

REVIEW

Electronic Nicotine Delivery Systems and Cardiovascular/Cardiometabolic Health

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ABSTRACT: The use of electronic nicotine delivery systems, specifically electronic cigarettes (e-cig), has risen dramatically within the last few years; the demographic purchasing these devices is now predominantly adolescents that are not trying to quit the use of traditional combustible cigarettes, but rather are new users. The composition and appearance of these devices has changed since their first entry into the market in the late 2000s, but they remain composed of a battery and aerosol delivery system that is used to deliver breakdown products of propylene glycol/vegetable glycerin, flavorings, and potentially nicotine or other additives. Manufacturers have also adjusted the type of nicotine that is used within the liquid to make the inhalation more palatable for younger users, further affecting the number of youth who use these devices. Although the full spectrum of cardiovascular and cardiometabolic consequences of e-cig use is not fully appreciated, data is beginning to show that e-cigs can cause both short- and long-term issues on cardiac function, vascular integrity and cardiometabolic issues. This review will provide an overview of the cardiovascular, cardiometabolic, and vascular implications of the use of e-cigs, and the potential short- and long-term health effects. A robust understanding of these effects is important in order to inform policy makers on the dangers of e-cigs use.

Key Words: aerosols ■ nicotine ■ propylene glycol ■ tobacco products ■ vaping

Smoking is the leading cause of preventable disease and death in the United States^{1,2} and has been responsible for ≈20 million deaths in the United States over the past 60 years.³ Electronic nicotine delivery systems (ENDS), including electronic cigarettes (e-cig), were invented as a healthier alternative to traditional cigarettes and were marketed as a smoking cessation aid. E-cigs are considered a modern technological replacement for traditional (combustible) tobacco cigarettes and were introduced for commercial use in 2004. The term e-cig includes a range of battery-powered devices that use a variety of aerosol delivery methods that have changed over time, as summarized in Figure 1. E-cig liquids or cartridges usually contain a mixture of propylene glycol (PG) and vegetable glycerin (VG) in combination (PG/VG), flavorants, and various nicotine concentrations, typically ranging from 1.6 to 19 mg/cartridge^{4,7} or potentially other additives. In August 2016, a World Health Organization report concluded that it was not currently possible to measure the relative risk of

ENDS in comparison to combustible tobacco products due to the large variances in e-cig ingredients and device elements.⁸ Therefore, it is not known to what extent the use of these products is safer, in terms of relative or absolute risk reduction, when compared with traditional cigarette smoking.

ENDS use is increasing rapidly among youth (age 18–24 years old) and adolescents (grades 6–12),^{9–11} with current e-cig use doubling among middle (grade 6–8) and high school (grade 9–12) students from 3.3% to 6.8%.¹² Current e-cig use is higher amongst adult occasional smokers and heavy smokers (20 cigarettes/d) than among daily smokers and less frequent smokers.¹³ About 6% of all US adults and 21% of US adult smokers have tried e-cigs¹⁴ and data from the United States, United Kingdom, Canada, and Australia shows that nearly half of current smokers and former smokers are aware of e-cigs. As expected, awareness is higher in countries where these products are legal (73% in the United States compared with 20% in Australia).¹³ Furthermore, >75%

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Nonstandard Abbreviations and Acronyms	
ALDH2	aldehyde dehydrogenase 2
ANS	autonomic nervous system
BP	blood pressure
CVD	cardiovascular disease
E-cig	electronic cigarettes
ENDS	electronic nicotine delivery systems
HR	heart rate
HRV	HR variability
LV	left ventricle
PG	propylene glycol
ROS	reactive oxygen species
VG	vegetable glycerin
WAT	white adipose tissue

of smokers and former smokers believe that e-cigs are less harmful than traditional cigarettes.

Compared with traditional combustible cigarettes that burn continuously during use, e-cigs release aerosols only upon inhalation. E-cig particles are small enough to penetrate the alveoli, allowing for nicotine absorption¹⁵ and deeper penetration into the lungs and systemic circulation.¹⁶ E-cig vapor may induce oxidative stress in lung endothelial cells¹⁷ and lead to serious inflammatory pulmonary diseases.¹⁸ In addition, e-cig aerosols may also increase the risk of acute coronary syndrome, particularly in patients with preexisting risk factors.¹⁹ Since e-cig ingredients, route of administration, and methods of use differ from those of conventional cigarettes, it is likely that their cardiopulmonary effects may also differ. Although there is evidence that carbon-based cigarette particles from conventional cigarettes are harmful when inhaled, little is known about the effects of inhaling liquid-based particles from e-cigs, as well as the health effects of heating these components. The process of heating the e-cig liquid may elicit chemical reactions, potentially leading to chemical changes differing from the original e-cig liquid, unintentionally leading to health effects different from those caused by cigarette smoke. The use of e-cigs has been shown to cause dysregulation of the autonomic nervous system (ANS) homeostasis via the stimulant effects of nicotine, and it has also been suggested that there may be a risk of cardiomyopathy.²⁰ Short-term e-cig usage also demonstrated effects on blood vessel tone, heart rate (HR), HRV (HR variability), and rhythm.²¹ A recent study showed that e-cig smoking affects arterial function in a similar manner to smoking traditional cigarettes in both nonsmokers and smokers.²² Therefore, this review intends to elucidate the current understanding of adverse cardiovascular and metabolic consequences of e-cig use.

EFFECTS ON MYOCARDIAL HEALTH

The number of clinical trials and prospective studies conducted to investigate the effects of e-cig use on myocardial function and structure is limited. However, data suggest that e-cig exposure and sequelae may combine as essential drivers of cardiovascular disease (CVD) in a similar manner by which traditional tobacco smoking and hypertension are essential drivers of CVD. E-cig-related sequelae include increased sympathetic activity and oxidative stress, altered metabolism, as well as increased vascular stiffness and endothelial dysfunction (discussed below). The combination of complications influences the development of CVD via aneurysm formation, atherosclerosis, and free radical formation.

A cross-sectional analysis has shown that daily e-cig use was independently associated with higher odds of myocardial infarction (odds ratio, 1.79 [95% CI, 1.20–2.66]), as was daily conventional cigarette smoking (odds ratio, 2.29 [95% CI, 2.29–3.24]).²³ This analysis further highlighted how underlying risk factors for CVD such as age, diabetes, hypertension, hypercholesterolemia, and chronic pulmonary, and vascular changes can influence alterations of the myocardium in e-cig users similarly to conventional cigarette smoking, albeit with decreased risk in this study. Acute clinical studies, using echocardiography, reported no changes in myocardial function in adult smokers using e-cigs²⁴; however, that is to be expected as short-term studies are not likely to detect cardiac remodeling and current cigarette smoking use may conceal changes induced by e-cigs. Animal studies further support chronic e-cig use as a potential driver for CVD with acute e-cig use yielding mixed results. Interestingly, animal models have provided insights into how the presence of comorbidities, e-cig flavorings, and other forms of e-cig exposure factor into structural and functional changes in cardiac tissues that serve as a pathway for CVD development.

