used to pull the e-liquid through a mesh to create a fine aerosol. Recently, analyses of commonly used vaping fluids have shown that e-cigarette fluids contain at least seven groups of potentially toxic compounds: nicotine, carbonyls, volatile organic compounds (VOCs, such as benzene and toluene), particles, trace metal elements according to flavor (14, 17), and bacterial endotoxins and β-glucans (18, 19) (Figure 1). Additive compounds without nicotine can cause lung damage by eliciting cellular toxicity. For example, two flavoring chemicals alone, diacetyl and 2,3-pentanediol, have been shown to perturb transcriptomic changes related to ciliogenesis and cytoskeletal structure in well-differentiated primary normal human bronchial epithelial (NHBE) cells (64). The literature contains many reports of acute lung disease caused by the vaping of nicotine-containing ENDS, including acute eosinophilic pneumonia, respiratory bronchiolitis-associated interstitial lung disease, and hypersensitivity pneumonitis (47, 55, 56). The heterogeneity in the response to inhaled insults is not unexpected, given the numerous chemicals contained in e-cigarette aerosols and variability in how hosts respond to different insults due to underlying genetic and environmental factors. However, some commonalities in acute lung injury pathology related to THC products containing VEA emerged during the outbreak (55).

Population-based data on individuals who vape e-liquids chronically are sparse. There are a number of reports on known toxic exposures to humans generated by actual products on the market and growing evidence of adverse human health effects (65). As with cigarette smoking, the inhalation of chemicals contained within ENDS aerosols can elicit inflammatory responses in the lungs. Vaping has thus far been associated with asthma (39, 66–69), bronchiolitis (55, 56), and alteration of airway defenses (64). In the study including the Population Assessment of Tobacco and Health (PATH) study Wave 4 data on 33,606 US adult participants who indicated ever using e-cigarettes, the risk of wheezing and other respiratory symptoms was greater in ENDS users as compared to nonusers and lower compared to smokers (70). Comparisons of adults who ever vaped without marijuana versus those who ever vaped with marijuana (at least sometimes or rarely) showed that self-reported respiratory symptoms over the past 12 months were significantly increased when vaping with marijuana, including wheezing/whistling in the chest, wheezing in the chest during or after exercise, and a dry cough at night (71). This study revealed that lifetime

use of e-cigarettes with marijuana associates with self-reported respiratory symptoms over the past 12 months among adults.

Studies of e-cigarette exposure in humans are limited but have demonstrated pulmonary and cardiac toxicities. In one study of healthy never smokers who were exposed to ENDS aerosols for a short time, 10 subjects were assessed at baseline with questionnaires, chest X-rays, lung function tests, plasma levels of endothelial microparticles, and bronchoscopy to obtain small airway epithelial cells and alveolar macrophages. One week later, subjects inhaled 10 puffs of Blu brand e-cigarettes two times. Following repeated exposure, both clinical and biological parameters were examined. Although no significant changes in clinical parameters were observed, biological changes were observed. Compared to baseline, inhalation of e-cigarette aerosol with nicotine caused altered transcriptomes of small airway epithelial cells and alveolar macrophages among all subjects and elevated plasma microparticle levels, providing in vivo human data demonstrating that acute inhalation of e-cigarette aerosols dysregulates normal human lung homeostasis in a limited cohort of healthy naïve individuals (72).

Human exposure studies have also generally shown increased sympathetic nerve activity, platelet hemostasis processes, reactive oxygen species (ROS) generation, and endothelial dysfunction. Studies with conflicts of interest vis-à-vis industry sponsorship were less likely to report such effects, whereas almost all nonconflicted studies did (20). Interestingly, adverse cardiac effects were also noted in a study of healthy, nonsmoking, and nonvaping adults who were exposed to secondhand vaping emissions (25). In this randomized, repeated measures cross-over study, total heart rate variability [measured by the standard deviation of beat-to-beat (NN) intervals (SDNN)], heart rate variability over short cycles [the average of SDNN (ASDNN)], and heart rate correct QT intervals (QTc) were assessed. Nicotine from these e-cigarette exposures were associated with a 7.8% decrease in SDNN, a 7.7% decrease in ASDNN, and a 3.8-ms decrease in QTc. Greater nicotine over a longer exposure (15–30 min) was associated with greater QTc reductions. These results were the first evidence of short-term, secondhand e-cigarette vapor-induced cardiac autonomic effects in healthy nonsmokers.

