

*Annual Review of Pharmacology and Toxicology*  
**Multisystem Toxicity of  
 E-Cigarettes in Preclinical  
 and Clinical Studies:  
 Pathophysiologic Effects of  
 E-Cigarette Aerosol Exposures  
 from Head to Toe**

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Annu. Rev. Pharmacol. Toxicol. 2026. 66:325–52

First published as a Review in Advance on August 19, 2025

The *Annual Review of Pharmacology and Toxicology* is online at [pharmtox.annualreviews.org](http://pharmtox.annualreviews.org)

<https://doi.org/10.1146/annurev-pharmtox-062124-022856>

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## Keywords

electronic cigarette, e-cig, vaping, nicotine, aerosol, oxidative stress

## Abstract

The global population breathes unsafe levels of pollutants. In recent years, electronic cigarettes (e-cigs) have become a significant source of particulate matter (PM), which causes injurious effects across organ systems. E-cig users and bystanders are exposed to concentrated aerosols, commonly called vapor, that we now know can have harmful long-term consequences due to PM and chemicals contained within. E-cigs are diverse in design, and e-liquids vary dramatically, making it difficult to draw broad conclusions from studies of different devices, brands, and flavors. With the rise in popularity of e-cigs, it is important to define the health effects across the body. In this comprehensive review, we dissect and summarize the known organ-specific effects of e-cigs, including underlying molecular mechanisms. Notably, e-cig aerosols broadly cause increased cytokine release and oxidative stress, which are associated with a heightened risk of organ dysfunction. We also highlight ways to minimize harmful e-cig constituents to develop safer products.

## INTRODUCTION

One of the main self-induced inhaled exposures is cigarette smoke. Cigarette smoking is the leading cause of preventable disease and death in the United States and has accounted for approximately 20 million deaths in the United States since 1964 (1, 2). While traditional cigarette smoking rates have declined in recent decades in the United States, an estimated 5.6 million American children alive today will die prematurely from a smoking-related disease. Cigarette smoke has high levels of particulate matter (PM)<sub>2.5</sub> (PM < 2.5 μm; considered fine PM), which are small enough to reach the alveoli, thus driving lung damage and inflammation throughout the lungs (3).

Electronic nicotine delivery systems (ENDS), including electronic cigarettes (e-cigs), were invented as a supposedly healthier alternative to conventional cigarettes and were introduced to the commercial market in 2007 (4). E-cigs include a wide range of battery-powered devices using an array of different e-liquids. E-liquids consist primarily of a mixture of propylene glycol (PG) and glycerol (commonly called vegetable glycerin or VG), chemical flavorants, and nicotine ranging from 1.6 to 59 mg/mL (5, 6). Different ratios of PG:VG, such as 70:30, 50:50, or 20:80, produce different quantities of PM, with increasing VG associated with the greatest production of PM<sub>2.5</sub> (7). Furthermore, wattage applied to e-liquids during aerosolization also impacts PM size (8). While e-cigs produce lower levels of toxins, they have been found to contain volatile organic compounds (VOCs), heavy metals, aldehydes, and diacetyl (9). Recently, the World Health Organization has commented that it is currently unclear whether ENDS products are dangerous as compared to traditional cigarettes.

E-devices use lithium batteries to heat e-liquids and create an aerosol for inhalation. E-cig aerosols consist of both fine PM<sub>2.5</sub> and ultrafine PM<sub>0.1</sub> (PM < 0.1 μm) that users and nonusers are exposed to via primary and secondary routes, respectively (10). Due to the rising rates of e-cig usage and the emerging variations of e-cigs, these concentrated particles will play a significant role in shaping air pollutants, and their influence will likely continue to grow in the future. Currently, multiple studies report alarming levels of nicotine and toxicants in the latest generation of e-cigs that pose significant harm to multiple organ systems. However, many studies have investigated the health effects of e-cig exposure in older adults who are former conventional cigarette smokers or concurrent conventional cigarette smokers, which introduces bias into the results. One way to

deal with this bias biologically is to focus studies on younger subjects (12–30 years old) who have had much less exposure to combustible tobacco (and other lung-altering inhalants, such as air pollution). Adolescents and young adults typically start vaping e-cigs before adding in or transitioning to combustible tobacco, making them an ideal population to study to define the biological effects of e-cig aerosol inhalation. Another challenge is that a wide variety of e-cigs have been studied, including differences in e-device, PG:VG ratio, nicotine type and concentration, and types of chemical flavorants, which makes drawing conclusions across studies difficult. This comprehensive review attempts to describe these phenomena and point out methodological issues impacting these findings.

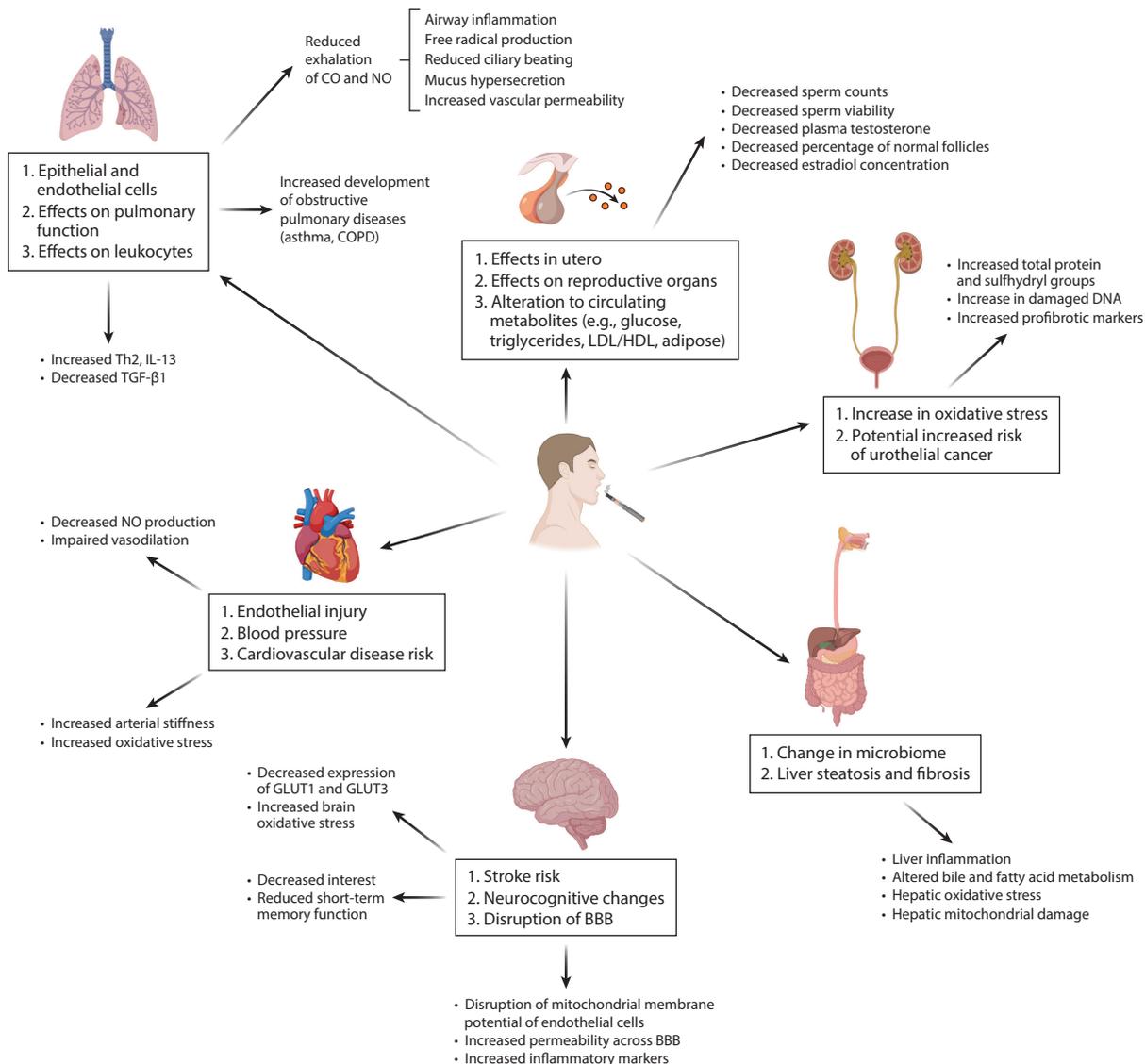
In this comprehensive review, we describe the organ-specific effects of e-cig exposure. There are numerous interactions between organ systems that we address throughout (**Figure 1**). Understanding how variations of e-cig exposures play a role in disease initiation and progression will allow us to design effective mitigation strategies, including a push for change in policy. As the use of e-cigs continues to rise in both indoor and outdoor settings, so does concern regarding the health effects of these readily available, novel, self-induced exposures.

## E-CIGARETTE OVERVIEW

The modern form of the e-cig was invented in 2003 as an alternative nicotine delivery device with the aim of helping combustible (conventional) cigarette smokers quit altogether. As e-cig use became popular in the 2010s, an inverse trend was seen with diminished rates of conventional cigarette smoking by children and young adults, but a concomitant increase in rates of e-cig vaping (11). However, while e-cigs have led to lower rates of initiation of combustible tobacco use in adolescents, e-cigs have had no overt impact on tobacco quit rates in adults, with the decline in adult tobacco use continuing at the same pace since 1964, when the first Surgeon General's Report of the harms of smoking was published (12). Approximately 40% of adult vapers also smoke conventional tobacco, which is known as dual use (13). Because e-cigs only entered the international market in 2007 and only became popular around 2013–2016, there is very little known about the long-term health effects of these products as compared to conventional tobacco and PM in general, which have been studied for decades. Further, dual use of e-cigs with conventional tobacco has been studied even less so, although there is growing concern that the combined impact of these inhalants on the respiratory tract may be more detrimental than either alone (14).

E-cigs got their name because these devices use electricity, from a battery, to heat and aerosolize nicotine-containing solutions. All e-cigs are composed of a battery, heating coil, resistor, and tank, pod, or cartridge to hold the e-liquid. While e-cigs were originally designed to look like a conventional cigarette, garnering the name cig-a-like, all generations of e-cigs since have been designed to look different from conventional tobacco cigarettes. While there exists a large variety of sizes, shapes, and colors of e-cigs on the market, e-cigs across all generations have the same core components.

The heating and aerosolization of e-liquids by e-cigs leads to the emission of aerosols containing harmful  $PM_{2.5}$  and  $PM_{0.1}$  (15). Different solvent compositions of e-liquids release different sizes of PM. For example, e-cigs with solvents containing lower ratios of PG:VG emit higher levels of  $PM_{2.5}$  (8). E-liquids were designed with one goal in mind: delivering nicotine via inhalable aerosols. Thus, the solvent PG became the main ingredient in e-liquids, as base nicotine is soluble in PG at high concentrations. VG became the second core ingredient due to its solvent properties and because it makes e-cig vapor clouds visually denser. Base nicotine (nicotine in its unprotonated form) was used in the first through third generations of e-cigs, but fourth-generation devices evolved to use nicotinic salts (e.g., protonated salts) because much higher concentrations are more



**Figure 1**

Overview of e-cig effects on organ systems. E-cig aerosol chemicals impact organ systems in different ways. Inflammation within each system is commonly driven through increased cytokine expression and release, which leads to organ system-specific disorders. While not entirely comprehensive, this figure highlights several effects that e-cig exposure may have on the pulmonary, cardiovascular, central nervous, gastrointestinal, renal, and endocrine systems with the associated mechanism(s) of action. Abbreviations: BBB, blood-brain barrier; CO, carbon monoxide; COPD, chronic obstructive pulmonary disease; GLUT, glucose transporter; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NO, nitric oxide; TGF- $\beta$ 1, transforming growth factor  $\beta$ 1. Figure adapted from images created in BioRender; Wold L. 2025. <https://BioRender.com/si8nbc2>.

palatable and can be inhaled with less severe throat burning sensations. While the first through third generations typically used nicotine concentrations of 3–12 mg/mL, fourth-generation devices typically use upwards of 59 mg/mL. Finally, chemicals added to contribute flavor are the other key ingredient to e-liquids, as unflavored e-liquids are highly unpalatable. With thousands of

flavors on the market, there are thousands of different flavor chemicals and combinations. Beyond these intentionally added ingredients, many other chemicals have been found within e-liquids and the aerosols generated during vaping, including aldehydes, heavy metals, VOCs, nitrosamines, and polycyclic aromatic hydrocarbons. Thus, while e-cig aerosols typically contain fewer toxic chemicals than conventional cigarette smoke, toxicological findings indicate that e-cig aerosols contain harmful substances (16, 17).

