Preventive Medicine 127 (2019) 105770

Contents lists available at ScienceDirect

Preventive Medicine

journal homepage: www.elsevier.com/locate/ypmed

Review Article

The cardiovascular effects of electronic cigarettes: A systematic review of experimental studies

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A B S T R A C T

Background: Smoking is responsible for substantial cardiovascular morbidity and mortality. Electronic cigarettes have been advocated as a means to reduce this disease burden; by reducing exposure to harmful substances in smokers who are unable to quit. Concerns have been raised however, about cardiovascular effects of their use, with inhalants containing carbonyls and fine particulate matter. We systematically reviewed experimental studies of *in vitro*, animal, and human cardiovascular effects associated with electronic cigarette use.

Methods: A literature search was conducted using Ovid MEDLINE & Embase databases, identifying experimental studies investigating cardiovascular effects of electronic cigarette use. Subsequently, Cochrane Risk of Bias tools were used to assess study quality. Any differences in outcomes by conflict of interest and risk of bias status were sought.

Results: 38 studies were included, investigating animals (n = 6), humans (n = 24) and human cardiovascular cells *in vitro* (n = 8). 74.3% of studies found potentially harmful effects. Increased sympathetic nerve activity was observed in human studies, whilst platelet haemostatic processes, reactive oxygen species production and endothelial dysfunction were reported across all study types. Studies with conflicts of interest or median-high risk of bias were less likely to identify potentially harmful effects (p = 0.0007, p = 0.04 respectively).

Discussion: Most studies suggest potential for cardiovascular harm from electronic cigarette use, through mechanisms that increase risk of thrombosis and atherosclerosis. Notably, studies with conflicts of interest are significantly less likely to identify concerning cardiovascular effects. Included studies examine healthy, adult participants, limiting generalisation to potential high-risk groups including individuals with established cardiovascular disease or young, non-smokers.

1. Introduction

Conventional and electronic (e-) cigarettes deliver nicotine to the bloodstream, resulting in significant production of its primary metabolite – cotinine (Etter, 2016; Etter and Bullen, 2011). Nicotine is known to affect the cardiovascular system through sympathetic nervous system activation. This increases myocardial contractility, heart rate, blood pressure and coronary vasoconstriction (Benowitz and Burbank, 2017; United States Department of Health and Human Services, 2014). Clinical studies into nicotine primarily focus on nicotine replacement therapy (NRT) use, which typically produces nicotine concentrations half those of smoking, vaping or using smokeless tobacco. Whilst reviews have not found an association between NRT use and cardiovascular morbidity (Ford and Zlabek, 2005; Lindson-Hawley et al., 2016) studies into smokeless tobacco use have found associations with fatal coronary artery disease (Gupta et al., 2018); with mortality rates halving in individuals who quit product use after a myocardial infarction (Arefalk et al., 2014). It is not possible however to determine whether this mortality is attributable to nicotine.

Other constituents of conventional and electronic cigarettes have raised more concern. Cigarettes produce carbon monoxide (CO) which contributes to carboxyhaemoglobin formation, increasing blood viscosity and contributing to thrombogenesis. Both products deliver fine ($PM_{2.5}$) and ultra-fine ($PM_{0.1}$) particulate matter (Fuoco et al., 2014; Zhang et al., 2013). These may trigger pathophysiological processes including vascular inflammation and platelet activation (Fernandez et al., 2015; Geiss et al., 2015; Tianrong, 2013; Soule et al., 2017; Pellegrino et al., 2012), with chronic exposure constituting a cardiovascular risk factor (Brook et al., 2010). Thermal degradation of e-cigarette solvent carriers glycerol and propylene glycol can also produce carbonyls, such as formaldehyde, acetaldehyde, and acrolein (Tianrong, 2013; Hecht et al., 2015), that may cause pathophysiological changes once broken down into reactive oxidant species (Lerner et al., 2015a; Goel et al., 2015; Zhao et al., 2017; Lerner et al., 2015b), potentially contributing to cardiomyopathy (Henning et al., 2017). E-cigarette liquids have also been manufactured with numerous flavorings, such as

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https://doi.org/10.1016/j.ypmed.2019.105770
Received 14 December 2018; Received in revised form 18 June 2019; Accepted 12 July 2019
Available online 22 July 2019

cinnamaldehyde, which may have cardiotoxic effects (Farsalinos et al., 2013). Heavy metals such as cadmium and lead have been detected in certain e-cigarette aerosols (Hess et al., 2017), which have been associated with hypertension (Navas-Acien et al., 2007) and coronary artery disease respectively (Tellez-Plaza et al., 2013). It is worth noting however that mere detection of toxicants in aerosols does not mean they will reach the bloodstream in toxic quantities.

Middlekauff recently developed a model illustrating four mechanisms by which e-cigarettes may increase the risk of cardiovascular disease: (i) sympathetic nerve activation; (ii) oxidative stress; (iii) endothelial dysfunction and (iv) platelet activation. These mechanisms may induce arrhythmias, atherosclerosis and acute ischaemia. Whilst investigation of these long-term sequelae is problematic due to the inchoate nature of e-cigarettes, their inducing mechanisms can be investigated through various biomarkers including (i) haemodynamic changes; (ii) oxidant and antioxidant levels; (iii) measures of arterial stiffness and (iv) platelet aggregation, respectively (Middlekauff, 2019).

This study systematically reviews the evidence of physiological and pathophysiological cardiovascular effects after direct exposure to e-cigarettes and discusses the implications for cardiovascular disease.

2. Methods

Four researchers conducted the review applying Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher et al., 2009).

2.1. Search strategy

A literature search was conducted on 17 July 2017 and updated on 12th June 2019 using Ovid MEDLINE and Embase databases from 1996 to 11 June 2019. The following search terms were utilised: 'e-cig*' or 'electronic cig*' or 'e-liquid' or 'e-juice' or 'electronic nicotine delivery system' or 'vape' combined with 'cardi*' or 'myocardi*' or 'coronary' or 'heart' or 'vascular' or 'endotheli*'. Reference lists of included articles and pertinent policy papers were examined for additional citations and a secondary literature search was conducted through Web of Science.

2.2. Inclusion, exclusion and study eligibility criteria

Experimental studies pertaining to (human) *in vitro*, animal, or human cardiovascular effects of e-cigarette use were included. Full details are presented in web Appendix 1. Studies had to report quantifiable biomarkers of cardiovascular effects or cardiovascular pathology. Non-experimental studies were excluded but are summarised in web Appendix 2. Human studies: Eligibility criteria: adults with or without cardiovascular disease, independent of smoking status and age.

2.3. Data extraction and synthesis

Extraction tables collated data on study, participant, and intervention characteristics together with study results. Despite the publication of a recent meta-analysis of haemodynamic outcomes from e-cigarette use (Skotsimara et al., 2019), we decided to synthesise extracted data narratively due to concerns about study heterogeneity. We organised our findings based on a conceptual model of potential pathways that draws on previous papers, including that developed by *Middlekauff* (Fig. 1) (Middlekauff, 2019).

2.4. Conflict of interest in studies

To assess for any influence of conflicts of interest (COI), which is not generally captured by traditional quality assessment tools, one reviewer extracted outcome data and conclusions verbatim from included papers and another blindly judged whether results and/or conclusions were supportive of e-cigarette use. COI status was based on evidence (obtained from statements in the paper concerned and/or other papers or presentations by the individuals involved) that authors or studies received funding or other assistance from tobacco and/or e-cigarettes manufacturers. Chi-squared with two-tailed Fisher's Exact Test assessed significance of the relationship between COI status against potentially harmful cardiovascular outcomes and conclusions supportive of e-cigarette use.

2.5. Quality assessment

Quality of studies was assessed using Cochrane Risk of Bias (RoB) tools (Higgins et al., 2016). RoB status was then assessed against outcome data and conclusions using Chi-squared tests, with significance measured using two-tailed Fisher's Exact Test because of the small numbers of studies. Study heterogeneity precluded assessing publication bias by means of a funnel plot. RoB was also compared in studies that we did or did not identify as having potential conflict of interest (Appendix 6).

3. Results

3.1. Study selection

The electronic search identified 766 records with an additional 10 from reference lists (Fig. 2). After removal of duplicates, 563 records were screened for inclusion by title and abstract, leaving 82 full-text articles to be assessed for eligibility, when 44 articles were excluded due to: inappropriate study designs (non-experimental or lacking control/comparators) (n = 10), no relevant outcome measures (n = 1), inappropriate study population (n = 4) or had no full-text articles associated with their abstracts (n = 29) (web Appendix 2). 38 articles were included in the review.

3.2. Study characteristics

This review included randomised controlled-trials (n = 8) (Barber et al., 2017; Cravo et al., 2016; D'Ruiz et al., 2017; Hom et al., 2016; Cooke et al., 2015; Olfert et al., 2017; Farsalinos et al., 2016; Lee et al., 2018); randomised crossover studies (n = 10) (Yan and D'Ruiz, 2015; Antoniewicz et al., 2016; Fogt et al., 2016; Moheimani et al., 2017a; Franzen et al., 2018; Ikonomidis et al., 2018; Chaumont et al., 2018; Sumartiningsih et al., 2019; Biondi-Zoccai et al., 2019; Antoniewicz et al., 2019); non-randomised controlled trials (n = 13) (Anderson et al., 2016; Schweitzer et al., 2015; Shi et al., 2019; Lee et al., 2019; Espinoza-Derout et al., 2019; Teasdale et al., 2016; Putzhammer et al., 2016; Farsalinos et al., 2014a; Vlachopoulos et al., 2016; Kaisar et al., 2017; Taylor et al., 2017; Qasim et al., 2018; Chatterjee et al., 2019) and non-randomised crossover studies (n = 7) (Carnevale et al., 2016; Eissenberg, 2010; Szoltysek-Boldys et al., 2014; Vansickel et al., 2010; Nocella et al., 2018; Kerr et al., 2018; Pywell et al., 2018). These articles studied human subjects (n = 24) (Cravo et al., 2016; D'Ruiz et al., 2017; Cooke et al., 2015; Farsalinos et al., 2016; Yan and D'Ruiz, 2015; Antoniewicz et al., 2016; Fogt et al., 2016; Moheimani et al., 2017a; Franzen et al., 2018; Ikonomidis et al., 2018; Chaumont et al., 2018; Sumartiningsih et al., 2019; Biondi-Zoccai et al., 2019; Antoniewicz et al., 2019; Farsalinos et al., 2014a; Vlachopoulos et al., 2016; Chatterjee et al., 2019; Carnevale et al., 2016; Eissenberg, 2010; Szoltysek-Boldys et al., 2014; Vansickel et al., 2010; Nocella et al., 2018; Kerr et al., 2018; Pywell et al., 2018), animal subjects (n = 6) (Olfert et al., 2017; Lee et al., 2018; Shi et al., 2019; Espinoza-Derout et al., 2019; Kaisar et al., 2017; Qasim et al., 2018) and a range of human cardiovascular cells types and platelets (n = 8) (Barber et al., 2017; Hom et al., 2016; Anderson et al., 2016; Schweitzer et al., 2015; Lee et al., 2019; Teasdale et al., 2016; Putzhammer et al., 2016; Taylor et al., 2017). The total duration of exposure of cells to e-cigarette aerosol extract (eCAE) in *in vitro* studies ranged from 4 h to 72 h