In another study (26), exhaled breath was collected from 17 e-cigarette vapers and analyzed for nicotine, PG, VG, formaldehyde, acetaldehyde, acrolein, tobacco-specific nitrosamines, and heavy metals. Among the analytes in exhaled breath, levels of nicotine, PG, tobacco-specific nitrosamines, and copper were increased. Based upon the initial assessment of toxicants in exhaled breath, bystander exposure was estimated for two different exposure scenarios. Each of the two scenarios simulated daily exposures during either a daily commute in a small unventilated car with two e-cigarette users or a daily office hour in an office-sized space with one e-cigarette user. Results showed that bystanders may experience irritation of the respiratory tract and systemic effects, including palpitations and increased systolic blood pressure. The irritation of the respiratory tract was associated with exposure to PG and VG, and systemic effects were associated with exposure to nicotine (26).

Early life exposure to e-cigarettes/ENDS is a public health concern. There are no human studies, asyet, of maternal ENDS use and birth/development outcomes, but the

principal toxicants delivered by these devices raise considerable public health concern (73). Exposure of female mice to e-cigarettes in early pregnancy significantly impaired embryo implantation, as evidenced by the nearly complete absence of implantation sites in e-cigarette-exposed animals at day 5, despite exhibiting high levels of progesterone, an indicator of pregnancy (74). Effects of nicotine, a key substance of concern but by no means the only one in ENDS, on the fetus are well known (75–78). Hence, first- and second-hand e-cigarette aerosols may well pose significant hazards to the fetus. How these adverse fetal responses impair pulmonary function and disease risk in later life is a subject of ongoing research.

5. MODELS AND MECHANISMS OF LUNG INJURYAND INFLAMMATION

Clinical observations and epidemiological studies confirming the adverse biological effects of e-cigarettes on human health, and increasing cases of EVALI-related deaths, emphasize the urgent need to understand the pathophysiologic mechanisms of EVALI and acute and chronic effects of e-cigarette use. However, due to the rapid growth of e-cigarette use worldwide, these mechanisms and the long-term consequences of e-cigarette use remain unknown. Unknown pathophysiologic mechanisms result in a lack of biomarkers, which are needed as diagnostic tools. To identify potential biomarkers and targeted treatments and to prevent vaping-induced diseases including EVALI, both in vitro and in vivo approaches are required to understand the causes and pathophysiologic mechanisms of EVALI.

E-cigarettes elicit adverse health effects through direct contact of aerosols (also called e-cigarette vapor) with tissues or cells in the oral cavity and lung or through systemic effects on multiple organs including the heart, brain, eyes, and kidneys (65, 79, 80, 81–86) (Figure 3). Because the most substantial toxicity of e-cigarettes is expected in the lung, and systemic effects may be propagated by injured lung, many studies have focused on the lung using both in vitro and in vivo models (Figure 2). In vitro models (82, 87) are less likely to replicate real-life exposure to e-cigarettes but provide mechanistic insight into molecular and cellular pathways impacted by specific chemicals contained in e-cigarette aerosols. Despite the known limitations of in vitro models, multiple studies have found similarities of transcriptomic profiles in airway epithelial cells from e-cigarette users and cultured NHBE cells exposed to e-cigarette aerosols (72, 88, 89). In the earliest in vitro studies, cells were exposed to e-liquids, which do not recapitulate the chemical composition of aerosols generated by ENDS. Recently, researchers transitioned to systems in which mammalian cells were directly exposed to e-cigarette aerosols, increasing the relevance of these studies to real-life exposures.

In vivo exposures have been done primarily in rodents, with early models also using e-liquids instead of aerosols (90, 91). Researchers have now universally transitioned to nose-only or whole-body exposures of animals to freshly generated e-cigarette aerosols. Use of commercially available e-cigarettes has increased the translatability of these studies to the general population but has made it difficult for researchers to keep up as new generations of devices emerge every 2–4 years. Studies focused on flavors popular in the vaping community also increase the relevance of results to e-cigarette users. Alternatively, some researchers focus on core components of e-liquids (PG, VG, and nicotine) that are

present in all e-liquid solutions and thus have high relevance to the entire vaping community. As specific chemicals have been identified as harm inducing, such as VEA as the causal agent of EVALI, researchers introduce these into vaping exposure systems to rapidly assess for lung injury and inflammation. Because inhalants can alter the immune and inflammatory state of the lungs and body (e.g., tobacco smoke), researchers also assess for alterations in the responses of e-cigarette-exposed animals to common clinical challenges using models of acute lung injury, bacterial and viral pneumonia, and airway reactivity.

