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A review of the effects of e-cigarette exposure on physiology

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Thesis

A REVIEW OF THE EFFECTS OF E-CIGARETTE EXPOSURE ON PHYSIOLOGY

by

MADELEINE ELIZABETH REYNOLDS

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Approved by

First Reader

Jessica L. Fetterman, Ph.D.
Assistant Professor of Medicine

Second Reader

Christopher M. Schonhoff, Ph.D.
Assistant Professor of Pharmacology, Physiology, and Biophysics

A REVIEW OF THE EFFECTS OF E-CIGARETTE AND NICOTINE EXPOSURE ON PHYSIOLOGY

MADELEINE ELIZABETH REYNOLDS

ABSTRACT

Electronic cigarettes (e-cigarettes) have emerged as an alternative to combustible cigarette smoking with claims that they are a safer alternative to traditional cigarettes. Over the years the use of e-cigarettes has greatly increased worldwide without much regulation. The use of e-cigarettes became a health crisis when youth started using them at alarming rates. Emerging studies have suggested e-cigarette exposure may have harmful health effects to organ systems and physiological processes. E-cigarette use and exposure affect the cardiovascular and respiratory systems as well as elicit neurological effects. There is also evidence that e-cigarette liquids and aerosols contain carcinogenic chemicals, which and are detrimental to oral health, gastrointestinal health, the urinary system, and reproductive health. Overall, the evidence indicates e-cigarettes have the potential to harm various physiology.

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LIST OF ABBREVIATIONS

ACE2.....Angiotensin converting enzyme II

CACs..... Circulating angiogenic cells

CCL2.....Chemokine ligand 2

CXCL2..... Chemokine C-X-C motif ligand 2

D1R.....Dopamine D1 receptor

E-cigarettes .....Electronic cigarettes

EGR-1 .....Early growth response protein 1

EMT .....Epithelial to mesenchymal transition

ERK1-2 ..... Extracellular signal-regulated kinase 1-2

EVALI.....Electronic vaping associated lung injury

FDA.....Food and Drug Administration

nAChRs.....Nicotinic acetylcholine receptors

SOX2.....Sex determining region Y-box 2

TCA.....Tobacco Control Act

TCEP..... Tris (2-chloroethyl) phosphate

TRAIL.....Tumor necrosis factor related apoptosis-inducing ligand

TRPA1 .....Transient receptor protein ankyri

INTRODUCTION

E-cigarettes emerged as an alternative to traditional combustible cigarette smoking after the causal relationships between lung diseases and cigarette smoke became known to the public. The most well-known health risk of combustible cigarette smoking is its association with lung cancer- roughly 85% of lung cancer cases are attributed to combustible cigarette smoking (Warren & Cummings, 2013). The knowledge of the effects of combustible cigarette smoking drove individuals to switch to harm reduction methods that were marketed as a healthier option, but still contained nicotine, like e-cigarettes.

E-cigarettes were first introduced to the United States in 2007 as an alternative to combustible cigarette smoking with promises of decreased health risks on an individual (Dinardo & Rome, 2019). Many big tobacco companies are the parent companies to some of these e-cigarette brands. Marketing of e-cigarettes targeted current smokers as a gateway method that could be used to quit smoking all together (Palazzolo, 2013). There were also claims that e-cigarette companies targeted youth specifically. A widely popular e-cigarette brand Juul settled a multi-million dollar lawsuit that found them liable for marketing their products aggressively to young adults (Jewett & Creswell, 2023). Studies have shown that e-cigarettes may lead to initiation of traditional combustible cigarette smoking in non-smokers and relapse in ex-smokers due to nicotine dependence (Sayed et al., 2021). Based on the initial claims made by e-cigarette companies, they appeared to be a safer alternative to combustible cigarette smoking.

As they grew in popularity over the years, the designs and tactics of use of e-cigarettes changed as shown in Figure 1 below. The solution of e-cigarettes usually consists of nicotine, a humectant, and added flavorings that are aerosolized and inhaled by the consumer (Walley et al., 2019). Most e-cigarettes create an aerosol by heating and aerosolizing nicotine, vegetable glycerin, and propylene glycol (Esteban-Lopez et al., 2022). While propylene glycol and vegetable glycerin are deemed safe for ingestion, when heated and inhaled they produce toxic byproducts (Gordon et al., 2022a).

When compared to combustible cigarette smoke, aerosol from an e-cigarette tends to be clearer, smell like a particular flavor, and is easier to inhale as the components of the aerosol are less irritating than combustible cigarette smoke. Specific e-cigarettes that contain a metal heating coil also contain various metals (iron, nickel, chromium, and aluminum to name a few) that when heated can induce toxicity (Gordon et al., 2022b). Overall, e-cigarette devices tend to have a healthier appearance when compared to traditional odorous, darker cigarette smoke. Over the years the terms for e-cigarettes have changed and with some individuals who use e-cigarettes referring to them as “vapes” and the action of “vaping.” For this thesis we will use the term e-cigarette to be consistent with most of the research literature on the subject.

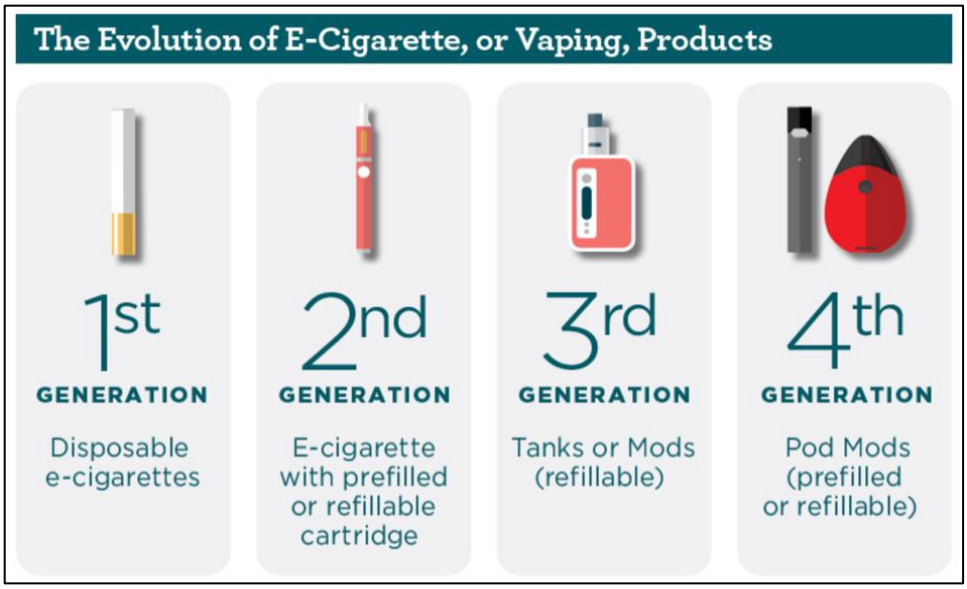

Figure 1: The evolution of vaping devices through generations. The beginning devices were disposable and looked like traditional cigarettes. As time went on these devices modified to become non-disposable with refillable tanks or disposable pods. Figure taken from (*What Is Vaping? | Texas DSHS*, n.d.)

Since e-cigarette use has increased in recent years and has become a relatively new health concern, there is limited data about the effects of these products on the human body. There has been some difficulty in conducting research due to the diversity of e-cigarette products and the varying components of e-liquids (Walley et al., 2019). However, in recent years, studies have come forth suggesting potential health risks of e-cigarette use. There has been debate as to whether they provide aid to someone trying to quit smoking, or if they sustain tobacco addiction.

E-cigarettes gained public attention when it was known that youth were using these devices, despite laws requiring them to be 18 or 21 to purchase (Dinardo & Rome, 2019). As of 2023, e-cigarettes are the most used tobacco product among middle and high school students where 10.0% of high school students and 4.6% of middle school students

admit to using e-cigarettes (Birdsey, 2023). Flavored e-cigarettes are preferred by youth and young adult users, particularly fruit, mint or menthol, and desserts/candy flavors (Hamberger & Halpern-Felsher, 2020). E-cigarette devices can be sold at various venues including smoke shops, grocery stores, gas stations, convenience stores, and on the internet, which contributes to their accessibility to underage users (Walley et al., 2019). The rapid increase in e-cigarette use among youth and young adults raises concerns that youth are being exposed to the negative effects of high doses of nicotine and inhaled toxins at a young age. Additionally, many studies have shown that e-cigarettes can serve as gateway to combustible cigarette smoking among youth and young adults (Chapman et al., 2018). This public health concern involving youth and addiction to e-cigarettes appears to be a large driving factor in the conduction of research studies on the potential health effects of e-cigarettes.

These factors made e-cigarette use an overall concern that the United States government felt needed further regulation. In 2009, the Tobacco Control Act (TCA) gave the Food and Drug Administration authority to regulate the manufacturing, distribution, and marketing of tobacco products (Green, n.d.). Originally, regulation of e-cigarettes were not covered under this act and the FDA tried to ban the import and sale of e-cigarettes by classifying them as drug delivery devices under the Food, Drug, and Cosmetic Act (Gholap & Halquist, 2020). This was challenged in court by e-cigarette companies, and in 2010 it was ruled that the FDA could not regulate e-cigarettes as drug devices and could not prevent the import or sale of e-cigarettes.

It was not until 2016 that the FDA gained ability to thoroughly regulate e-cigarettes under the TCA, which meant there were six years where e-cigarettes were unregulated in the United States of America (Gholap & Halquist, 2020). In 2019, the White House signed legislation raising the minimum age to purchase tobacco and e-cigarette products from 18 to 21 years old (Bonner et al., 2021). That same year the FDA also issued a flavor ban on cartridge-based and pod-based e-cigarette products. This ban does not apply to flavors such as tobacco or menthol and does not apply to disposable e-cigarettes or refillable e-cigarette products (Bonner et al., 2021). E-cigarette use among high school students did decline from 14.1% to 10.0% from 2022 to 2023 (Birdsey, 2023), which could, in part, be the results of the flavor ban.

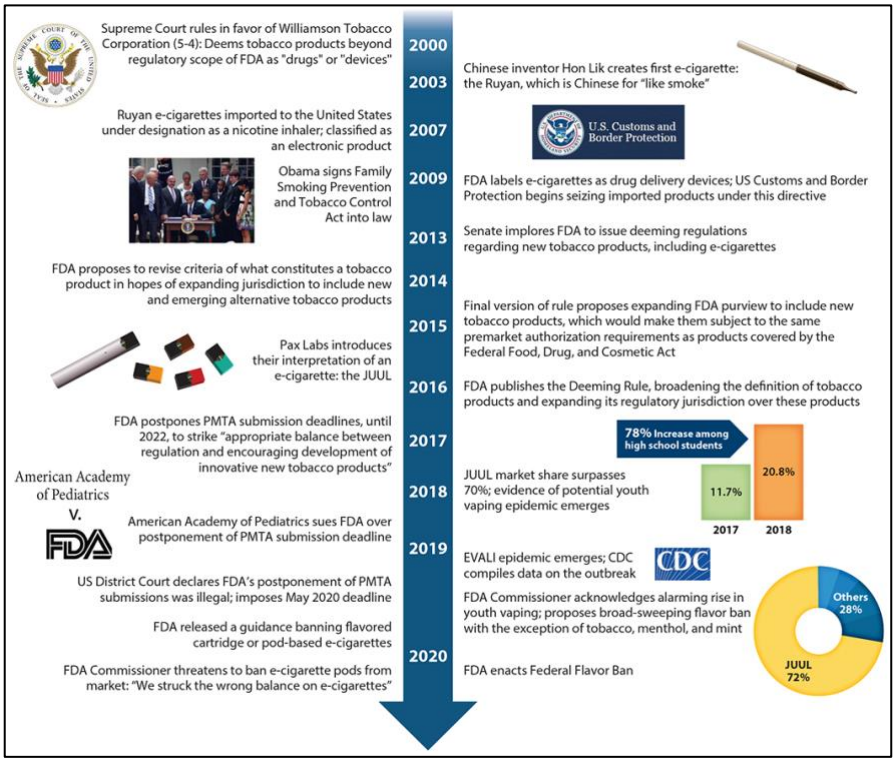

Figure 2: Timeline of e-cigarette existence and regulatory actions in the United States of America. Figure taken from (Gordon et al., 2022b).