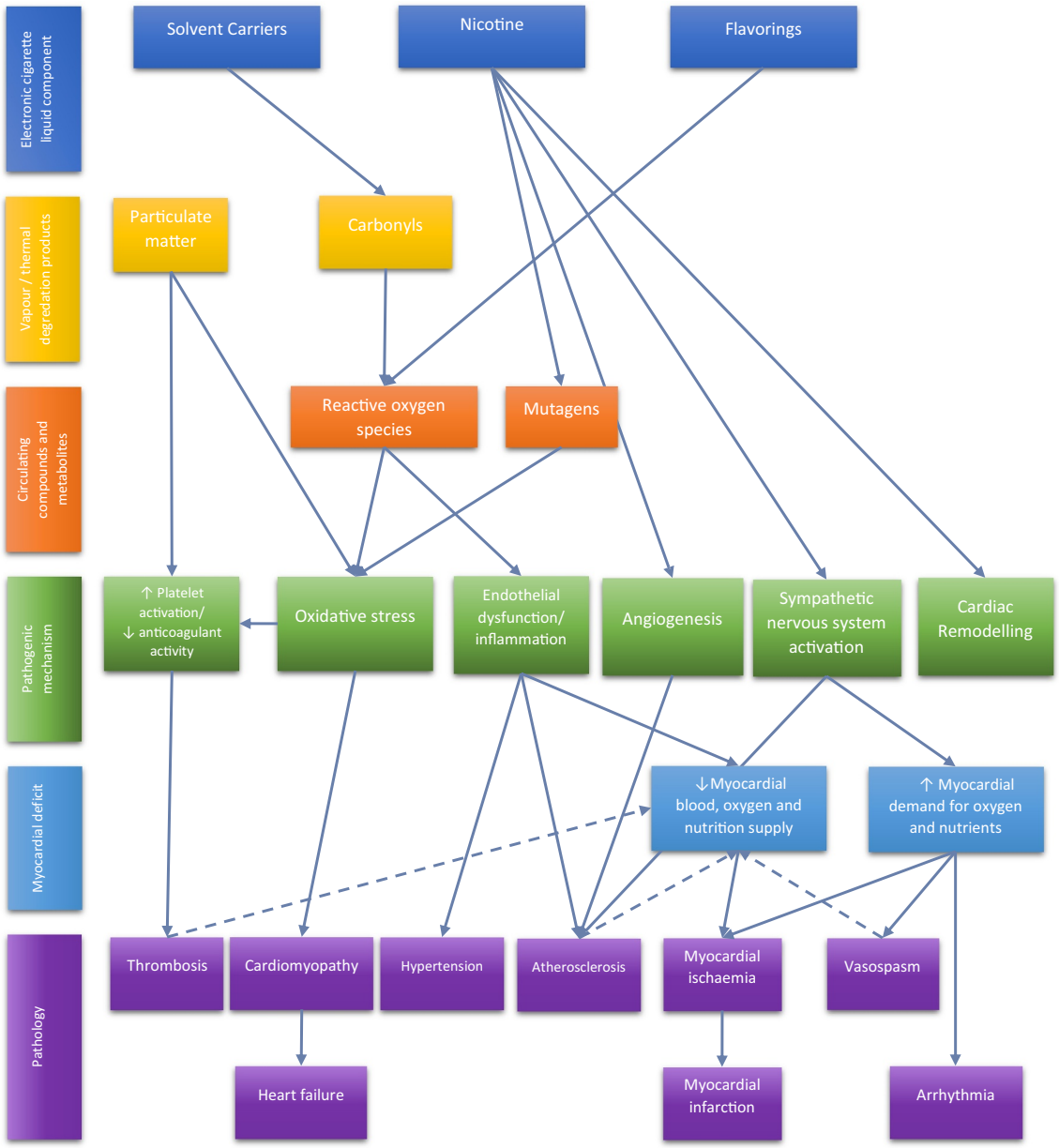

Fig. 1. Potential pathways via which e-cigarettes may affect the cardiovascular system.

(Table 1, Appendix 3). Sample size in human studies ranged from 10 to 408 participants, with attrition ranging from 0% to 39%. In human cross-over studies the washout period ranged from 1 h to 4 weeks (Table 2).

3.3. Participant characteristics

Only 11% of human studies investigated solely non-smoking populations (Cooke et al., 2015; Fogt et al., 2016; Moheimani et al., 2017a; Chatterjee et al., 2019). 45.8% of human studies included subjects without prior use of e-cigarettes and understanding of vaping topography ($n = 11$) (D'Ruiz et al., 2017; Cooke et al., 2015; Antoniewicz et al., 2016; Fogt et al., 2016; Moheimani et al., 2017a; Chatterjee et al., 2019; Eissenberg, 2010; Vansickel et al., 2010; Nocella et al., 2018; Kerr et al., 2018; Pywell et al., 2018), whilst a further 33.3% of studies did not state whether subjects had previously used these devices (n = 8) (Farsalinos et al., 2016; Yan and D'Ruiz, 2015; Chaumont et al., 2018; Sumartiningsih et al., 2019; Biondi-Zoccai et al., 2019; Antoniewicz et al., 2019; Vlachopoulos et al., 2016; Carnevale et al., 2016). Only 45% of studies chemically verified baseline smoking abstinence (n = 11) (Farsalinos et al., 2016; Yan and D'Ruiz, 2015; Nocella et al., 2018; Antoniewicz et al., 2016; Franzen et al., 2018; Chaumont et al., 2018; Biondi-Zoccai et al., 2019; Antoniewicz et al., 2019; Eissenberg, 2010; Szoltysek-Boldys et al., 2014; Vansickel et al., 2010). Mean age of human subjects, who were healthy volunteers, ranged from 22.9 to 46.6 years old.

3.4. Intervention characteristics

Interventions in *in vitro* studies are summarised in Table 3 and in human and animal studies in Table 4. The brand and generation of e-cigarettes reported varied widely – if reported at all. Only three studies reported utilising newer generation devices (Olfert et al., 2017; Franzen et al., 2018; Chaumont et al., 2018). Only 44.7% of studies reported any electrical characteristics of devices ($n = 17$), with voltage varying from 3.0 to 5.0 V and resistance varying from 0.4 to 2.4 Ω. Few studies included independent chemical analyses of e-liquids ($n = 6$) (D'Ruiz et al., 2017; Antoniewicz et al., 2016; Chaumont et al., 2018;

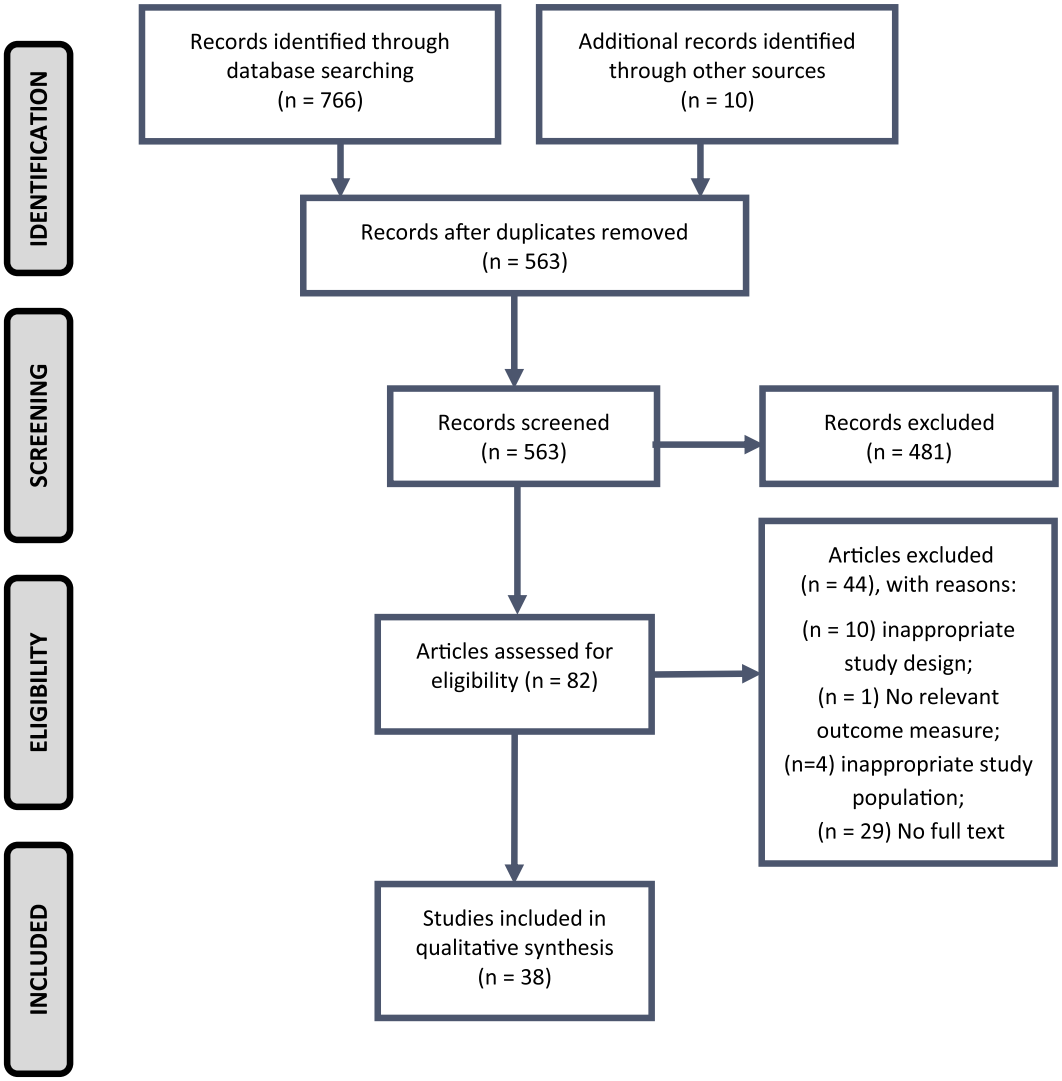

Fig. 2. PRISMA study selection process.