5.1. Inflammatory Cytokines and Mediators

Overall, exposure to e-cigarettes induces secretion of proinflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α), from epithelial cells and immune cells in the upper airway and lung (80, 92–96). A particular pattern of neutrophil signals has been detected across studies. In the sputum of e-cigarette users, the neutrophilic granule proteins neutrophil elastase, proteinase 3, azurocidin 1, and myeloperoxidase are significantly increased (97),suggesting activation of neutrophils by e-cigarette exposure. Exposure of neutrophils to e-cigarette vapor extract markedly increases expression of CD11b and CD66b, which play a critical role in the activation of neutrophils (95). Furthermore, exposure to e-cigarette vapor extract leads to increased IL-8 secretion and protease activity, including that of neutrophil elastase and matrix metalloproteinase 9 (95). Increased proteases can damage lung basement membrane and extracellular matrix, leading to emphysema (98, 99). In one of the first studies of VEA using alveolar type II epithelial cells cultured in an air-liquid interface, VEA was incorporated into the cells with subsequent release of monocyte and neutrophil chemokines, demonstrating the direct role of VEA in inducing inflammatory responses (96).

Across in vivo exposures, the main cells found to be recruited to the lungs in response to e-cigarette aerosol inhalation include macrophages (94, 100, 101), neutrophils (102), eosinophils, and T cells (101, 103). As evidence of the importance of both e-device type and the composition of the heating element, Kleinman et al.'s (102) histologic analysis found acute lung injury in rats exposed to e-cigarette aerosols generated by a heating element made of nickel-chromium alloy (at 70 W) but not one with a stainless-steel atomizer. To assess how e-cigarette exposure alters airway inflammation, two distinct mouse models of allergic asthma had been used: house dust mite and ovalbumin (104–106). In a mouse model of allergic asthma using house dust mite, which induces type 2-low airway inflammation, exposure to e-cigarette aerosols containing 12 mg/mL nicotine suppressed allergic inflammatory responses (105).In a mouse model of allergic asthma using ovalbumin, which induces type 2-high airway inflammation, exposure to e-cigarette aerosols containing 18 mg/mL nicotine increased allergic inflammatory responses (106).Because these two groups used two different asthma models (type 2-low inflammation versus type-2 high inflammation), it is possible that e-cigarette aerosol effects vary based on asthma phenotype. Alternatively, differences could also be due to different e-devices and wattage applied (Joyetech eVic-VT e-cigarette with a maximum of 45 W versus the Kanger Mini ProTank 2 with 5.76 W). As mentioned above, much work has focused on the e-cigarette effects on the inflammatory state of the lungs. Investigators have identified changes in numerous cytokines within the airways and lung parenchyma of e-cigarette-exposed rodents, but consistent

patterns have not been identified, likely owing to the heterogeneity of exposure models, e-devices, and e-liquids used (65).

5.2. Cellular Damage

Vaping results in the generation of increased reactive aldehyde species (13, 107) causing the cellular accumulation of 4-hydroxynonenal, which induces apoptosis, mitochondria dysfunction, and protein inactivation (108–110).E-cigarette exposure also directly induces cellular damage by driving increased ROS generation during oxidative burst (92,111,112) and DNA damage (80,88,113). In multiple epithelial cell lines, e-cigarette exposure increases comet tail length and the accumulation of gamma H2AX (γH2AX) foci, suggesting single-strand and double-strand DNA breaks caused by e-cigarettes (113). E-cigarette-exposed cells also show increased rates of apoptosis and necrosis, regardless of nicotine content (113). Higher levels of apoptosis in lung cells of mice exposed to e-cigarette aerosols daily have also been observed, which suggests an increased risk of developing emphysema (94), as well as apoptosis within cardiac tissue (114). Finally, Canistro et al. (115) demonstrated the comutagenic and cancer-initiating effects of e-cigarette aerosol exposures in a rat model. They found that e-cigarette aerosol inhalation caused a boost in phase I carcinogen-bioactivating enzymes, including activators of polycyclic aromatic hydrocarbons, and increased oxygen free radical production and DNA oxidation to 8-hydroxy-2′-deoxyguanosine.