The emergence of lung disorders and disease, such as electronic vaping associated lung injury (EVALI), has raised concerns regarding the contents of these e-cigarette products and their overall health risk. However, more recently studies are starting to show correlations between e-cigarette use and health risks to multiple organ systems. Clinical evidence suggests that e-cigarette use may have detrimental effects on the cardiovascular and pulmonary systems. Emerging data also suggests potential effects of e-cigarette use on neuronal, oral, urinary, gastrointestinal, and reproductive health. Thus, the purpose of this thesis is to summarize and assess recent health reports and studies on e-cigarette effects on each organ system. This will provide readers a framework to judge whether e-cigarettes provide a safer alternative to traditional tobacco smoking.

CARDIOVASCULAR

CARDIOVASCULAR FUNCTION

The detrimental effects of traditional combustible cigarette smoking on the cardiovascular system have long been known. Emerging studies suggest that e-cigarette devices can also generate harmful toxins that affect the cardiovascular system. Inhalation of e-cigarette aerosol containing nicotine increased mean arterial pressure and heart rate to a similar degree as traditional combustible cigarette smoking (Gonzalez & Cooke, 2021). One study compared individuals exposed to traditional tobacco smoke and e-cigarette aerosol and found that heart rate and systolic blood pressure increased from baseline before exposure for both the traditional tobacco group and the e-cigarette group (Vlachopoulos et al., 2016). A study performed showed that individuals who used traditional cigarettes and individuals who used e-cigarettes with nicotine had higher peripheral and central blood pressures compared to the control group consisting of e-cigarettes without nicotine (Franzen et al., 2018). This suggests nicotine could be a component in e-cigarettes that contribute to increases in blood pressure.

The increased heart rate and blood pressure associated with e-cigarette use likely results from increased sympathetic nerve traffic to blood vessels and the heart (Dimitriadis et al., 2022). Individuals who chronically used e-cigarettes had elevated sympathetic nervous system activation that led to increased heart rate variability, which is a known risk factor for cardiovascular disease (MacDonald & Middlekauff, 2019). The effects of sustained sympathetic activation of the heart and vasculature overtime could lead to cardiovascular disease. Such effects of over-stimulation by the sympathetic

nervous system could be particularly detrimental in people who have impaired baroreflex function, altering the ability to regulate blood pressure in response to physiological changes (Gonzalez & Cooke, 2021).

One study compared vascular function between individuals who used combustible cigarettes, e-cigarettes, both forms of cigarettes, and individuals who did not use either and found that e-cigarette use either alone or in conjunction with combustible cigarette use was associated with impairment of arterial stiffness (Fetterman et al., 2020). A study comparing individuals who used traditional combustible cigarettes, e-cigarettes containing nicotine, and a control group of e-cigarettes without nicotine showed elevated arterial stiffness in the combustible cigarette group and e-cigarette group containing nicotine (Franzen et al., 2018). Arterial stiffness is associated with coronary artery disease and cardiovascular events.

ATHEROSCLEROSIS AND ENDOTHELIAL DISRUPTION

Atherosclerosis and subsequent thromboses are a frequent cause of cardiovascular disease. Atherosclerosis is mediated by various components such as fats, cholesterol, fibrous material, endothelial cells, and immune cells that create plaques on the walls of arteries as depicted in Figure 3 (Falk, 2006). A study used magnetic resonance imaging and positron emission tomography to evaluate the vasculature of individuals who used traditional cigarettes only, e-cigarettes only, and a control group of individuals who did not use either and found that the e-cigarette group as well as traditional tobacco group

experienced carotid plaque burden, whereas the control group did not (Sahota et al., 2021).

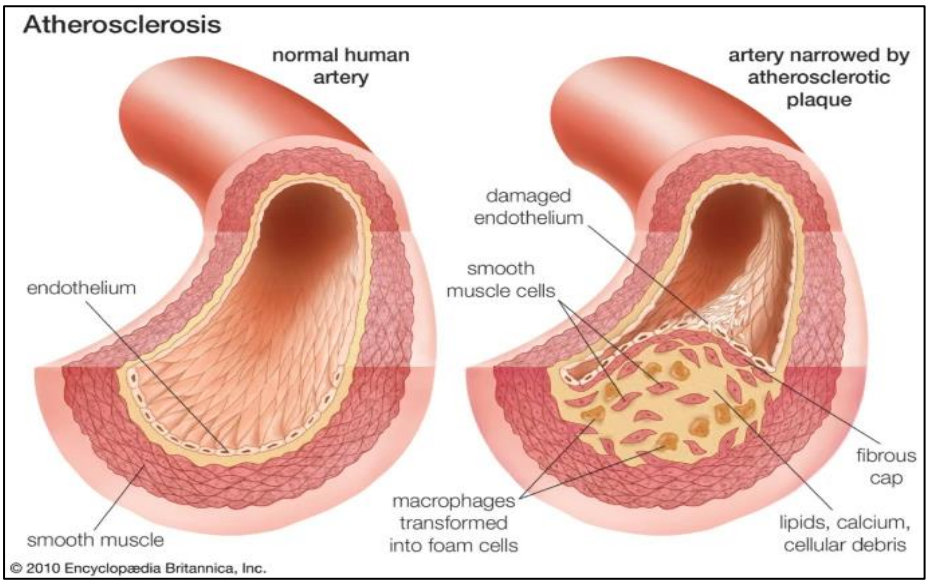

Figure 3: Atherosclerotic artery compared to normal artery. The key elements of atherosclerosis formation are macrophage transformation to foam cells, lipid debris buildup, smooth muscle cell infiltration, fibrous cap formation, and endothelial damage. Figure taken from (Subbotin, 2012).

E-cigarette use is associated with a higher immune response, specifically in proinflammatory monocytes and lymphocytes (Kelesidis et al., 2020). This proinflammatory response is suspected to be one contributor to atherosclerotic disease, especially elevations of CD14+ and CD16+ cells (Kelesidis et al., 2020). CD14+ and CD16+ are molecular markers for monocytes and macrophages, and neutrophils respectively. While this study does also show that individuals who used traditional tobacco cigarettes had more elevated immune responses than individuals who use e-cigarette, using e-cigarettes is not without potential cardiac risk.

Circulating angiogenic cells (CACs) are biomarkers that can be used to evaluate vascular health. A study compared CACs levels of individuals who use traditional

cigarettes, individuals who use e-cigarettes, and a control group of individuals who do not use any tobacco products and found that the e-cigarette group had similar levels of CACs with an inflammatory/hematopoietic phenotype to the traditional cigarette group and higher levels than the control group (Amraotkar et al., 2023). This data supports the evidence that e-cigarette use is associated with vascular dysfunction.

One study tested the effects of differing e-cigarette components and flavorings such as; propylene glycol and vegetable glycerol mixture without nicotine salts, propylene glycol and vegetable glycerin mixture with nicotine salts, mango flavoring, menthol flavoring, mint flavoring, and Virginia tobacco flavoring on human aortic endothelial cells *in vitro*. Each tested group showed decreased cell viability when compared to the non-treated control group cells, indicating that other components besides nicotine can disrupt cell viability (Majid et al., 2023). In this study, only the menthol flavored group increased reactive oxygen species compared to the non-treated control group (Majid et al., 2023). Certain flavorings of e-cigarettes could be promoting oxidative stress and endothelial cell dysfunction, which has implications for the development of cardiovascular disease.

A study exposed platelets taken from healthy individuals who did not use tobacco products to traditional cigarette smoke extracts and e-cigarette aerosol extracts and found that platelet aggregation and activity increased after e-cigarette exposure when compared to the non-treated control group and to similar levels as the traditional cigarette group

(Hom et al., 2016). This increase in platelet aggregation can lead to thrombus formation as depicted in Figure 4 below (Lyytinen et al., 2023).

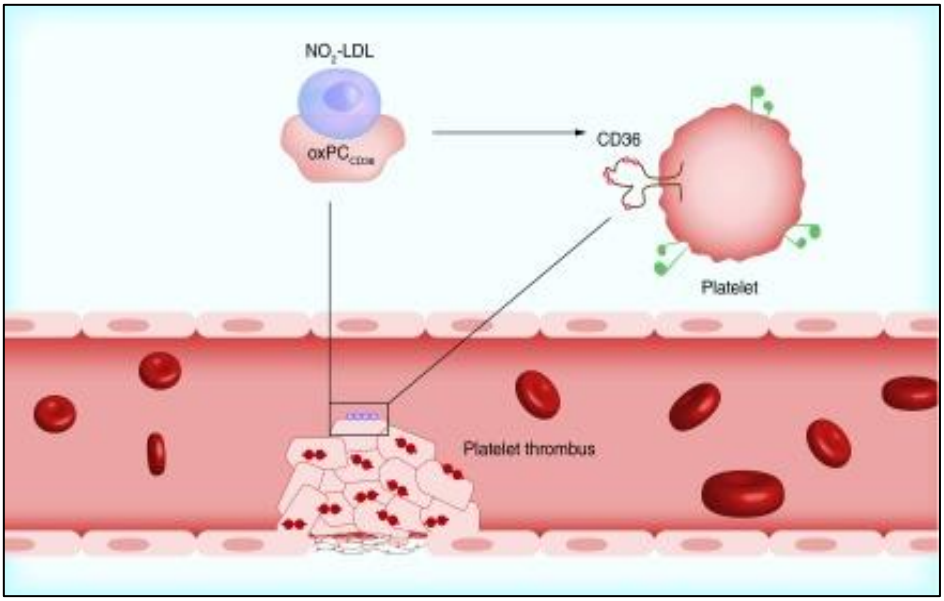

Figure 4: Platelet aggregation on the walls of arteries leading to thrombus formation. Figure taken from (Karagkiozaki et al., 2016).

In a crossover design study, individuals inhaled e-cigarette aerosol with and without nicotine and blood samples were collected at baseline before inhaling, 2, 4, and 6-hours post-inhalation. The results showed that extracellular vesicles of platelet origin and endothelial cell origin increased following e-cigarette with nicotine aerosol inhalation, but the e-cigarette without nicotine group did not show a significant increase (Mobarrez et al., 2020). Platelet and endothelial cell extracellular vesicles are recognized as biomarkers for vascular injury and atherosclerosis (J. Zhang, 2022).