Schweitzer et al., 2015; Farsalinos et al., 2014a; Kerr et al., 2018) and only *Schweitzer* et al. tested resultant vapour constituents for presence of newly formed oxidation products.(Schweitzer et al., 2015) Only one *in vitro* study measured e-cigarette heating coil temperature (Putzhammer et al., 2016) whilst only two considered high coil temperatures (Table 3) (Teasdale et al., 2016; Putzhammer et al., 2016). Reported nicotine concentration in eCAE solution varied from 0 to 36 mg/mL with only 37.5% of human and animal studies estimating nicotine delivery, through plasma nicotine and/or urinary cotinine concentrations ($n = 9$) (Olfert et al., 2017; Yan and D'Ruiz, 2015; Moheimani et al., 2017a; Chaumont et al., 2018; Biondi-Zoccai et al., 2019; Qasim et al., 2018; Eissenberg, 2010; Vansickel et al., 2010; Nocella et al., 2018). Notably, both *Eissenberg* et al. and *Vansickel* et al. reported no statistically significant increase in blood nicotine concentration after e-cigarette use, with participants being under-exposed (Eissenberg, 2010; Vansickel et al., 2010).

Only 50% of human studies chemically verified abstinence ($n = 12$) (D'Ruiz et al., 2017; Cooke et al., 2015; Farsalinos et al., 2016; Yan and D'Ruiz, 2015; Antoniewicz et al., 2016; Franzen et al., 2018; Chaumont et al., 2018; Biondi-Zoccai et al., 2019; Antoniewicz et al., 2019; Szoltysek-Boldys et al., 2014; Vansickel et al., 2010; Nocella et al., 2018). Most studies did not report frequency of abstinence testing, with Farsalinos et al. (2016) having periods up to 24 weeks without assessing abstinence (Farsalinos et al., 2016) There was significant inter-study variation in inhalation regime. Some studies controlled for duration and intensity of 'vaping' whilst others allowed *ad libitum* use. Notably, *Pywell* et al. utilised a vaping protocol based on smoking protocols used in the literature but abandoned it because of nausea (Pywell et al., 2018). Only *Chaumont* et al. assessed subjects' tolerance to vaping prior to investigation (Chaumont et al., 2018).

3.5. Study results: in vitro studies

These are summarised in Table 5.

3.5.1. Oxidative stress

Three studies found statistically significant increases in reactive oxygen species (ROS) associated with endothelial injury (Anderson et al., 2016; Lee et al., 2019; Putzhammer et al., 2016). *Teasdale* et al. did not however find significant upregulation in expression of genes involved in the oxidative stress pathway (Teasdale et al., 2016).

3.5.2. Endothelial cellular function

Four studies reported statistically significant reductions in endothelial cell viability when exposed to certain eCAE (Anderson et al., 2016; Lee et al., 2019; Putzhammer et al., 2016; Taylor et al., 2017), whilst *Lee* et al. identified significant impairment in endothelial cell viability after exposure to serum from e-cigarette and cigarette smokers

Table 1
Characteristics of *in vitro* studies (n = 8).

Author (year)	Conflict of interest	Type of study	Cell type	Length of exposure	Outcome measures
Anderson et al. (2016)	✓	Non-randomised, controlled trial	Umbilical vein endothelial cells	72 h	Cell viability, Reactive oxygen species, DNA damage
Barber et al. (2017)	✗	Randomised, controlled trial	Umbilical vein endothelial cells	48 h	Cell viability, Cell metabolic activity, Complement deposition, gC1qR & cC1qR expression, Complement inhibitor expression
Hom et al. (2016)	✗	Randomised, controlled trial	Platelets	4 h	Platelet aggregation, Platelet adhesion, Complement deposition, C1q receptor expression
Lee et al. (2019)	✗	Non-randomised, controlled trial	Pluripotent stem cell–derived endothelial Cells	48 h, (16 h)[&]	Cell viability, Reactive oxidative species generation, Apoptosis, Endothelial function (tube formation, LDL and lipid uptake and cell migration), cross-talk with macrophages, transcriptomic profile
Putzhammer et al. (2016)	✗	Non-randomised, controlled trial	Umbilical vein endothelial cells	48 h	Cell viability, Inhibition of cell proliferation, Reactive oxidative species, Morphological alterations
Schweitzer et al. (2015)	✗	Non-randomised, controlled trial	Pulmonary microvascular cells	10 h	Endothelial barrier disruption*
Taylor et al. (2017)	✓	Non-randomised, controlled trial	Umbilical vein endothelial cells	20 h	Inhibition of cell migration
Teasdale et al. (2016))	✗	Non-randomised, controlled trial	Coronary artery endothelial cells	64 h	Genetic markers of oxidative stress#

*Measured by trans-endothelial electrical resistance & exposure for tube formation.
#HMOX1, GCLM, OSLIN1, PAR4, CYP1A1, CYP1B1, IL8, NTPX1.

compared to non-smokers (Lee et al., 2019). *Lee* et al. also identified increased endothelial cell tube formation, reflective of increased angiogenesis (DeCicco-Skinner et al., 2014). Other cardiotoxic effects identified included DNA damage (Anderson et al., 2016), cell morphological changes (Putzhammer et al., 2016) and reduced cell metabolic activity (Barber et al., 2017). These changes may constitute a mechanism for endothelial dysfunction *in vivo,* however caution should be taken when extrapolating from *in vitro* findings.

Statistically significant reductions in endothelial cell density (Barber et al., 2017) and proliferation (Putzhammer et al., 2016) (recognised indicators of endothelial injury and dysfunction) were detected in eCAE exposures in one study each. *Lee* et al. found significant inhibition of endothelial cell migration (Lee et al., 2019), whilst *Taylor* et al. found no significant inhibition after eCAE exposure. This inhibition has been associated with impaired vascular repair after endothelial dysfunction induced by smoking (Taylor et al., 2017).

Schweitzer et al. identified increased endothelial cell barrier disruption (Schweitzer et al., 2015) after eCAE exposure. In vascular pathologies, endothelial barrier disruption is caused by pro-inflammatory stimuli destabilising endothelial intracellular junctions. The resultant barrier disruption permits migration of immune cells into the arterial intima – inducing vascular inflammation (Chistiakov et al., 2015).

3.5.3. Endothelial-complement interactions

Barber et al. investigated the effect of eCAE on deposition of complement factors on endothelial cell surfaces, endothelial expression of gC1qR and cC1qR, and endothelial complement inhibitors. All eCAE exposures were associated with statistically significant increases in C1q and C4d complement deposition and expression of gC1qR and cC1qR cellular proteins, with some extracts causing statistically significant complement inhibitor expression (Barber et al., 2017). Interestingly, endothelial C1q deposition did not increase when cells were exposed to smoke extract from conventional cigarettes. *In Vivo,* these endothelial-complement interactions have been associated with increased endothelial dysfunction - contributing to atherosclerosis (Yin et al., 2008; Yin et al., 2015).

3.5.4. Platelet function

Hom et al. reported significant increases in platelet aggregation, adhesion, activation and complement deposition after exposure to eCAE (Hom et al., 2016). These changes have been invoked as a mechanism for increased risk of thrombosis after cigarette smoking (Hung et al., 1995).

3.5.5. Study results: animal studies.

These are summarised in Table 6.

3.5.6. Cardiac function

Lee et al. reported statistically significant increases in two mutagens (O^6-methyldeoxyguanosines and γ-hydroxy-1,N2-propano-deoxyguanosine) in cardiac tissue of mice exposed to eCAE (Lee et al., 2018). *Espinoza-Derout* et al. identified cardiomyocyte mitochondrial nuclear damage and cytoplasmic abnormalities; as well as intramyocardial lipid accumulation and reduced expression of a cardioprotective gene after exposure to eCAE (Espinoza-Derout et al., 2019). *Olfert* et al. reported statistically significant increases in left ventricular mass of mice after chronic exposure to e-cigarette vapour but not those exposed to cigarette smoke (Olfert et al., 2017). *Espinoza-Derout* et al. however observed no significant change (Espinoza-Derout et al., 2019). *Olfert* et al. observed no significant decreases in fractional shortening and ejection fraction in mice exposed to e-cigarette vapour (Olfert et al., 2017), whilst *Espinoza-Derout* et al. observed both of these findings (Espinoza-Derout et al., 2019). *Shi* et al. found no significant effects of vaping on cardiac contractility, fibrosis or geometric properties (Shi et al., 2019).

Table 2
Characteristics of human (n = 24) and animal experimental studies (n = 6).

Author & Year	Conflict of interest	Type of study	Sample size	Attrition rate	Comparator	washout period	Outcome measures
Antoniewicz et al. (2019)[48]	×	Randomized, double-blinded, crossover design	17	0%	nicotine-free e-cigarette	1 week	Heart rate, systolic pressure, diastolic pressure, arterial stiffness.
Antoniewicz et al. (2016)[40]	×	Randomised crossover study	16	12.5%	complete cessation	1 week	Endothelial progenitor cells, Microvesicles
Biondi-Zoccai et al. (2019)[47]	×	Randomized, blinded, crossover design	20	0%	complete cessation/ Cigarette	1 week	Systolic pressure, diastolic pressure, markers of oxidative stress, antioxidant reserve, endothelial dysfunction
Carnevale et al. (2016)[62]	×	Non-randomised crossover	40 (48?)*	0%	cigarette / sham smoking*	1 week	Markers of oxidative stress+
Chatterjee et al (2019)[61]	×	Non-randomised controlled trial	10 (6?)**	0%	complete cessation	NA	Markers of oxidative stress and inflammation
Chaumont et al. (2018)[45]	×	Randomised crossover study	25	16%	Nicotine-free e / sham vaping	1 week	microcirculatory function, arterial stiffness, hemodynamic parameters and oxidative stress
Cooke et al. (2015)[35]	×	Randomised controlled trial	20	0%	Placebo e-cigarette	NA	Heart rate, Systolic pressure, Diastolic pressure
Cravo et al. (2016)[32]	✓	Randomised controlled trial	408	5.1%	Cigarette	NA	Heart rate, Systolic pressure, Diastolic pressure
D'Ruiz et al. (2017)[33]	✓	Randomised controlled trial	105	1%	complete cessation	NA	Heart rate, Systolic pressure, Diastolic pressure
Eissenberg et al. (2010)[63]	×	Non-randomised Crossover	16	0.5%	cigarette / sham smoking	48 hours	Heart rate
Farsalinos et al. (2014)[56]	✓	Non-randomised controlled trial	76	0%	Cigarette	NA	Heart rate, Systolic pressure, Diastolic pressure, Myocardial function
Farsalinos et al. (2016)[37]	✓	Randomised controlled trial	300	39%	nicotine-free e-cigarette	NA	Heart rate, Systolic pressure, Diastolic pressure
Fogt et al. (2016)[41]	×	Randomised crossover study	20	0%	nicotine-free e-cigarette	≥1 week	Heart rate, Systolic pressure (resting & exercising), Diastolic pressure (resting & exercising)

(*continued on next page*)

Table 2 (*continued*)