Inflammation and DNA damage are also associated with compromised oral health (80).In both human periodontal ligament fibroblasts and human gingival epithelium progenitors, exposure to e-cigarettes increases secretion of inflammatory mediators [IL-8 and prostaglandin E2 (PGE2)] and DNA damage as marked by γH2AX (80). E-cigarette exposure increases generation of ROS that induces inflammatory responses in both human epithelial cells and mouse models (116). In H292 cells maintained in air-liquid interface culture, exposure to flavored e-cigarette aerosols induces cellular toxicity and ROS generation (111). In primary NHBE cells differentiated in air-liquid interface culture, exposure to e-cigarette aerosols induces a marker of ROS, 8-isoprostane, in a dose-dependent manner (88). Gene ontology analysis suggests that cellular regeneration and differentiation are impaired, while DNA damage and ROS generation are increased in NHBE cells. In small airway epithelial cells, exposure to e-cigarette emissions generates eight times more ROSthanincontrolcells(117).Murineexposuresarealsoassociatedwithgeneexpressionchanges consistent with increased oxidative burst and apoptosis in cardiac tissue in particular, raising concern for the development of cardiomyopathy (114), and e-cigarette exposures lead to increased lipid peroxidation, which is evidence of oxidative stress (118).

E-cigarette aerosols also impact mitochondria, cilia, and fibrosis. E-cigarette exposure induces mitochondria dysfunction, resulting in reduced ATP production, which is linked to compromised ciliary functions (119). Mitochondrial dysfunction may be due to increased ROS within mitochondria and may lead to insufficient energy production in cells. Chronic exposure to e-cigarette aerosols can be associated with the development of organ fibrosis, with increases in both profibrotic and oxidative stress markers (93).

5.3. Transcriptional and Proteomic Modifications

E-cigarette aerosols induce transcriptional alterations in both oral and airway epithelial cells (64, 80, 88, 89, 94, 120–122). As identified by RNA sequencing analysis and confirmed by quantitative polymerase chain reaction (qPCR), two tumor suppressor genes (*NOTCH1* and *HERC2*) are downregulated in e-cigarette users (120). Transcriptional analysis of oral epithelial cells from e-cigarette users suggests that cancer risk is increased. E-cigarette exposure reduces the ciliated-cell marker gene (*FOXJ1*) while increasing expression of the genes involved in xenobiotic metabolism (*CYP1A1* and *CYP1B1*) and oxidative stress (*DNAH10*) (88).

Proteomic analysis of airway epithelial cells from biopsies revealed unique protein expression profiles in vapers compared to analysis in nonsmokers (89). In airway epithelial cells from vapers, expressions of MUC5AC, MUC4, and CYP1B1 proteins were higher than in those from nonsmokers. These findings from human tissues were further validated in NHBE cells after exposure to aerosolized PG/VG. Exposure to PG/VG for four days induced MUC5AC protein in well-differentiated NHBE cells. These data suggest that e-cigarette exposure may cause airway obstruction by increased secretion of gel-forming mucin in vapers (89).Interestingly, e-cigarette exposure reduces ribosomal proteins and subsequent protein biogenesis in NHBE cells (121), indicating the potential detrimental effect of e-cigarette smoke on ribosomes and the associated protein synthesis in the airway epithelium. In NHBE cells, the phospholipid and fatty acid triacylglycerol metabolism pathways are found among the cellular pathways with the most significantly enriched gene expression following e-cigarette exposure (122). These data suggest that alterations in cellular glycerophospholipid biosynthesis are an important consequence of e-cigarette exposure.

5.4. Impaired Host Defense: Barrier Dysfunctions, Mucociliary Clearance,Bacterial Clearance, and Viral Defense

Epithelial integrity is the first line of lung defense. However, exposure to e-cigarette aerosols not only causes sloughing of epithelial cells, but it also disrupts epithelial barrier integrity (88, 93, 96, 111, 112). In mice, VEA inhalation leads to lung damage similar to that seen in humans (54). Specifically, VEA inhalation causes lung edema, neutrophilia, epithelial cell death, and lymphocyte-predominant perivascular inflammation (96) and also reduces the production of surfactant protein A (96, 112).