ELECTROPHYSIOLOGY

One study used human induced pluripotent stem cell (a type of somatic stem cell)-derived cardiomyocytes and compared exposure to e-cigarette aerosol and to traditional cigarette smoke. It was shown that e-cigarette aerosol and traditional cigarette smoke produced similar toxic effects on cardiomyocytes, even though the nicotine concentration in e-cigarettes was much lower (Basma et al., 2020). This could indicate that there are other components of e-cigarette aerosol besides nicotine that could be associated with affecting cardiomyocytes such as aldehydes, metals, and flavorings (Basma et al., 2020). The cardiomyocytes exposed to e-cigarette aerosol produced reactive oxygen species, had negative chronotropic effects, altered myocardial gene expression, and decreased cellular survival that were similar to the cardiomyocytes exposed to traditional cigarette smoke (Basma et al., 2020). This shows that e-cigarette aerosol could have a negative impact on cardiomyocytes in a similar manner that traditional cigarette smoking does and as a result could alter the electrophysiology of the heart.

A study consisting of individuals who use e-cigarettes and a control group of individuals who did not use tobacco or nicotine containing products showed prolonged Tp-e intervals and higher Tp-e/QT and Tp-e/QTc ratios of the e-cigarette group compared to the control group (Demir et al., 2020). An electrocardiogram showing alterations in electrophysiology from this study is below in Figure 5. This shows that e-cigarette use could disrupt ventricular repolarization and result in ventricular arrhythmias.

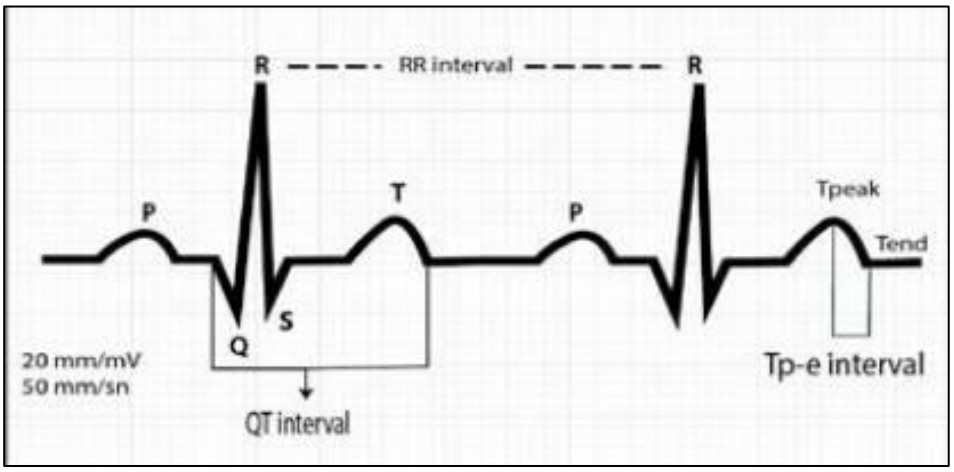

Figure 5: Altered electrocardiogram in individuals exposed to e-cigarette aerosol. Figure taken from (Demir et al., 2020).

Increased ventricular tachyarrhythmias was observed in mice exposed to nicotine-containing menthol solution as well as nicotine-free propylene glycol solution compared to filtered air exposed mice (Carll et al., 2022). This study also showed menthol containing e-cigarette solution caused changes in atrial conduction, showing that various components of e-cigarette aerosol could alter electrophysiology. These results suggest e-cigarettes could increase cardiovascular disease by altering heart electrophysiology and promoting cardiac arrhythmias (Carll et al., 2022). Mice exposed to e-cigarette aerosol had altered electrophysiology of the heart, including prolongation of the QT interval and alterations in action potential durations (Abouassali et al., 2021). These alterations are an associated risk for cardiac arrythmias.

PULMONARY

AIRWAY IMPAIRMENT

In addition to the cardiovascular system, another organ system affected by e-cigarettes is the pulmonary system. A study measured airway parameters in healthy individuals after inhalation of e-cigarette aerosol at baseline before inhalation, 2 hours, and 4 hours post inhalation. Airway obstructing features, such as increased flow resistance, were shown after inhalation of e-cigarette aerosol and raises concerns to the effect e-cigarettes have on pulmonary health (Antoniewicz et al., 2019). One study examined normal human bronchial epithelial cells *in vitro* when exposed to e-cigarette aerosol and found that significant ribosomal transcriptomic changes and disruptions in translation occurred that decreased overall protein synthesis when compared to the control group exposed to only air (H.-R. Park et al., 2021). Protein synthesis is essential for cell growth and development and disruptions to these processes could impair airway epithelium and increase the risk for lung disease.

E-cigarette liquids contain propylene glycol and vegetable glycerin that help to aerosolize and solubilize nicotine (Woodall et al., 2020). These two compounds were shown to negatively impact glucose uptake by human airway epithelial cells and modify glucose metabolism (Woodall et al., 2020). Glucose is one of the most essential compounds needed for cellular function and disruptions to these processes negatively impact cell viability. Propylene glycol and vegetable glycerin also were shown to increase airway epithelial permeability (Woodall et al., 2020). Airway permeability is critical to protect against pathogens in the air and to perform normal lung function.

A survey study showed an association between individuals who use e-cigarettes and asthma disease in an adult population who had never previously been traditional cigarette smokers (Perez et al., 2019). There is also evidence that e-cigarette use induces higher reports of wheezing and respiratory symptoms compared to individuals who do not use tobacco products (Li et al., 2019). In this same study, it was also shown that these respiratory symptoms were more frequently reported in individuals who quit smoking and had switched to e-cigarettes compared to those who had quit combustible cigarettes and did not use e-cigarettes (Li et al., 2019). The cellular changes and changes to the airway following e-cigarette aerosol exposure could be factors that contribute to future lung disease.

RESPIRATORY IMMUNE FUNCTION

A study tested effects of e-cigarettes containing nicotine aerosol on both *in vitro* human bronchial epithelial cells and *in vivo* female sheep. E-cigarette aerosol containing nicotine impacted both the *in vitro* and *in vivo* groups airway mucociliary function by activating transient receptor protein ankyrin 1 (TRPA1) and allowing calcium influx that creates a cascade of events leading to increased viscosity of mucus and impaired mucociliary clearance (Chung et al., 2019). Nicotine was found to instigate this process by activating TRPA1 in both groups. The cascade effect that leads to impaired mucociliary clearance is depicted in Figure 6 below.

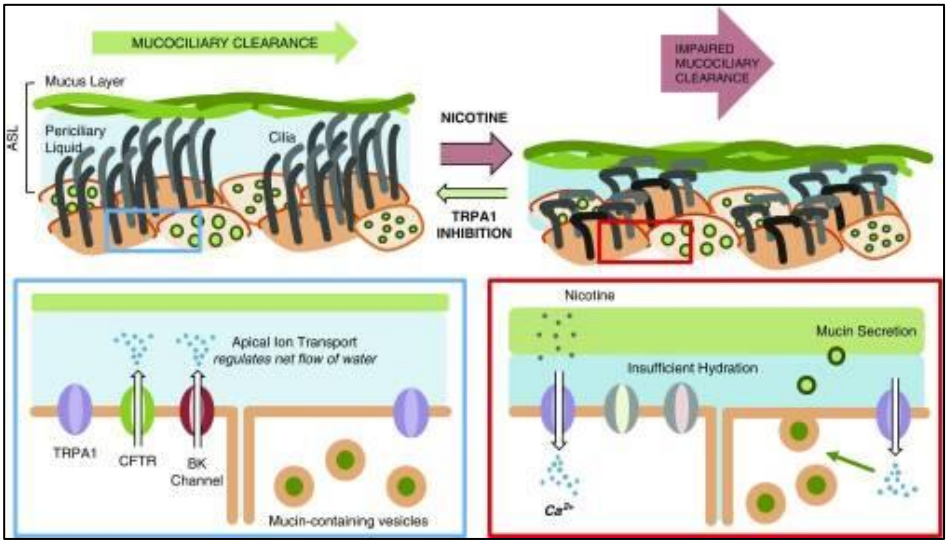

Figure 6: Impaired mucociliary clearance caused by nicotine activation of TRPA1 channel. Figure taken from (Chung et al., 2019).

A study performed testing of diacetyl and 2,3-pentanedione (common e-cigarette flavorings) on human bronchial epithelial cells and found that these specific compounds induced transcriptomic changes that directly impaired the morphology of respiratory cilia and decreased the number of ciliated cells after 48 hours of exposure (H.-R. Park et al., 2019). This shows that flavoring chemicals of e-cigarettes could also cause disturbance in cilia function. This is important for immune health as cilia are responsible for clearing debris from the lung and any disruption to that process can lead to impaired lung function.

In a study that measured lung parameters in individuals who did not normally use tobacco products when they inhaled e-cigarettes with and without nicotine, alterations to small airway epithelium occurred as well as impairment to alveolar macrophages in both the nicotine positive and nicotine negative groups (Staudt et al., 2018). Alveolar macrophages are immune cells that are critical to immune defenses given that the lungs

come in direct contact with outside air containing pathogens and pollutants. Human bronchial epithelial cells and macrophages when exposed to 2,3-pentanedione were found to produce reactive oxygen species (Morris et al., 2021). The production of reactive oxygen species can induce damage to various cell types by affecting DNA and RNA. A study of 30 e-cigarette flavoring chemicals showed that the most cytotoxic flavorings in lung epithelial cells and pulmonary macrophages were alpha-pinene (cedarwood; pine), decanal (citrus), eugenol (clove; spice), hexanal (fruity; green), nonanal (green; lemon), and trans-2-hexen-1-al (green; fruity) (Morris et al., 2021). Any disruption to alveolar macrophage function could lead to a decreased immune response.

A study specifically tested JUUL brand e-cigarette aerosol exposure on alveolar type II cells and found that the e-cigarette exposed cells had downregulation of genes involved in immune signaling, specifically chemokine ligand 2 (CCL2), chemokine C-X-C motif ligand (CXCL2), and early growth response protein 1 (EGR-1), when compared to the control group cells exposed to only air (Wick et al., 2022). These chemokines and proteins are important for regulating immune response and disruption to them can affect protection against pulmonary infection.

COVID-19 virus enters cells in the respiratory tract via their surface spike proteins binding with Angiotensin Converting Enzyme II (ACE2) on epithelial cells throughout the respiratory tract (McAlinden et al., 2021). In relation to COVID-19 disease and e-cigarette use, a study exposed bronchial epithelial cells and small airway epithelial cells to traditional cigarette smoke and e-cigarette aerosol with and without nicotine to see if ACE2 levels were affected. It was shown that e-cigarette aerosol with

and without nicotine increased protein expression of ACE2 to a similar degree as traditional cigarette smoke when compared to the non-treated control group exposed to air (McAlinden et al., 2021). Individuals who use e-cigarettes could be more susceptible to contracting the COVID-19 virus given the increase in ACE2 levels.

Nasal mucosal IgA levels is an indicator of immune response to infection by the influenza virus (Rebuli et al., 2021). One study inoculated participant groups of individuals who use traditional cigarettes, individuals who use e-cigarettes, and individuals who do not use any tobacco products with live-attenuated influenza virus and measured IgA levels in nasal lavage fluid. It was shown that IgA levels did not increase in groups that used e-cigarettes and traditional cigarettes when compared to the non-tobacco control group (Rebuli et al., 2021). It was also shown that cytokines that regulated antiviral host-defense responses were reduced in the e-cigarette group compared to the non-tobacco group (Rebuli et al., 2021). This shows that e-cigarette use could potentially alter host-defense mechanisms and alter defense against viral infection.

Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) is thought to mediate apoptosis of cells infected with viruses as an immune attack on the virus (Agraval et al., 2023). A study tested human lung slices that were exposed to e-cigarette liquid and influenza A virus and found that these specimens had increased viral load of influenza A and dysregulation of TRAIL expression when compared to the control group of human lung slices exposed only to influenza A virus (Agraval et al., 2023). The dysregulation of immune response mechanisms in response to e-cigarette use could worsen viral infections in the lungs.