Acute exposures (2 weeks) to e-cigs showed cardiac function changes via echocardiography.²⁵ This study showed that e-cig exposure for 3 hours a day for 14 days, similar to a casual-use model,²⁶ found no significant effects on ejection fraction, but did elicit significantly increased angiogenesis in mouse heart tissue and significantly increased collagen content, but not tissue fibrosis.²⁵ This study implies that acute e-cig exposure had no significant effect on contractile function or fibrosis but did induce cardiac angiogenesis. Although it is known that traditional combustible cigarettes inhibit angiogenesis, nicotine alone stimulates angiogenesis, and increased angiogenesis may be beneficial in instances of myocardial infarction (MI)^{25,27}; however, the role of increased angiogenesis following e-cig exposure necessitates further study to fully understand risks and benefits of increased angiogenesis.

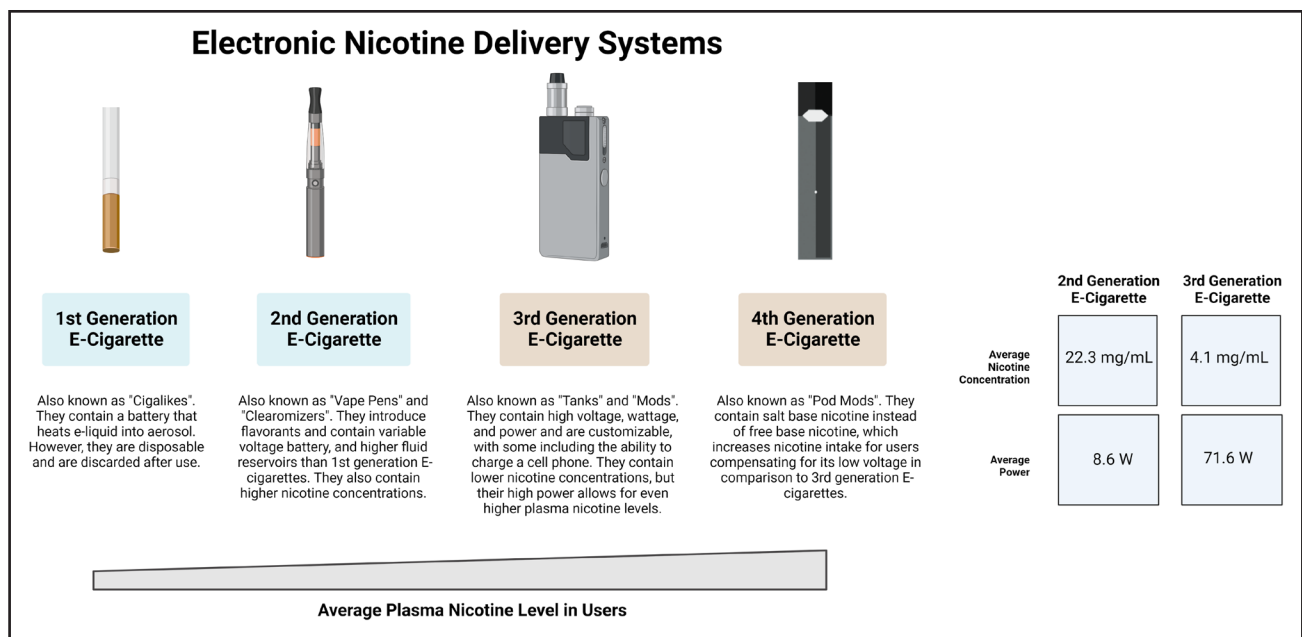


Figure 1. Electronic nicotine delivery systems.

First generation electronic cigarettes (e-cig) or cigalike, as well as representative second to fourth generations of e-cigs with design characteristics.^{4–6} Created with BioRender.com.

In contrast, a chronic exposure (ie, 12-weeks) study in mice found that e-cig use concomitant with a high-fat diet resulted in decreased left ventricle (LV) fractional shortening, ejection fraction, and velocity of circumferential fiber shortening in mice exposed to nicotine-containing e-cig, compared with no nicotine and saline-exposed mice.²⁸ High-fat diet mice exposed to nicotine-containing e-cigs also exhibited LV structural abnormalities such as lipid accumulation (ventricular steatosis), myofibrillar derangement and destruction, and mitochondrial hypertrophy on microscopy.²⁸ These structural changes were likely due to the accompanied increases in oxidative stress, plasma free fatty acids, cardiomyocyte apoptosis, and inactivation of AMP-activated protein kinase and activation of its downstream target, acetyl-coenzyme A-carboxylase.

Similarly, another 12-week study in apolipoprotein-E knockout (ApoE^{-/-}) mice (used as a model of lipid metabolism dysregulation, atherosclerosis, and obesity) demonstrated that nicotine-containing e-cig exposure resulted in decreased LV fractional shortening and ejection fraction compared with nicotine-free and saline-exposed ApoE^{-/-} mice.²⁹ ApoE^{-/-} mice also showed changes in ventricular transcriptomic analysis of genes related to metabolism, circadian rhythm, and inflammation as well as increased oxidative stress and mitochondrial DNA mutations. Electron microscopy also showed ultrastructural myocyte abnormalities potentially indicative of cardiomyopathy, or the development of cardiomyopathy.²⁹ These myocyte abnormalities included nuclear abnormalities such as shrunken nuclei, chromatin condensation and fragmentation, and nuclear malformation with convoluted nuclear membranes as well as cytoplasmic

abnormalities such as myofibrillar derangement, thinning, and destruction alongside intramyocardial lipid accumulation and mitophagy. Chronic combustible tobacco smoking has previously been associated with the development of cardiomyopathy³⁰ with Gvozdiak et al³¹ coining the term smoker's cardiomyopathy in 1987. More recent data has shown that cigarette smoke exposure resulted in increased LV end diastolic and systolic diameters,³² which are associated with the development of cardiomyopathy.³³ The extent to which e-cigs may induce these structural changes requires more investigation. However, chronic studies using ApoE^{-/-} mice over the span of 6 months found that the cardiovascular effects of e-cig exposure were reduced relative to combustible tobacco smoking.³⁴ These findings indicate that e-cig use alongside other comorbidities (ie, obesity) may induce or potentiate e-cig-associated CV adverse effects.