There are also tetrahydrocannabinol (THC)- and terpene-containing e-cigs as well. Users tend to refer to these e-cigs containing marijuana concentrates with different terminology, including dabbing or dab pens. Since THC has different solubility characteristics relative to nicotine, these THC devices typically use coconut oil, polyethylene glycol, medium-chain triglycerides, and other solvents. They also have been found to contain or produce toxic chemicals, including benzenes, vitamin E acetate (VEA), pesticides, and heavy metals (18). THC-containing e-cigs were the culprits behind the e-cig- or vaping product-associated lung injury (EVALI) epidemic of 2019, with VEA being identified as the chemical leading to acute lung injury and subsequent respiratory failure (19). Beyond THC vapes, consuming cannabis via vaporizers is also becoming common and is also referred to as vaping. Typically, a vaporizer heats a solid or semisolid version of cannabis to similar temperatures as e-devices to create an inhalable vapor. While we have begun to understand some of the rapidly toxic effects of some chemicals within e-cig devices and aerosols, much work remains to be done to understand the ongoing cases of EVALI and the chronic diseases that will occur with long-term use (20).

As of 2016, the US Food and Drug Administration (FDA) has regulatory authority over all tobacco products sold in the United States, including e-cigs. All e-cig manufacturers are required to submit a premarket tobacco product application to the FDA to obtain authorization to sell their products. Requirements include submitting ingredient lists and including health warnings on packages, among several others. Currently, e-cigs are not FDA approved as smoking cessation devices or authorized to make claims regarding risk reduction relative to cigarettes. Following a congressional hearing in 2019, the FDA issued a warning regarding youth-facing advertisement strategies employed by JUUL Labs. Subsequently, in 2020, the FDA passed legislation to reduce sales of e-cigs to underage (less than 18 years old) users, banning the sale of most flavored cartridge pods. However, this legislation did not apply to e-cigs using synthetic tobacco free nicotine, which became widespread in the months following. This loophole was closed with legislation passed in March 2022.

## **PUBLIC HEALTH RELEVANCE OF SECOND- AND THIRDHAND E-CIGARETTE AEROSOL EXPOSURE**

While the chemical contents of numerous e-cig aerosols have been identified, including toxins, multiple studies have demonstrated that their usage results in the emission of high concentrations of PM<sub>2.5</sub>. One study mimicked e-cig use in a thoroughly ventilated office space, where three study subjects vaped a tobacco-flavored nicotine-free e-cig from 10 AM to 12 PM. The study found that after vaping, the average mass concentration for PM<sub>2.5</sub> increased from 6 µg/m<sup>3</sup> to 197 µg/m<sup>3</sup>, with maximum values reaching 514 µg/m<sup>3</sup> (21). In another study examining second-hand exposure, PM<sub>2.5</sub> levels were measured among a group of 59–86 e-cig users at an e-cig event (10). Initially, PM<sub>2.5</sub> levels were measured at a median range of 1.92–3.20 µg/m<sup>3</sup>. However, these levels skyrocketed to alarmingly high concentrations of 819 µg/m<sup>3</sup> as the event progressed. It is evident that e-cigs have the potential to contribute heavily to PM<sub>2.5</sub> exposure; therefore, e-cigs are highly likely to contribute to increasing mortality rates and other major health impacts, making them a major public health concern.

Specific for indoor air quality, there is concern for second- and thirdhand exposures to e-cig aerosols. Several studies have established that e-cig use contributes to worsening air quality through emission of highly concentrated PM<sub>2.5</sub> and other toxicants, such as nicotine, following user exhalation (22). These emitted particulates can be directly inhaled (secondhand) and have also been found to significantly deposit on surfaces (thirdhand), including windows, walls, floors, wood, and metal (23). Studies are needed to further define the extent to which e-cigs contribute to worsening indoor air quality when accounting for the variation in device design, e-liquid composition, and patterns of use.

Utilizing a model of a business as usual emission scenario, mortality from air pollution is predicted to double by 2050 (24). These projections, while alarming considering current mortality rates, are not surprising given the popularity and availability of e-cig devices. These devices possess the potential to emit highly toxic pollutants, and the market is witnessing an increasing emergence of new variants of ENDS that seem to produce more and more dangerous chemicals. However, preventative measures could blunt this projection. A 2012 projection study estimated that worldwide implementation of 14 emission control measures could prevent 0.64 to 4.92 million premature deaths annually after 20 years; these projections are based on seemingly unrealistic worldwide synergy, but they clearly highlight the potential for significant mitigation from existing technologies and policies (25).

## **E-CIGS AND THE RESPIRATORY SYSTEM**

Due to the relative novelty, variability, and the rapid evolution of e-cig devices and e-liquid compositions, even animal data may not be rapidly applicable to current e-cig users. Here we focus on physiological aspects of the use of e-cigs, as well as susceptibility to respiratory infections and their effect on conditions such as asthma and chronic obstructive pulmonary disease (COPD).

### **Physiological Effects of E-Cigs on the Respiratory System**

E-cig aerosols can lead to inflammatory dysregulation in the airways and lungs, which has immediate implications in the physiology and function of the lungs. For instance, data show that inhalation of e-cig aerosols for even 5 min can increase airway flow resistance and decrease vital capacity, indicating obstruction of the conducting airways (26–28). Similarly, another study showed that vaping mint-flavored e-cigs with 5% nicotine for 20 min increases breathing frequency and decreases blood oxygenation levels, although the forced vital capacity was not affected (29). Moreover, reductions of exhaled carbon monoxide levels and fractional exhaled nitric oxide (NO) have also been observed (28). Related to this, NO in exhaled breath is primarily produced by airway epithelial cells and promotes bronchial hyperreactivity, mucus hypersecretion, vascular permeability, free radical production, airway inflammation, and tissue damage and reduces ciliary beat frequency (30). These findings are aligned with the observation that airway mucus is altered in e-cig users, concomitantly with elevated concentrations of mucin MUC5AC in nasal mucosa (31). Therefore, it is not surprising that in two large longitudinal observational cohorts, chronic usage of e-cigs was associated with greater loss of lung function and a higher risk of COPD exacerbations (32), although larger longitudinal studies are needed before final conclusions can be made about this claim, as well as expanded studies to assess other variability in e-cig design and contents. Similar to what has been determined in humans, murine models have shown that e-cig aerosols can result in respiratory dysfunction and modified pulmonary vasculature hemodynamics (33, 34).

## Respiratory Infections

It is well established how smoking of conventional cigarettes can lead to susceptibility to infections and bacterial colonization of the airways. Similarly, e-cigs have been shown to render the host prone to worse respiratory infections due to alterations in host defenses and inflammatory responses caused by e-cigs. Although several studies have shown that e-cig aerosols can increase the virulence of specific bacteria (35–37), just a few studies have addressed the direct effect of e-cigs in susceptibility to infections in vivo. For instance, in the context of human infection, a study showed that infection of e-cig users with live-attenuated influenza virus leads to higher viral loads in nasal lavage fluid (31). In the context of mouse models, a study showed that exposure to a commercial e-cig (NJOY menthol bold, containing 1.8% nicotine) for 2 weeks leads to increased colony-forming units in the lungs upon challenge with *Streptococcus pneumoniae* (38). Finally, mice exposed for 3 months to e-cig aerosols (PG:VG with 33 mg/mL of nicotine) showed increased mortality after challenge with influenza A (39). In aggregate, these few studies demonstrate the need for future studies addressing how e-cig use can impact susceptibility to infections and colonization of potential pathogenic bacteria in the airways.

## Asthma

Multiple studies have associated asthma and respiratory symptoms with e-cig use or secondhand e-cig exposure in humans, although most of these studies are survey based. For instance, adolescent surveys assessing inhaler use and asthma found an association of current e-cig use (within 30 days) with current asthma and previous asthma (40). Another study utilizing the 2017 Youth Risk Behavior Survey data identified an association between current e-cig use and self-reporting of asthma, with 29% of the e-cig cohort reporting a diagnosis of asthma, although they found ever-use of an e-cig to be 42% and current use to be 13%, with most current users being dual users of both combustible tobacco and e-cigs (41). Similarly, a large study surveying 16–19-year-olds reported asthma to be more common in current e-cig users and an association between secondhand exposure to e-cigs and incidence of wheeze and uncontrolled asthma symptoms in general (42).

To better define the effect of e-cigs in asthma, several studies have used murine models. A study exposing mice to e-cig aerosols concomitantly with ovalbumin-induced allergic inflammatory airway disease, a mouse model of asthma, found that the inflammatory response in mice exposed to e-cig aerosols had increased airway recruitment of immune cells and higher levels of the Th2 cytokine IL-13 but reduced transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) levels (43). In addition, a different study assessing house dust mite-induced asthma in mice exposed to e-cig aerosols with and without different flavorants showed reduced airway inflammation in all mice exposed to e-cig aerosols containing nicotine. In addition, they also found an increase in peripheral airway hyper-responsiveness with specific flavorants but not with others, suggesting that flavored e-cigs without nicotine had significant but heterogeneous effects on features of allergic airways disease (44). Further research elucidating the effects of various e-cig components, such as chemical flavorants, on asthma is necessary to better characterize the associated pathophysiology.

## COPD

A common disease in chronic smokers is COPD, which is a chronic inflammatory lung disease that causes obstructed airflow from the lungs. A significant association has been found between e-cig use and COPD among former combustible cigarette smokers and those who reported never using combustible cigarettes, where the odds of having COPD were significantly greater for daily e-cig users, occasional users, and former users compared with never e-cig users (45). Moreover, it has

been found that COPD patients that use e-cigs have higher neutrophil and lower macrophage cell counts in sputum as compared to those COPD patients using conventional cigarettes only (46). Finally, in a mouse model of COPD, it was observed that exposure to e-cig aerosols can increase airway enlargement, mucus secretion, and fibrogenesis. In addition, multiple cytokines such as macrophage colony stimulating factor (M-CSF), IL-1ra, IL-10, and TGF- $\beta$ 1 were increased in bronchoalveolar lavage (47). Thus, it is important to consider that switching to e-cigs may not necessarily help reduce the effects of smoking on COPD symptoms, although more studies are necessary to determine this.

### **Respiratory System Summary**

The respiratory tract is the primary entry for e-cig aerosols and cigarette smoke (including secondhand exposures), which are well-known causes of numerous deleterious effects on respiratory systems. Our understanding of the mechanisms by which e-cig aerosol exposure affects the respiratory system is paramount to determining their impact on other coexposures, lung diseases, and lung injury. The use of e-cigs has significant implications for pulmonary physiology and function. Furthermore, the effects of e-cig aerosols in lungs remain mostly reliant on animal studies. Due to the known effects of e-cigs on physiology and inflammation, there is a need for more studies addressing susceptibility to infections as well as to inflammatory conditions such as asthma and COPD, our current knowledge of which is primarily based on observational studies. Since asthma and COPD are diseases with both airway obstruction and inflammation, it is likely that e-cig use by patients with either disease will worsen the severity of their underlying lung disease. The clinical and physiologic effects caused by e-cig vaping may be less than those caused by tobacco cigarette smoking, but we cannot draw concrete conclusions without more longitudinal studies and further investigations into the effects of the various e-cig components and e-liquid contents.

### **E-CIGS AND THE CARDIOVASCULAR SYSTEM**

Conventional cigarette smoking is well-known to cause a multitude of harmful effects on the human cardiovascular system (48–50). Whether the more recent nicotine delivery devices (e-cigs) will do the same remains unknown. Current prospective data are limited to short-term e-cig use or acute effects and focus on young, healthy adults who have a lower incidence of cardiovascular disease. Furthermore, e-cigs vary greatly in their chemical composition, and new e-cig development has outpaced research. Therefore, to what degree the available data we have are applicable to these newer devices is unknown.

### **Systemic Blood Pressure and Arterial Stiffness**

Current evidence demonstrates that e-cig use causes acute increases in systemic blood pressure (51–54). A systematic review found that e-cigs increased systolic and diastolic blood pressure by an average of 2 mm Hg in a pooled analysis (55). These findings are mostly attributed to sympathetic nervous system activation by nicotine (51); however, one study did show an increase in blood pressure with nicotine-free e-cigs (56). In people who switch from conventional cigarettes to e-cigs, there is a reduction in systemic blood pressure (57) by a mean of 7 mm Hg (55). Conventional cigarette smokers who use an e-cig do not have an increase in blood pressure, unlike when they smoke conventional cigarettes (53). Thus, it appears that e-cigs increase systemic blood pressure, but switching from conventional cigarettes to e-cigs decreases blood pressure.

Several clinical studies have shown that e-cigs increase arterial stiffness (56, 58–60), although whether this effect is nicotine dependent is unclear. However, there is some variability in the way arterial stiffness is measured, and some studies do not show an increase (53). A cross-sectional

study did not find an increase in arterial stiffness in e-cig users versus non-e-cig users (61). Thus, data are currently mixed.