ELECTRONIC VAPING ASSOCIATED LUNG INJURY (EVALI)

EVALI was termed the disease for patients who showed critical respiratory symptoms that were ruled out to be caused by anything else other than e-cigarettes (Jonas & Raj, 2020). Symptoms of EVALI are most commonly reported are dyspnea, cough, tachycardia, and high blood pressure followed by less frequently reported symptoms such as vomiting, nausea, and diarrhea (Tituana et al., 2023). Vitamin E acetate is a component of some e-cigarette products and has been suggested to be the main cause for EVALI outbreaks (J.-A. Park et al., 2022). Vitamin E acetate incorporates into phospholipids of surfactant, resulting in increased surface tension of alveoli (Tituana et al., 2023). Functional surfactant is a critical component to lung function and detriments to surfactant negatively impact breathing ability.

A common observation in chemical-induced pneumonitis is the presence of lipid-laden alveolar macrophages, which were also observed in cases of EVALI (J.-A. Park et al., 2022). Lipid-laden macrophages are frequently associated with on-going e-cigarette use (Jonas & Raj, 2020). The presence of propylene glycol and vegetable glycerin in e-cigarette aerosol showed deposition of lipids in alveolar macrophages in mice exposed to e-cigarette aerosol for 4 months in the presence and absence of nicotine (Figure 7) (Madison et al., n.d.). This suggests that propylene glycol and vegetable glycerin in e-cigarettes could be the compounds affecting lipid deposition, not nicotine. Lipid deposition is known to disrupt normal lung function by impairing alveolar macrophages and altering surfactant levels.

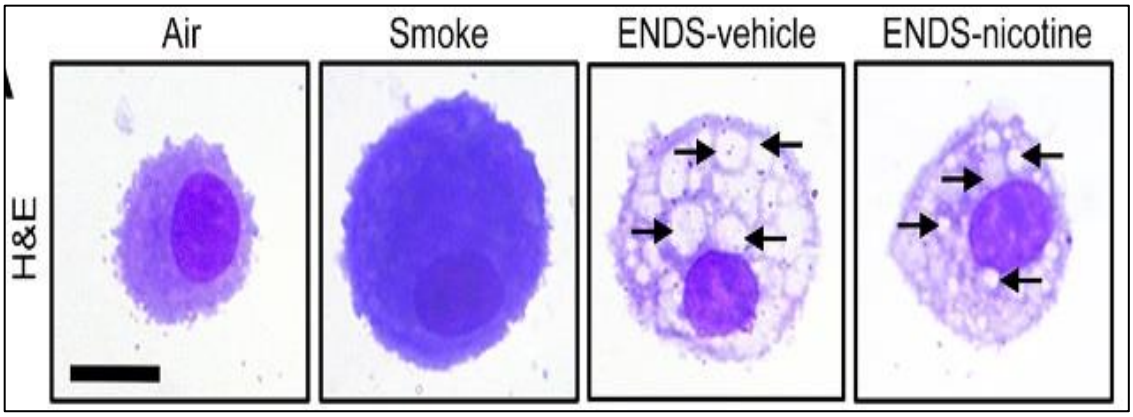

Figure 7: Lipid deposition in alveolar macrophages (black arrows) exposed to electronic nicotine delivery systems (ENDS) aerosol with and without nicotine compared to air exposed control group and smoke group. Figure modified from (Madison et al., n.d.).

The histological images below from EVALI patients show prominent hyaline membranes, foamy macrophages, pneumocyte hyperplasia, fibroblast proliferations within airways and alveoli, eosinophilic infiltration, and intrabronchial inflammation (Figure 8) (Reagan-Steiner et al., 2020). Histological findings of EVALI patients were consistent with presentations of acute lung injury.

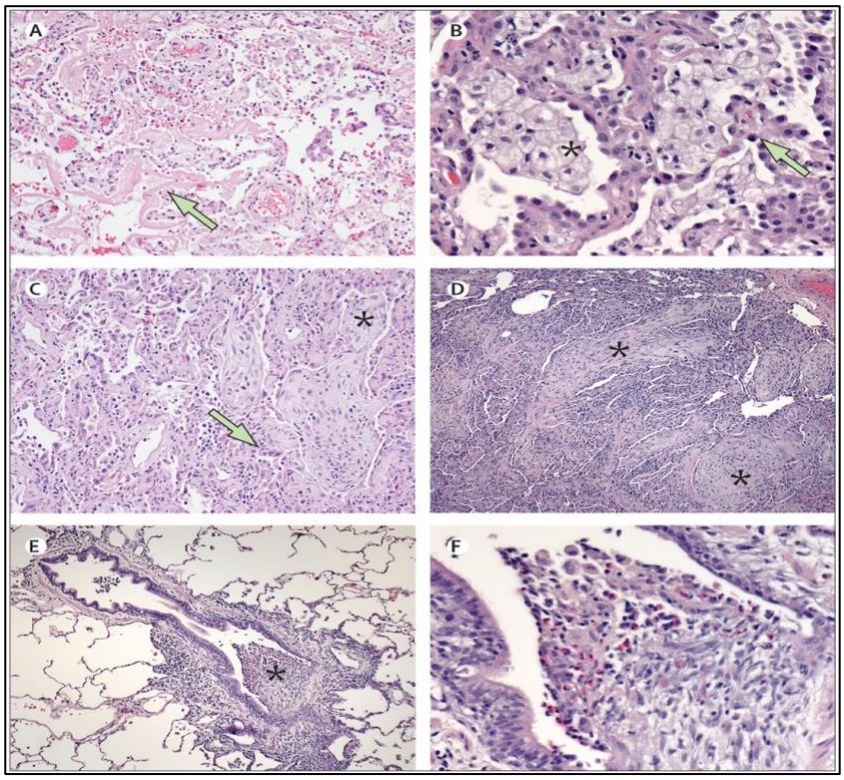

Figure 8: Histology changes from EVALI individuals. (A) Prominent hyaline membranes (green arrow). (B) Foamy macrophages (asterisk) and pneumocyte hyperplasia (green arrow). (C) Pneumocyte hyperplasia (green arrow) and fibroblastic proliferations (asterisk). (D) Fibroblastic proliferations within airways (asterisk). (E) Airway inflammation and intrabronchiolar fibroblastic plugs (asterisk). (F) Infiltration of eosinophils and macrophages. Figure taken from (Reagan-Steiner et al., 2020).

LUNG CANCER

Lung cancer accounts for the highest number of cancer-related deaths mostly due to metastasis (Zahedi et al., 2018). While combustible tobacco use is a major cause of lung cancer, there is increasing evidence that nicotine also plays a role in promoting tumor growth. Nicotine activates the alpha subunit on nicotinic acetylcholine receptors and causes a cascade effect that leads to an upregulation of sex determining region Y-box 2 (SOX2) expression (Schaal et al., 2018). Overexpression of SOX2 promotes

proliferation and metastasis of cancer (Schaal et al., 2018). Exposure of human adenocarcinoma alveolar basal epithelial cells to either menthol or tobacco flavored e-cigarette liquids and aerosols promoted epithelial to mesenchymal transition (EMT) based on changes in cell morphology, cell-to-cell adhesion, molecular markers, and motility (Zahedi et al., 2018). The process of EMT is depicted in Figure 9 below.

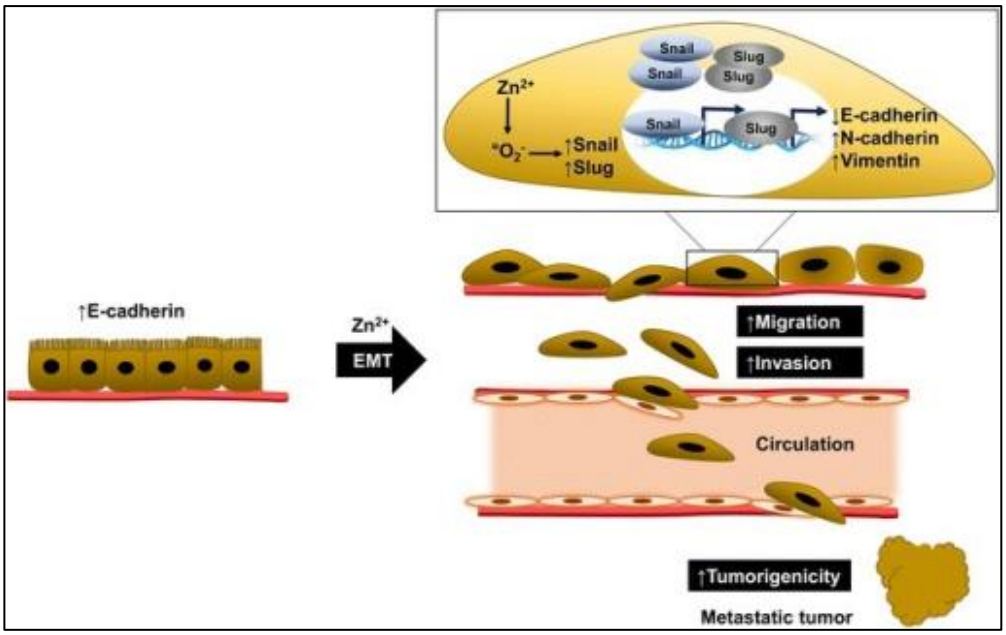

Figure 9: EMT transition in lung cancer cells promoting metastasis. Cancer cells increase migration and invasion into circulation through upregulation of markers Snail, Slug, N-cadherin, and Vimentin and down regulation of E-cadherin causing reorganization of adhesion and cytoskeleton proteins. Figure taken from (Ninsontia et al., 2016).

Epithelial to mesenchymal transitions are how cancer cells begin to metastasize and is associated with poorer prognosis. In mice models, nicotine from e-cigarettes is metabolized into certain nicotine-derived nitrosamine ketones, which are not only carcinogenic to lung epithelium, but also impair DNA repair activity (Lee et al., 2018). Cytochrome P450 is known to be associated with tumor development and progression of

lung cancer (Oyama et al., 2007). In rat lung it was found that e-cigarette aerosol exposure increased risk of lung cancer by increasing CYP-linked monooxygenases, such as P450, compared to tissue from the control group of air exposed rats (Canistro et al., 2017). These studies suggest that e-cigarettes may promote lung cancer development and metastasis.

NEUROLOGICAL

YOUTH

The increasing prevalence of e-cigarette use among youth has prompted research into the neurological side effects of nicotine on the adolescent brain. Studies have shown that brain sites, such as the nucleus accumbens, amygdala, frontal cortex, and ventral tegmental area, are directly affected by nicotine (Markou, 2008). A study done on rats explored the effects nicotine has on nicotinic acetylcholine receptors (nAChRs) in adolescent vs. adult rats. It was found that the adolescent rat group exposed to nicotine had increased nAChR number and activity in brain regions that correspond to reward effects when compared to the adult rat group exposed to nicotine (Doura et al., 2008). Another study on the effect of nicotine on adolescent and adult rat brains showed that nicotine reduced reward thresholds to a greater extent in female adolescent rats when compared to male adolescent rats or adult rats (Xue et al., 2018). This shows that the reward effect of nicotine could be enhanced in youth more so than in adults, which could enhance the susceptibility to which youth depend on e-cigarettes.