Secondary forms of e-cig exposures exist such as perinatal nicotine exposure, which has recently been shown to reduce viability of human embryonic stem cells and induce minor changes in cell-type distribution upon nicotine-containing e-cig exposure when analyzed via single-cell RNA sequencing.³⁵ Human embryonic stem cell exposure to nicotine resulted in disrupted intracellular Ca²⁺ handling, as characterized by increased gene expression of *HMGB1* (high motility group box 1) and *TLR4* in human embryonic stem cell-derived myocytes. Increased expression of HMGB1 proteins resulted in impaired cardiac excitation-contraction via sarcoplasmic reticulum leakage of Ca²⁺ through TLR4-reactive oxygen species (ROS) signaling, increasing the risk for Ca²⁺-associated arrhythmias.³⁵ Further, perinatal ENDS

exposure, and other forms of nicotine exposure, may cause gender-dependent increases in cardiovascular adverse events. Male offspring from pregnant rats exposed to nicotine via subcutaneous osmotic minipumps (at concentrations representative of ENDS and traditional tobacco smoking use) from gestational day 4 through postnatal day 10 developed cardiac dysfunction in adulthood³⁶; this was not seen in female offspring. Male offspring displayed enhanced I/R-induced cardiac dysfunction and infarction associated with the overexpression of miR-181a in LV tissues, which was not seen in female offspring. The downstream effects of miR-181a overexpression altered target genes, such as enhanced cardiac angiotensin receptor expression, upregulated TGF- β (transforming growth factor beta protein)/Smads proteins, upregulated autophagy-related protein, and decreased cardiac long non-coding RNA H19 levels.³⁶ The disruption of these target genes has been shown to alter regulation of cardiac remodeling, hypertrophy, and interstitial fibrosis, as well as increased expression of ischemia-sensitive signaling proteins and facilitation of cell death.^{36–40}

Sex-dependent differences have also been observed in adolescent mice following 3-month e-cig exposure, with male mice exhibiting greater reductions of LV fractional shortening.²⁶ Adolescent mice exposed to e-cigs had reduced end-systolic elastance independent of nicotine concentration and reduced preload-recruitable stroke work in e-cigs with nicotine, indicative of reduced contractile capacity.²⁶ Male adolescent mice following 3 months of e-cig use showed increased perivascular fibrosis in e-cig liquid without nicotine, further supported by increased, although nonsignificant, type I collagen.²⁶ This same study also showed that gene expression of *Col1a1* and *Col3a1* was increased following e-cig use with nicotine at 3 weeks, but was reversed by 3 months, suggesting that perhaps nicotine may play a protective role.

Interestingly, the female adolescent mice exposed in the same study did not show alterations in cardiac function and serum biomarker profiles only showed elevated IFN- γ (interferon- γ) levels, whereas males had increased IL-18 (interleukin-18), CCL2 (chemokine C-C motif ligand 2), macrophage inflammatory protein 1 β , stem cell factor, and vascular endothelial growth factor A levels.²⁶ Although e-cig exposure concentrations of nicotine were consistent between male and female mice, the 3-hydroxycotinine to cotinine ratio was increased in female mice.²⁶ The 3-hydroxycotinine /cotinine ratio is a measure of cytochrome P450 2A5 (CYP2A5) activity and is responsible for nicotine metabolism. These elevations suggest that female mice metabolize nicotine at a higher rate than male mice and were cardioprotective of the deleterious effects of nicotine observed in the male counterparts potentially due to upregulation of CYP2A5 activity or expression. Further research on sex-based

differences in e-cig nicotine metabolism are required to better understand how health outcomes may be influenced by sex.

Direct changes in cardiac tissues following conventional cigarette smoking have been well documented, but research on e-cig effects concerning cardiac tissue changes remain sparse. Mouse models using subcutaneous nicotine and angiotensin II pump infusions for 4 weeks demonstrated that nicotine augmented cardiac remodeling via increased MMP-2 (matrix metalloproteinase 2) activity and resulted in cardiac growth and increased aortic wall thickness, indicating a possible cardiotoxic interaction between nicotine and systemic hypertension.⁴¹ Research on zebrafish embryos in nontissue culture plates containing e-cig extracts investigated the effects of e-cigs on cardiac structure changes.⁴² It was discovered that exposure to both cigarettes and e-cigs are associated with pericardial edema, reduced heart function, and cardiac fibrosis. E-cig exposure in rats revealed increased expression of TGF- β in cardiac tissue.⁴³ TGF- β is responsible for several molecular changes that promote fibrotic changes in the heart, including activation of collagen synthesis, up-regulation of CTGF (connective tissue growth factor) expression, and increased matrix protein production,⁴⁴ leading to increased cardiac fibrosis. Although specific changes in the pericardium have not been thoroughly investigated, changes in TGF- β expression are a reliable indicator of cardiovascular pathology such as arterial hypertension, atherosclerosis, and coronary artery disease.

Furthermore, while more research is needed to understand the mechanisms behind these adverse cardiac structural changes, this same study showed reductions in transcriptional levels of cardiac myosin light chain 2 (*cmlc2*; the zebrafish orthologue of *MYL7*), cardiac muscle troponin T (*tnnt2*), and gap junction protein connexin-43 (*cx43*), which may be of importance for understanding how e-cigs influence cardiac contractile function, as these proteins are of great importance in maintaining cardiac function.⁴² However, more research is needed concerning structural changes.

Similarly, in vitro e-cig aerosol exposure in human embryonic stem cells showed reduced expression of sarcomere genes *MLC2v* and *MYL6* that regulate myosin light chains in a similar fashion to the *cmlc2* gene in zebrafish.⁴² These studies collectively provide evidence that important cardiac regulatory genes are downregulated after e-cig exposure, suggesting that e-cigs have the potential to severely reduce both sarcomere assembly and cardiac contractility contributing to CVD, such as hypertrophic cardiomyopathy.⁴⁵ More studies are needed to bridge the connection between e-cigs and CVD, as well as the mechanisms that occur through the numerous transcriptional changes that have been proven.

EFFECTS ON CARDIOMETABOLIC HEALTH, ADIPOSE TISSUE, AND METABOLIC ACTIVITY

Tobacco smoking and nicotine use are known to impact metabolic health and adipose tissue function.⁴⁶ Nicotine suppresses appetite, and cessation of tobacco use is associated with increased body weight. Nicotine affects metabolism in various tissues, including adipose tissue.⁴⁷ Nicotine consumption decreases lipogenesis and increases adipose tissue lipolysis, increasing the risk of developing type 2 diabetes.⁴⁶ However, the effects of e-cigs on metabolic function and adipose tissue have not been thoroughly investigated.