### **Heart Rate and Cardiac Function**

Multiple studies have shown an increase in heart rate with e-cig use (62). In a pooled analysis, heart rate was increased by a mean of 2 bpm, although there is significant variability in these data (55). Only a single clinical study has examined the effect on cardiac function using echocardiography and did not identify any changes (63). This study was examining acute effects of e-cig use, but in animals, no change was seen in heart rate, stroke volume, or cardiac output after chronic (8 months) e-cig use (64).

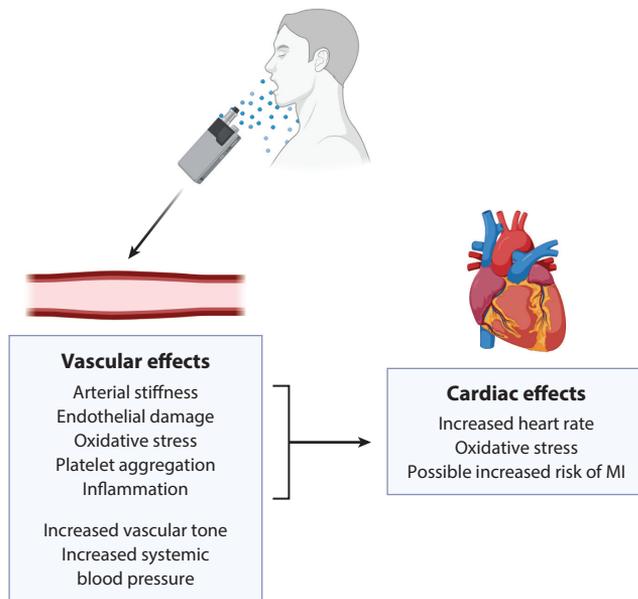
### **Vascular Endothelium**

Many studies show that e-cigs cause acute endothelial dysfunction, including impaired vasodilation (56, 60, 65, 66), decreased bioavailability of NO (61), and decreased NO production from endothelial cells (66). Whether these effects are nicotine dependent remains unclear. In vitro, exposure to e-cig extract increases endothelial permeability (67), and while nicotine itself damages the endothelium, other e-cig constituents, including acrolein, PG, and glycerol, also damage the endothelium (67).

In humans, the data are mixed. In a study of never smokers, nicotine-free e-cig use did not cause acute changes in endothelial function, as measured by flow-mediated vasodilation (68). Furthermore, a study of traditional cigarette smokers who were randomized into four groups to quit with the aid of behavioral therapy, nicotine-replacement therapy, and nicotine-containing and nicotine-free e-cigs found that there were no differences in cardiovascular outcomes (mean arterial pressure, flow-mediated dilation) between groups (69). Yet, another study found that cigarette smokers who switch to e-cigs have lower blood pressure and heart rate and some markers of improved endothelial function (70). One possible explanation for these conflicting data is the variation of nicotine content within e-cig devices. These studies were conducted with users of second- and third-generation e-cigs, which had far lower concentrations of nicotine (2–6 mg/mL) relative to both conventional cigarettes and the e-cigs on the market today (59 mg/mL). Several studies show that e-cig use increases biomarkers related to endothelial damage (66, 71, 72) and biomarkers of oxidative stress (51, 56, 59, 65, 66). These effects were seen in e-cigs with and without nicotine, although the effects are less than those seen in traditional cigarettes (59, 65, 66). Yet again, a large observational study did not demonstrate an increase in markers of oxidative stress and inflammation in sole e-cig users, unlike in traditional cigarette or dual users (73).

### **Long-Term Cardiovascular Effects and Risk of Myocardial Infarction**

Long-term effects of e-cigs in humans are not yet known due to their recent introduction into the market. In mice, 8 months of nicotine-containing e-cig exposure increased arterial stiffness, enhanced sensitivity to vasoconstriction, and reduced simulated vascular relaxation, similar to conventional cigarettes (64). Animal studies demonstrate that nicotine-containing e-cigs cause early atherosclerotic changes in mice. Both nicotine-containing and nicotine-free e-cigs cause increased platelet activation, reactivity, and aggregation (74, 75). However, in general, the prothrombotic effects of e-cigs are less than those of traditional cigarettes (76). Results from clinical studies are mixed. In a retrospective study using UK National Health Service self-reported data between 2014 and 2016, daily e-cig use was independently associated with increased odds of having a myocardial infarction (77). However, many of the e-cig users were former conventional smokers, and thus separating specific effects of e-cigs is difficult. A study of the same data set between 2016 and



**Figure 2**

Effects of e-cigs on the cardiovascular system. E-cig use has been associated with significant vascular alterations, including arterial stiffness, endothelial damage, oxidative stress, platelet aggregation, and inflammation. These changes can contribute to increased heart rate, heightened oxidative stress, and increased risk of myocardial infarction (MI). Additionally, e-cig-induced vascular changes are characterized by increased vascular tone and systemic blood pressure. Figure adapted from images created in BioRender; Wold L. 2025. <https://BioRender.com/jgc1s2a>.

2017 did not show any association (78), and a review of US self-reported data from 2013–2019 did not demonstrate a difference in cardiovascular disease risk in sole e-cig users versus nonusers of tobacco products; however, nonusers included former smokers. Further, the risk of dual use (e-cigs and conventional cigarettes) was similar to pure cigarette use (79).

### Cardiovascular System Summary

Despite the multitude of both clinical and nonclinical studies investigating the effects of e-cigs on cardiovascular outcomes, the data remain incomplete. Many clinical studies include former conventional cigarette smokers, and few have examined the effects of e-cig use on people with preexisting cardiovascular conditions. Many studies examining the acute effects of e-cigs on cardiovascular measures demonstrate adverse effects, including increases in systemic blood pressure, heart rate, arterial stiffness, and markers of endothelial damage (**Figure 2**). Whether the acute effects of e-cigs will translate into a higher risk of cardiovascular disease remains to be seen, and prospective clinical trials of long-term e-cig use and variations in e-cig products are needed.

### CENTRAL NERVOUS SYSTEM

When compared to studies on tobacco smoking, there is a paucity of knowledge regarding the effects of ENDS on the central nervous system. With thousands of vapers in their 40–60s, it is important to understand the impact on cerebral vasculature and stroke risk. Furthermore, the impact of e-cig use on the blood-brain barrier (BBB) is important to unpack. Finally, given that their core

ingredients are highly addictive, their impact on mood, behavior, memory, and neurocognition is of great interest.

## Cerebrovascular Effects

Several animal studies have investigated mechanisms by which e-cigs may affect cerebral vasculature and stroke pathophysiology. Mice exposed to e-cig aerosols and subsequently to oxygen-glucose deprivation to simulate ischemic stroke conditions were noted to have decreased expression of glucose transporters GLUT1 and GLUT3 and overall decreased glucose utilization in brain tissue, thought to enhance brain injury following ischemic stroke (80). Complementary results were found by Kaiser et al. (81) via transient middle cerebral artery occlusion (tMCAO), a model of ischemic stroke. Mice exposed to e-cig aerosols had increased brain oxidative stress following stroke conditions with decreased activation of Nrf2, a transcription factor involved in antioxidant defenses. Another murine study utilizing tMCAO found that ischemic brain injury was significantly worsened after JUUL exposure, also with downregulation of Nrf2, although the impact was not as great as that seen after tobacco smoking (80). Both tobacco smoke and e-cig aerosols were found to disrupt the mitochondrial membrane potential of brain microvascular endothelial cells, which may increase the risk of cerebrovascular disease. E-cig aerosols also seem to affect the brain lipidome, with rats exposed to e-cig aerosols having significantly increased saturated fatty acids and decreased polyunsaturated fatty acids; fatty acid composition determines an atherogenic index as well as a thrombogenic index (82).

Data on human studies regarding stroke and e-cig use are conflicting and currently limited to cross-sectional observational work. Several studies have shown an increased risk of stroke in humans utilizing e-cigs (83–85). However, a 2022 meta-analysis showed that the increase in stroke risk due to e-cig use was no longer statistically significant when a subgroup analysis removed individuals who had previously used combustible tobacco (86). Another human cross-sectional study found that sole e-cig users did not have increased risk of stroke compared to nonsmokers; however, the findings were not age matched, with sole e-cig smokers being much younger than conventional cigarette smokers (87). As a 2023 viewpoint article states, understanding the relationship between e-cig use and stroke in humans requires further high-quality, longitudinal, epidemiological data, especially given adolescent usage and reverse causality in current cross-sectional data (88).

## Effects on the Blood-Brain Barrier

E-cigs may also impact the BBB. Kaiser et al. (81) demonstrated that tobacco smoke and e-cig aerosol exposures both decrease the expression of tight junctional proteins involved in the BBB, increase the permeability of dextran across the BBB, and increase inflammatory markers at the same site. Subsequent studies have shown that inhalation of e-cig aerosols alters the transcriptome of the BBB (89). Interestingly, this effect was more pronounced after exposure to nicotine-free e-cig aerosols than with nicotine-containing e-cigs. This study also utilized a dextran tracer as a marker of BBB permeability and found that while e-cig exposure without nicotine increased BBB permeability, e-cig exposure with nicotine did not, suggesting a potential neuroprotective role of nicotine (89). While these studies conclude that e-cigs have adverse effects on the BBB, the role of aerosol components remains incompletely understood.

## Neurocognitive Effects

Another area of interest is the neurocognitive effects of e-cigs. Broadly, studies have identified decreased interest and reduced short-term memory function associated with e-cigs (90), as well

as more compulsive withdrawal behavior relative to tobacco smoke (91), with changes in visual attention, depressive behavior, compulsive behavior, and anhedonia. E-cig flavors may modulate addictive behavior, independent of other e-cig constituents (for a full review of neurocognitive effects, please see the **Supplemental Appendix**).

### Central Nervous System Summary

E-cig exposure can potentially lead to increased stroke risk and deterioration of the BBB. Furthermore, various rodent studies show that e-cig exposure leads to changes in neurocognitive function. Different components of e-cigs show variable effects in the central nervous system, and further studies on individual components and flavors of e-cigs are needed.

### DIGESTIVE SYSTEM

There are relatively few studies on the effects of e-cigs on the digestive system, most of which focus on the liver, and almost all are preclinical studies. There are very few clinical data linking e-cigs to digestive health, even though 77% of patients with EVALI present with gastrointestinal (GI) complaints, including nausea, vomiting, diarrhea, and abdominal pain, and 47% have elevated liver enzymes (92). There are case reports of non-EVALI-associated liver damage (93). Despite this, a retrospective review of National Health and Nutrition Examination Survey (NHANES) data from 2015–2016 showed increased, but not significant, reports of GI symptoms in e-cig users, including nausea, vomiting, constipation, and diarrhea (94).

### Hepatic Effects

E-cigs have been shown to have multiple detrimental effects on the liver in preclinical studies, including inducing liver inflammation (95), oxidative stress (96), and altered lipid metabolism (97). Chronic (3–6 months) daily exposure to e-cig aerosols containing nicotine induces hepatic fibrosis in mice (98). E-cig (with nicotine) aerosol exposure has also been shown to increase hepatic steatosis in animal models of metabolic-associated fatty liver disease (99). The exact constituents of the e-cigs that are responsible for these findings are not clear. Certain ingredients, such as glycerol and PG, are well-documented hepatotoxins (100). However, the roles of other ingredients, including nicotine itself, are less well-defined. For instance, in some studies, the addition of nicotine to the e-cig vape was necessary to alter bile acid and fatty acid metabolism and cause histologic liver damage (101), hepatic oxidative stress, and mitochondrial damage (102). However, specific flavors such as mango and mint from JUUL e-cigs did not induce liver fibrosis at 1 or 3 months (103). Thus, while most data suggest that e-cigs have harmful effects on the liver, more research is needed to assess how variations of e-cigs induce liver pathology.