Franzen et al. (2018)[43]	✗	Randomised crossover study	15	0%	Nicotine-free e-cigarette, cigarette	48 hours	Peripheral blood pressure, central blood pressure, arterial stiffness
Ikonomidis et al. (2018)[44]	✗	Randomised crossover study	70	0%	Sham smoking, nicotine-free e-cigarette, cigarette	1 hour	Aortic stiffness (augmentation index; Pulse wave velocity); Oxidative stress (malondialdehyde (MDA) plasma concentration)
Kerr et al. (2018)[67]	✗	Non-randomised crossover	20	0%	Cigarette	24 hours	Heart rate, blood pressure, reactive hyperaemia index (microvascular hyperactivity), augmentation index (arterial stiffness)
Moheimani et al. (2017)[42]	✗	Randomised crossover study	39	26%	nicotine-free e-cigarette, sham vaping	4 weeks	Heart rate, Heart rate variability, PON-1 (marker of oxidative stress)
Nocella et al. (2018)[66]	✗	Non-randomised crossover	40	0%	cigarette	1 week	Platelet aggregation, soluble CD40-ligand, soluble P-selectin
Pywell et al. (2018)[68]	✗	Non-randomised crossover	15	0%	Nicotine-free e-cigarette	Unclear	Superficial microcirculation of the hand, deep microcirculation of the hand
Sumartiningsih et al. (2019)[46]	✗	randomized crossover study	24	0%	nicotine-free e-cigarette	3 days	Heart rate Systolic pressure, Diastolic pressure Heart rate variability
Szołtysek-Bołdys et al. (2014)[64]	✓	Non-randomised crossover	15	0%	Cigarette	24 hours	Arterial stiffness#, Systolic pressure, Diastolic pressure
Vansickel et al. (2010)[65]	✗	Non-randomised crossover	48	33.3%	cigarette / sham smoking	48 hours	Heart rate
Vlachopoulos et al. (2016)[57]	✗	Non-randomised controlled trial	24	0%	cigarette / sham smoking	Length not specified	Arterial stiffness, Systolic pressure, Diastolic pressure, Heart rate
Yan et al. (2014)[39]	✓	Randomised crossover study	23	0%	Cigarette	36 hours	Heart rate, Systolic pressure, Diastolic pressure

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Table 2 (*continued*)

Espinoza-Derout et al. (2019)[53]	×	Non-randomised controlled trial	Unclear	0%	Nicotine-free e-cigarette/ saline aerosol	NA	Cardiac function, gene activation (apoptotic, inflammatory, fibrotic and remodelling genes), Reactive oxygen species production, DNA damage, atherosclerosis
Kaisar et al. (2017)[58]	×	Non-randomised controlled trial	18	0%	Cigarette	NA	Vascular inflammation^ Thrombomodulin
Lee et al. (2018)[38]	×	Randomised controlled trial	20	0%	Filtered air control	NA	Cardiac mutagens (O^6-methyldeoxyguanosines, γ-hydroxy-1,N2-propano-deoxyguanosines)
Olfert et al. (2017)[36]	×	Randomised controlled trial	45	17.8%[$]	cigarette; filtered air	NA	Arterial stiffness; Arterial response to vasoactive compounds; Cardiac function
Qasim et al. (2018)[60]	×	Non-randomised controlled trial	>16	0%	filtered air	NA	Haemostasis, platelet count, platelet activation/adhesion, thrombogenesis
Shi et al. (2019)[51]	×	Non-randomised controlled trial	35	0%	Room air	NA	Heart rate, Heart weight, angiogenic markers, vascular fibrosis markers

*Subjects who underwent sham smoking not mentioned in methods – Unclear if part of 40 original subjects or an additional 8 subjects.
**Study design referred to enrolment of 6 regular e-cigarette smokers also but limited information is provided about these participants.
[#]Arterial stiffness measured by Stiffness Index (SI) and Reflection Index (RI).
+ serum NOX2-derived peptide, serum nitric oxide and 8-Iso-prostaglandin F2a, serum vitamin E, flow mediated dilatation (FMD).
^ Measured *via* PECAM-1, VCAM-1 and ICAM-1 markers.
$ Subject attrition due to expected deaths associated with long-term murine studies.
Grey highlight indicates animal study.

3.5.7. Vascular function

Kaisar et al. reported significant increases in three markers of vascular inflammation (PECAM-1, VCAM-1, ICAM-1) after e-cigarette vapour inhalation (Kaisar et al., 2017). *Espinoza-Derout* et al. identified increased expression of inflammatory and apoptotic genes (Espinoza-Derout et al., 2019) associated with atherosclerotic lesion formation (Morton and Barnes, 1982) and ROS-induced heart failure (Nojiri et al., 2006). *Olfert* et al. reported significant increases in pulse wave velocity (a measure of arterial stiffness associated with endothelial dysfunction (Ohkuma et al., 2017)) in mice after long-term e-cigarette vapour inhalation. Furthermore, vapour inhalation led to an increased aortic vasoconstrictive response to (the vasoconstrictor) phenylephrine and a reduced aortic vasodilatory response to (the vasodilator) methacholine compared to mice exposed to filtered air as a control. These vascular dysfunctions may also be associated with increased risk of hypertension (Tzemos et al., 2015). No significant difference in aortic vasodilation was identified however in response to (the vasodilator) nitroprusside between mice exposed to e-cigarette vapour and filtered air. Urine cotinine (a nicotine biomarker) level in mice exposed to e-cigarette vapour was approximately half that of those exposed to cigarette smoke, yet vascular damage was similar, suggesting a role for mechanisms other than those involving nicotine (Olfert et al., 2017). *Shi et al*, identified a significant increase in angiogenesis, which could ultimately contribute to atherogenesis (Shi et al., 2019). Most notably, *Espinoza-Derout* et al. identified statistically significant increases in atherosclerotic plaque formation in mice exposed to eCAE compared to

Table 3
Interventions in *in vitro* studies (n = 8).

Author (year)	E-cigarette brand	Cigarette brand	Generation of device	Electrical characteristics of device	Coil temperature	Measured chemical profile	Flavours	Nicotine concentration in solution	Intervention regimen	Use of filter
Anderson et al. (2016)	Green smoke, Blu, Njoy, Vuse	3R4F*	1st	Not Measured	Not measured	Not measured	+	500 μM	2 x (2 s puff / min), Total vol = 55 ml	0.22 μm
Barber et al. (2017)	NJoy OneJoy, eGO OKC	Marlboro (1.2% nicotine)	1st	Not Measured	Not measured	Not measured	+	1.2%, 1.8%, 0 mg, 12 mg, 18 mg	2 x (5 s puff / min) 5 min total length	No
Hom et al. (2016)	Njoy OneJoy, eGO OKC	NS	1st & Non-1st	Not Measured	Not measured	Not measured	+	1.2%, + 1.8%, + 0 mg, 12 mg, + 18 mg, +	NS	No
Lee et al. (2019)	Freedom smoke USA, Johnson Creek, and E liquid market E-cigarette Containing RY4-flavored e-liquid (Changning Dekang) with 16 mg/ml nicotine	Marlboro	NS	Not measured	Not measured	Not measured	+	0 mg/ml, 6 mg/ml, 18 mg/ml	e-cig: 10 min (1 puff every 30 s, each puff lasting 2 s) Combustible cigarette: 10 min or Until the cigarette went out, by taking 2 puffs/min, Each puff lasting 2 s	No
Putzhammer et al. (2016)	Unknown brand High-nicotine	NS	1st & non-1st?	Not measured	116 +/− 3.9 °C In 10 s Of activation	Not measured	+	0 mg/ml 6 mg/ml, 9 mg/ml, 12 mg/ml, 18 mg/ml, 24 mg/ml, Not specified	1 s coil pre-activation, 20 x (2 s puff / 30 s)	0.2 μm
Schweitzer et al. (2015)	iClear v6 Filtered	2R4F*	NS	Not measured	Not measured	Propylene glycol, glycerol, Flavours, Acrolein#	NS	2.5 mM, 3.5 mM, 5 mM, 10 mM	1 cigarette / min, Similar rate for e-cigarette	0.2 μm
Taylor et al. (2017)	Vype ePen, Vype eStick,	3R4F*	1st (eStick) & 2nd (ePen)	3.7v (eStick), 4.0v (ePen)	Not measured	Not measured	+	36 mg/mL, 18 mg/mL	E-cigarette: 2 s puff /30s Vol = 55 mL Cigarette: 3 s puff /30s Vol = 55 mL	No
Teasdale et al. (2016)	iStick, Aerotank, Haven fluid	Marlboro gold (0.6 mg nicotine)	NS	4.2v, 10.8w, 1.8 Ω	Not measured (constant flow rate to minimise temperature spikes)	Not measured	–	18 mg/mL	5 x (5 s / 15 s) Air rate 70 ml/min (same as cigarette)	0.2 μm

*Research reference cigarette # confirmed by Nuclear Magnetic Resonance (NMR) and high-resolution mass spectroscopy + Platelets were exposed to nicotine concentrations similar to those delivered blood concentration from electronic cigarette use.

aerosol control (Espinoza-Derout et al., 2019).

3.5.8. Platelet function and haemostasis

Qasim et al. reported statistically significant increases in platelet aggregation, alpha particle secretion, dense particle secretion, platelet-integrin activation and platelet resistance to inhibition by prostacyclin but not platelet count following eCAE exposure. They also identified significant decreases in bleeding time (indicative of increased haemostasis) and occlusion time (indicative of increased thrombogenesis) (Qasim et al., 2018), whilst *Kaisar* et al. reported statistically significant decreases in circulating thrombomodulin in mice - a molecule protective against thrombosis (Kaisar et al., 2017).

3.6. Study results: human studies

3.6.1. Sympathetic nerve activation

18 studies measured heart rate as a biomarker of high sympathetic nerve activation – a state associated with increased cardiovascular risk (Middlekauff et al., 2014). Most studies reported increases (n = 14) (Cooke et al., 2015; Yan and D'Ruiz, 2015; Fogt et al., 2016; Moheimani et al., 2017a; Franzen et al., 2018; Ikonomidis et al., 2018; Chaumont et al., 2018; Sumartiningsih et al., 2019; Antoniewicz et al., 2019; Farsalinos et al., 2014a; Vlachopoulos et al., 2016; Szoltysek-Boldys et al., 2014; Vansickel et al., 2010; Kerr et al., 2018), and some decreases (n = 2) (D'Ruiz et al., 2017; Farsalinos et al., 2016) after e-cigarette use. Seven of these studies reported statistically insignificant changes (Cravo et al., 2016; Farsalinos et al., 2016; Fogt et al., 2016;

Table 4
Interventions in human (n = 24) and animal experimental studies (n = 6).