Mucociliary clearance is critical for protection of the respiratory tract against inhaled toxic substances (123). Impaired mucociliary clearance results in chronic inflammation by establishing a favorable environment for pathogenic bacterial colonization and growth (123, 124). In both in vitro and in vivo models, e-cigarette exposures compromise mucociliary clearance by reducing ciliary beating frequency (94, 119, 125, 126). In NHBE cells, e-cigarette exposure reduces not only the number of ciliated cells (64, 94) but also ciliary beating frequency (94, 119, 125, 126). Exposure to aerosolized, nicotine-containing e-cigarette fluids reduces ion conductance and mucociliary function in human bronchial epithelial cells and induces airway hyperreactivity and air space enlargement in exposed mice (note that e-liquids were aerosolized with a medication nebulizer, not an e-device)

(94). A transient reduction of ciliary beating frequency was also observed after exposure to cinnamaldehyde-containing e-liquid, vaped aerosol, or cinnamaldehyde alone (119). Reduced ciliary beating frequency occurred secondary to reduced ATP production, which resulted from dysregulated mitochondria (119). In well-differentiated NHBE cells, exposure to e-cigarette vapor reduced airway surface liquid hydration and increased mucus viscosity in a nicotine-dependent manner (126). Impaired mucociliary clearance appears to be mediated by TRPA1, an ion channel, and not nicotinic acetylcholine receptors.

Multiple cells in the lungs contribute to host defense through direct antimicrobial activities. E-cigarette vapor exposure has been found to inhibit antibacterial function of epithelial cells, macrophages, and neutrophils (63, 93, 127). Inhibition of phagocytosis in macrophages (92, 128) is one mechanism by which vaping impacts bacterial clearance. The other impaired host defense mechanism is inhibition of neutrophil extracellular trap formation (NETosis) (127). Exposure of NHBE cells to e-liquids reduces the expression of SPLUNC1,a host defense molecule,providing further evidence of the impaired bacterial host defenses in e-cigarette users (129). In particular, NHBE cells were isolated from young healthy donors aged 8–10 years. Thus, these in vitro studies support epidemiological studies demonstrating the increased rates of chronic bronchitis symptoms in adolescent e-cigarette users compared to nonsmokers (130). Finally, e-cigarette aerosol exposure has been found to promote biofilm formation and virulence of common bacterial colonizers and pathogens (63, 131), which indicates that vapers, like conventional tobacco smokers before them, may develop higher rates of invasive bacterial infections.

In vitro e-cigarette aerosol exposure causes apoptosis, secondary necrosis, and necrosis in lung epithelial cells and apoptosis and inflammatory caspase–mediated cell death in macrophages (132). Exposure to e-cigarette aerosols containing nicotine inhibits phagocytic and efferocytic abilities of primary macrophages, leading to decreased bacterial clearance when challenged with a bacterial pathogen (118). Exposure of neutrophils, which are the first cells recruited to the site of infection, to e-cigarette aerosols with and without nicotine leads to decreased phagocytosis and decreased bactericidal activity (63, 127). Suppression of antimicrobial functions of both macrophages and neutrophils by e-cigarette aerosols in vitro and ex vivo supports the concept that e-cigarette use damages host defenses and will lead to increased susceptibility to pulmonary infections.

Alveolar macrophages cultured with either e-liquid or e-cigarette vapor condensate result in a dose-dependent reduction in cell viability (92). E-cigarette vapor condensate is significantly more toxic to alveolar macrophages than nonvaped e-liquid. Excessive production of ROS, inflammatory cytokines, and chemokines induced by e-cigarette aerosols may induce an inflammatory state in alveolar macrophages within the lung that is partly dependent on nicotine. Inhibition of phagocytosis also suggests that users may suffer from impaired bacterial clearance. The two primary chemicals found in e-liquids are PG and VG. These additives act as vehicles and carriers for nicotine, which is highly insoluble in water, as well as for flavorings. Although some researchers view these chemicals as unimportant, both have been found to have toxic effects at both the cellular and host levels. PG and VG reduce glucose uptake in NHBE cells in an air-liquid interface culture (133). This is relevant in that glucose transports move extracellular glucose into airway cells to

maintain luminal surface with a low concentration of glucose in which bacterial growth is prohibited. Thus, reduced glucose uptake by PG/VG suggests compromised innate immunity against bacterial infections in e-cigarette users (134). In NHBE cells in vitro, PG/VG also reduce membrane fluidity and impaired protein diffusion, suggesting that PG/VG could alter cellular endocytosis and exocytosis (89).