### Effects on the Gut

Most of the data we have on the effects of e-cigs on the gut come from animal models and are focused on the colon. E-cig aerosol inhalation causes inflammation and reduces gene expression associated with the epithelial barrier in the colon of mice after chronic (3 months) exposure (104). In vitro studies using human colon organoids similarly show a disruption of epithelial barrier function and increased inflammation, as well as increased susceptibility to infection with *Escherichia coli* (104). What is interesting about this study is that these findings are only seen with the nicotine-free e-cig vape. When nicotine is included, these findings are reversed (104), which is not completely unexpected given that nicotine has been shown to have barrier-tightening and anti-inflammatory effects in the large bowel (105, 106). However, another study found that 4 weeks of nicotine-containing e-cig vape exposure induced colonic damage in rats (107), and in

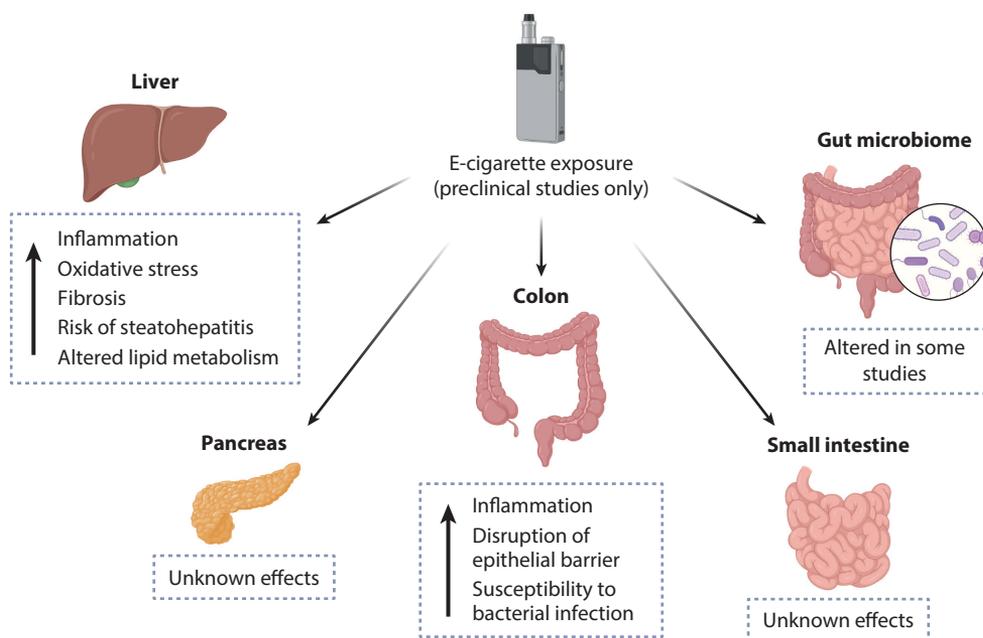
a study examining two different flavors of JUUL e-cigs, subacute (1 month) vape exposure led to increased inflammatory cytokines in the colons of mice, but this effect disappeared at 3 months (103). Thus, these contradictory effects on colon inflammation are likely due to differences in exposure, duration, and e-cig constituents.

## Gut Microbiome

In mice, e-cig exposure has variable effects on the gut microbiome, with some studies showing no effect (97) and others showing a shift in diversity with e-cig exposure independent of whether they contained nicotine. Human data are limited, and in a small study ( $n = 30$ ), e-cigs did not alter the gut microbiome, although this cohort was mostly men (108). Of note, e-cigs appear to adversely alter the oral microbiome (109).

## Summary of the Digestive System

Preclinical data suggest that e-cigs cause liver damage, including inflammation, oxidative stress, altered lipid metabolism, increased metabolic-associated liver diseases, and even fibrosis (Figure 3). E-cigs may also cause colonic inflammation and disruption in the epithelial barrier, although the impact of nicotine in e-cigs is unclear. Effects of e-cigs on the gut microbiome are not well studied, and effects on other GI organs, including the pancreas, have not been investigated. Furthermore,



**Figure 3**

Effects of e-cigs on the gastrointestinal system. Preclinical studies into the effects of e-cig exposure have revealed adverse impacts on both the liver and colon. E-cig exposure is known to elicit inflammation, oxidative stress, and fibrosis in the liver, heightening the risk of steatohepatitis and altering lipid metabolism. Similarly, in the colon, e-cig exposure has been associated with inflammation, compromised epithelial barrier integrity, and increased susceptibility to bacterial infections. Studies exploring alterations in the gut microbiome have yielded conflicting findings, and studies investigating effects on the pancreas and small intestine remain unclear. Figure adapted from images created in BioRender; Wold L. 2025. <https://BioRender.com/hd40u0g>.

there are few clinical data on the role of e-cigs in digestive health, despite GI complaints being relatively common among e-cig users.

## ENDOCRINE SYSTEM

### Systemic E-Cig Effects

Given the systemic effects of e-cigs in extrapulmonary tissues, there is a need to review the impact of e-cigs on circulating metabolites, particularly regarding systemic inflammation, dyslipidemia, and glucose intolerance that may alter the risk for disease development.

Several cross-sectional studies have investigated the association between e-cig use and glucose intolerance (110–113). Dual users of e-cigs and conventional tobacco had significantly higher HbA1c than nonsmokers (110). Female dual users were more likely to have high fasting plasma glucose levels compared to nonsmokers [odds ratio (OR) 2.73, 95% confidence interval (CI) 1.02–7.31] (111). Further, current e-cig users were more likely to have self-reported prediabetes compared to never e-cig users (OR 1.97, 95% CI 1.26–3.10), especially male e-cig users (OR 2.36, 95% CI 1.26–4.40) (112). However, no significant differences between e-cig users and nonsmokers in glucose tolerance and insulin sensitivity were found (113).

Although human data remain inconclusive, animal data support an association between e-cig exposure and impaired glucose tolerance. A study of *ApoE* knockout mice exposed to e-cigs with and without nicotine, conventional cigarettes, and ambient air found that mice exposed to either e-cigs with nicotine or conventional cigarettes had significantly decreased insulin sensitivity. However, exposure to e-cigs without nicotine did not have this effect, suggesting nicotine may drive glucose dysregulation (114). Conversely, intraperitoneal administration of e-cig liquids with and without nicotine in rats had a negative impact on glucose metabolism, resulting in higher plasma glucose levels and reduced hepatic glycogen storage (102), suggesting that non-nicotine e-cig components may also contribute to the development of metabolic syndrome (MetS).

One in vivo study investigated the effect of switching from conventional cigarettes to e-cigs during pregnancy. Dams were exposed to conventional cigarette smoke and half the dams were switched to e-cig aerosol exposure during mating, pregnancy, and feeding until pups were weaned. In dams, glucose metabolism was altered after exposure to conventional cigarettes compared to air, as evidenced by lower plasma insulin levels and higher hepatic glucose metabolism markers (GLUT4, PPAR- $\gamma$ , PGC-1 $\alpha$ , and FOXO1) (115). Switching to e-cigs normalized plasma insulin levels and all hepatic glucose metabolism markers except GLUT4. Offspring mice demonstrated decreased glucose tolerance in the pups of dams exposed to conventional cigarettes, which persisted even when dams were switched to e-cig exposure (115), thus reinforcing complete smoking and vaping cessation during pregnancy.

An examination of the plasma of e-cig users compared to never smokers and vapers found e-cig use to be associated with decreased concentrations of several tricarboxylic acid (TCA) cycle metabolites: (2*R*,3*S*)-2, 3-dimethylmalate, D-glucose, (R)-2-hydroxyglutarate ((*R*)-2-HG), O-phosphorylethanolamine, malathion, D-threo-isocitrate, malic acid, and 4-acetamidobutanoic acid (*N*-acetyl-GABA) (116). Assessment of the plasma metabolome of mice found alterations to TCA metabolites as well (117): specifically, alterations to amino acid metabolism in relation to the  $\alpha$ -ketoglutarate in the TCA cycle. Interestingly, the dysregulated TCA metabolites in humans were unique to e-cig users, highlighting the differences in exposures between e-cigs and conventional cigarettes.

### Circulating Lipid Mediators

Cross-sectional studies, using Korean National Health and Nutrition Examination Survey (KHANES) data, support an association between e-cig use and altered lipid metabolism (111,

118). Conventional cigarette smoking and dual use in females were found to have an increased risk of developing MetS compared to nonsmoking females (OR 1.80, 95% CI 1.02–3.18 and OR 4.02, 95% CI 1.48–10.93, respectively). Female dual users were also more likely to have elevated triglycerides (OR 3.90, 95% CI 1.54–9.89) (111). Current e-cig users were shown to have significantly greater waist circumference, higher triglycerides, and higher diastolic blood pressure compared to nonsmokers, contributing to a higher risk of MetS (118). Indeed, e-cig users were more likely to have MetS (OR 1.40, 95% CI 1.08–1.81); however, most e-cig users included in this study were dual users, obscuring the effects of e-cigs alone (118). A secondary analysis of the 2015–2018 US NHANES found data in agreement with findings from the KHANES data. Among the included NHANES participants there was increased prevalence of MetS reporting among previous [percentile rank (PR) 1.15, 95% CI 1.03–1.28] and current (PR 1.30, 95% CI 1.13–1.50) e-cig users compared to never users (119). The authors found similar elevations in triglycerides in current e-cig users (PR 1.42, 95% CI 1.15–1.75) and former (PR 1.23, 95% CI 1.07–1.41) e-cig users. However, these associations may be driven in part by dual users who were even more likely to report MetS (PR 1.53, 95% CI 1.22–1.91) and elevated triglycerides (PR 1.40, 95% CI 1.11–1.75) compared to never e-cig users as no significant elevation was found among exclusive e-cig users that never used conventional tobacco. Another clinical study analyzing various lipid species in smokers demonstrated that e-cig use did not significantly alter the plasma lipidome when compared to nonsmokers (120). These findings are notable in highlighting the added risk of combining conventional tobacco smoking with e-cig use, with higher rates of MetS and elevated triglycerides, in particular. Also highlighted was a lack of benefit in switching from conventional tobacco to e-cigs in terms of lipid profile effects (120).

Animal models of the effects of e-cigs on lipid metabolism have conflicting results. *ApoE* knockout mice exposed to e-cig aerosols, with and without nicotine, and conventional cigarette smoke had significantly higher total cholesterol (TC), triglycerides, and low-density lipoprotein (LDL) and lowered high-density lipoprotein (HDL) compared to control mice exposed to ambient air, suggesting that dyslipidemia may be induced by e-cig exposure regardless of nicotine content and to a similar degree as with conventional cigarettes (114). However, in rats, e-cig exposure, with and without nicotine, showed a favorable impact on lipid metabolism, with lowered TC and LDL. Rats also had lower TC/HDL and LDL/HDL ratios, suggesting decreased cardiovascular and atherogenic risk (102). These data may represent species-specific responses to inhalants.

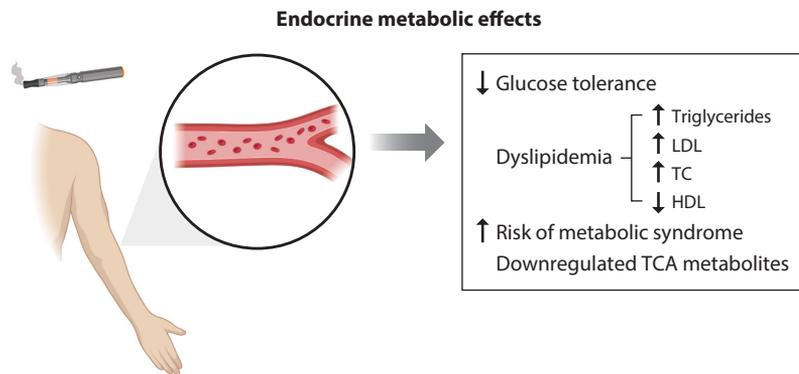
A comprehensive survey of inflammatory mediators found increased IL-1 $\beta$ , IL-6, IL-8, IL-13, interferon (IFN)- $\gamma$ , matrix metalloproteinase-9, and intercellular cell adhesion molecule-1 in e-cig users (121). The survey also found significantly decreased concentrations of the pro-resolving lipid mediators resolvin D<sub>1</sub> and D<sub>2</sub> in plasma from e-cig users compared to nonusers (121).

## Summary of E-Cig Systemic Endocrine Effects

E-cig aerosols have been associated with increased insulin resistance, altered glucose tolerance, increased reactive oxygen species production, and adipose inflammation that may contribute to risk for development of subsequent cardiometabolic diseases (Figure 4).