There are conflicting results on body weight and glucose tolerance following e-cig use in rodents. Multiple studies have shown that 6 to 12 weeks of varying amounts of e-cig exposure reduced body weight and improved glucose tolerance in male mice fed either a control chow⁴⁸ or high-fat diet.^{28,48} These effects are mediated in part by adaptations to the liver, including increased expression of GLUT2 and GLUT4 (glucose transporters), fatty acid synthesis,⁴⁸ and cytokines (IL-10).⁴⁹ In contrast, an earlier study found that 12 weeks of e-cig exposure did not affect body weight or glucose tolerance in male chow-fed mice.⁵⁰ These studies all used different exposure chambers, sources of e-cig vapors, duration of exposure, and strains of mice, which could contribute to the different experimental outcomes; therefore, future studies should take each of these factors into consideration. The role of diet and adiposity in interacting or compounding e-cig exposure has not been fully investigated and is likely a contributing factor to effects on glucose metabolism. Finally, all previous studies were performed in male mice; investigating the metabolic effect of e-cigs in female mice will be important to determine sex-specific differences.

Recent studies have investigated the effects of e-cigs on white adipose tissue (WAT). Acute exposure to e-cigs in male and female mice decreased their respiratory exchange ratio, suggesting acute e-cigs increase whole-body lipid metabolism.⁵¹ Only 6 weeks of e-cig exposure has a direct effect on WAT. Expression of numerous inflammatory chemokines were increased in the serum of mice and gene expression of these chemokines were increased in visceral WAT.⁴⁸ The investigators hypothesized that e-cigs could increase whole-body inflammation by activating inflammatory resident macrophages in adipose tissue, but this has not been thoroughly investigated. Interestingly, this increase in chemokines was observed in mice exposed to nicotine-free e-cigs, indicating that the inflammatory effects of e-cig vapor exposure may not be directly caused by nicotine consumption, but by the other

substances produced by the e-cig vaporization. In addition to the more commonly studied flavorants and PG/VG base, e-cigs may contain additional chemicals that are not well characterized and are triggering an inflammatory response. Future investigations may investigate which chemicals of the e-cig vapor are responsible for the increase in inflammation. In vitro studies using 3T3-L1 cells, an adipocyte cell line, showed that while tobacco smoke decreased cell viability and impaired adipocyte differentiation, e-cig exposure does not directly impact cell proliferation and differentiation into mature adipocytes, which contrasts with what is seen following exposure to cigarette smoke.⁵² Overall, these limited data would suggest that e-cig exposure does not directly affect WAT,⁵² but instead alters the resident immune cells within WAT.⁴⁹ Further research is required to determine what immune cells are affected, if e-cig exposure affects WAT function in vivo, and the underlying mechanisms for these adaptations. It is essential to determine if changes are due to intrinsic changes in adipocytes or if e-cig exposure influence adipose tissue to immune cell crosstalk.

In humans, recent studies showed that chronic e-cig exposure has a direct impact on whole-body metabolism. The effects of e-cig use on glucose metabolism in humans have shown conflicting results.^{50,53,54} E-cig use is associated with an increased odds ratio of self-reported prediabetes,⁵³ and higher fasting glucose,⁵⁴ while data analysis from the annual cross-sectional National Health and Nutrition Examination Survey showed no effect of sole e-cig consumption in glucose tolerance and HOMA-IR (homeostatic model assessment of insulin resistance).⁵⁰ It is unclear why there are discrepancies between these observational studies; however, only 0.88% (30/3415) of the subjects from the National Health and Nutrition Examination Survey cohort were e-cig users. E-cig usage impacts the lipid profile of humans^{54–56} and is also correlated with decreased serum levels of ω -6 fatty acids, including linoleic and arachidonic acid, as well as their bioactive metabolites, the oxylipins 9-hydroxyoctadecadienoic acid and 13-hydroxyoctadecadienoic acid.⁵⁵ However, e-cig use does not affect total levels of triglycerides, LDL (low-density lipoprotein), and HDL (high-density lipoprotein).^{54,56} These initial data suggest that e-cig use affects the metabolic profile of humans by altering the levels of different free fatty acids and their metabolites. Altered levels of oxylipins suggest that WAT function may be affected by e-cig exposure in humans, since WAT is the main source of fatty acid and lipid storage and production. Future studies are required to determine if e-cig usage alters whole-body metabolic function, as well as to directly study their effect on WAT function. The effects of e-cigs on adipose tissue and CV dysfunction are summarized in Figure 2.

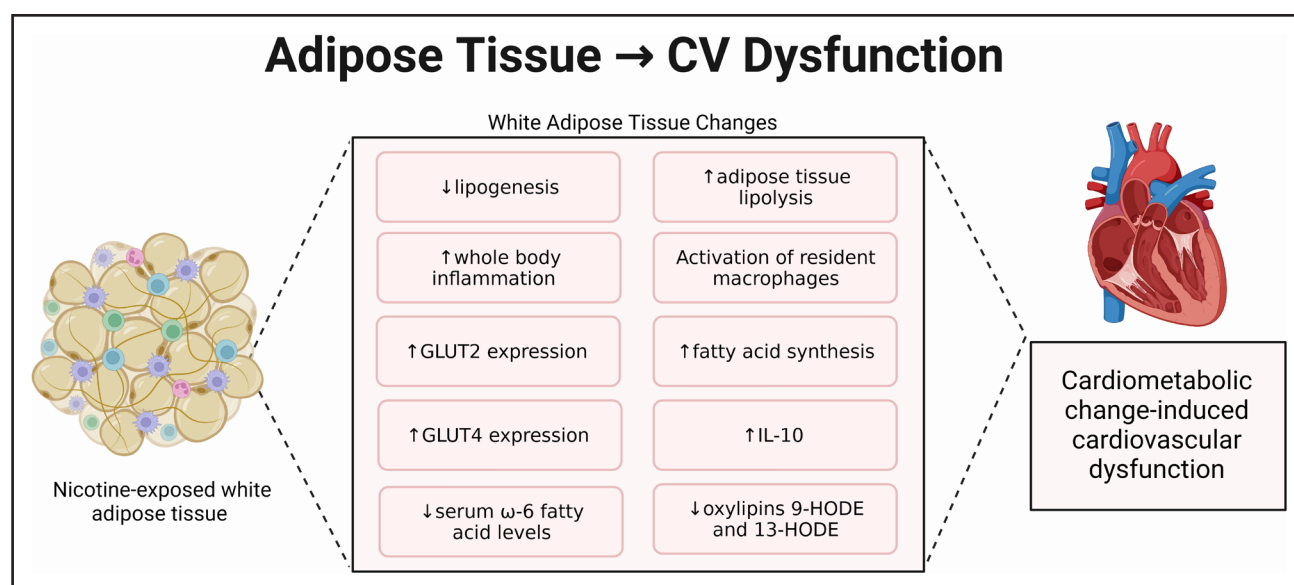


Figure 2. Electronic cigarettes (e-cig)-induced adipose tissue changes and cardiovascular (CV) dysfunction.