Early adolescent, late adolescent, and adult rats were exposed to nicotine for 7 days and then 35 days later were evaluated on morphine reward responses. It was shown that early adolescent rats had increased behavioral response to morphine compared to the late adolescent rats and adult rats (Kota et al., 2018). This suggests that exposure to nicotine during early adolescence could have long-term effects that impact drug-use behavior later in life. A survey study compared substance use among adolescents who use e-cigarettes, traditional tobacco cigarettes, both traditional cigarettes and e-cigarettes, and

no tobacco products to see which groups had also used other forms of substances. It was found that adolescents who used e-cigarettes also tended to use other substances such as alcohol, oral tobacco, marijuana, and amphetamines more often than individuals who did not use any tobacco products (Kristjansson et al., 2015). This study suggests that youth who use e-cigarettes are more susceptible to other substance use.

Another study performed in rats showed that adolescent rats who were exposed to nicotine showed signs of long-lasting deficits in sociability and social recognition memory when compared to the adult rat group (Jobson et al., 2019). This same study also showed that adolescent nicotine-exposed rats had downregulation of prefrontal cortex dopamine D1 receptor (D1R) expression and increased extracellular signal-regulated kinase 1-2 (ERK1-2) phosphorylation states that lasted into adulthood. Such molecular changes are known to contribute to mood disorders, including anxiety (Jobson et al., 2019). This study suggests that nicotine exposure in youth can have lasting effects on mental health into adulthood.

A review found that individuals who use e-cigarettes were more likely to experience increased depression symptoms and suicidal ideations compared to individuals who did not use e-cigarettes (Khan et al., 2023). A theory called Tobacco-Induced Neurotoxicity of Adolescent Cognitive Development postulates that nicotine affects the prefrontal cortex causing increased impulsivity and inattention in adolescents (Leslie, 2020). There is conflicting evidence on whether the use of nicotine causes anxiety and depression or if nicotine use is a coping mechanism for mental health issues (Leslie, 2020). An analysis done of high school students showed that the use of e-

cigarettes caused the acceleration of growth in depressive symptoms (Lechner et al., 2017).

BLOOD-BRAIN-BARRIER

An *in vitro* study tested a model blood-brain-barrier using stem cell derived brain microvascular endothelial cells exposed to traditional tobacco cigarette smoke and e-cigarette aerosol over a 24-hour period. Both the traditional cigarette group and the e-cigarette group were shown to impact the distribution of tight junction proteins ZO-1 and occludin and downregulate expression levels of claudin-5 (Kadry et al., 2021). These proteins are vital to maintaining the blood-brain-barrier (Figure 10) and dysregulation can cause increased permeability.

In vivo studies in male mice were preformed to determine if nicotine influenced the blood-brain-barrier status post ischemia, as occurs in a stroke. It was found that nicotine exposure caused a proinflammatory response by increasing the expression of inflammatory cytokines and chemokines compared to the mice group not treated with nicotine (Bradford et al., 2011). These responses worsened ischemic injury to the brain. Another study provided additional evidence for this by concluding that nicotine caused oxidative stress and promoted the onset of stroke in mice exposed to traditional tobacco smoke and e-cigarette aerosol when compared to the non-treated air control group (Kaisar et al., 2017).

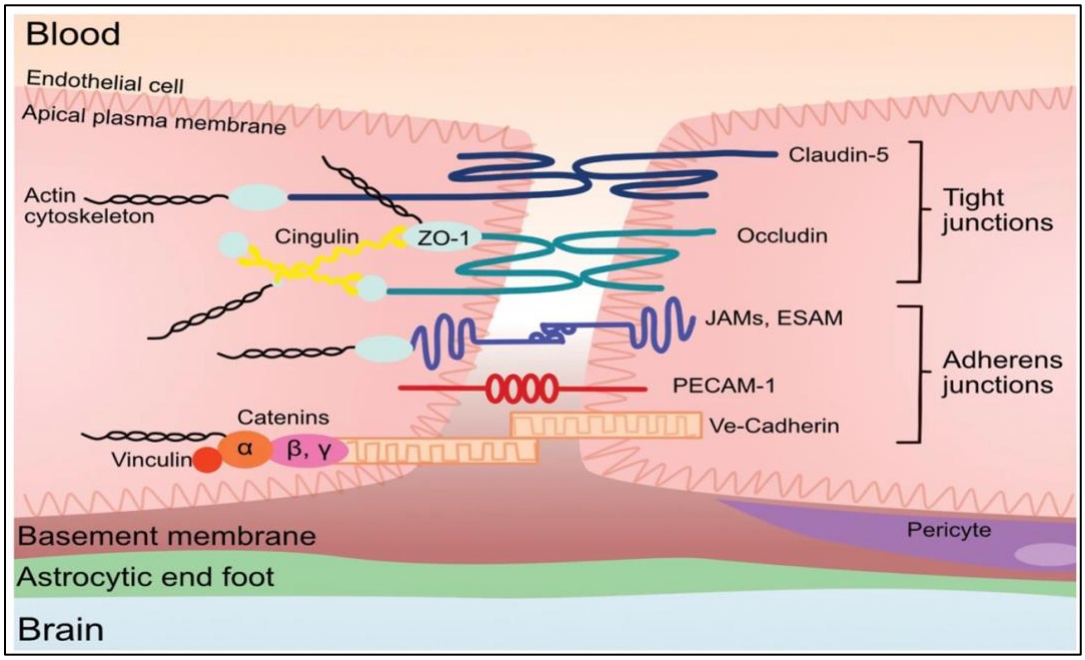

Figure 10: Diagram of the blood-brain-barrier and the important tight junction proteins involved in maintaining the blood-brain-barrier such as ZO-1, Occludin, and Claudin-5. Figure taken from (Knox et al., 2022)

SPINAL HEALTH

There have been studies to show that nicotine has an impact on intervertebral disc health. While these studies looked at nicotine itself, not specifically through e-cigarette delivery, the amount of nicotine used in the studies was representative of nicotine amounts that can be achieved by e-cigarette use. In a study where rabbit groups were exposed to nicotine or saline, it was shown that the nicotine exposed groups had necrosis of the nucleus pulposus and disturbance of annulus fibrosus cells when compared to the saline exposed control group (Iwahashi et al., 2002). This creates an environment for degenerative disc disease (Fiani et al., n.d.).

Bovine chondrocytic intervertebral disc cells exposed to nicotine were shown to have disrupted cell architecture and extracellular matrix when compared to the nicotine-free control group (Akmal et al., 2004). Immunohistochemistry from this study also revealed that the nicotine treated group had increased presence of type I collagen in the extracellular matrix and reduced type II collagen (Akmal et al., 2004). The replacement of type II collagen with type I is suggestive of disc degeneration. Further studies are needed in this area focusing on whether other components of e-cigarette aerosol affect spinal health and whether adolescent vs adult exposure results in different susceptibilities to nicotine-exposure-induced spinal toxicity.

ORAL HEALTH

Use of e-cigarettes is associated with less oral health risks when compared to traditional cigarette smoking, but is associated with higher risk compared to individuals who do not use tobacco products (Holliday et al., 2021). Traditional combustible cigarette smoking is associated with effecting oral health and studies have been done to evaluate whether e-cigarette use poses similar dangers. One study extracted human epithelial gingival cells of healthy individuals who did not use tobacco products and exposed the gingival epithelial cells to e-cigarette aerosol. The study showed that exposure of the gingival cells to e-cigarette aerosol was associated with morphological cell changes from small cuboidal shape to a large undefined shape when compared to the non-treated control group (Rouabhia et al., 2017). Morphological changes of cells typically indicate cellular damage, suggesting that e-cigarette aerosol is associated with oral cell damage.

This study also found that lactate dehydrogenase release from cells, an indicator of apoptosis, was increased in gingival epithelial cells exposed to e-cigarette aerosol compared to the non-treated control group (Rouabhia et al., 2017). Another study done that also tested human gingival cells found that only e-cigarette aerosol containing nicotine had resulted in increased lactate dehydrogenase levels in human gingival fibroblast cells while e-cigarette aerosol that did not contain nicotine had no effects on lactate dehydrogenase levels (Sancilio et al., 2017). This suggests the nicotine in e-cigarette-generated aerosols induces toxicity in oral cells.

E-cigarettes are shown to alter the oral microbiome and it is thought that glycerol and propylene glycol are the main contributors to this shift (Ganesan et al., 2020). The oral cavity of individuals who were exposed to e-cigarette aerosol had higher levels of microbial communities of *Porphyromonas* and *Veillonella* compared to that of individuals who did not use e-cigarettes. These microbial increases likely increases susceptibility to infections (Pushalkar et al., 2020). The change in the oral microbiome was associated with inflammatory diseases such as periodontitis (Figure 11), and *Veillonella* is often the dominant species identified in chronic periodontitis (Chopyk et al., 2021).

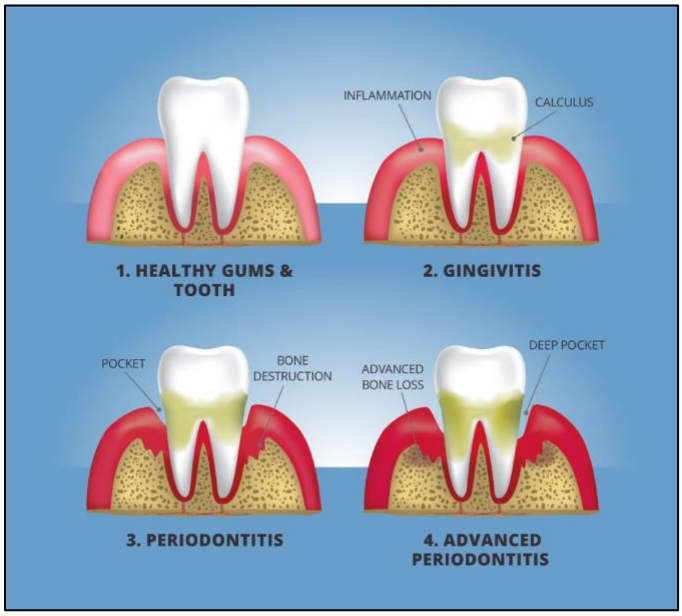

Figure 11: Periodontitis progression starting with inflammation of the gums and hardened dental plaque that progresses to deep pockets and bone loss. Figure taken from (Diabetes, Gum Disease, & Other Dental Problems - NIDDK, n.d.)

In *in vitro* studies it was also found that e-cigarette aerosol was associated with lower levels of *Streptococcus sanguinis* and *Streptococcus gordinii*, two types of bacteria that are known to be commensals to the oral microbiome, while allowing the flourishing

of *Streptococcus mutans* that is known to be an opportunistic pathogen to the oral microbiome (Catala-Valentin et al., n.d.). This shifts the microbiome in favor of pathogens and can lead to increased risk of infection. When oral epithelial cells were exposed to e-cigarette aerosol, *Staphylococcus aureus* biofilm formation was enhanced when compared to the only air treated control group. It was also noted that the oral epithelial cells exposed to e-cigarette aerosol had a reduced ability to clear the *Staphylococcus aureus* infection, likely due to the decrease in secretion of proinflammatory cytokines (Cátala-Valentín et al., 2022). Imbalance of *Staphylococcus aureus* can lead to increased risk of infections.