In terms of viral immunity, airway epithelial cells from vapers were found to have decreased expression of Toll-like receptor 3, suggesting that viral immunity is impaired by e-cigarette use (89).Infectionofe-cigarette-exposedmicewithinfluenzaleadstoincreasedlunginflammationand injury, consistent with an inability to control the viral infection and the potential to develop an immunomodulated state leading to excessive lung inflammation in response to viral infection (118, 135). Due to the COVID-19 pandemic, some groups have assessed specifically for the effects of vaping on molecules that play a role in SARS-CoV-2 infections, finding that female (but not male) mice exposed to e-cigarette aerosols containing nicotine had increased angiotensin-converting enzyme 2 (ACE2) levels in the lung (101).

6. CONCLUSION

Since the appearance of ENDS on the market, the prevalence of e-cigarette use has become a growing public health concern. Despite the expected toxicity of inhaled nicotine and various chemical additives (Figure1), the impact on human health has been controversial. To date, a growing body of evidence indicates that e-cigarettes cause lung inflammation and injury (Figure 2) as well as systemic adverse effects in multiple organs (Figure 3). However, the pathophysiological mechanisms by which the lung and various organs are damaged remain unknown. Thus far, most evidence was collected in observational studies, some of which showed contradictory outcomes. Incongruous outcomes may be attributable to multiple factors, including frequency of vaping, e-device type, e-liquid composition, age, sex, and underlying health conditions. Henceforth, we should prioritize our efforts toward controlled studies to elucidate the pathophysiologic mechanisms behind the adverse health effects caused by e-cigarettes. Advanced knowledge will allow us to develop biomarkers and treatments of vaping-related diseases.

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Glossary

E-cigarette	electronic cigarette
EVALI	electronic cigarette or vaping use-associated lung injury
VEA	vitamin E acetate
ENDS	electronic nicotine delivery systems
THC	tetrahydrocannabinol

CBD	cannabidiol
BAL	bronchoalveolar lavage
PG	propylene glycol (propane-1,2-diol)
VG	vegetable glycerine (propane-1,2,3-triol)
NHBE cells	normal human bronchial epithelial cells
ROS	reactive oxygen species

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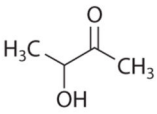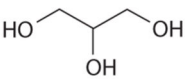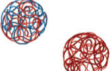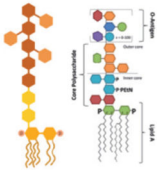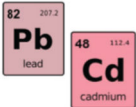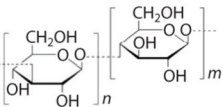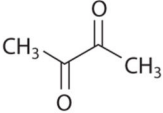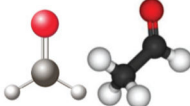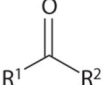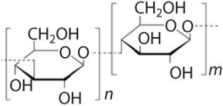
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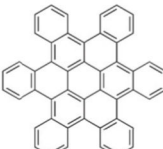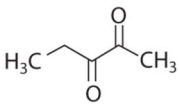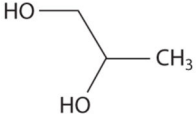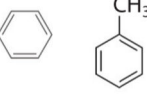
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Allen et al. 2016[1]	Klager et al. 2017[2]	Lee et al. 2017[1]	Lee et al. 2019[2]	Fowles et al. 2020[3]	Lee & Christiani 2020[2]
Acetoin	Glyercol	Nanoparticles	Endotoxins	Heavy metals	β-Glucans
Diacetyl	Formaldehyde and acetaldehyde	Carbonyls	β-Glucans	Polycyclic aromatic hydrocarbons (PAHs)	
2,3-Pentanedione	Propylene glycerol	Volatile organic compounds (VOCs)			

[1]Study based on aerosols
[2]Study based on e-liquids
[3]Study based on aerosols and e-liquids

Figure 1.
Toxic substances detected in e-cigarette aerosols and e-liquids. Toxic substances detected in e-cigarettes include toxicants (chemicals, nanoparticles, and heavy metals) and toxins (endotoxin and β-glucans). Figure adapted from images created with BioRender.com.