Nicotine exposure leads to changes in white adipose tissue (WAT). These changes include decreased lipogenesis, serum levels of ω-6 fatty acids, and oxylipins 9- and 13- hydroxyoctadecadienoic acid (HODE), as well as increased adipose tissue lipolysis, fatty acid synthesis, whole body inflammation, GLUT2 and GLUT4 (glucose transporters) expression, IL-10 levels, and activation of resident macrophages. Created with BioRender.com.

EFFECTS ON VASCULAR HEALTH

Due to the chemical makeup of inhaled toxins in the e-cig aerosols, endothelial dysfunction is a common contributor to the development of CVD within the heart and corresponding vessels. E-cig users display diminished flow-mediated vasodilation, often resulting in endothelial dysfunction even after a single use of e-cigs.⁵⁷ Studies also demonstrate that NO bioavailability plays a significant role in vascular health, and reductions in NO bioavailability can induce the release of inflammatory factors, leading to endothelial dysfunction and dysregulation of blood pressure (BP) in in vivo randomized control trials.^{58,59} E-cig liquid (e-liquid) composition and flavorings also elicit adverse effects on endothelial membranes.⁶⁰ Apple/mint and tobacco flavored e-cigs induce cytotoxic effects on endothelial cells that can eventually lead to cell death and heart disease.⁶⁰ Similarly, a recent study that used human induced pluripotent stem cell-derived endothelial cells found that cinnamon-flavored e-cigs exhibited potent cytotoxicity, leading to decreased cell viability, increased ROS and activation of ROS stress-related pathways, caspase 3/7 activity, and LDL uptake, and impaired tube formation and migration following acute e-cig exposure.⁶¹ These findings suggest that acute exposure to flavored e-cigs may induce or exacerbate endothelial dysfunction. E-cigs have also been demonstrated to increase arterial stiffness following acute use of e-cigs.⁶² Arterial stiffness and endothelial dysfunction often occur in concert with one another.⁶³ Using pulse wave technology, one study of 70 current adult smokers showed that both nicotine-free and nicotine-containing

e-cig use resulted in increased arterial stiffness, but relatively less than conventional cigarettes.⁶³

Exposure to e-cig vapor has been shown to increase the risk of thrombosis with short-term usage, with platelet dysfunction being a common explanation for this increase. The brand JUUL e-cigs have been used specifically to test the potential effects of e-cigs on thrombosis. The results, analyzing blood taken directly from the heart, showed decreased duration in occlusion time within mice exposed to JUUL, indicating thrombosis.⁶⁴ ADP- or thrombin-induced (1 μmol/L and 0.1 U/mL, respectively) platelet aggregation was also significantly increased when compared with controls. Mice exposed to JUUL e-cigs had enhanced ADP- and thrombin-induced P-selection expression and increased agonist-mediated activation of GPIIb/IIIa (glycoprotein IIb/IIIa). These factors both play a large role in platelet adhesion and coagulation,⁶⁵ negatively impacting the cardiovascular system. When these results were compared with the levels of GPIIb/IIIa in other e-cigs, the findings demonstrated that JUUL had greater detrimental effects on ADP-triggered aggregation.⁶⁴ Although it is not entirely clear, it is conceivable that endothelial dysfunction and oxidative stress likely contribute to this increase in platelet aggregation.⁶⁶

Oxidative stress may play a role in endothelial dysfunction and thrombosis.⁶⁶ A study showed decreased prostaglandin production in the vascular endothelium as well as microvascular endothelial dysfunction following exposure to e-cigs containing nicotine.⁶⁶ This study concludes that endothelial dysfunction could be induced by a NO-independent mechanism or plasma myeloperoxidase,

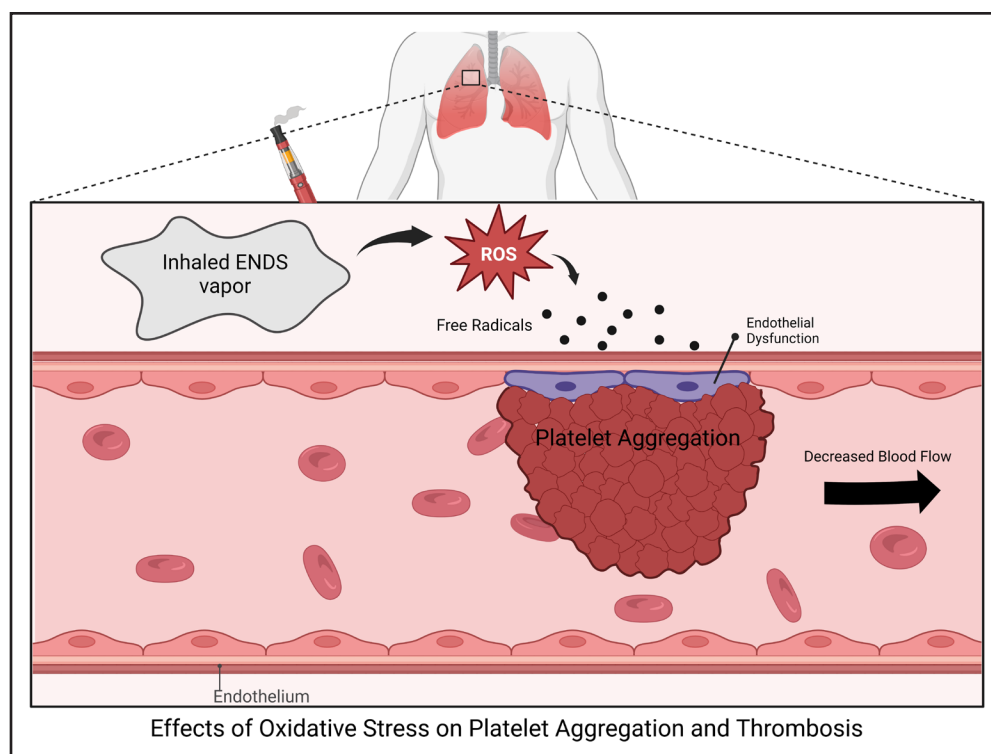


Figure 3. Effects of electronic cigarettes (e-cig)-induced oxidative stress on platelet aggregation and thrombosis.