A correlation between *Staphylococcus aureus* and e-cigarette aerosol in potentiating malignant cells was also studied. It was shown that oral epithelial cells cultured with *Staphylococcus aureus* and exposed to e-cigarette aerosols had an increase in cyclooxygenase-2 (COX2) expression compared to the non-treated control group (Cátala-Valentín et al., 2022). COX2 is an inflammatory marker that is known to promote cancer development. Limited studies have been done to show a correlation between e-cigarette aerosol and a direct cause to cancer, but evidence suggests that e-cigarette exposure may promote an environment that can lead to oral cancer. In an *in vitro* study, e-cigarette exposure of head and neck squamous cell cancer cells demonstrated that, regardless of nicotine content, e-cigarette aerosol resulted in the accumulation of double stranded DNA breaks (Yu et al., 2016). Double stranded breaks are associated with carcinogenic potential of cells. Histology comparing normal oral mucosa to oral cancer mucosa is depicted in Figure 11 below.

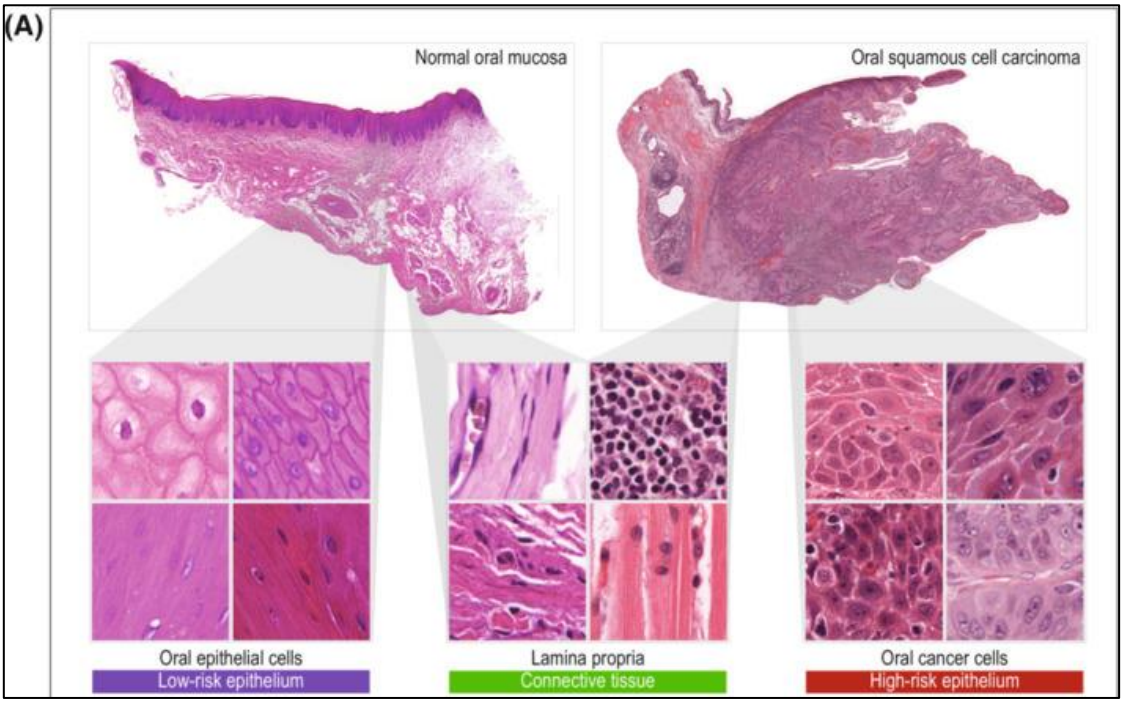

Figure 12: Histology images of oral squamous cell carcinoma compared to normal oral mucosa. Figure modified from (Zhang et al., 2023).

Most head and neck cancers are treated with a chemotherapy drug called Cisplatin. It was shown that e-cigarette use was associated with a greater likelihood of resistance of head and neck squamous cell carcinoma to Cisplatin (Manyanga et al., 2021). This is notable given that about 9.5% of all cancer patients report using e-cigarettes, according to a survey done in 2019 (Sanford et al., 2019).

GASTROINTESTINAL

Emerging evidence suggests that e-cigarettes pose a threat to the gastrointestinal system, although the effects of e-cigarettes on the gastrointestinal system is understudied. A retrospective study of individuals who use e-cigarettes showed that e-cigarettes could potentially alter the motility of the gastrointestinal tract and cause irritation and inflammation, usually resulting in clinical symptoms such as vomiting and diarrhea (Debnath et al., n.d.). Another study specifically testing JUUL brand mango and mint e-cigarettes in mice showed that e-cigarette exposure caused an increase in pro-inflammatory cytokines such as tumor necrosing factor alpha, interleukin-6, and interleukin-8 in the colon after one month of exposure compared to the control group exposed to air (Moshensky et al., n.d.). Chronic inflammation of the colon could lead to infection and diseases such as inflammatory bowel disease.

One study of e-cigarette exposure of rats showed distorted colonic mucosa with loss of folding and decreased goblet cells (Figure 13) (Mohammed et al., 2021). E-cigarette aerosol exposure also increased intestinal permeability, inflammatory cell infiltration, levels of tumor necrosis factor-alpha, suggestive of ulcerative colitis (Mohammed et al., 2021).

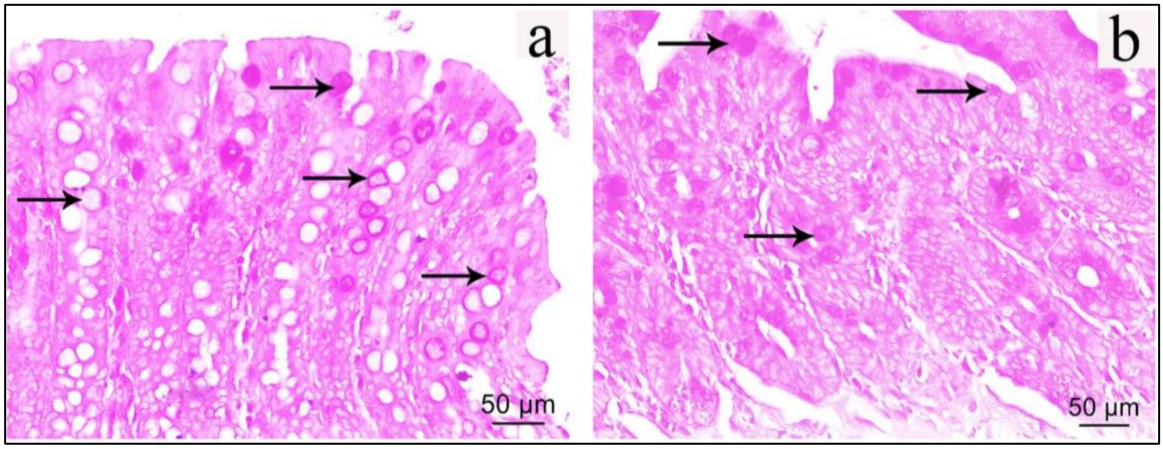

Figure 13: a) Control group with a normal goblet cell (black arrows) population. b) E-cigarette aerosol exposure decreased goblet cell (black arrows) population. Figure modified from (Mohammed et al., 2021).

Traditional cigarette smoking is known to increase esophageal disease risk, such as Barret's esophagus and gastro-intestinal reflux disease (GERD) (Pasricha & Kochar, 2021). A case study of a 25-year-old male with well controlled GERD for years was unable to tolerate swallowing and had chronic inflammation and granulation tissue of the esophagus after 2 months of heavy e-cigarette use (Pasricha & Kochar, 2021). GERD, left untreated, could disrupt the esophageal epithelium and increase the risk for esophageal cancer. The compound nitrosonornicotine has been studied in animal models as an esophageal carcinogen and is found in traditional tobacco cigarette smoke as a result of nitrosation of nicotine (Bustamante et al., 2018). A study of individuals who use e-cigarettes suggests that nitrosonornicotine is formed endogenously, which has the potential to increase the risk for esophageal cancer (Bustamante et al., 2018).

URINARY HEALTH

BLADDER

Traditional combustible tobacco smoking increases the risks of bladder cancer (Fuller et al., 2018); however, studies now suggest that e-cigarette use is associated with exposure to several carcinogens with implications for urinary health. Individuals exposed to e-cigarette aerosols have higher levels of some volatile organic chemicals in their urine; however, it was noted that the levels of volatile organic compounds are lower than have been observed in urine from individuals who use traditional cigarettes (Lorkiewicz et al., 2018). This is suggestive that e-cigarettes can be a risk to increasing risk for developing bladder cancer (Figure 14).

Biomarkers for carcinogens have been noted in the urine of individuals who use e-cigarettes (Bjurlin et al., 2021). O-toluidine and 2-napthylamine are two chemicals that are known to cause bladder cancer and were present in the urine of 92% of e-cigarette users (Molony et al., 2023). Certain carcinogens such as aromatic amines, aldehydes, and polyaromatic hydrocarbons are associated with bladder cancer and have been identified as components of e-cigarette liquids (Fuller et al., 2018). The presence of these compounds, whether as metabolites found in urine or in e-cigarette liquid, shows the carcinogenic potential of e-cigarettes.

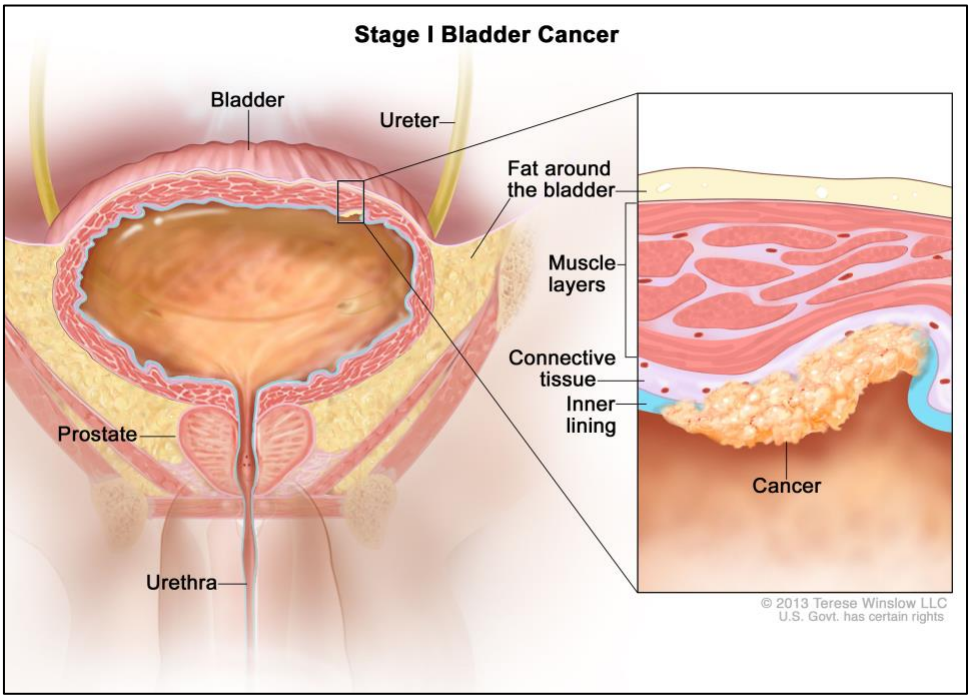

Figure 14: Stage 1 bladder cancer. Cancer cells are confined to the epithelial and connective tissue layers but have not penetrated the muscle layers. Figure taken from (Bladder Cancer Treatment (PDQ®) - NCI, 2023)

A study also compared traditional combustible cigarette smoking with e-cigarette use and concluded that both caused alteration of proteins in extracellular vesicles that could promote DNA damage in urothelial cells (Molony et al., 2023). This effect has been shown to lead to increased risk of non-malignant urothelial cells. (Molony et al., 2023). Limited study has been done on the effect of Tris (2-chloroethyl) phosphate (TCEP) in humans, but animal studies have shown that TCEP could be a potential carcinogen (Bandara et al., 2023). TCEP metabolite levels appear higher in human studies of individuals who use e-cigarettes compared to individuals who use combustible cigarette and individuals who do not use tobacco products. (Bandara et al., 2023).