## Effects of E-Cigs on Hormones

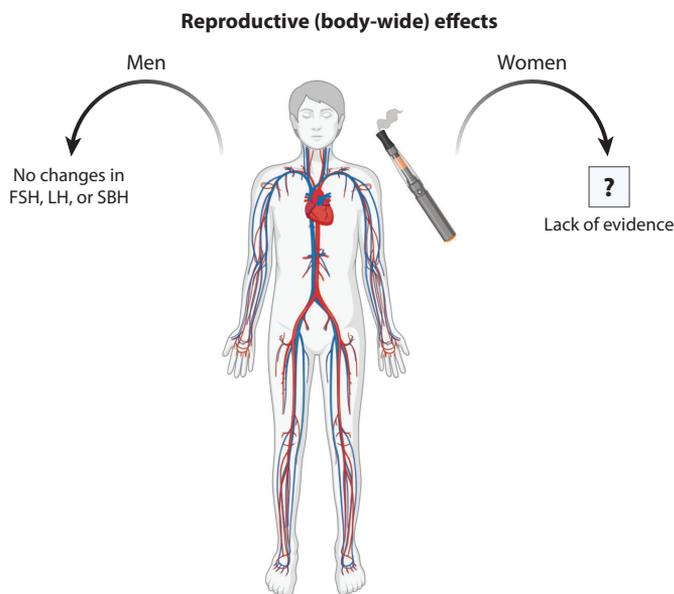
In addition to circulating metabolites, there are several hormones produced by the endocrine system that exert varying effects on their target tissues. Currently, data on the effects of e-cigs on the endocrine system are limited, highlighting a need for more research that includes population



**Figure 4**

Impact of e-cigs on the endocrine axis. E-cigs induce a variety of endocrine metabolic effects, including decreased glucose tolerance, dyslipidemia, downregulation of TCA metabolites, and an increased risk of developing metabolic syndrome. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TCA, tricarboxylic acid. Figure adapted from images created in BioRender; Wold L. 2025. <https://BioRender.com/y1gsod0>.

studies. Epidemiological data on the effects of e-cig use on reproductive hormones are limited. A cross-sectional study that analyzed 1,221 Danish men found that there were no differences in plasma concentrations of reproductive hormones [follicle-stimulating hormone (FSH), luteinizing hormone (LF), sex-hormone binding globulin (SBH), testosterone, estradiol, or inhibin-B] between e-cig users and nonusers (122) (Figure 5). However, e-cig users did have significantly



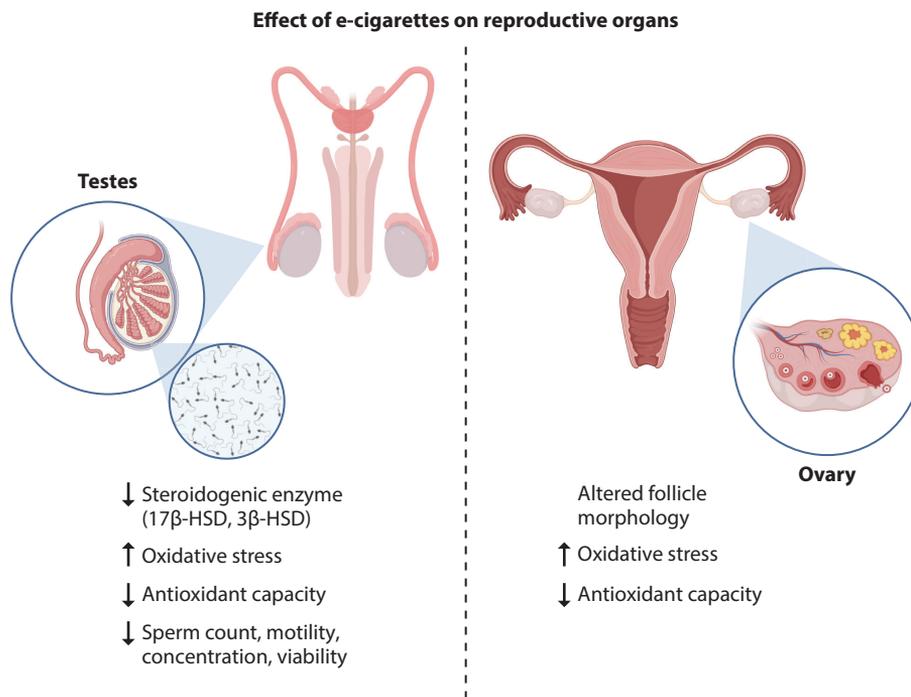
**Figure 5**

Impact of e-cigs on hormones. Currently, no effects have been found from e-cig exposure on male levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex-hormone binding globulin (SBH). The effects of e-cig exposure in females remain largely understudied. Figure adapted from images created in BioRender; Wold L. 2025. <https://BioRender.com/1iel70o>.

lower sperm concentrations and total sperm counts. Notably, a third of e-cig users included in this study were dual users, so it is unclear whether these effects can be attributed to e-cigs alone (122).

### E-Cig Effects on Reproductive Organs, Gestation, and Development

Animal studies suggest that e-cigs have an adverse effect on the reproductive organs. Intraperitoneal injection of e-cig liquid, with and without nicotine, into male rats resulted in significantly decreased sperm count and sperm viability, as well as decreased plasma testosterone levels (102). Additionally, altered morphology of the testes was observed based on disorganized tubular contents, germ cell desquamation, and decreased expression of testosterone synthesis genes and enzymes involved in testosterone production (102). Similarly, inhalation of e-cig aerosol in rats was associated with decreased catalytic activity of  $3\beta$ -hydroxysteroid dehydrogenase (HSD),  $17\beta$ -HSD, and glucose-6-phosphate dehydrogenase and testicular toxicity evidenced by lower testicular weight and gonadosomatic index (123). The activity of sorbitol dehydrogenase, a testosterone-dependent enzyme, was also decreased (123). Both studies hypothesized oxidative stress as a mechanism of testicular toxicity, supported by the increased activity of antioxidant enzymes, presence of sulfhydryl groups, and increased protein carbonyl formation and lipid oxidation products (102, 123) (Figure 6).



**Figure 6**

E-cig effects on reproductive organs. E-cig exposure in males has been shown to decrease steroidogenic enzymes, antioxidant capacity, and sperm count, motility, concentration, and viability, as well as increase oxidative stress within the testes. E-cig exposure in females has been shown to alter ovarian follicle morphology as well as increase ovarian oxidative stress and decrease ovarian antioxidant capacity. Abbreviation: HSD, hydroxysteroid dehydrogenase. Figure adapted from images created in BioRender; Wold L. 2025. <https://BioRender.com/459zwkv>.

Morphological assessment of rat ovaries cultured in vitro and exposed to e-cig liquids containing low and high nicotinic salts or flavor demonstrated decreased percentages of normal follicles and decreased estradiol concentrations (124). Further analysis of the exposed ovaries found increased markers of oxidative stress, including impaired super oxide dismutase (SOD), catalase, and glutathione peroxidase activity (124). Thus, oxidative stress may serve as a common mechanism underlying altered reproductive organ function (**Figure 6**).

### Reproductive Organs Summary

Within the male reproductive system, e-cig exposures correlate with decreased sperm motility, sperm concentration, and sperm viability and overall testicular toxicity and infertility. In the female reproductive system, effects of e-cig exposure on oocytes, ovaries, and infertility are largely unknown (**Figure 6**). Most existing studies investigate associations observed in animal studies, highlighting a need for longitudinal studies to determine causality and mechanisms underlying these changes. Data thus far have shown that prenatal exposure to e-cigs alters the physiology of every organ system, indicating that e-cig use is not safe during pregnancy, particularly for the developing fetus (for a full review of in utero effects, please see the **Supplemental Appendix**).

### E-CIGS AND THE URINARY SYSTEM

Traditional cigarettes are associated with reduced renal function, particularly with a background of hypertension or diabetes, but there are limited data on the effects of e-cigs on the kidney (125, 126). E-cigs are thought to potentially impact chronic kidney disease pathophysiology via ultrafine particles and flavoring, not just nicotine (127).

Rats treated with intraperitoneal e-liquid without nicotine for 28 days had no increase in creatinine and no histological evidence of damage to the glomeruli. However, kidneys from the rats showed dark nuclei in the collecting ducts and increased total protein and sulfhydryl groups, indicating oxidative stress (102). Another study found that daily inhalation of e-cig aerosols for 3–6 months led to an increase in profibrotic genes and decreased glomerular filtration, raising concerns regarding renal-toxic effects of e-cigs with long-term use (98). Interestingly, a study combining the impact of a high-fat diet and nicotine e-cigs in a mouse model showed that after 5 weeks of exposure, there was an increase in damaged DNA and profibrotic markers in the kidney. Even with the high-fat diet and nicotine-free e-cig vapor there was a suppression of kidney mitochondrial oxidative phosphorylation complexes (128). In humans, a cross-sectional study of young volunteers showed that 94% had albuminuria, which is higher than among conventional tobacco smokers (129).

The next area of concern is risk of urothelial cancer, given the existing association with nicotine. There are limited murine studies; however, findings showed increased levels of mutagenic  $O^6$ -methyldeoxyguanosines and  $\gamma$ -hydroxy-1, $N^2$ -propano-deoxyguanosines in the bladders of mice exposed to e-cigs (130). Furthermore, 58% of mice exposed to e-cig aerosols daily for 54 weeks showed bladder urothelial hyperplasia, versus 6% of controls (131). There is evidence that there are increased levels of carcinogens in the bladder after e-cig use. However, only one cross-sectional human study showed an increase in bladder cancer in e-cig users compared to nonsmokers (132).

To summarize, murine studies on the effects of e-cig exposure on the urinary system are scarce, with only a few investigating mechanisms of action through changes in gene expression, oxidative stress, and increased markers of renal tubule injury. Although it may still take several years to obtain human epidemiologic data on the incidence of bladder cancer following exposure to e-cigs, these studies suggest that e-cigs are carcinogenic and drive pathologic changes in the urothelium.

Further investigation that focuses on the various constituents of e-cigs may also elucidate the pathophysiology driving the carcinogenic effects on the urinary system.

## E-CIGS AND THE MUSCULOSKELETAL SYSTEM

Conventional cigarettes have negative effects on muscles, cartilage, tendons, and ligaments and reduce bone density, leading to increased risk of fracture (133). The potential for e-cigs to have similar adverse effects is especially important, given the high prevalence of e-cig use in adolescents who still have maturing bones.

Most data on e-cigs and the skeletal system come from in vitro studies. E-liquid causes reduced cell viability in osteoblast-like cells, with variations between different flavorants (134–136). However, there are conflicting results regarding whether these effects are independent of nicotine content (134, 136). E-liquid appears to be cytotoxic to these cells, as well as to impair bone marrow-derived mesenchymal stem cell differentiation into osteoblasts (137). E-cig vapor also impairs osteoblast function (138). The exact e-cig constituents responsible for these findings have yet to be identified, although certain chemicals, including acetaldehyde and acrolein, have been shown to impair bone health in vitro (139, 140). Despite these in vitro data, only one in vivo study has been performed, where 6 months of e-cig vapor exposure did not decrease bone density in *ApoE*<sup>-/-</sup> mice, but it did negatively impact bone architecture, with microfractures noted in the femurs. Of note, this study was performed in *ApoE*-deficient mice in order to best model cardiovascular outcomes; however, these mice have increased bone mass when compared to wild-type mice, so it is unclear whether these results are applicable to other models (141).

Clinical data are limited regarding bone health and e-cig use. A 2021 study using pooled 2017–2018 NHANES data showed a significantly higher prevalence of self-reported fragility fracture in e-cig users compared with nonusers (142). More clinical studies are needed to further assess the relationship between bone health and e-cig use, as was recently highlighted in a systematic review (143).

Less is known regarding the effects of e-cigs on muscles, tendons, and cartilage. In vivo, skeletal muscle from mice exposed to e-cig aerosols containing nicotine showed increased oxidative stress and poorer muscle health (144). Nicotine-containing e-cig exposure impairs muscle force development, running speeds, and regeneration from injury in mice (145). E-cigs alters tendon properties, and rats exposed to e-cigs have impaired tendon healing (146).

To summarize, preclinical data suggest that e-cigs negatively impact osteoblast differentiation and functioning, leading to compromised bone health; however, more in vivo experiments and clinical studies are needed to investigate this further. Effects on tendons and skeletal muscle are similarly negative, with animal data suggesting reductions in running speeds and recovery from injury. More studies, especially in humans, are needed to further define possible adverse effects.

## METHODOLOGICAL CHALLENGES

Understanding the health effects of e-cigs is complicated by several methodological issues. One challenge is that many e-cig users in epidemiological studies are current or former cigarette smokers. This overlap is problematic, as health effects observed among e-cig users may partially result from prior or concurrent cigarette use. To address this issue, it is important that studies adjust for cigarette use and, where possible, analyze groups separately (e.g., e-cig users, dual users, never smokers) (147–150). Additionally, longitudinal studies that follow never smokers who begin using e-cigs can help clarify the independent health effects of e-cigs (147–150).

Another methodological challenge in interpreting the health effects of e-cigs is the diversity of e-cig devices and e-liquid composition (e.g., PG to VG ratios, nicotine concentration, chemical

flavorants) (147–150). These variations lead to significant differences in chemical exposures and may lead to different health outcomes in e-cig users. Thus, grouping all e-cig users together risks overlooking these important differences. To address this challenge, it is important for future studies to incorporate detailed e-device and e-liquid characteristics. Translational and basic science studies should prioritize the standardization of these variables whenever possible. Additionally, meta-analyses may be particularly useful, as they may be able to categorize studies by e-device type or e-liquid composition, leading to more precise conclusions.