Author & Year	e-cigarette brand	Generation of device	Electrical characteristics of device	Measured Chemical profile	Declared Chemical profile	Nicotine concentration in solution	Nicotine delivery to bloodstream	Intervention regimen	Method of assessing in-trial abstinence
Antoniewicz et al. (2019)[48]	variable mod, eVic-VT, Shenzhen Joyetech Co., Ltd., China	3rd	32 W	Not measured	Propylene glycol, vegetable glycerin, ethanol without flavorings	19 mg/ml, 0 mg/ml	Not measured	30 x 3 s puffs / 30 min	Urinary cotinine
Antoniewicz et al. (2016)[40]	Valeo laboratories (aerosol), eGO XL (device)	2nd	3.7 V	Propylene glycol, glycerol, ethanol	-	12 mg/ml	Not measured	10 x (10 puffs / min) (adjusted to cigarette)	Urinary cotinine
Biondi-Zoccai et al. (2019)[47]	Blu Pro, Fontem, Netherlands	NS	NS	-	-	16mg	Serum cotinine	9 puffs	Serum cotinine
Carnevale et al. (2016)[62]	NS	NS	NS	Not measured	Tobacco flavour	16mg	Not measured	9 puffs	Not measured
Chatterjee et al (2019)[61]	E-puffer eco disposable e-cigs	NS	3.7 V	Not measured	Pharma-grade propylene glycol, vegetable glycerine	0 mg/ml	Not measured	16-17 x 2 s puffs / 3 min	Not measured
Chaumont et al. (2018)[45]	Smok Alien 220 box mod	4th	60w	Propylene glycol, vegetable glycerin10i buse10e (Purpose-made liquid)	Propylene glycol, vegetable glycerin	3mg/ml	Serum nicotine assessment	25 puffs (4s puff / 30 s)	Urinary cotinine & eCO < 5 ppm
Cooke et al. (2015)[35]	Green Smart Living, Clean Electronic Cigarettes	1st; NS	NS	Not measured	NS	18 mg; 0 mg	Not measured	20 x (1 puff / 30 s)	Urinary cotinine
Cravo et al. (2016)[32]	Fontem Ventures	NS	3.0-4.2V	Not measured	Propylene glycol, Glycerol, Water, Menthol & Tobacco flavours	2.0%	Not measured	Expected 40 – 60 puffs	Not measured
D'Ruiz et al. (2017)[33]	Blu	NS	NS	Glycerol, Propylene glycol, tobacco & cherry flavour	-	24 mg/mL	Estimated from nicotine % and volume of aerosol used	ad libitum use & 15 min prior to tests	eCO < 12 ppm
Eissenberg et al. (2010)[63]	Njoy NPRO, Crown Seven Hydro	1st	NS	Not measured	Menthol flavour, Tobacco flavour	16 mg, 18mg	No significant change from baseline,	10 x (Puff ad libitum / 30 s), continuous heart rate monitoring	Not measured
Farsalinos et al. (2014)[56]	e-Go Nobacco, e-Go Alter Ego	2nd	3.5V	propylene glycol, linalool, tobacco essence, methyl vanillin	-	11 mg/ml	Not measured	E-cigarette: 7 min ad 11i buse Cigarette: smoke 1 cigarette	Not measured
Farsalinos et al. (2016)[37]	Categoric model 401	1st	3.7V	-*	Sweet tobacco aroma	2.4%, 1.8%, 0%	Not measured	Week 1 -12: Vape Ad libitum	eCO ≤ 7 ppm
								Week 13 – 52: Smoke and vape ad libitum	
Fogt et al. (2016)[41]	Green Smart Living	1st	NS	Not measured	NS	18 mg/ml; 0mg/ml	Estimated by urine cotinine	Inhaled every 30s / 10 minute	Not measured
Franzen et al. (2018)[43]	eGo-T CE4 vaporizer	3rd	3.3V, 1.5 ohms, 7.26w	Not measured	Propylene glycol, glycerine, tobacco flavouring	24 mg/ml; 0mg/ml	Not measured	1 puff every 30s / 10 puffs	eCO < 6 ppm
Ikonomidis et al. (2018)[44]	NOBACCO eGo Epsilon BDC 1100	NS	3.9V	Not measured	Propylene glycol, glycerine, flavouring	12 mg/ml	Not measured	NS	Self-reported

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Table 4 (*continued*)

Kerr et al. (2018)[67]	SmokeMax	2nd	3.3V	Propylene glycol, glvcerine, vanillin, furaneol, ethyl vanillin	Propylene glycol, glvcerine, vanillin, furaneol, ethyl vanillin	17.27 mg/ml	Not measured	15 puffs	Self-reported
Moheimani et al. (2017)[42]	Greensmoke cig-a-like, e-Go One	1st, 2nd	NS; 1 ohm	Not measured	Vegetable glycerine, propylene glycol, tobacco flavouring, strawberry flavouring	0%, 1.2%	calculated by plasma nicotine	3s inhale, 3s hold and 3s exhale / 30s	Not measured
Nocella et al. (2018)[66]	NS	NS	NS	Not measured	Tobacco flavouring	0.6 mg	Serum cotinine analysis	9 puffs	Serum cotinine
Pywell et al. (2018)[68]	NS	NS	NS	NS	NS	0 mg 24 mg	Not measured	1 puff / 30 seconds for 5 minutes (not tolerated so switched to ab libitum	Not assessed
Sumartiningsih et al. (2019)[46]	NS	NS	NS	Not measured	NS	0 mg/mL 3 mg/mL	Not measured	NS	Not measured
Szołtysek-Bołdys et al. (2014)[64]	Volish e-Go 3	2nd	3.4V, 2.4 ohms	Not measured	NS	24 mg/mL	Not measured	15x (1.8 s puff – 17 s interval)	eCO < 7 ppm
Vansickel et al. (2010)[65]	Njoy NPRO; Crown Seven Hydro	1st	NS	Not measured	Propylene glycol, Glycerol, Ethanol, Water, Acetylpyrazine Guaiacol, Mysomine, Cotinine, Vanillin, Tobacco flavour	16 mg, 18mg	No significant change from baseline	10 puffs, 30 s interval	eCO ≤10 ppm
Vlachopoulos et al. (2016)[57]	NS	NS	NS	Not measured	NS	NS	Not measured	5 mins e-cigarette use or 30 mins e-cigarette use or smoke 1 cigarette	NS
Yan et al. (2014)[39]	Blu disposable, Blu rechargeable	NS	NS	Not measured	Glycerol, Propylene glycol, Flavours, Distilled water, Citric acid	1.6%, 2.4%	Increased plasma nicotine in e-cigarette A,B,C & E.	e-cigarette: 50 x (5 s puffs / 30 s) & ad lib use / 1 hr 1 cigarette:	eCO ≤ 12 ppm+
							Insignificant increase in e-cigarette D	(normal puff duration / 30s)	
Espinoza-Derout et al. (2019)[53]	BluCig PLUS	NS	NS	Not measured	Propylene glycol, glycerol, classic tobacco flavour (nicotine-containing e-cigarette), gold leaf flavour (nicotine-free e-cigarette)	2.4%, 0%	Estimated via plasma cotinine	24 x 4s puff with 25s break / 12 hours for 12 weeks	NA
Kaisar et al. (2017)[58]	Blu	NS	NS	Not measured	NS	24 mg/mL	Not measured	35 ml total vol, 2s puffs / 60s 6 times / day for 2 weeks.*	NA
Lee et al. (2018)[38]	NJOY	NS	4.2V	Not measured	Propylene glycol, vegetable glycerine mixture	10 mg/mL	Not measured	35-mL puff volumes of 4-s duration at 30-s intervals	NA
Olfert et al. (2017)[36]	eGrip OLED, Joyetech	3rd	4.8V	Not measured	Cappuccino Flavour	18 mg/mL	Estimated via urine cotinine	(5s puff every 99s for 1hr) 4 x day /8 months	NA

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Table 4 (*continued*)

Qasim et al. (2018)[60]	Absolute Zero e-liquid	NS	5V, 0.4 ohm	Not measured	Propylene glycol, vegetable glycerine, menthol flavour	18mg/mL	Serum cotinine analysis	2 x 200 puffs/day 5 days/1 week	NA
Shi et al. (2019)[51]	NA	NA	NS	Not measured	propylene glycol, glycerin	24 mg/ml	plasma cotinine analysis	1 puff per minute and duration of 10 seconds per puff 3 hours per day, 10 min break every hour for 14 days	NA

*Paper includes broken hyperlink to toxicological report # measured every 2 weeks for 6 weeks, then after 12 weeks and finally after another 24 weeks + subject removal left to discretion of investigator.

Grey highlight indicates animal study.

Table 5
Outcome results for *in vitro* studies (n = 8).

Author (year)	Outcome measure	E-cigarette	Significance of E-cigarette	Cigarette	Significance of cigarette
Hom et al. (2016))	Platelet aggregation	Increased	$P < 0.05$	Increased	$P < 0.05$
	Platelet adhesion	Increased	$P < 0.05$	Increased	$P < 0.05$
	Platelet activation	Increased	$P < 0.05$	Increased	$P < 0.05$
	Platelet c1q complement deposition	Not increased	P = NS	–	–
	Platelet c3b complement deposition	Increased	$P < 0.05$	–	–
	Platelet c4d complement deposition	Not increased	P = NS	–	–
	Platelet c5b-9 complement deposition	Not increased	P = NS	–	–
Barber et al. (2017)	Endothelial C1q complement deposition	Increased	$P < 0.05$	Increased	P = NS
	Endothelial C3b complement deposition	Increased	(2/5) $P < 0.05$	Increased	P = NS
	Endothelial gC1qR expression	Increased	$P < 0.05$	Increased	$P < 0.05$
	Endothelial cC1qR expression	Increased	$P < 0.05$	Increased	$P < 0.05$
	Endothelial complement inhibitor (CD35) expression	Increased	(2/5) $P < 0.05$	Increased	$P < 0.05$
	Endothelial complement inhibitor (CD55) expression	Increased	(1/5) $P < 0.05$	Increased	P = NS
	Endothelial complement inhibitor (CD59) expression	Increased	(3/5) $P < 0.05$	Increased	P = NS
Anderson et al. (2016)	Cell viability	Reduced	$P < 0.001$	Reduced	$P < 0.001$
Barber et al. (2017)	Cell viability	Reduced	(4/5) $P < 0.05$	Reduced	P = NS
Lee et al. (2019)	Cell viability	Reduced	$P < 0.05$	–	–
	Reactive oxidative species (H_2O_2 levels)	Increased	$P < 0.05$	Increased	$P < 0.05$
	Apoptosis (caspases 3/7 activity)	Increased	$p < 0.05$	–	–
	Endothelial function (tube formation)	Increased	$P < 0.05$	Increased	$P < 0.05$
	Endothelial function (LDL and lipid uptake)	Increased	p = ???	–	–
	Endothelial function (cell migration)	Decreased	$p < 0.001$	–	–
	Cross-talk between endothelial cells & macrophages (macrophage dual polarisation)	Increased	$P < 0.05$	–	–
	Cross-talk between endothelial cells & macrophages (macrophage cytokine production)	Increased	$P < 0.05$ (Marcado e-liquid)	–	–
	Cross-talk between endothelial cells & macrophages (ROS production)	Increased (for e-liquid	$P < 0.05$ (Marcado e-liquid)	–	–
	Transcriptome Of iPSC-ECs	Affected	$P < 0.05$ (Marcado e-liquid)	–	–
Putzhammer et al. (2016)	Cell viability	Reduced	(5 / 11) $P < 0.05$	Reduced	$P < 0.001$
Anderson et al. (2016))	DNA damage	Detected	–	Detected	–
Putzhammer et al. (2016)	Cell morphological alterations	Detected	–	Detected	–
Barber et al. (2017)	Cell density	Reduced	$P < 0.05$	Reduced	$P < 0.05$
Putzhammer et al. (2016)	Cell proliferation	Reduced	(4 / 11) $P < 0.001$	Reduced	$P < 0.001$
Taylor et al. (2017)	Cell migration	Reduced	P = NS	Reduced	$P < 0.05$
Anderson et al. (2016)	Reactive oxygen species	Increased	$P < 0.001$	Increased	$P < 0.001$
Putzhammer et al. (2016)	Reactive oxygen species	Increased	(1 / 11) $P < 0.001$	Increased	$P < 0.001$
Teasdale et al. (2016)	Upregulation of genetic markers of oxidative stress*	No	P = NS	Yes	$P < 0.05$
Barber et al. (2017))	Cellular metabolic activity	Reduced	$P < 0.05$	Reduced	$P < 0.05$
Schweitzer et al. (2015))	Endothelial barrier disruption#	Yes	$P < 0.0001$	Yes	$P < 0.0001$