Overall effects on the lung
- Increased cytokines and chemokines
- Increased infiltration of inflammatory cells
- Increased activity of inflammatory cells
- Increased ROS and DNA damage (γH2AX)
- Altered proteomics and transcriptomic profiles
- Altered cellular metabolism

Effects on airway physiology
- Hyperreactivity
- Increased airway resistance
- Mucus hypersecretion
- Impaired ciliary beating
- Epithelial cell sloughing

Effects on host defense
- Disrupted epithelial layer integrity
- Reduced macrophage phagocytosis
- Reduced bacterial clearance
- Reduced antiviral immunity

Effects on alveolar compartment
- Impaired gas exchange
- Hypersensitive pneumonitis
- Alveolar edema
- Reduced surfactant protein
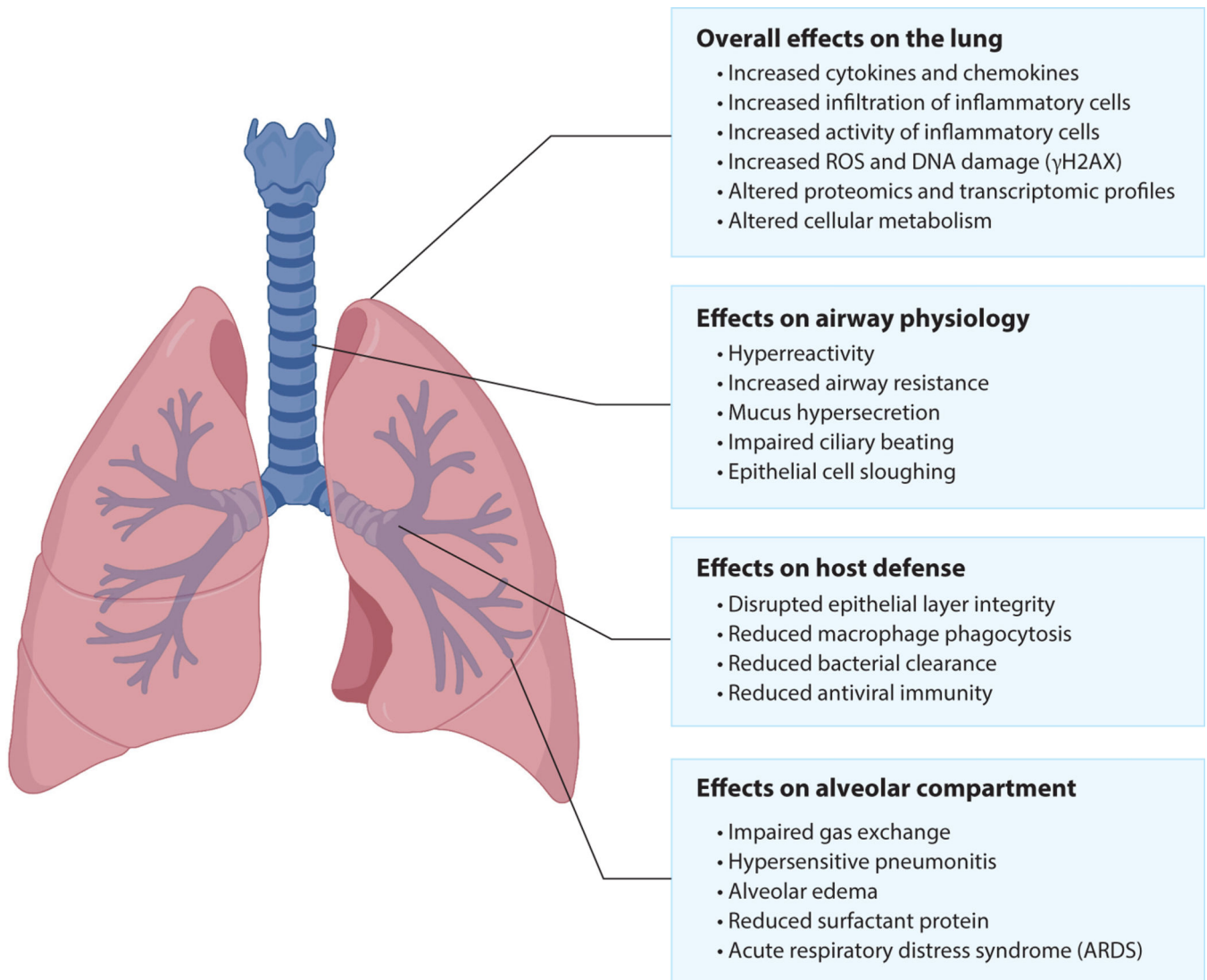- Acute respiratory distress syndrome (ARDS)