## CONCLUSIONS

The impacts of e-cigs are vast and encompass nearly all organ systems. While many have been extensively studied (e.g., cardiovascular and pulmonary systems) (147–150), many others are not well-described (e.g., musculoskeletal system). The most common effects of e-cig exposure occur through increased oxidative stress and inflammatory responses, which are seen in almost every organ system. However, the mechanisms underlying these changes in each organ system need further study. Paradoxically, a protective role of nicotine is observed in many organ systems, and with the increase in nicotine concentrations among new generations of e-cigs, it is important to define the cellular and molecular mechanisms underlying these effects along with other e-cig constituents, such as e-device type and flavors. With many similarities between general PM and e-cig exposure effects, there is a concerning potential for a notable increase of these health effects as e-cigs continue to become a large source of general PM. Further work must be done to educate the public, policymakers, and health-care providers about the health effects of e-cigs.

### SUMMARY POINTS

1. Electronic cigarette (e-cig) usage has grown substantially over their 15 years on the market, and inhalation of the aerosol produced, commonly called vapor, is now known to cause organ-specific effects.
2. E-cig vapor is an avoidable exposure.
3. E-cig aerosols are a significant source of particulate matter and have diverse effects across the body.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## AUTHOR CONTRIBUTIONS

M.J.M., P.B., E.W.N., M.K.B., M.Z., M.W.G., J.A.M.-S., L.B., A.P., A.K., J.M.H., and A.L.F. conducted literature reviews, wrote sections, created figures, and edited the manuscript. L.E.C.A. and L.E.W. supervised and edited the manuscript and wrote the Introduction and Conclusions.

## ACKNOWLEDGMENTS

The authors thank the sponsors of research in the Wold laboratory, including the National Institutes of Health (NIH) (R01 HL139348, R01 AG057046) and the American Heart Association (20YVNR35490079) to L.E.W., and the sponsors of research in the Crotty Alexander

laboratory, including the NIH (R01 HL147326, R01 HL137052, and K24 HL155884), VA (Merit 1I01BX004767, Merit 1I01BX006447), and California State Funding Program: Tobacco-Related Disease Research Program (TRDRP) (T33IR6633, T32SR5359, T32KT4896, T31IP1740, T29IR0475, and T30IP0965). Finally, the authors would like to thank the TRDRP for the postdoctoral fellowship grant (T33FT6629) awarded to J.A.M.-S.

## LITERATURE CITED

1. Mathers CD, Loncar D. 2006. Projections of global mortality and burden of disease from 2002 to 2030. *PLOS Med.* 3:e442
2. Samet JM. 2013. Commentary: Secondhand smoke causes disease everywhere, including mental health care settings. *Int. J. Epidemiol.* 42:894–95
3. Crotty Alexander LE, Shin S, Hwang JH. 2015. Inflammatory diseases of the lung induced by conventional cigarette smoke: a review. *Chest* 148:1307–22
4. Perez MF, Crotty Alexander LE. 2020. Why is vaping going up in flames? *Ann. Am. Thorac. Soc.* 17:545–49
5. Travis N, Knoll M, Cook S, Oh H, Cadham CJ, et al. 2023. Chemical profiles and toxicity of electronic cigarettes: an umbrella review and methodological considerations. *Int. J. Environ. Res. Public Health* 20:1908
6. US Dep. Health Hum. Serv. 2016. *E-cigarette use among youth and young adults: a report of the Surgeon General*. Rep., Cent. Dis. Control Prev. [https://archive.cdc.gov/www\\_cdc\\_gov/tobacco/sgf/e-cigarettes/index.htm](https://archive.cdc.gov/www_cdc_gov/tobacco/sgf/e-cigarettes/index.htm)
7. Close A, Blackerby J, Tunnell H, Pender J, Soule E, Sousan S. 2023. Effects of e-cigarette liquid ratios on the gravimetric filter correction factors and real-time measurements. *Aerosol Air Qual. Res.* 23:230011
8. Eversole A, Crabtree M, Spindle TR, Baassiri M, Eissenberg T, Breland A. 2021. E-cigarette solvent ratio and device power influence ambient air particulate matter. *Tob. Regul. Sci.* 7:177–83
9. Lee MS, LeBouf RF, Son YS, Koutrakis P, Christiani DC. 2017. Nicotine, aerosol particles, carbonyls and volatile organic compounds in tobacco- and menthol-flavored e-cigarettes. *Environ. Health* 16:42
10. Soule EK, Maloney SF, Spindle TR, Rudy AK, Hiler MM, Cobb CO. 2017. Electronic cigarette use and indoor air quality in a natural setting. *Tob. Control* 26:109–12
11. Levy DT, Warner KE, Cummings KM, Hammond D, Kuo C, et al. 2019. Examining the relationship of vaping to smoking initiation among US youth and young adults: a reality check. *Tob. Control* 28:629–35
12. Natl. Cancer Inst. 2024. *Adult tobacco use*. Rep., Natl. Cancer Inst. [https://progressreport.cancer.gov/prevention/tobacco/adult\\_smoking](https://progressreport.cancer.gov/prevention/tobacco/adult_smoking)
13. Mayer M, Reyes-Guzman C, Grana R, Choi K, Freedman ND. 2020. Demographic characteristics, cigarette smoking, and e-cigarette use among US adults. *JAMA Netw. Open* 3:e2020694
14. Mears MJ, Hookfin HL, Bandaru P, Vidal P, Stanford KI, Wold LE. 2023. Electronic nicotine delivery systems and cardiovascular/cardiometabolic health. *Circ. Res.* 132:1168–80
15. Li L, Nguyen C, Lin Y, Guo Y, Fadel NA, Zhu Y. 2021. Impacts of electronic cigarettes usage on air quality of vape shops and their nearby areas. *Sci. Total Environ.* 760:143423
16. Kosmider L, Spindle TR, Gawron M, Sobczak A, Goniewicz ML. 2018. Nicotine emissions from electronic cigarettes: individual and interactive effects of propylene glycol to vegetable glycerin composition and device power output. *Food Chem. Toxicol.* 115:302–5
17. St. Helen G, Liakoni E, Nardone N, Addo N, Jacob P III, Benowitz NL. 2020. Comparison of systemic exposure to toxic and/or carcinogenic volatile organic compounds (VOC) during vaping, smoking, and abstention. *Cancer Prev. Res.* 13:153–162
18. Guo W, Vrdoljak G, Liao VC, Moezzi B. 2021. Major constituents of cannabis vape oil liquid, vapor and aerosol in California vape oil cartridge samples. *Front. Chem.* 9:694905
19. Neczypor EW, Mears MJ, Ghosh A, Sassano MF, Gumina RJ, et al. 2022. E-cigarettes and cardiopulmonary health: review for clinicians. *Circulation* 145:219–32
20. Crotty Alexander LE, Ware LB, Calfee CS, Callahan SJ, Eissenberg T, et al. 2020. E-cigarette or vaping product use-associated lung injury: developing a research agenda. An NIH workshop report. *Am. J. Respir. Crit. Care Med.* 202:795–802

21. Schober W, Szendrei K, Matzen W, Osiander-Fuchs H, Heitmann D, et al. 2014. Use of electronic cigarettes (e-cigarettes) impairs indoor air quality and increases FeNO levels of e-cigarette consumers. *Int. J. Hyg. Environ. Health* 217:628–37
22. Fernandez E, Ballbe M, Sureda X, Fu M, Salto E, Martinez-Sanchez JM. 2015. Particulate matter from electronic cigarettes and conventional cigarettes: a systematic review and observational study. *Curr. Environ. Health Rep.* 2:423–29
23. Goniewicz ML, Lee L. 2015. Electronic cigarettes are a source of thirdhand exposure to nicotine. *Nicotine Tob. Res.* 17:256–58
24. Lelieveld J, Evans JS, Fnais M, Giannadaki D, Pozzer A. 2015. The contribution of outdoor air pollution sources to premature mortality on a global scale. *Nature* 525:367–71
25. Anenberg SC, Schwartz J, Shindell D, Amann M, Faluvegi G, et al. 2012. Global air quality and health co-benefits of mitigating near-term climate change through methane and black carbon emission controls. *Environ. Health Perspect.* 120:831–39
26. Antoniewicz L, Brynedal A, Hedman L, Lundback M, Bosson JA. 2019. Acute effects of electronic cigarette inhalation on the vasculature and the conducting airways. *Cardiovasc. Toxicol.* 19:441–50
27. Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN, Behrakis PK. 2012. Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. *Chest* 141:1400–6
28. Song Y, Li X, Li C, Xu S, Liu Y, Wu X. 2022. What are the effects of electronic cigarettes on lung function compared to non-electronic cigarettes? A systematic analysis. *Int. J. Public Health* 67:1604989
29. McClelland ML, Sesoko CS, MacDonald DA, Davis LM, McClelland SC. 2021. The immediate physiological effects of e-cigarette use and exposure to secondhand e-cigarette vapor. *Respir. Care* 66:943–50
30. Escamilla-Gil JM, Fernandez-Nieto M, Acevedo N. 2022. Understanding the cellular sources of the fractional exhaled nitric oxide (FeNO) and its role as a biomarker of type 2 inflammation in asthma. *Biomed. Res. Int.* 2022:5753524
31. Rebuli ME, Glista-Baker E, Hoffman JR, Duffney PF, Robinette C, et al. 2021. Electronic-cigarette use alters nasal mucosal immune response to live-attenuated influenza virus. A clinical trial. *Am. J. Respir. Cell Mol. Biol.* 64:126–37
32. Bowler RP, Hansel NN, Jacobson S, Barr RG, Make BJ, et al. 2017. Electronic cigarette use in US adults at risk for or with COPD: analysis from two observational cohorts. *J. Gen. Intern. Med.* 32:1315–22
33. Kastratovic N, Markovic V, Harrell CR, Arsenijevic A, Stojanovic MD, et al. 2024. Effects of combustible cigarettes and electronic nicotine delivery systems on the development and progression of chronic lung inflammation in mice. *Nicotine Tob. Res.* 26:704–14
34. Snoderly HT, Alkhadrawi H, Panchal DM, Weaver KL, Vito JN, et al. 2023. Short-term exposure of female BALB/cj mice to e-cigarette aerosol promotes neutrophil recruitment and enhances neutrophil-platelet aggregation in pulmonary microvasculature. *J. Toxicol. Environ. Health A* 86:246–62
35. Bagale K, Paudel S, Cagle H, Sigel E, Kulkarni R. 2020. Electronic cigarette (e-cigarette) vapor exposure alters the *Streptococcus pneumoniae* transcriptome in a nicotine-dependent manner without affecting pneumococcal virulence. *Appl. Environ. Microbiol.* 86:e02125–19
36. Corriden R, Moshensky A, Bojanowski CM, Meier A, Chien J, et al. 2020. E-cigarette use increases susceptibility to bacterial infection by impairment of human neutrophil chemotaxis, phagocytosis, and NET formation. *Am. J. Physiol. Cell Physiol.* 318:C205–14
37. Hwang JH, Lyes M, Sladewski K, Enany S, McEachern E, et al. 2016. Electronic cigarette inhalation alters innate immunity and airway cytokines while increasing the virulence of colonizing bacteria. *J. Mol. Med.* 94:667–79
38. Sussan TE, Gajghate S, Thimmulappa RK, Ma J, Kim JH, et al. 2015. Exposure to electronic cigarettes impairs pulmonary anti-bacterial and anti-viral defenses in a mouse model. *PLOS ONE* 10:e0116861
39. Madison MC, Landers CT, Gu BH, Chang CY, Tung HY, et al. 2019. Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine. *J. Clin. Investig.* 129:4290–304
40. Schweitzer RJ, Wills TA, Tam E, Pagano I, Choi K. 2017. E-cigarette use and asthma in a multiethnic sample of adolescents. *Prev. Med.* 105:226–31