*(HMOX1, GCLM, OSGIN1, PAR4, CYP1A1, CYP1B1, IL8 and NTPX1) # only for 5 mM and 10 mM not 2.5 mM or 3.5 mM solution. ^ 2 samples also showed statistically significant increases.

Table 6
Outcomes in human (n = 24) and animal experimental studies (n = 6).

Author (Year)	Outcome measure	E-cigarette	Significance of e-cigarette	Cigarette	Significance of cigarette	Control
Antoniewicz et al. (2019)[48]	Heart rate	Increased	P = 0.001	-	-	No change
Chaumont et al. (2018)[45]	Heart rate	Increased	P < 0.0001	-	-	-
Cooke et al. (2015)[35]	Heart rate	Increased	P ≤ 0.03 +	-	P = NS	Decreased
Cravo et al. (2016)[32]	Heart rate	No raw data	P = NS	Not stated	-	-
D'Ruiz et al. (2017)[33]	Heart rate	Decreased	(1/3) P = 0.0207	-	P = 0.0483	Decreased
Eissenberg et al. (2010)[63]	Heart rate	No raw data	P = NS	No raw data	P < 0.05	-
Farsalinos et al. (2014)[56]	Heart rate	Increased	P = 0.001 +	-	P < 0.001	Increased
Farsalinos et al. (2016)[37]	Heart rate	Decreased	P = NS	-	-	-
Fogt et al. (2016)[41]	Heart rate	Increased	P=NS+	-	-	Increased
Franzen et al. (2018)[43]	Heart Rate	Increased	P < 0.05	Increased	P < 0,05	No change
Ikonomidis et al. (2018)[44]	Heart Rate	Increased	P = NS	Increased	P = NS	No change
Kerr et al. (2018)[67]	Heart Rate	Increased	P < 0.001	Increased	P < 0.001	-
Moheimani et al. (2017)[42]	Heart rate	Increased	P=0.03, P=0.01+ P=0.002@	-	-	Increased
Sumartiningsih et al. (2019)[46]	Heart Rate	Increased	P < 0.05	Increased	P < 0.05	Increased
Szołtysek-Bołdys et al. (2014)[64]	Heart rate	Increased	P = NS	No raw data	P = NS	-
Vansickel et al. (2010)[65]	Heart rate	Increased	P = NS	Increased	P < 0.05	No raw data
Vlachopoulos et al. (2016)[57]	Heart rate	Increased	(5-minute use) P = 0.57 (30-minute use) P < 0.05	Increased	P < 0.05	-
Yan et al. (2014)[39]	Heart rate	Increased	(2/5) P < 0.01	Increased	P = 0.001	-
Moheimani et al. (2017)[42]	Cardiac vagal tone (HRV)	Decreased	P = 0.03+ P = 0.009@	-	-	Decreased
	Sympathetic tone (HRV)	Increased	P = 0.003+ P = 0.01	-	-	Decreased
Sumartiningsih et al. (2019)[46]	SDNN (ms) (exercising HRV)	Increased	P < 0.05+	Increased	P < 0.05+	-
	RMSSD (ms) (exercising HRV)	Increased	P < 0.05+	Increased	P < 0.05+	-
Farsalinos et al. (2014)[56]	Early diastolic peak velocity $	Increased	P = NS	Decreased	P < 0.001	-
	Early diastolic strain rate $	Increased	P = NS	Decreased	P < 0.001	-
	Isovolumetric relaxation time – HR corrected $	Decreased	P = NS	Increased	P < 0.001	-
	Myocardial Performance Index $	Decreased	P = NS	Increased	P = 0.002	-
Franzen et al. (2018)[43]	Central systolic pressure	Increased	P = NS	Increased	P = NS	No change
Ikonomidis et al. (2018)[44]	Central systolic pressure	Decreased	P = NS	Decreased	P = NS	No change

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Table 6 (*continued*)

Antoniewicz et al. (2019)[48]	Systolic pressure	Increased	P = 0.227	-	-	Increased
Biondi-Zoccai et al. (2019)[47]	Systolic pressure	Increased	P < 0.001	Increased	P < 0.001	-
Chaumont et al. (2018)[45]	Systolic pressure	Increased	P < 0.0001	-	-	-
Cooke et al. (2015)[35]	Systolic pressure	Increased	P ≥ 0.05	-	-	Decreased
Cravo et al. (2016)[32]	Systolic pressure	No raw data	P = NS	No raw data	Not stated	-
D'Ruiz et al. (2017)[33]	Systolic pressure	Decreased	(1/3) P = 0.0079	-	-	Decreased
Farsalinos et al. (2014)[56]	Systolic pressure	Increased	P = NS	Increased	P < 0.001	-
Farsalinos et al. (2016)[37]	Systolic pressure	Decreased	P = 0.001	-	-	-
Fogt et al. (2016)[41]	Systolic pressure	Decreased	P = 0.04+	-	-	Increased
Franzen et al. (2018)[43]	Systolic pressure	Increased	P < 0.05	Increased	P < 0.05	No change
Kerr et al. (2018)[67]	Systolic pressure	Decreased	P = NS	Increased	P = NS	-
Moheimani et al. (2017)[42]	Systolic pressure	Increased	P = NS	-	-	Decreased
Sumartiningsih et al. (2019)[46]	Systolic pressure	Increased	P < 0.05	Increased	P < 0.05	Increased
Szołtysek-Bołdys et al. (2014)[64]	Systolic pressure	Increased	P = NS	Increased	P = NS	-
Vlachopoulos et al. (2016)[57]	Systolic pressure	Increased	(5-minute use) P < 0.05 (30-minute use) P < 0.01	Increased	P < 0.01	-
Yan et al. (2014)[39]	Systolic pressure	Increased	(1/5) P = 0.02	Increased	P < 0.04	-
Fogt et al. (2016)[41]	Exercising systolic pressure	Increased	P=NS+	-	-	Increased
Franzen et al. (2018)[43]	Central diastolic pressure	Increased	P = NS	Increased	P = NS	Decreased
Antoniewicz et al. (2019)[48]	Diastolic pressure	Increased	P = 0.062	-	-	Increased
Biondi-Zoccai et al. (2019)[47]	Diastolic pressure	Increased	P < 0.001	Increased	P < 0.001	-
Chaumont et al. (2018)[45]	Diastolic pressure	Increased	P < 0.0001	-	-	-
Cooke et al. (2015)[35]	Diastolic pressure	Increased	P = 0.001	-	-	Decreased
Cravo et al. (2016)[32]	Diastolic pressure	No raw data	P = NS	No raw data	Not stated	-
D'Ruiz et al. (2017)[33]	Diastolic pressure	Decreased	P < 0.0417	-	-	Decreased
Farsalinos et al. (2014)[56]	Diastolic pressure	Increased	P < 0.001	Increased	P < 0.001	-
Farsalinos et al. (2016)[37]	Diastolic pressure	Decreased	P = 0.02	-	-	-
Fogt et al. (2016)[41]	Diastolic pressure	Increased	P=0.04+	-	-	Increased
Franzen et al. (2018)[43]	Diastolic pressure	Increased	P = NS	Increased	P < 0.05	Decreased
Kerr et al. (2018)[67]	Diastolic pressure	No change	P = NS	Increased	P = NS	-
Moheimani et al. (2017)[42]	Diastolic pressure	Increased	P = NS	-	-	Decreased
Sumartiningsih et al. (2019)[46]	Diastolic pressure	No change	P = NS	Increased	P < 0.05+	No change
Szołtysek-Bołdys et al. (2014)[64]	Diastolic pressure	Decreased	P = NS	Increased	P = NS	-
Vlachopoulos et al. (2016)[57]	Diastolic pressure	Increased	Not stated	Increased	Not stated	

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Table 6 (*continued*)

Yan et al. (2014)[39]	Diastolic pressure	Increased	P < 0.005	Increased	P = 0.00014	-
Fogt et al. (2016)[41]	Exercising diastolic pressure	Increased	P=0.02+	-	-	Increased
Pywell et al. (2018)[68]	Superficial microcirculation of the hand	Decreased	P < 0.05#	-	-	Increased
	Deep microcirculation of the hand	Decreased	P < 0.05#	-	-	Increased
Chaumont et al. (2018)[45]	(endothelial-dependent) vasodilatory response to acetylcholine	Decreased	P < 0.0001+	-	-	-
	(endothelial-independent) vasodilatory response to nitroprusside	Decreased	P = NS+	-	-	-
	Thermal hyperemia	No change	P = NS	-	-	-
	Augmentation Index 75 (Arterial Stiffness)	Increased	P = 0.013	-	-	-
Antoniewicz et al. (2019)[48]	Augmentation Index 75 (Arterial Stiffness)	Increased	P = 0.006	-	-	No change
Franzen et al. (2018)[43]	Augmentation Index 75 (Arterial Stiffness)	Increased	P = 0.001	Increased	P < 0.01	No change
Ikonomidis et al. (2018)[44]	Augmentation Index 75 (Arterial Stiffness)	Increased	P < 0.05	Increased	P < 0.05	No change
Szołtysek-Bołdys et al. (2014)[64]	Stiffness Index (Arterial Stiffness)	Increased	P = NS	Decreased	P = 0.0056	-
	Reflective Index (Arterial Stiffness)	Decreased	P = NS	Decreased	P = 0.01	-
Antoniewicz et al. (2019)[48]	Pulse wave velocity (Arterial Stiffness)	Increased	P = 0.037	-	-	No change
Chaumont et al. (2018)[45]	Pulse wave velocity (Arterial Stiffness)	Increased	P < 0.0001	-	-	-
Franzen et al. (2018)[43]	Pulse wave velocity (Arterial Stiffness)	Increased	P < 0.05	Increased	P < 0.01	No change
Ikonomidis et al. (2018)[44]	Pulse wave velocity (Arterial Stiffness)	Increased	P < 0.05	Increased	P < 0.05	No change
Vlachopoulos et al. (2016)[57]	Pulse wave velocity (Arterial Stiffness)	Increased	(5-minute use) P = NS (30-minute use) P = 0.002	Increased	P < 0.001	-
Antoniewicz et al. (2016)[40]	Endothelial progenitor cells	Increased	P = 0.003	-	-	No change
	All circulating Microvesicles	Increased	P = NS	-	-	Increased
	E-selectin positive microvesicles	Increased	P = 0.038	-	-	Increased