KIDNEY

E-cigarettes have been shown to also disrupt the renal system. Even though e-cigarettes lack combustion, the e-cigarette heating process releases various chemicals such as nicotine, flavorings, and ultrafine particles that can reach the kidneys. The renin-angiotensin-aldosterone system regulates filtration rate of the glomerulus as well as reabsorption and secretion of various ions. Nicotine has been shown to affect the renin-angiotensin-aldosterone system which can alter control on blood pressure perfusing the kidneys and lead to inflammation (Scharf et al., 2022) and cause filtration dysfunction.

Cinnamaldehyde is a type of flavoring agent used in e-cigarettes and has been shown to activate TRPA1 channels that exist in renal tubular cells (Hoi et al., 2020)(Wu et al., 2019). TRPA1 channel activation is known to promote inflammatory response (Figure 15) (Wu et al., 2019). In mice studies, TRPA1 activation showed higher reactive oxygen species production in renal tubular cells (Wu et al., 2021). The flavorings of e-cigarettes could potentially cause disruption to the kidney by promoting inflammation of renal tubules. The byproducts of the e-cigarette heating process can promote inflammatory processes, activate cell death pathways in renal tubular cells, and decrease glomerular filtration rate that ultimately could promote the development of chronic kidney disease (Scharf et al., 2022).

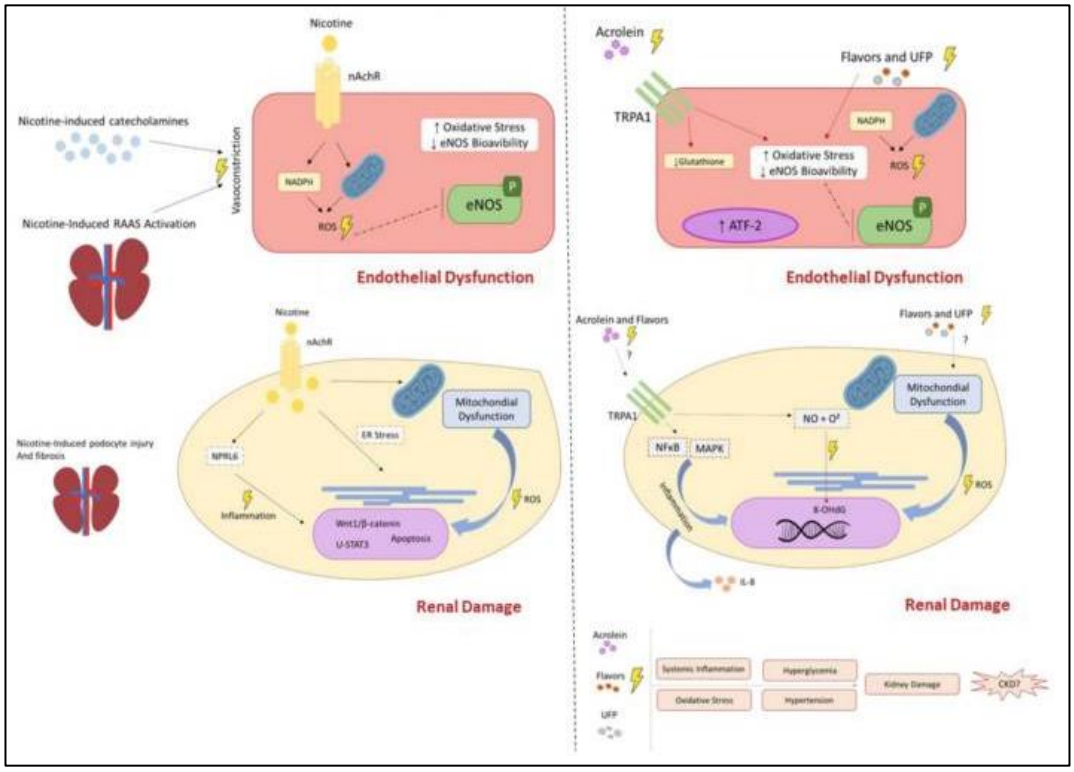

Figure 15: Endothelial dysfunction as a result of oxidative stress in renal cells by activation of TRPA1. Figure modified from (Scharf et al., 2022).

Albumin in the urine is a sign of a diseased kidney, whereas typically albumin is a protein that does not get filtered out of the blood under normal, healthy conditions. Albuminuria is a sign of vascular damage in glomeruli as well as other areas of the body (Podzolkov et al., 2020). Levels of albuminuria appeared higher in individuals who use e-cigarettes compared to those who use traditional combustible cigarettes (Podzolkov et al., 2020). This study noted that the reason for higher albuminuria among individuals who use e-cigarettes is not clear but the evidence suggests it is unrelated to the nicotine concentration (Podzolkov et al., 2020). This suggests there could be other substances, or

substances that appear in higher concentrations, in e-cigarette liquids and aerosols that differ from traditional combustible cigarettes that could elicit harmful health effects.

REPRODUCTIVE HEALTH

Little is known about the effects of e-cigarette aerosol on reproductive health in both males and females. Most research available was performed using rodent models. One study found that e-cigarette liquid exposure to rat ovaries significantly changed the morphology of ovarian follicles and decreased the total number of normal follicles when compared to the non-treated ovary control group (Chen et al., 2022). This study also found that e-cigarette aerosol induced nuclear pyknosis in granulosa cells, which likely disrupted follicle growth (Chen et al., 2022). Granulosa cells play a major role in supporting follicle growth and producing reproductive hormones.

E-cigarette aerosol was also shown to affect the male reproductive system in rats. A study showed that e-cigarette aerosol free of nicotine was detrimental to the germinal epithelial layer of the seminiferous tubules in rats, the main site of sperm production, in rats (Vivarelli et al., 2019). Exposure of male rats to e-cigarettes produced aerosol resulted in exposure to toxic aldehydes that affected enzymes involved in steroidogenesis (Vivarelli et al., 2019).

Traditional combustible cigarette smoking in pregnancy is associated with low birth weight and other effects on fetal development, but not a lot of data is available on the effects of e-cigarette use on fetal development in pregnancy. A study on mice found that e-cigarette aerosol impaired and delayed implantation of embryos in pregnant mice by altering essential ion transport and signaling pathways such as JAK/STAT signaling (Wetendorf et al., 2019). This study also found that *in utero* e-cigarette aerosol exposed female offspring, while no different in size at birth, were smaller than the control groups

at around 8.5 months of age (Wetendorf et al., 2019). This indicates that the effects of e-cigarette exposure during development could be long lasting.

A study showed that pregnant mice that underwent chronic exposure to e-cigarettes containing nicotine had altered vascular hemodynamics through the maternal uterine and fetal umbilical arteries, which may contribute to intrauterine growth restriction (Orzabal et al., 2019). Consistent with this, the weights and crown-rump length of both the fetal and born pups were lower in the group exposed to e-cigarettes aerosol compared to non-exposed control group (Orzabal et al., 2019).

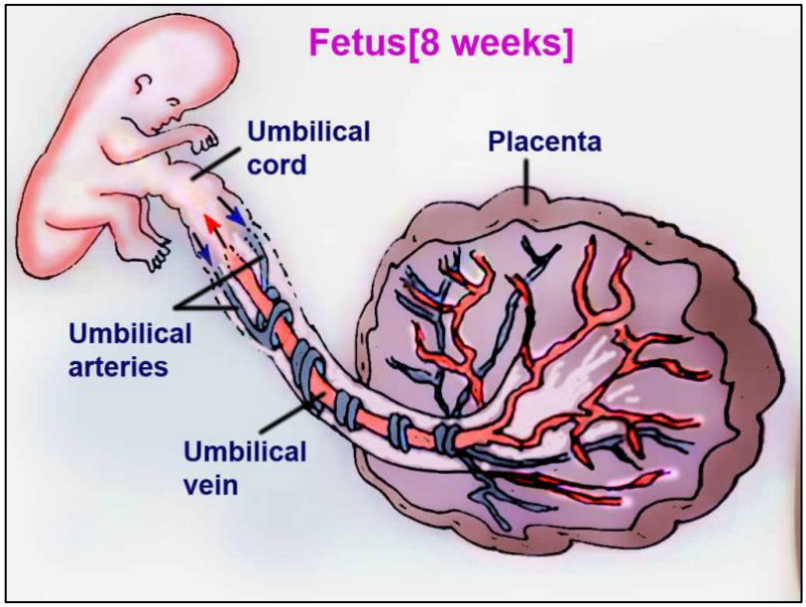

Figure 16: Arterial and venous blood flow between placenta and human fetus. Figure taken from (Sidhu & Lui, 2024).

Similar findings of the effects of e-cigarette exposure on the placenta and fetus have been shown to occur regardless of the presence or absence of nicotine, suggesting that other components in the e-cigarette aerosol are implicated in fetal growth restriction (Greene & Pisano, 2019).

DISCUSSION

This thesis provided a summary of possible health-risks associated with e-cigarette use on various physiological systems. E-cigarette exposure altered cardiovascular parameters such as blood pressure and heart rate, likely from the increase in sympathetic activity due to nicotine (Dimitriadis et al., 2022). Individuals who used e-cigarettes also had elevated arterial stiffness that is associated with coronary artery disease (Franzen et al., 2018). These impacts can increase the risk for cardiovascular events and diseases. When compared to traditional combustible cigarettes, e-cigarettes elicited similar responses in carotid plaque buildup and inflammatory responses that are risk factors for atherosclerosis (Sahota et al., 2021), (Kelesidis et al., 2020). Various flavorings and specific compounds such as propylene glycol and vegetable glycerin are components of e-cigarettes that altered cell viability in human aortic endothelial (Majid et al., 2023). These studies provide evidence that e-cigarette use could increase risk for developing atherosclerosis.

E-cigarette aerosol exposure to platelets elicited similar aggregation patterns when compared to traditional cigarette exposure (Hom et al., 2016) and e-cigarette exposure increased extracellular vesicles of platelet and endothelial origin (J. Zhang, 2022). E-cigarette use could increase the risk for platelet aggregation and thrombus formation. Exposure to e-cigarettes also affected cardiac electrophysiology and altered QT intervals and actional potentials that could result in cardiac arrythmias (Demir et al., 2020), (Abouassali et al., 2021). The cardiovascular responses elicited by e-cigarette

aersol show that e-cigarettes have the potential to harm the cardiovascular system in similar manners as traditional combustible cigarettes.

The pulmonary system also is affected in various way by e-cigarette exposure. E-cigarettes affect pulmonary airways by increasing flow resistance (Antoniewicz et al., 2019) and altering protein synthesis of bronchial epithelial cells (H.-R. Park et al., 2021). Glucose uptake and metabolism, as well as airway permeability, were negatively impacted by propylene glycol and vegetable glycerin compounds found in e-cigarette liquids (Woodall et al., 2020). Respiratory symptoms, such as wheezing, were more frequently reported in individuals who switched from traditional tobacco cigarette smoking to e-cigarette smoking in comparison to individuals who stopped using tobacco products all together (Li et al., 2019). Respiratory symptoms are signs of inflammation and narrowing of airways. The evidence of these studies suggest that e-cigarettes affect pulmonary airways and bronchial cells that could impact airway function in the lung.