41. Wills TA, Choi K, Pagano I. 2020. E-cigarette use associated with asthma independent of cigarette smoking and marijuana in a 2017 national sample of adolescents. *J. Adolesc. Health* 67:524–30
42. Alnajem A, Redha A, Alroumi D, Alshammasi A, Ali M, et al. 2020. Use of electronic cigarettes and secondhand exposure to their aerosols are associated with asthma symptoms among adolescents: a cross-sectional study. *Respir. Res.* 21:300
43. Taha HR, Al-Sawalha NA, Alzoubi KH, Khabour OF. 2020. Effect of e-cigarette aerosol exposure on airway inflammation in a murine model of asthma. *Inhal. Toxicol.* 32:503–11
44. Chapman DG, Casey DT, Ather JL, Aliyeva M, Daphtary N, et al. 2019. The effect of flavored e-cigarettes on murine allergic airways disease. *Sci. Rep.* 9:13671
45. Antwi GO, Rhodes DL. 2022. Association between e-cigarette use and chronic obstructive pulmonary disease in non-asthmatic adults in the USA. *J. Public Health* 44:158–64
46. Higham A, Beech A, Jackson N, Lea S, Singh D. 2022. Sputum cell counts in COPD patients who use electronic cigarettes. *Eur. Respir. J.* 59:2103016
47. Han H, Peng G, Meister M, Yao H, Yang JJ, et al. 2021. Electronic cigarette exposure enhances lung inflammatory and fibrotic responses in COPD mice. *Front. Pharmacol.* 12:726586
48. Ding N, Sang Y, Chen J, Ballew SH, Kalbaugh CA, et al. 2019. Cigarette smoking, smoking cessation, and long-term risk of 3 major atherosclerotic diseases. *J. Am. Coll. Cardiol.* 74:498–507
49. Teo KK, Ounpuu S, Hawken S, Pandey MR, Valentin V, et al. 2006. Tobacco use and risk of myocardial infarction in 52 countries in the INTERHEART study: a case-control study. *Lancet* 368:647–58
50. Mons U, Muezzinler A, Gellert C, Schottker B, Abnet CC, et al. 2015. Impact of smoking and smoking cessation on cardiovascular events and mortality among older adults: meta-analysis of individual participant data from prospective cohort studies of the CHANCES consortium. *BMJ* 350:h1551
51. Moheimani RS, Bhetraratana M, Peters KM, Yang BK, Yin F, et al. 2017. Sympathomimetic effects of acute e-cigarette use: role of nicotine and non-nicotine constituents. *J. Am. Heart Assoc.* 6:e006579
52. Oncken CA, Litt MD, McLaughlin LD, Burki NA. 2015. Nicotine concentrations with electronic cigarette use: effects of sex and flavor. *Nicotine Tob. Res.* 17:473–78
53. Szołtysek-Bołdys I, Sobczak A, Zielinska-Danch W, Barton A, Koszowski B, Kosmider L. 2014. Influence of inhaled nicotine source on arterial stiffness. *Przegl. Lek.* 71:572–75
54. Vlachopoulos C, Ioakeimidis N, Abdelrasoul M, Terentes-Printzios D, Georgakopoulos C, et al. 2016. Electronic cigarette smoking increases aortic stiffness and blood pressure in young smokers. *J. Am. Coll. Cardiol.* 67:2802–3
55. Skotsimara G, Antonopoulos AS, Oikonomou E, Siasos G, Ioakeimidis N, et al. 2019. Cardiovascular effects of electronic cigarettes: a systematic review and meta-analysis. *Eur. J. Prev. Cardiol.* 26:1219–28
56. Chaumont M, de Becker B, Zaher W, Culie A, Deprez G, et al. 2018. Differential effects of e-cigarette on microvascular endothelial function, arterial stiffness and oxidative stress: a randomized crossover trial. *Sci. Rep.* 8:10378
57. D’Ruiz CD, O’Connell G, Graff DW, Yan XS. 2017. Measurement of cardiovascular and pulmonary function endpoints and other physiological effects following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers. *Regul. Toxicol. Pharmacol.* 87:36–53
58. Franzen KF, Willig J, Cayo Talavera S, Meusel M, Sayk F, et al. 2018. E-cigarettes and cigarettes worsen peripheral and central hemodynamics as well as arterial stiffness: a randomized, double-blinded pilot study. *Vasc. Med.* 23:419–25
59. Ikonomidis I, Vlastos D, Kourea K, Kostelli G, Varoudi M, et al. 2018. Electronic cigarette smoking increases arterial stiffness and oxidative stress to a lesser extent than a single conventional cigarette: an acute and chronic study. *Circulation* 137:303–6
60. Caporale A, Langham MC, Guo W, Johncola A, Chatterjee S, Wehrli FW. 2019. Acute effects of electronic cigarette aerosol inhalation on vascular function detected at quantitative MRI. *Radiology* 293:97–106
61. Fetterman JL, Keith RJ, Palmisano JN, McGlasson KL, Weisbrod RM, et al. 2020. Alterations in vascular function associated with the use of combustible and electronic cigarettes. *J. Am. Heart Assoc.* 9:e014570
62. Nides MA, Leischow SJ, Bhattar M, Simmons M. 2014. Nicotine blood levels and short-term smoking reduction with an electronic nicotine delivery system. *Am. J. Health Behav.* 38:265–74

63. Farsalinos KE, Tsiapras D, Kyrzopoulos S, Savvopoulou M, Voudris V. 2014. Acute effects of using an electronic nicotine-delivery device (electronic cigarette) on myocardial function: comparison with the effects of regular cigarettes. *BMC Cardiovasc. Disord.* 14:78
64. Olfert IM, DeVallance E, Hoskinson H, Branyan KW, Clayton S, et al. 2018. Chronic exposure to electronic cigarettes results in impaired cardiovascular function in mice. *J. Appl. Physiol.* 124:573–82
65. Biondi-Zoccai G, Sciarretta S, Bullen C, Nocella C, Violi F, et al. 2019. Acute effects of heat-not-burn, electronic vaping, and traditional tobacco combustion cigarettes: the Sapienza University of Rome–Vascular Assessment of Proatherosclerotic Effects of Smoking (SUR-VAPES) 2 randomized trial. *J. Am. Heart Assoc.* 8:e010455
66. Carnevale R, Sciarretta S, Violi F, Nocella C, Loffredo L, et al. 2016. Acute impact of tobacco versus electronic cigarette smoking on oxidative stress and vascular function. *Chest* 150:606–12
67. Schweitzer KS, Chen SX, Law S, Van Demark M, Poirier C, et al. 2015. Endothelial disruptive proinflammatory effects of nicotine and e-cigarette vapor exposures. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 309:L175–87
68. Haptonstall KP, Choroomi Y, Moheimani R, Nguyen K, Tran E, et al. 2020. Differential effects of tobacco cigarettes and electronic cigarettes on endothelial function in healthy young people. *Am. J. Physiol. Heart Circ. Physiol.* 319:H547–56
69. Klonizakis M, Gumber A, McIntosh E, Brose LS. 2022. Medium- and longer-term cardiovascular effects of e-cigarettes in adults making a stop-smoking attempt: a randomized controlled trial. *BMC Med.* 20:276
70. George J, Hussain M, Vadiveloo T, Ireland S, Hopkinson P, et al. 2019. Cardiovascular effects of switching from tobacco cigarettes to electronic cigarettes. *J. Am. Coll. Cardiol.* 74:3112–20
71. Mobarrez F, Antoniewicz L, Hedman L, Bosson JA, Lundback M. 2020. Electronic cigarettes containing nicotine increase endothelial and platelet derived extracellular vesicles in healthy volunteers. *Atherosclerosis* 301:93–100
72. Antoniewicz L, Bosson JA, Kuhl J, Abdel-Halim SM, Kiessling A, et al. 2016. Electronic cigarettes increase endothelial progenitor cells in the blood of healthy volunteers. *Atherosclerosis* 255:179–85
73. Stokes AC, Xie W, Wilson AE, Yang H, Orimoloye OA, et al. 2021. Association of cigarette and electronic cigarette use patterns with levels of inflammatory and oxidative stress biomarkers among US adults: population assessment of tobacco and health study. *Circulation* 143:869–71
74. Lyytinen G, Brynedal A, Anesater E, Antoniewicz L, Blomberg A, et al. 2023. Electronic cigarette vaping with nicotine causes increased thrombogenicity and impaired microvascular function in healthy volunteers: a randomised clinical trial. *Cardiovasc. Toxicol.* 23:255–64
75. Hom S, Chen L, Wang T, Ghebrehwet B, Yin W, Rubenstein DA. 2016. Platelet activation, adhesion, inflammation, and aggregation potential are altered in the presence of electronic cigarette extracts of variable nicotine concentrations. *Platelets* 27:694–702
76. Nocella C, Biondi-Zoccai G, Sciarretta S, Peruzzi M, Pagano F, et al. 2018. Impact of tobacco versus electronic cigarette smoking on platelet function. *Am. J. Cardiol.* 122:1477–81
77. Alzahrani T, Pena I, Temesgen N, Glantz SA. 2018. Association between electronic cigarette use and myocardial infarction. *Am. J. Prev. Med.* 55:455–61
78. Farsalinos KE, Polosa R, Cibella F, Niaura R. 2019. Is e-cigarette use associated with coronary heart disease and myocardial infarction? Insights from the 2016 and 2017 National Health Interview Surveys. *Theor. Adv. Chronic Dis.* 10:2040622319877741
79. Berlowitz JB, Xie W, Harlow AF, Hamburg NM, Blaha MJ, et al. 2022. E-cigarette use and risk of cardiovascular disease: a longitudinal analysis of the PATH study (2013–2019). *Circulation* 145:1557–59
80. Sifat AE, Vaidya B, Kaiser MA, Cucullo L, Abbruscato TJ. 2018. Nicotine and electronic cigarette (E-Cig) exposure decreases brain glucose utilization in ischemic stroke. *J. Neurochem.* 147:204–21
81. Kaiser MA, Villalba H, Prasad S, Liles T, Sifat AE, et al. 2017. Offsetting the impact of smoking and e-cigarette vaping on the cerebrovascular system and stroke injury: Is Metformin a viable countermeasure? *Redox. Biol.* 13:353–62
82. Cardenia V, Vivarelli F, Cirillo S, Paolini M, Canistro D, Rodriguez-Estrada MT. 2018. The effect of electronic-cigarettes aerosol on rat brain lipid profile. *Biochimie* 153:99–108
83. Awad K, Mohammed M, Martin SS, Banach M. 2023. Association between electronic nicotine delivery systems use and risk of stroke: a meta-analysis of 1,024,401 participants. *Arch. Med. Sci.* 19:1538–40

84. Shi J, Xiong L, Guo J, Yang Y. 2023. The association between combustible/electronic cigarette use and stroke based on national health and nutrition examination survey. *BMC Public Health* 23:697
85. Bricknell RAT, Ducaud C, Figueroa A, Schwarzman LS, Rodriguez P, et al. 2021. An association between electronic nicotine delivery systems use and a history of stroke using the 2016 behavioral risk factor surveillance system. *Medicine* 100:e27180
86. Zhao K, Li J, Zhou P, Xu L, Yang M. 2022. Is electronic cigarette use a risk factor for stroke? A systematic review and meta-analysis. *Tob. Induc. Dis.* 20:101
87. Parekh T, Pemmasani S, Desai R. 2020. Risk of stroke with e-cigarette and combustible cigarette use in young adults. *Am. J. Prev. Med.* 58:446–52
88. Elser H, Vijayaraghavan M, Kasner SE. 2023. E-cigarettes and stroke risk—present uncertainties and future directions. *JAMA Neurol.* 80:883–84
89. Heldt NA, Seliga A, Winfield M, Gajghate S, Reichenbach N, et al. 2020. Electronic cigarette exposure disrupts blood-brain barrier integrity and promotes neuroinflammation. *Brain Behav. Immun.* 88:363–80
90. Prasedya ES, Ambana Y, Martyasari NWR, Aprizal Y, Nurrijawati, Sunarpi. 2020. Short-term E-cigarette toxicity effects on brain cognitive memory functions and inflammatory responses in mice. *Toxicol. Res.* 36:267–73
91. Ponzoni L, Moretti M, Sala M, Fasoli F, Mucchietto V, et al. 2015. Different physiological and behavioural effects of e-cigarette vapour and cigarette smoke in mice. *Eur. Neuropsychopharmacol.* 25:1775–86
92. Layden JE, Ghinai I, Pray I, Kimball A, Layer M, et al. 2020. Pulmonary illness related to e-cigarette use in Illinois and Wisconsin—final report. *N. Engl. J. Med.* 382:903–16
93. Van Roey G, Goos W, Claessens C, Hoorens A, Verlinden W, Schouten J. 2024. Acute grade IV toxic hepatitis due to the e-cigarette. *Acta Gastroenterol. Belg.* 87:44–47
94. Debnath M, Debnath D, Singh P, Wert Y, Nookala V. 2022. Effect of electronic cigarettes on the gastrointestinal system. *Cureus* 14:e27210
95. Rubenstein DA, Hom S, Ghebrehiwet B, Yin W. 2015. Tobacco and e-cigarette products initiate Kupffer cell inflammatory responses. *Mol. Immunol.* 67:652–60
96. Granata S, Canistro D, Vivarelli F, Morosini C, Rullo L, et al. 2023. Potential harm of IQOS smoke to rat liver. *Int. J. Mol. Sci.* 24:12462
97. Chen H, Burke C, Donovan C, Faiz A, Saad S, Oliver BG. 2023. E-cigarette vapour alters high-fat diet-induced systemic inflammatory responses but has no effect on high-fat diet-induced changes in gut microbiota. *Nutrients* 15:1783
98. Crotty Alexander LE, Drummond CA, Hepokoski M, Mathew D, Moshensky A, et al. 2018. Chronic inhalation of e-cigarette vapor containing nicotine disrupts airway barrier function and induces systemic inflammation and multiorgan fibrosis in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 314:R834–47
99. Hasan KM, Friedman TC, Shao X, Parveen M, Sims C, et al. 2019. E-cigarettes and western diet: important metabolic risk factors for hepatic diseases. *Hepatology* 69:2442–54
100. Lechasseur A, Mouchiroud M, Tremblay F, Bouffard G, Milad N, et al. 2022. Glycerol contained in vaping liquids affects the liver and aspects of energy homeostasis in a sex-dependent manner. *Physiol. Rep.* 10:e15146
101. Phillips B, Titz B, Kogel U, Sharma D, Leroy P, et al. 2017. Toxicity of the main electronic cigarette components, propylene glycol, glycerin, and nicotine, in Sprague-Dawley rats in a 90-day OECD inhalation study complemented by molecular endpoints. *Food Chem. Toxicol.* 109:315–32
102. El Golli N, Jrad-Lamine A, Neffati H, Rahali D, Dallagi Y, et al. 2016. Impact of e-cigarette refill liquid with or without nicotine on liver function in adult rats. *Toxicol. Mech. Methods* 26:419–26
103. Moshensky A, Brand CS, Alhaddad H, Shin J, Masso-Silva JA, et al. 2022. Effects of mango and mint pod-based e-cigarette aerosol inhalation on inflammatory states of the brain, lung, heart, and colon in mice. *eLife* 11:e67621
104. Sharma A, Lee J, Fonseca AG, Moshensky A, Kothari T, et al. 2021. E-cigarettes compromise the gut barrier and trigger inflammation. *iScience* 24:102035
105. McGilligan VE, Wallace JM, Heavey PM, Ridley DL, Rowland IR. 2007. The effect of nicotine in vitro on the integrity of tight junctions in Caco-2 cell monolayers. *Food Chem. Toxicol.* 45:1593–98