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Table 6 (*continued*)

Biondi-Zoccai et al. (2019)[47]	Levels of sNox2-dp, pg/mL (oxidative stress)	Increased	P < 0.001	Increased	P < 0.001	-
	H_2O_2 production, µlmol/L (oxidative stress)	Increased	P < 0.001	Increased	P < 0.001	-
	Levels of 8-iso-PGF2a, pmol/mL ((oxidative damage)	Increased	P < 0.001	Increased	P < 0.001	-
	Levels of vitamin E, µlmol/mmoL (antioxidant status)	Decreased	P < 0.001	Decreased	P < 0.001	-
	Levels of HBA, % (antioxidant status)	Decreased	P < 0.001	Decreased	P < 0.001	-
	Levels of sCD40L, ng/mL (platelet activation)	Increased	P < 0.001	Increased	P < 0.001	-
	Levels of soluble P-selectin, ng/mL (platelet activation)	Increased	P < 0.001	Increased	P < 0.001	-
	Flow mediated dilatation, % (endothelial dysfunction)	Decreased	P < 0.001	Decreased	P < 0.001	-
	NO bioavailability (antioxidant status)	Decreased	P = 0.006	Decreased	P = 0.006	-
Chatterjee et al (2019)[61]	C-reactive protein (inflammation)	Increased	P < 0.05	-	-	-
	Nitrogen oxide metabolites	Decreased	P < 0.005	-	-	-
	Soluble ICAM-1	Increased	P < 0.05	-	-	-
	Endothelium ICAM-1 expression (oxidative stress)	Increased	P < 0.001	-	-	-
	Endothelium ROS generation (oxidative stress)	Increased	P < 0.001	-	-	-
Kerr et al. (2018)[67]	Microparticles	Decreased	P = NS	Increased	P < 0.001	-
	Endothelial microparticles	No change	P = NS	Increased	P < 0.001	-
	Platelet microparticles	Increased	P < 0.001	Increased	P < 0.001	-
	P-selectin	Decreased	P = 0.026	Decreased	P = NS	-
	E-selectin	Decreased	P = NS	Decreased	P = NS	-
	Reactive hyperaemia index (endothelial function)	Increased	P = 0.006	Increased	P = NS	-
Nocella et al. (2018)[66]	Platelet aggregation	Increased	P ≤ 0.01	Increased	P ≤ 0.01	-
	P-selectin	Increased	P ≤ 0.01	Increased	P ≤ 0.01	-
	CD40L	Increased	P ≤ 0.01	Increased	P ≤ 0.01	-

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Table 6 (*continued*)

Chaumont et al. (2018)[45]	Plasma myeloperoxidase (oxidative stress)	Increased	P = 0.001	-	-	-
	Protein-bound 3-chlorotyrosine (oxidative stress)	Increased	P = NS	-	-	-
	Protein-bound homocitrulline (oxidative stress)	Increased	P = NS	-	-	-
Carnevale et al. (2016)[62]	sNOX2-dp, pg/mL (oxidative stress)	Increased	P < 0.001	Increased	P < 0.001	-
	8-iso-PGF2a, pmol/L (oxidative stress)	Increased	P < 0.001	Increased	P < 0.001	-
	NO bioavailability, μM (oxidative stress)	Decreased	P < 0.001	Decreased	P < 0.001	-
	Vitamin E, μmol/mmol (oxidative stress)	Decreased	P < 0.001	Decreased	P < 0.001	-
	FMD, % (endothelial dysfunction)	Decreased	P < 0.001	Decreased	P < 0.001	-
Ikonomidis et al. (2018)[44]	MDA (oxidative stress)	Increased	P < 0.05	Increased	P < 0.05	No change
Kerr et al. (2018)[67]	PECAM-1 &	Decreased	P = NS	Decreased	P = 0.028	-
	VCAM-1 &	Decreased	P = NS	Increased	P = NS	-
	ICAM-1 &	Decreased	P = NS	Decreased	P = NS	-
Moheimani et al. (2017)[42]	PON-1 &	Decreased	P = NS	-	-	Decreased

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Table 6 (*continued*)

	Left Ventricular Ejection Fracture	Decreased	P < 0.05+	-	-	No change
	Left Ventricular Fractional Shortening	Decreased	P < 0.01+	-	-	No change
	Velocity of Circumferential Fibre Shortening	Decreased	P < 0.01+	-	-	No change
	Left Ventricular Mass	Decreased	P = NS+	-	-	No change
	Left Ventricular Diastolic Functions	Decreased	P = NS+	-	-	No change
	Cardiac expression of CoI5a3 (inflammatory gene)	Increased	P < 0.05+	-	-	No change
Espinoza-Derout et al. (2019)[53]	Cardiac expression of TNFRF12A/Fn14 (ROS gene)	Increased	P < 0.05+	-	-	No change
	Cardiac expression of Selectin E (inflammatory gene)	Increased	Not Stated	-	-	No change
	Leucocyte extravasation signalling	Increased	Not Stated	-	-	No change
	Cardiac expression of Harakiri mRNA (pro-apoptotic gene)	Increased	P < 0.01+	-	-	No change
	Cardiac expression of Wisp2/CCN5 (cardioprotective gene)	Decreased	P < 0.05+	-	-	No change
	Collagen type I/III ratio mRNA (fibrotic marker)	Not present	NA	-	-	Not present
	Cardiomyocyte nuclear abnormalities	Present	NA	-	-	Not present
	Cardiomyocyte cytoplasmic abnormalities	Present	NA	-	-	Not present
	Intramyocardial lipid accumulation	Present	NA	-	-	Not present
	MDA (oxidative stress)	Increased	P < 0.05+	-	-	No change
	Cardiac mitochondrial DNA damage	Increased	P < 0.01+	-	-	No change
	Atherosclerotic lesion formation	Increased	P < 0.01+			Increased
Kaisar et al. (2017)[58]	PECAM-1 &	Increased	P < 0.05	Increased	P < 0.05	-
	VCAM-1 &	Increased	P < 0.05	Increased	P < 0.05	-
	ICAM-1 &	Increased	P < 0.05	Increased	P < 0.01	-
	Thrombomodulin (anticoagulant)	Decreased	P < 0.0001	Decreased	P < 0.0001	-
Lee et al. (2018)[38]	O^6-methyldeoxyguanosines (cardiac mutagen)	Increased	P < 0.001+	-	-	-
	γ-hydroxy-1,N2-propano-deoxyguanosines (cardiac mutagen)	Increased	P < 0.0001+	-	-	-

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Table 6 (*continued*)

Olfert et al. (2017)[36]	Pulse Wave Velocity (Arterial Stiffness)	Increased	P < 0.05+	Increased	P < 0.05+	-
	Aortic vasoconstrictive response to phenylephrine	Increased	P < 0.05+	Increased	P < 0.05+	-
	Aortic vasodilatory response to methacholine	Decreased	P < 0.05+	Reduced	P < 0.05+	-
	Aortic vasodilatory response to nitroprusside	Normal response	P = NS+	Normal response	P = NS+	-
	Left ventricular mass	Increased	P<0.05¶	No change	-	-
	% Fractional shortening	Decreased	P=NS	Decreased	P < 0.04	-
	% Ejection fracture	Decreased	P=NS	Decreased	P < 0.01	-
Qasim et al. (2018)[60]	Bleeding time (haemostasis)	Decreased	P < 0.01+	-	-	-
	Occlusion time (thrombogenesis)	Decreased	P < 0.01+	-	-	-
	Platelet count	No change	P = NS+	-	-	-
	Platelet aggregation	Increased	Not stated+	-	-	-
	Platelet alpha particle secretion	Increased	P < 0.01+	-	-	-
	Platelet dense particle secretion	Increased	P < 0.01+	-	-	-
	Platelet integrin activation	Increased	P < 0.05+	-	-	-
	Platelet resistance to inhibition by prostacyclin	Increased	P < 0.001+	-	-	-

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Table 6 (*continued*)

Shi et al. (2019)[51]	End diastolic dimension (EDD)	Unchanged	P = NS	-	-	Unchanged
	End systolic dimension (ESD)	Unchanged	P = NS	-	-	Unchanged
	Heart rate	Decreased	P < 0.01	-	-	Unchanged
	Ejection fraction	Decreased	P = NS	-	-	Unchanged
	Aorta dimension	Unchanged	P = NS	-	-	Unchanged
	Heart weight	Decreased (Female > Male)	P = NS +	-	-	-
	Collagen I protein and α-SMA expression (cardiac fibrosis)	Mixed picture	P = NS	-	-	-
	Masson's Trichrome staining for cardiac fibrosis	% (male and female)	P = NS	-	-	-
	Immuno-fluorescent staining of CD31 (heart tissue angiogenesis).	Increased	P = 0.01+	-	-	-
	Immuno-fluorescent staining of CD34 (heart tissue angiogenesis).	Increased	P = 0.03+	-	-	-
	ELISA measurement of plasma VEGF	Unchanged	P = NSNS	-	-	-

Significant for smokers but not for non-smokers + Significance against control ^ Dual use $ Myocardial function & Markers of vascular inflammation. Grey highlight indicates animal study. ¶ Significance against cigarette @analysis includes only those with measurable cotinine levels.

Ikonomidis et al., 2018; Eissenberg, 2010; Szoltysek-Boldys et al., 2014; Vansickel et al., 2010), and one reported clinically insignificant changes (Cravo et al., 2016).