Inhaled e-cig vapor increases levels of reactive oxygen species (ROS). This increase in ROS affects the buffering capacity of antioxidants, inducing oxidative stress.⁶⁷ More specifically, within endothelial cells, NO is the primary antioxidant.⁶⁷ When NO reacts with superoxide anion, free radicals form to disrupt proteins within the endothelium,⁶⁷ leading to endothelial dysfunction and platelet aggregation, further predisposing individuals to thrombosis, and decreased blood flow beyond the site of platelet aggregation. ENDS indicates electronic nicotine delivery systems. Created with BioRender.com.

affecting heart rhythm, HR, and BP.⁶⁶ Interestingly, e-cig-induced endothelial dysfunction could be rescued with antioxidants which increased flow-mediated vasodilation, indicating that issues with oxidative stress could be an underlying cause for the condition.⁶⁶ Similarly, acute e-cig usage is associated with other serum markers of oxidative stress, such as increased 8-isoprostanes and NOX2 (NADPH oxidase) levels, suggesting that NO may be an underlying mechanism for e-cig-induced endothelial dysfunction, affecting HR, BP, and arterial pressure.⁵⁷ The effects of e-cig-induced oxidative stress and subsequent platelet aggregation and thrombosis are summarized in Figure 3.

OXIDATIVE STRESS AND POTENTIAL BIOMARKERS

Although some research has shown that ENDS products induce fewer mediators of oxidative stress than traditional cigarettes, e-cig use may still produce some absolute risk for oxidative stress-induced adverse health effects. E-cigs contain a variety of components that contribute to their diversity across thousands of different e-cig designs, possessing many different cofactors that contribute to the degree of oxidative stress produced by e-cigs. These factors include the following:

vapor volume, voltage setting, type of heating system, nicotine concentration, type of atomizers, battery size, e-liquid composition, and the type of refillable flavoring being used.⁶⁸ The effects of e-cig-induced oxidative stress, and sequential inflammatory markers, may impact cardiometabolic and cardiovascular function. The individual effects of each of these components remains understudied. However, many studies have shown that the type of e-liquid used in e-cigs has several detrimental effects on oxidative stress, such as the release of ROS. One study investigated the impact that e-cig flavorings have on ROS levels from analyzing ROS handling proteins. They found that 3 flavors (tobacco, apple/mint, and vanilla) induced cytotoxic behavior alongside varied nicotine concentrations (2.5%, 2.5%, and 5%, respectively).⁶⁰ Vanilla and tobacco flavored e-cig liquids showed a greater overall increase in ROS levels than apple/mint flavored e-liquid. Vanilla and apple/mint e-cig flavorings, both containing nicotine concentrations of 12 mg/mL, resulted in increased glutathione levels by 110% and 107%, respectively.⁶⁰ Glutathione is an antioxidant that is released in response to oxidative stress⁶⁹ and increased glutathione levels following e-cig exposure suggest that e-cigs may elicit oxidative stress. A similar study also displayed increased ROS levels following e-cig exposure containing 20 different flavors.⁷⁰

However, the increases in ROS levels varied by e-cig flavorant. The effects of different e-cig flavorants on ROS levels from this study are summarized in Table 1.

Thiobarbituric acid reactive substance functions as another biomarker of oxidative stress, as it is involved in lipid peroxidation.⁶⁰ Tobacco and vanilla flavored e-cigs with nicotine concentrations of 12 mg/mL increased thiobarbituric acid reactive substance levels by 38% and 71%, respectively, while tobacco flavored e-cigs without nicotine increased thiobarbituric acid reactive substance levels by 40%, when compared with controls.⁶⁰ These results indicate that flavored e-cigs elicit oxidative stress mediator release and increased ROS handling proteins with and without nicotine. Although many studies have shown that e-cig flavorings are associated with an increase in ROS, some studies have shown that e-cig flavorings may also provide some protective benefits. Apple/mint flavored e-cigs (12 mg/mL nicotine concentration) decreased thiobarbituric acid reactive substance levels by 22%.⁶⁰ The addition of the chemical ethyl vanillin to e-cig flavorings has also been shown to decrease ROS levels compared with baseline data.⁷⁰ Still, the overall usage of e-cigs has been shown to induce oxidative stress.

Clinical trials have shown that following e-cig exposure, plasma levels of myeloperoxidase were increased.⁶⁶

Table 1. E-cig Flavorant-Induced Increases in ROS Levels

Commercial E-cig flavorants	ROS handling enzyme increases
Vanilla custard	122%
Cotton candy	114%
Butterscotch	106%
Subtle cinnamon	105%
Rainbow candy	102%
Real watermelon	94%
Ripe strawberry	80%
Bubblegum	76%
Dark raz	74%
Raspberry	72%
Tootie fruitie cereal	80%
Pear	70%
Real honey	67%
Kiwi	66%
Sweet tea	65%
Root beer	61%
Coffee	58%
Grape	56%
Blue raz	49%
Lemon	46%

The effects of 49 different commercially available e-cig flavorants on lung cells were captured and analyzed for their effects on free radical generation via measurements of secondary lipid peroxidation of TBARS assay.⁷⁰ Of the 49 e-cig flavorants investigated, 20 e-cig flavorants showed significant increases in ROS handling enzymes, expressed as % increase in TBARS levels. E-cig indicates electronic cigarettes; ROS, reactive oxygen species; and TBARS, thiobarbituric acid reactive substance.

Myeloperoxidase is an enzyme normally released to induce oxidative stress and inflammation,⁶⁶ suggesting that inhaled e-cigs containing nicotine and flavorings activate leukocytes to promote the toxic release of myeloperoxidase. Additionally, NOX2, an enzyme involved in the quenching of oxidative stress,⁶⁶ was increased following e-cig exposure, as were hydrogen peroxide (H₂O₂) and 8-iso- PGF2α.⁵⁸ However, levels of vitamin E and activity of enzymes responsible for H₂O₂ breakdown were reduced, demonstrating that e-cig use increased oxidative stress while also depleting antioxidant levels.⁵⁸ It is hypothesized that reduction in most antioxidant proteins is due to oxidative DNA damage caused by e-cig use.⁷¹ More research is still needed to identify which components of e-cigs are responsible for inducing oxidative stress and depleting antioxidant levels. However, it remains apparent that these devices pose a risk for inducing oxidative stress, predisposing for negative impacts on cardiometabolic health and cardiovascular function.