106. Wang H, Zhao JX, Hu N, Ren J, Du M, Zhu MJ. 2012. Side-stream smoking reduces intestinal inflammation and increases expression of tight junction proteins. *World J. Gastroenterol.* 18:2180–87
107. Mohammed HO, Alaa El-Din EA, Farag AI. 2021. Impact of e-cigarettes on colonic mucosa and the role of recovery: involvement of oxidative and inflammatory pathway. *Environ. Sci. Pollut. Res. Int.* 28:64561–71
108. Stewart CJ, Auchtung TA, Ajami NJ, Velasquez K, Smith DP, et al. 2018. Effects of tobacco smoke and electronic cigarette vapor exposure on the oral and gut microbiota in humans: a pilot study. *PeerJ* 6:e4693
109. Pushalkar S, Paul B, Li Q, Yang J, Vasconcelos R, et al. 2020. Electronic cigarette aerosol modulates the oral microbiome and increases risk of infection. *iScience* 23:100884
110. Choi DW, Jeon J, Lee SA, Han KT, Park EC, Jang SI. 2018. Association between smoking behavior patterns and glycated hemoglobin levels in a general population. *Int. J. Environ. Res. Public Health* 15:2260
111. Oh SS, Jang JE, Lee DW, Park EC, Jang SI. 2020. Cigarette type or smoking history: Which has a greater impact on the metabolic syndrome and its components? *Sci. Rep.* 10:10467
112. Atuegwu NC, Perez MF, Oncken C, Mead EL, Maheshwari N, Mortensen EM. 2019. E-cigarette use is associated with a self-reported diagnosis of prediabetes in never cigarette smokers: results from the behavioral risk factor surveillance system survey. *Drug Alcohol Depend.* 205:107692
113. Orimoloye OA, Uddin SMI, Chen LC, Osei AD, Mirbolouk M, et al. 2019. Electronic cigarettes and insulin resistance in animals and humans: results of a controlled animal study and the National Health and Nutrition Examination Survey (NHANES 2013–2016). *PLOS ONE* 14:e0226744
114. Lan K, Zhang G, Liu L, Guo Z, Luo X, et al. 2020. Electronic cigarette exposure on insulin sensitivity of ApoE gene knockout mice. *Tob. Induc. Dis.* 18:68
115. Li G, Chan YL, Wang B, Saad S, George J, et al. 2020. E-cigarettes damage the liver and alter nutrient metabolism in pregnant mice and their offspring. *Ann. N. Y. Acad. Sci.* 1475:64–77
116. Wang Q, Ji X, Rahman I. 2021. Dysregulated metabolites serve as novel biomarkers for metabolic diseases caused by e-cigarette vaping and cigarette smoking. *Metabolites* 11:345
117. Ren X, Lin L, Sun Q, Li T, Sun M, et al. 2022. Metabolomics-based safety evaluation of acute exposure to electronic cigarettes in mice. *Sci. Total Environ.* 839:156392
118. Kim T, Choi H, Kang J, Kim J. 2020. Association between electronic cigarette use and metabolic syndrome in the Korean general population: a nationwide population-based study. *PLOS ONE* 15:e0237983
119. Cai J, Bidulescu A. 2023. Associations between e-cigarette use or dual use of e-cigarette and combustible cigarette and metabolic syndrome: results from the National Health and Nutrition Examination Survey (NHANES). *Ann. Epidemiol.* 85:93–99.e92
120. Middlekauff HR, William KJ, Su B, Haptonstall K, Araujo JA, et al. 2020. Changes in lipid composition associated with electronic cigarette use. *J. Transl. Med.* 18:379
121. Singh KP, Lawyer G, Muthumalage T, Maremanda KP, Khan NA, et al. 2019. Systemic biomarkers in electronic cigarette users: implications for noninvasive assessment of vaping-associated pulmonary injuries. *ERJ Open Res.* 5:00182-2019
122. Holmboe SA, Priskorn L, Jensen TK, Skakkebaek NE, Andersson AM, Jorgensen N. 2020. Use of e-cigarettes associated with lower sperm counts in a cross-sectional study of young men from the general population. *Hum. Reprod.* 35:1693–701
123. Vivarelli F, Canistro D, Cirillo S, Cardenia V, Rodriguez-Estrada MT, Paolini M. 2019. Impairment of testicular function in electronic cigarette (e-cig, e-cigs) exposed rats under low-voltage and nicotine-free conditions. *Life Sci.* 228:53–65
124. Chen T, Wu M, Dong Y, Kong B, Cai Y, et al. 2022. Effect of e-cigarette refill liquid on follicular development and estrogen secretion in rats. *Tob. Induc. Dis.* 20:36
125. Chuahirun T, Wesson DE. 2002. Cigarette smoking predicts faster progression of type 2 established diabetic nephropathy despite ACE inhibition. *Am. J. Kidney Dis.* 39:376–82
126. Regalado M, Yang S, Wesson DE. 2000. Cigarette smoking is associated with augmented progression of renal insufficiency in severe essential hypertension. *Am. J. Kidney Dis.* 35:687–94

127. Scharf P, Rizzetto F, Xavier LF, Farsky SHP. 2022. Xenobiotics delivered by electronic nicotine delivery systems: potential cellular and molecular mechanisms on the pathogenesis of chronic kidney disease. *Int. J. Mol. Sci.* 23:10293
128. Feng M, Bai X, Thorpe AE, Nguyen LT, Wang M, et al. 2023. Effect of e-vaping on kidney health in mice consuming a high-fat diet. *Nutrients* 15:3140
129. Podzolkov VI, Bragina AE, Druzhinina NA, Vasil'eva LV, Osadchiy KK, et al. 2020. Relation between tobacco smoking/electronic smoking and albuminuria/vascular stiffness in young people without cardiovascular diseases. *Kidney Blood Press. Res.* 45:467–76
130. Lee HW, Park SH, Weng MW, Wang HT, Huang WC, et al. 2018. E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. *PNAS* 115:E1560–69
131. Tang MS, Wu XR, Lee HW, Xia Y, Deng FM, et al. 2019. Electronic-cigarette smoke induces lung adenocarcinoma and bladder urothelial hyperplasia in mice. *PNAS* 116:21727–31
132. Bandara NA, Zhou XR, Alhamam A, Black PC, St-Laurent MP. 2023. The genitourinary impacts of electronic cigarette use: a systematic review of the literature. *World J. Urol.* 41:2637–46
133. Al-Bashaireh AM, Haddad LG, Weaver M, Kelly DL, Chengguo X, Yoon S. 2018. The effect of tobacco smoking on musculoskeletal health: a systematic review. *J. Environ. Public Health* 2018:4184190
134. Nicholson T, Davis L, Davis ET, Newton Ede M, Scott A, Jones SW. 2022. E-cigarette vapour condensate reduces viability and impairs function of human osteoblasts, in part, via a nicotine dependent mechanism. *Toxics* 10:506
135. Wavreil FDM, Heggland SJ. 2020. Cinnamon-flavored electronic cigarette liquids and aerosols induce oxidative stress in human osteoblast-like MG-63 cells. *Toxicol. Rep.* 7:23–29
136. Otero CE, Noeker JA, Brown MM, Wavreil FDM, Harvey WA, et al. 2019. Electronic cigarette liquid exposure induces flavor-dependent osteotoxicity and increases expression of a key bone marker, collagen type I. *J. Appl. Toxicol.* 39:888–98
137. Shaito A, Saliba J, Husari A, El-Harakeh M, Chhoury H, et al. 2017. Electronic cigarette smoke impairs normal mesenchymal stem cell differentiation. *Sci. Rep.* 7:14281
138. Rouabhia M, Alanazi H, Park HJ, Goncalves RB. 2019. Cigarette smoke and e-cigarette vapor dysregulate osteoblast interaction with titanium dental implant surface. *J. Oral Implantol.* 45:2–11
139. Pereira ML, Carvalho JC, Peres F, Fernandes MH. 2010. Simultaneous effects of nicotine, acrolein, and acetaldehyde on osteogenic-induced bone marrow cells cultured on plasma-sprayed titanium implants. *Int. J. Oral Maxillofac. Implants* 25:112–22
140. Hoshi H, Hao W, Fujita Y, Funayama A, Miyauchi Y, et al. 2012. Aldehyde-stress resulting from Aldh2 mutation promotes osteoporosis due to impaired osteoblastogenesis. *J. Bone Miner. Res.* 27:2015–23
141. Reumann MK, Schaefer J, Titz B, Aspera-Werz RH, Wong ET, et al. 2020. E-vapor aerosols do not compromise bone integrity relative to cigarette smoke after 6-month inhalation in an ApoE<sup>-/-</sup> mouse model. *Arch. Toxicol.* 94:2163–77
142. Agoons DD, Agoons BB, Emmanuel KE, Matawalle FA, Cunningham JM. 2021. Association between electronic cigarette use and fragility fractures among US adults. *Am. J. Med. Open* 1–6:100002
143. Armstrong ML, Smith N, Tracey R, Jackman H. 2022. The orthopedic effects of electronic cigarettes: a systematic review and pediatric case series. *Children* 9:62
144. Rivera JC, Espinoza-Derout J, Hasan KM, Lao C, Wilson JB, Shao XM, Tintut Y, Jordan M, Roos KP, Sinha-Hikim AP, Friedman TC. 2023. SAT639 Electronic cigarettes-exposed mice have impairments in skeletal muscle mediated by impaired Ampk activation. *J. Endocr. Soc.* 7(Suppl. 1):bvad114.1078
145. Nogueira L, Zemljic-Harpf AE, Yusufi R, Ranjbar M, Susanto C, et al. 2022. E-cigarette aerosol impairs male mouse skeletal muscle force development and prevents recovery from injury. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 323: R849–60
146. Kennedy P, Saloky K, Yadavalli A, Barlow E, Aynardi M, et al. 2021. Nicotine exposure via electronic cigarettes significantly impedes biomechanical healing properties of tendon healing in a rat model. *Arthroscopy* 37:3170–76

147. Tsai M, Byun MK, Shin J, Crotty Alexander LE. 2020. Effects of e-cigarettes and vaping devices on cardiac and pulmonary physiology. *J. Physiol.* 598:5039–62
148. Benowitz NL, Liakoni E. 2022. Tobacco use disorder and cardiovascular health. *Addiction* 117:1128–38
149. Echeagaray O, Savko C, Gallo A, Sussman M. 2022. Cardiovascular consequences of vaping. *Curr. Opin. Cardiol.* 37:227–35
150. Park JA, Crotty Alexander LE, Christiani DC. 2022. Vaping and lung inflammation and injury. *Annu. Rev. Physiol.* 84:611–29