E-cigarette aerosol containing nicotine impacted mucociliary function (Chung et al., 2019) and e-cigarette flavorings diacetyl and 2,3-pentanedione directly affected morphology of respiratory cilia (H.-R. Park et al., 2019). Bronchial epithelial cells and pulmonary macrophages when exposed to 2,3- pentanedione and multiple different e-cigarette flavorings produced reactive oxygen species (Morris et al., 2021). Exposure to e-cigarette aerosol showed downregulation of genes involved in immune signaling that could lead to disruption of immune responses (Wick et al., 2022). The impairment to pulmonary cilia and macrophages show that e-cigarette components such as nicotine and 2,3-pentanedione can impact lung function and alter immune responses.

E-cigarettes also affect the ability for the lung to fight against viral infections such as COVID-19 (McAlinden et al., 2021) and influenza A (Rebuli et al., 2021), (Agraval et al., 2023). These findings show that e-cigarette use does not come without risk to lung function and immune response.

Vitamin E acetate, a component of various e-cigarettes, has been linked to EVALI disease in individuals who use e-cigarettes by affecting surfactant composition and levels (J.-A. Park et al., 2022). Propylene glycol and vegetable glycerin, two components of e-cigarette liquid, exposure affected alveolar macrophages by altering their composition and disrupting their function (Madison et al., n.d.). The emergence of EVALI cases and pathology associated with EVALI gives reason to believe that e-cigarette use can induce lung injury and disease.

E-cigarette liquids and aerosols promoted EMT events in lung cancer cells by altering cell morphology and cell-to-cell adhesion (Zahedi et al., 2018). E-cigarette aerosol exposure also increased CYP-linked monooxygenases, which are known to be associated with tumor development (Canistro et al., 2017). Traditional combustible cigarette smoking has been known to increase risk of lung cancer and this evidence shows that e-cigarettes also have carcinogenic and metastatic potential in pulmonary cells.

Recent studies show that e-cigarettes, by exposing the central nervous system to nicotine, can affect the brain and spine. E-cigarettes are being used by youth and are therefore exposing developing brains to nicotine. In the brain, nicotine has been shown to alter nAchR expression in rats and enhance the reward response when exposed to

nicotine (Doura et al., 2008), (Xue et al., 2018). Animal and human studies have shown that nicotine exposure to young brains can increase the risk for other substance use later in life (Kota et al., 2018), (Kristjansson et al., 2015). This provides evidence that nicotine exposure in youth could have lasting effects that can affect the brain later in life. Nicotine exposure was associated with molecular changes that contribute to various mood disorders (Jobson et al., 2019). Individuals who used e-cigarettes experienced increased depression symptoms and suicidal ideations compared to individuals who did not use e-cigarettes (Leslie, 2020). The associations between nicotine and mental health in youth should raise caution to the potential risks of e-cigarette use on the developing brain.

In vitro studies showed e-cigarette exposure impacted the tight junctions involved in maintaining the blood-brain-barrier (Kadry et al., 2021). Nicotine exposure was found to worsen ischemic injury in the brain of mice by promoting oxidative stress and inflammatory responses (Bradford et al., 2011). Nicotine was also shown to impact spinal health by disrupting nucleus pulposus and annulus fibrosis cells (Iwahashi et al., 2002) as well as altering the extracellular matrix (Akmal et al., 2004). These studies show that e-cigarettes with nicotine have effects on various areas of cerebral physiology and spinal health.

E-cigarette aerosol effects the health of the oral cavity by inducing morphological changes of oral epithelial cells (Rouabhia et al., 2017) and inducing apoptosis (Sancilio et al., 2017). Components of e-cigarettes such as propylene glycol are also shown to alter the oral microbiome and make the oral cavity more susceptible to infections and disease (Pushalkar et al., 2020), (Chopyk et al., 2021). The imbalance of *Staphylococcus aureus*

from e-cigarette aerosol exposure could create an inflammatory environment in the oral cavity that promotes cancer development (Cátala-Valentín et al., 2022). Exposure to e-cigarette aerosol, with and without nicotine, was also associated with accumulation of double-stranded DNA breaks that could increase carcinogenic potential of cells (Yu et al., 2016). The evidence of damage to oral cells from e-cigarette exposure could lead to disruptions in oral health and increased risk of oral cancer.

E-cigarette aerosol was also shown to affect the gastrointestinal system by promoting an inflammatory environment and decreasing motility through the gastrointestinal tract (Debnath et al., n.d.), (Moshensky et al., n.d.). The colonic mucosa when exposed to e-cigarette aerosol decreased goblet cell population and altered mucosal folding as well as promoted inflammatory cell infiltration and increased permeability (Mohammed et al., 2021). These changes are indicative of diseased states and individuals should be cautioned on the use of e-cigarettes affecting the gastrointestinal tract. E-cigarette use was shown to exacerbate GERD disease (Pasricha & Kochar, 2021) and endogenously form nitrosonornicotine, which is a possible esophageal carcinogen (Bustamante et al., 2018). Both situations could increase the risk for esophageal cancer and show that e-cigarette use negatively impacts the gastrointestinal system.

E-cigarette exposure has negative implications for bladder health. Volatile organic compounds and known bladder carcinogens were present in the urine of individuals who use e-cigarettes (Lorkiewicz et al., 2018), (Molony et al., 2023). The liquids of e-cigarettes are made up of compounds that have bladder carcinogenic potential such as polyaromatic hydrocarbons, aromatic amines, and various aldehydes (Fuller et al., 2018).

Urothelial cells that were exposed to e-cigarette aerosol exhibited DNA damage and protein alterations that could potentiate malignancy (Molony et al., 2023), (Bandara et al., 2023). There is reason shown here that e-cigarettes can be harmful to bladder health and potentially be a carcinogen to bladder cancer.

Renal mechanisms were also shown to be affected by e-cigarette exposure. Albuminuria appeared higher in individuals who used e-cigarettes, suggesting vascular damage and filtration disruption (Podzolkov et al., 2020). The renin-angiotensin-aldosterone system was shown to be disrupted by e-cigarette exposure (Scharf et al., 2022). The cinnamaldehyde flavoring additive of e-cigarettes was shown to elicit inflammatory response in the kidney by activation of certain ion channels and promotion of reactive oxygen species (Hoi et al., 2020), (Wu et al., 2019). Byproducts of e-cigarette aerosol could promote inflammation and alter cell death pathways in renal tubular cells that ultimately could lead to kidney disease and kidney injury (Scharf et al., 2022). Negative impact to the renal system as shown could increase the risk for developing kidney disease and should not be excluded when evaluating potential harmful effects of e-cigarettes.

Although not as thoroughly studied, e-cigarette use is shown to affect reproductive health in both males and females. In female rodent models, e-cigarette use affected follicular growth and concentration as well as induced nuclear pyknosis in granulosa cells (Chen et al., 2022). Sperm production and steroidogenesis was also affected in male rats when exposed to e-cigarette aerosol (Vivarelli et al., 2019). E-cigarette exposure appears to harm reproductive mechanisms and hormone production in

both females and males. E-cigarette aerosol exposure also impaired implantation of mice embryos (Wetendorf et al., 2019) and altered vascular hemodynamics between maternal and fetal arteries (Orzabal et al., 2019). Based on the evidence provided in these studies, the public should be cautioned on the use of e-cigarettes in pregnancy and more studies are needed on the harm of e-cigarettes to reproductive health.

E-cigarettes are shown to affect many organ systems by nicotine exposure, flavoring additives, or other components of e-cigarette liquid. Various studies show e-cigarette use mimics physiological outcomes seen in traditional combustible cigarette use. Further investigation is needed to determine the specific components and compounds of e-cigarettes that promote these effects. Overall, evidence shows that e-cigarette use has many effects on differing physiological processes and systems and is not a healthy alternative to traditional combustible cigarette smoking. E-cigarette use should be done with caution and further studies are needed to determine long-term effects of e-cigarette use.

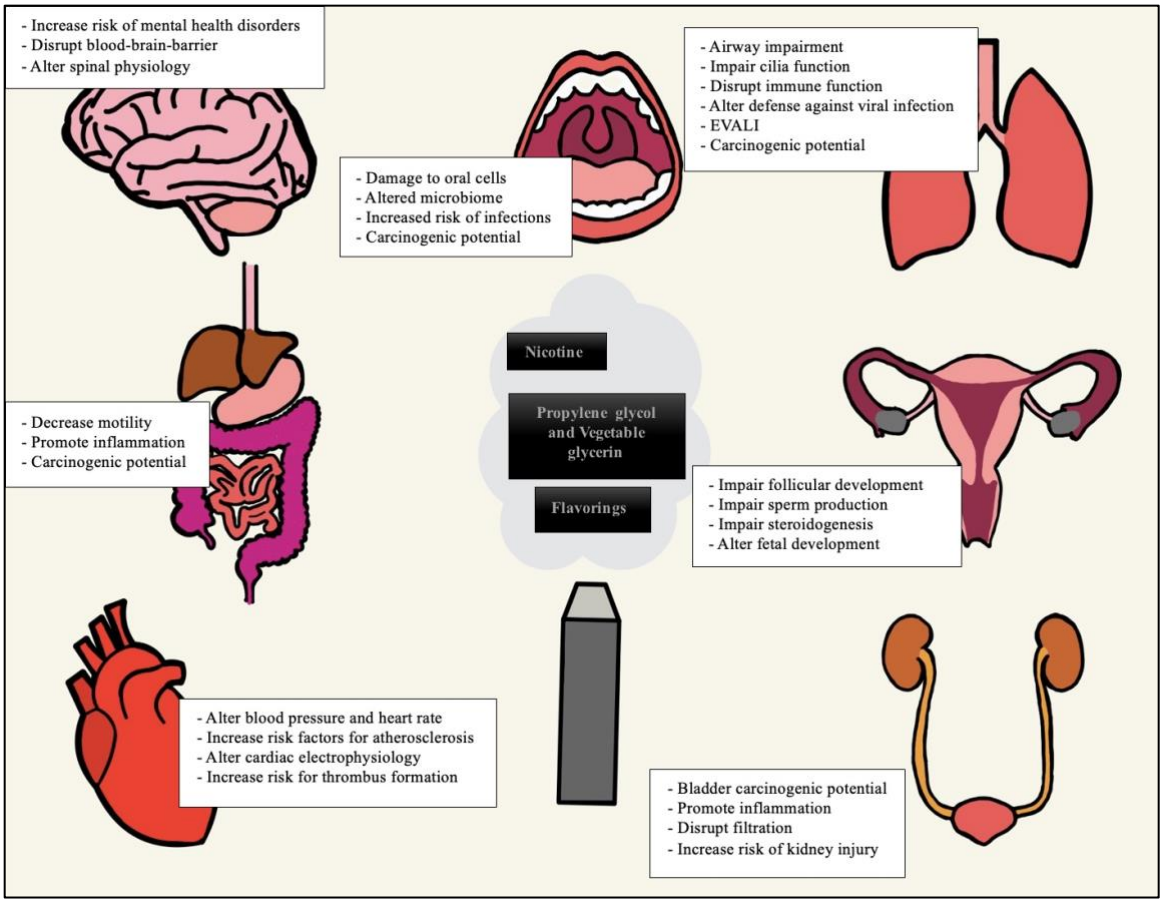

Figure 17: Summary of e-cigarette components effects found in these studies on various physiology including effects to the cardiovascular system, pulmonary system, gastrointestinal system, nervous system, urinary system, oral health, and reproductive health.

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