EFFECTS ON NEUROLOGICAL ACTIVATION OF THE CARDIOVASCULAR SYSTEM

The ANS is responsible for the involuntary physiological processes that maintain HR and BP among other autonomic functions. The ANS is comprised of the parasympathetic (parasympathetic or vagal) and the sympathetic nervous systems, which are largely responsible for maintaining cardiovascular homeostasis. Extensive research has shown that e-cig use, with and without nicotine, alters the balance between parasympathetic and sympathetic, resulting in skewed sympathetic overactivation, posing a risk for HR and BP elevations as well as arrhythmias. Extensive clinical research regarding the effects of vaping on HR have yielded mixed results, with several clinical trials showing that acute e-cig use results in significantly elevated HR,^{58,62,66,72–74} while other clinical trials and exposure methods showed no significant changes following acute e-cig use.^{62,63,73} Trials that demonstrated significantly increased HR following e-cig use showed that HR increases exist almost independently of nicotine concentration, with HR elevations present at 0,⁶⁶ 1.5,⁶² 3,⁶⁶ 18,⁷⁴ and 24 mg/mL⁷² of nicotine. However, the increases in HR following nicotine-free e-cig use were short lived.⁶⁶ Further, these same trials showed elevations in systolic BP^{58,62,66,72,73} and diastolic BP.^{58,66,73} Other studies focusing on e-cig effects on HRV and sympathetic tone found that acute e-cig exposure resulted in decreased HRV⁷⁵ and increased sympathetic tone⁷⁶; both studies further suggest an acute shift towards sympathetic predominance and blunting of vagal activity. Animal models support these epidemiological findings and further suggest that nicotine and e-cig liquid alone may not be the only constituents that alter sympathetic activation of the cardiovascular system and that

protonation of nicotine and e-cig flavorings may impact ANS homeostasis.

The effects of e-cig devices on ANS function have more recently been compared with oral nicotine packs (nicotine lozenges) and traditional tobacco smoke in a small clinical trial ($n=17$), with observed alterations in HRV as well as increased biomarkers of physiological stress (ie, salivary cortisol and serum catecholamines).⁷⁷ It was shown that HRV following nicotine lozenge use changed the least when compared with both traditional tobacco and e-cig, suggesting that hydrophilicity of nicotine salts may play a role in the variable ANS reactivity. E-cigs, such as JUUL, often contain protonated nicotine salts, which are more hydrophilic and closer to physiological pH; this allows for slower binding to nicotinic receptors and slower diffusion than unprotonated nicotine.^{77,78} HRV remained unchanged in participants during the first hour of e-cig exposure; however, after 1.5 hours, HRV decreased to nearly 20% of the value of smoking traditional cigarettes, and 50% at 2 hours.⁷⁷ Traditional tobacco heating systems showed HRV decreased by 80% compared with baseline almost instantaneously, whereas e-cig changes in HRV were subtle and gradual in onset.⁷⁷ These findings suggest that while e-cigs may have lowered relative risks for instantaneous changes in HRV when compared with traditional tobacco smoke, they also have increased relative risk compared with lozenges and absolute risks compared with abstaining from e-cig use, potentially because of nicotine salt protonation.

Preclinical studies using animal models demonstrated that various e-cig flavorings similar to those discussed in the oxidative stress and potential biomarkers section alter ANS homeostasis. Exposure to vanillin aldehyde flavored e-cig vapor in mice over a 10-week span resulted in increased sympathetic predominance in HRV measurements.⁷⁹ When compared with control air groups at 5 and 10 weeks of chronic exposure to vanillin aldehyde flavored e-cigs, e-cig-exposed mice showed significantly decreased pNNO6⁷⁹; which is a measurement of cardiac parasympathetic activity via the percentage of adjacent NN intervals (pNN) that differ from one another in a given time frame (eg, 6 ms).^{80,81} Decreased pNNO6 values following exposure suggest that aldehyde-containing flavorings decrease cardiac parasympathetic activity leading to the predominance of sympathetic activity, which has been linked to poor cardiovascular outcomes. Given the use of aldehydes (eg, vanillin aldehyde) in e-cig flavorings and aldehyde byproducts of combustion (eg, acetaldehyde), the pathological mechanisms by which these compounds induce cardiac dysfunction is of great concern. Aldehydes are metabolized by ALDH2 (aldehyde dehydrogenase 2), with $\approx 8\%$ of the population having an inactivating ALDH2 genetic variant (ALDH2*2) that reduces their ability to metabolize aldehydes. ALDH2*2 mice showed greater increases in HR following 10 days of e-cig exposure when compared

with ALDH2 control mice exposed to e-cig and ALDH2 and ALDH2*2 exposed to air.⁸² These alterations in aldehyde metabolism led to cardiovascular oxidative stress and may have potentially altered sympathetic activity that could be a symptom of oxidative stress or an additive insult to e-cigs containing flavorings.

Interestingly, studies have also demonstrated that biological sex may be yet another determinant in sympathetic activation following e-cig exposure. Mice exposed to various e-liquid constituents and aerosols (PG, VG, PG/VG, and tobacco- and menthol-flavored commercial e-liquids) showed significantly decreased HR during flavored e-cig exposures.⁸³ PG/VG-derived e-liquids caused the greatest changes in HR in male mice and showed no changes in female mice. Male mice also showed significantly increased HRV during flavored e-cig exposures, again with no changes observed in female mice. These changes were then followed by increased HR and decreased HRV between exposures and postexposure in males with no observable changes in females.⁸³ A recent study from Nabavizadeh et al⁸⁴ suggested that no individual constituent of e-cigs or traditional cigarettes was responsible for endothelial dysfunction but that inhaled particles induce vagal nerve signaling initiated by airway irritation. These studies indicate that different components of e-cigs, including but not limited to e-liquid and nicotine, as well as biological characteristics, such as sex and metabolic comorbidities, all interact to predispose e-cig users to adverse cardiovascular outcomes. Thus, assessment of the physiological mechanisms that direct inhalants elicit as well as the interplay with social determinants of health and comorbidities could potentially provide greater benefit to public health, rather than research focusing solely on the toxic effects of individual e-cig components.

CONCLUSIONS, LIMITATIONS, AND FUTURE DIRECTIONS

Although it is well known that the usage of and exposure to e-cig devices is fundamentally different from that of traditional cigarette combustion, the differences in cardiac, vascular, and cardiometabolic outcomes requires more investigation to better discern relative and absolute risks of e-cig use, as well as the mechanisms by which e-cigs elicit adverse cardiovascular outcomes. Additionally, e-cig effects on the cardiovascular and metabolic outcomes of individuals with comorbidities are of paramount concern when considering the relative and absolute risks of e-cig use in these individuals. Further, many studies have focused on how the essential components of e-cigs (ie, delivery vehicle [PG, VG, PG/VG] with or without nicotine or other additives) elicit cardiovascular and metabolic effects; however, how additional e-cig constituents (eg, nicotine salts [protonation/hydrophilicity status] and