Figure 2.
Mechanistic overview of the adverse effects of electronic cigarettes on the lung. As a primary organ, the lung is damaged and impaired by electronic cigarette use. Figure adapted from images created with BioRender.com. Abbreviations: γH2AX, gamma H2AX; ROS, reactive oxygen species.
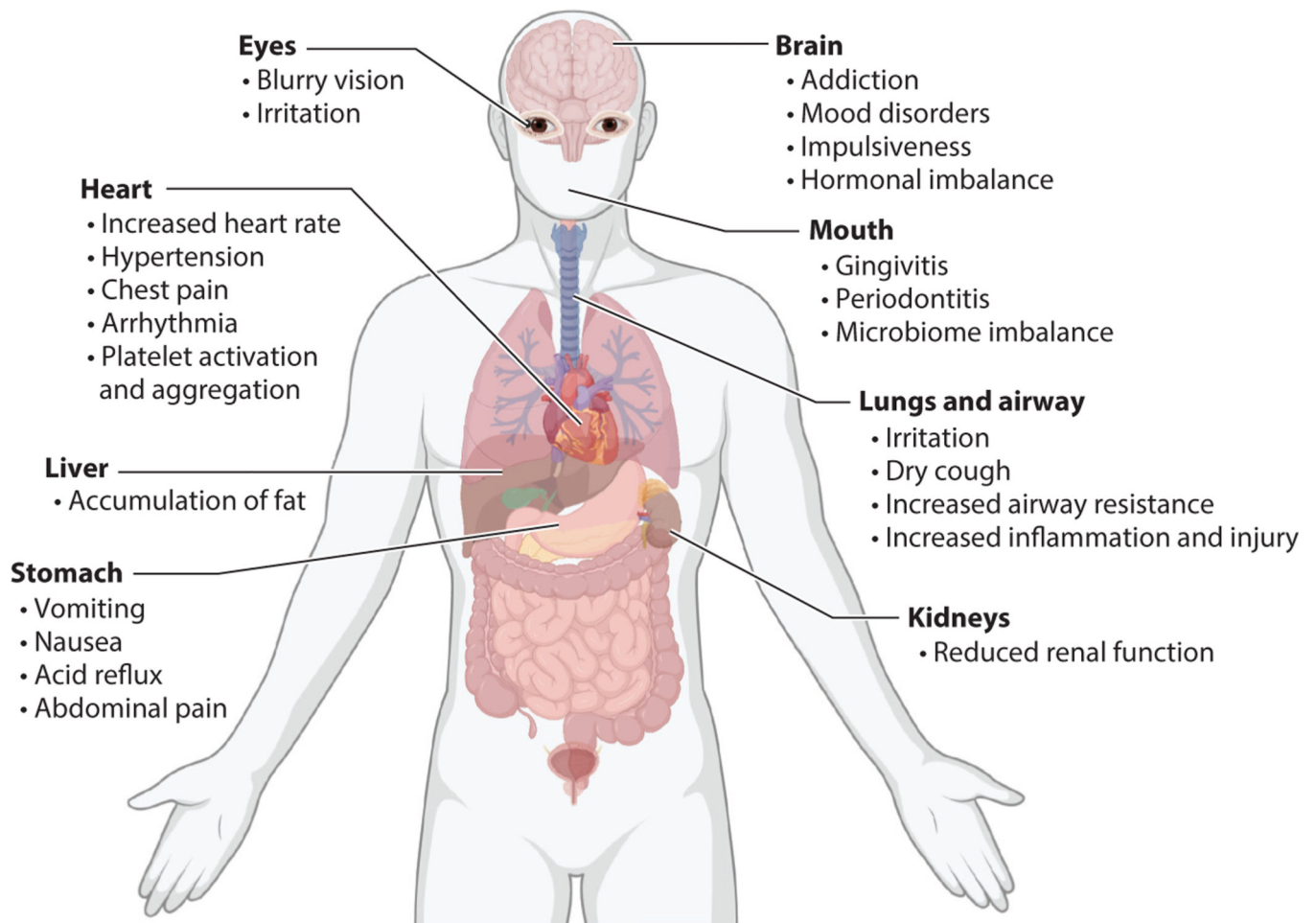
Figure 3.
Adverse effects of electronic cigarettes on human health. As the site of the contact with inhaled toxic chemicals, the lungs are directly damaged by electronic cigarette use, but multiple organs are damaged by systemic adverse effects. Figure adapted from images created with BioRender.com.