Table 2. Comprehensive Assessment of E-cigarette Induced Cardiometabolic Effects

Condition	Biomarker	Change	Device generation	Nicotine concentration, mg/mL	Flavor
Oxidative stress	GSH	↑ ⁶⁰	Unclear	12	Vanilla, apple/mint
		No change ⁶⁰	Unclear	0	Tobacco
				18	Apple/mint
	TBARS	↑ ⁶⁰	Unclear	0, 12, 18	Tobacco, vanilla
		No change ⁶⁰	Unclear	6	Vanilla, apple/mint
		↓ ⁶⁰	Unclear	12	Apple/mint
	MPO	↑ ⁶⁶	Third	3	None
		No change ⁶⁶	Third	0	None
	NOX2	↑ ⁵⁸	Second	16	Tobacco
	H ₂ O ₂	↑ ⁵⁸	Second	16	Tobacco
Sympathetic effects	HR	↑ ^{58,62,66,72-74}	First, ⁷³ second, ^{58,74} third ^{62,72}	0, ⁶⁶ 1.5, ⁶² 3, ⁶⁶ 16, ⁵⁸ 18, ⁷⁴ 24, ⁷² several ⁷³	None, ^{62,66} tobacco, ^{58,72,74} several ⁷³
		No change ^{62,63,73}	First, ⁷³ second, ⁶³ third ⁶²	0, ^{62,63} 12, ⁶³ several ⁷³	None, ⁶² several, ⁷³ unclear ⁶³
	Systolic BP	↑ ^{58,62,66,72,73}	First, ⁷³ second, ⁵⁸ third ^{62,66,72}	0, ^{62,66} 1.5, ⁶² 3, ⁶⁶ 16, ⁵⁸ 24, ⁷² several ⁷³	None, ^{62,66} tobacco, ^{58,72} several ⁷³
	Diastolic BP	↑ ^{58,66,73}	First, ⁷³ second, ⁵⁸ third ⁶⁶	0, ⁶⁶ 3, ⁶⁶ 16, ⁵⁸ several ⁷³	None, ⁶⁶ tobacco, ⁵⁸ several ⁷³
	HRV	↓ ^{75,77}	First, ⁷⁵ third, ⁷⁷ fourth ⁷⁷	18, ⁷⁵ unclear ⁷⁷	Tobacco, ⁷⁵ menthol, ⁷⁵ unclear ⁷⁷
	sympathetic tone	↑ ⁷⁶	First, second	12, unclear	Tobacco, strawberry
	pNN06	↓ ⁷⁹	Third	6	Vanilla custard
	Body weight	↓ ^{28,48}	Third	0, ⁴⁸ 24 ²⁸	Tobacco
		No change ^{28,48,50}	Third	0, ²⁸ 18, ⁴⁸ 36 ⁵⁰	Tobacco, ^{28,48} unclear ⁵⁰
	Glut2	↑ ⁴⁸	Third	0, 18	Tobacco
Adipose tissue effects	Glut4	↑ ⁴⁸	Third	0	Tobacco
	FASN	↑ ⁴⁸	Third	0, 18	Tobacco
	Cytokines (IL10)	↑ ⁴⁹	Third	0	Tobacco
	RER	↓ ⁵¹	Second	18	None
	serum ω-6 fatty acid levels	↓ ⁵⁵	Several	Several	Several
	Oxylipins 9-HODE and 13-HODE	↓ ⁵⁵	Several	Several	Several
	LDL, HDL	No change ^{54,56}	Fourth, ⁵⁴ unclear ⁵⁶	Several	Several
	End-systolic elastance	↓ ²⁶	Third	0, 20.2	None
	Preload-recruitable stroke work	↓ ²⁶	Third	20.2	None
	Perivascular Fibrosis	↑ ²⁶	Third	0	None
Myocardial Effects	COL1A1, COL3A1	↑(3 wks) ²⁶	Third	20.2	None
		No change (3 mo) ²⁶	Third	20.2	None
	EF	↓ (12 wks) ^{28,29}	Third	24	Tobacco
		No change (2 wks) ²⁵	Unclear	24	None
	LVFS	↓ ^{28,29}	Third	24	Tobacco
	VCF	↓ ^{28,29}	Third	24	Tobacco
	Arterial stiffness PWV	↑ ^{63,66}	Second, ⁶³ third ⁶⁶	0, ⁶³ 3, ⁶⁶ 12 ⁶³	None, ⁶⁶ unclear ⁶³
	Alx75	↑ ^{63,66}	Second, ⁶³ third ⁶⁶	0, ⁶³ 3, ⁶⁶ 12 ⁶³	None, ⁶⁶ unclear ⁶³
Vascular Effects	Flow-mediated vasodilation	↑ ²²	Unclear	16	Tobacco
	No bioavailability	↓ ^{22,58}	Second, ⁵⁸ unclear ²²	16	Tobacco

(Continued)

Table 2. Continued

Condition	Biomarker	Change	Device generation	Nicotine concentration, mg/mL	Flavor
Thrombosis Effects	Duration in occlusion time	↓ ⁶⁴	Fourth	18	Menthol
	ADP-induced platelet aggregation	↑ ⁶⁴	Fourth	18	Menthol
	ADP	↑ ⁶⁴	Fourth	18	Menthol
	P-selectin	↑ ⁶⁴	Fourth	18	Menthol
	GP1Ib/IIla	↑ ⁶⁴	Fourth	18	Menthol

8-iso-PGF₂α indicates urinary 8-isoprostane; ADP, adenosine diphosphate; Alx75, augmentation index corrected for heart rate; BP, blood pressure; COL1A1, collagen (type 1, alpha 1); COL3A1, collagen (type 3, alpha 1); EF, ejection fraction; FASN, fatty acid synthase; GP1Ib/IIla, glycoprotein 1Ib/IIla; GSH, glutathione; H₂O₂, hydrogen peroxide; HDL, high-density lipoprotein; HODE, hydroxyoctadecadienoic acid; HR, heart rate; HRV, heart rate variability; LDL, low-density lipoprotein; LVFS, left ventricle fractional shortening; MPO, myeloperoxidase; NOX2, NADPH oxidase; pNN06, the percentage of adjacent NN intervals (pNN) that differ from one another in >6 ms; PWV, pulse wave velocity; RER, respiratory exchange ratio; TBARS, thiobarbituric acid reactive substances; and VCF, velocity of circumferential fiber shortening.

added flavorings) influence cardiovascular and metabolic health remains understudied (summarized in Table 2). Although the United States Food and Drug Association banned the sale of flavored e-cig cartridges and pod-based in February 2020, there is still extensive use of menthol-flavored e-cig liquids amongst e-cig users.⁸⁵ Furthermore, the ban was implemented as a means of reducing the appeal of e-cigs to adolescent users; however, the ban does not prevent e-cig users from mixing unflavored e-liquids with other purchased flavorants and necessitates further study.

ARTICLE INFORMATION

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