17 studies investigated resting blood pressure as a proxy for sympathetic nerve activation. These found both increases (n = 10) (Cooke et al., 2015; Yan and D'Ruiz, 2015; Moheimani et al., 2017a; Franzen et al., 2018; Chaumont et al., 2018; Sumartiningsih et al., 2019; Biondi-Zoccai et al., 2019; Farsalinos et al., 2014a; Vlachopoulos et al., 2016; Szoltysek-Boldys et al., 2014) and decreases (n = 4) (D'Ruiz et al., 2017; Farsalinos et al., 2016; Fogt et al., 2016; Kerr et al., 2018) in systolic pressure and increases (n = 9) (Cooke et al., 2015; Yan and D'Ruiz, 2015; Fogt et al., 2016; Moheimani et al., 2017a; Franzen et al., 2018; Chaumont et al., 2018; Biondi-Zoccai et al., 2019; Farsalinos et al., 2014a; Vlachopoulos et al., 2016) and decreases (n = 3) (D'Ruiz et al., 2017; Farsalinos et al., 2016; Szoltysek-Boldys et al., 2014) in diastolic pressure, with differing degrees of significance. *Fogt* et al. assessed the effect of e-cigarette use on exercising peripheral blood pressure, identifying significant increases in systolic pressure compared to nicotine-free e-cigarettes (Fogt et al., 2016). *Pywell* et al. investigated the microcirculation of the hand following e-cigarette use, identifying statistically significant decreases in both superficial and deep flow, potentially associated with worse microvascular surgical outcomes (Pywell et al., 2018).

Assessments of acute changes in heart rate and blood pressure have limited prognostic value. Therefore, *Moheimani* et al. investigated measures of abnormal heart rate variability (HRV), (a better proxy for cardiac sympathetic nerve activity), associated with increased cardiovascular mortality in individuals with known and unknown cardiovascular morbidity (Kleiger et al., 1987; Hillebrand et al., 2013; Tsuji et al., 1996). They identified a statistically significant decrease in cardiac vagal tone and an increase in sympathetic tone after e-cigarette use (Moheimani et al., 2017a), whilst *Sumartiningsih* et al. identified significant increases in exercising HRV after vaping compared to the control group (Sumartiningsih et al., 2019). Finally, *Farsalinos* et al. identified no significant effect of vaping on myocardial function after very brief exposure (Farsalinos et al., 2014a).

3.6.2. Oxidative stress

Oxidative stress is an important mechanism in the development of atherosclerosis from cigarette smoking (Csordas and Bernhard, 2013). Two studies found significant increases in two ROS (sNOx2-dp and iso-PGF2a) and a significant decreases in vitamin E levels and nitrogen oxide bioavailability, which are protective against ROS (Biondi-Zoccai et al., 2019; Carnevale et al., 2016). *Biondi-Zoccai* et al. also identified significant increases in the ROS hydrogen peroxide (H_2O_2) and significant decreases in HBA% (protective H_2O_2 breakdown activity) (Biondi-Zoccai et al., 2019). *Ikonomidis* et al. found significant oxidative stress after e-cigarette use, as measured by malondialdehyde (MDA) (Ikonomidis et al., 2018); however *Moheimani* et al. did not find any significant acute effect of e-cigarette use on oxidative stress burden, as measured by paraoxonase-1 (PON-1) activity (Moheimani et al., 2017a). *Chaumont* et al. identified significant increases in plasma concentrations of myeloperoxidase, an enzyme involved in oxidative stress pathways (Chaumont et al., 2018), which has been associated with increased cardiovascular risk (Rudolph et al., 2012). However, no significant increases were identified in the oxidative stress-associated protein-bound 3-chlorotyrosine or homocitrulline (Chaumont et al., 2018). Two studies found significant increases in circulating CD40L

after e-cigarette use, which leads to endothelial cell activation and the production of ROS (Biondi-Zoccai et al., 2019; Nocella et al., 2018). Finally, *Chatterjee* et al. found significant increases in ROS generation and C-reactive protein (a biomarker of inflammatory processes including atherothrombosis) (Ridker, 2003), as well as significant decreases in (protective) NO metabolites after vaping (Chatterjee et al., 2019).

3.6.3. Endothelial function

Endothelial dysfunction is prognostic of atherosclerosis (Shechter et al., 2014). Studies reported various measures of arterial stiffness, indicative of endothelial dysfunction. *Carnevale* et al. measured arterial flow-mediated dilatation (FMD) finding significant impairment (Carnevale et al., 2016). However, *Szołtysek-Bołdys* et al. reported insignificant changes in arterial stiffness index (SI) and reflection index (RI) after e-cigarette use (Szoltysek-Boldys et al., 2014). Four studies identified significant increases in augmentation index normalised to a heart rate of 75 beats per minute (AIx75) (Franzen et al., 2018; Ikonomidis et al., 2018; Chaumont et al., 2018; Antoniewicz et al., 2019), whilst five studies reported significant increases in pulse wave velocity (PWV) after e-cigarette use (Franzen et al., 2018; Ikonomidis et al., 2018; Chaumont et al., 2018; Antoniewicz et al., 2019; Vlachopoulos et al., 2016).

Kerr et al. -found an increase in reactive hyperaemia index (Kerr et al., 2018), an indicator of endothelial dysfunction (Bonetti et al., 2004), whilst *Antoniewicz* et al. found significant increases in circulating reparative endothelial progenitor cells (EPCs) - suggesting vascular injury from vaping (Antoniewicz et al., 2016). *Chatterjee* et al. found significant increases in soluble and endothelial ICAM-1 (an adhesion molecule involved in endothelial activation and dysfunction) (Chatterjee et al., 2019), however *Kerr* et al. did not (Kerr et al., 2018). Finally, *Chaumont* et al. identified significantly reduced vasodilatory responses to the endothelial-dependent vasodilator acetylcholine but no significant reduction in vasodilatory response to the endothelial-independent vasodilator sodium nitroprusside after vaping, suggestive of endothelial dysfunction (Chaumont et al., 2018).

3.6.4. Platelet activation

Smoking can induce pathophysiological platelet activation, resulting in thrombosis and in turn ischaemia and potentially infarction (Csordas and Bernhard, 2013). *Kerr* et al. found significant increases in platelet microparticle secretion (Kerr et al., 2018), whilst *Nocella* et al. found significant increases in platelet aggregation after e-cigarette use (Nocella et al., 2018). Two studies found significant increases in soluble Platelet (P-) selectin (Biondi-Zoccai et al., 2019; Nocella et al., 2018) whilst *Kerr* et al. found a significant decrease in P-selectin (Kerr et al., 2018).

3.7. Summary of findings and proposed mechanisms

Based on the findings we propose mechanisms of the complex effects of e-cigarettes on the heart (Table 7).

3.8. Conflicts of interest in studies

21.1% of studies included in this review were deemed to have a potential COI ($n = 8$) (Cravo et al., 2016; D'Ruiz et al., 2017; Farsalinos et al., 2016; Yan and D'Ruiz, 2015; Anderson et al., 2016; Farsalinos et al., 2014a; Taylor et al., 2017; Szoltysek-Boldys et al., 2014) utilising funding, materials and/or researchers supplied by tobacco or e-cigarette manufacturers (web Appendix 4).

74.3% of all studies found a potentially harmful cardiovascular effect ($n = 29$). Only two of the eight papers (25%) deemed to have a potential COI reported a potentially harmful cardiovascular effect (Teasdale et al., 2016; Kaisar et al., 2017). In contrast, 27 of the 30 (90%) without apparent COI reported such an effect. The difference was significant (Fisher's Exact Test, $P = 0.0007$). Notably, two of the three studies without a COI that did not identify a cardiovascular effect appeared to have ineffective nicotine delivery to the bloodstream (Eissenberg, 2010; Vansickel et al., 2010).

Seven of the eight studies with a potential COI had conclusions that were supportive of electronic cigarette use (Cravo et al., 2016; D'Ruiz et al., 2017; Farsalinos et al., 2016; Yan and D'Ruiz, 2015; Anderson et al., 2016; Farsalinos et al., 2014a; Taylor et al., 2017) in addition to one study with no apparent COI (Teasdale et al., 2016). 24 of the 29 studies without a COI had conclusions that were unsupportive of e-cigarette use. This difference was highly significant (Fisher's Exact Test, $p < 0.0001$). Notably, six studies had conclusions that were neutral (Fogt et al., 2016; Sumartiningsih et al., 2019; Shi et al., 2019; Szoltysek-Boldys et al., 2014; Vansickel et al., 2010; Pywell et al., 2018), of which one had a COI (Szoltysek-Boldys et al., 2014).

3.9. Quality assessment

Details of the quality assessment undertaken are described in web Appendix 5. 34.2% of included studies were deemed to have a moderate-high risk of bias ($n = 13$). 11 of these studies formed conclusions on the implications of e-cigarettes use for health, with 6 (54.5%) having conclusions that were supportive of e-cigarette use (Appendix 6).

4. Discussion

4.1. Summary of results

38 experimental studies were identified. 90% of studies deemed to be without COI found potentially harmful effects on the cardiovascular system. Only two of eight studies deemed to have a potential COI reported a potentially harmful cardiovascular effect, whilst six of 11 studies with moderate-high risk of bias had conclusions that were supportive of e-cigarette use.

Human studies largely showed increases in heart rate and blood pressure as well as abnormalities in HRV, suggestive of sympathetic nerve activation. Both *in vitro* and *in vivo* studies showed an increase in ROS production and a reduction in anti-oxidants after e-cigarette exposure, constituting an atherosclerotic risk. This was evidenced in one murine study which found significantly greater atherosclerotic plaque development in mice exposed to e-cigarette vapour. *In vitro* studies identified disordered endothelial cellular structure, function and interactions; murine studies identified vascular inflammatory markers and angiogenesis, whilst human studies identified increased arterial stiffness - all suggestive of endothelial dysfunction. Platelet haemostatic processes were reported across murine, human *in vitro* and human *in vivo* studies, suggestive of an increased thrombotic risk.

Notably, vaping but not smoking increased endothelial (c)1q deposition, reactive hyperaemia and murine left ventricular mass. These changes may be suggestive of endothelial dysfunction and cardiac remodelling.

4.2. Consistency of findings with previous reviews

Benowitz undertook two literature reviews of the cardiovascular effects of nicotine (Benowitz and Burbank, 2017) and e-cigarettes (Benowitz and Fraiman, 2017) respectively but neither were conducted systematically nor limited to experimental studies. They noted the pharmacological plausibility of adverse cardiovascular outcomes of nicotine (and other vaporised e-cigarette compounds), particularly in those with primary cardiovascular disease.

Qasim et al. also reviewed this literature but did not use a systematic methodology or restrict studies to experimental designs. It focused on hypothetical effects of individual constituents, identifying carbonyls and their breakdown products as potential sources of oxidative stress and arguing that fine particulate matter in e-cigarette vapour could

Table 7
Summary of findings: the proposed complex pathogenic mechanisms of e-cigarettes' effect on the heart.

	Proposed pathogenic mechanisms					
	Angiogenesis	Oxidative stress	Endothelial dysfunction	Sympathetic nerve system activation	Platelet activation / anticoagulation inhibition	Cardiac remodelling
Biomarkers	● ↑ CD31 immunostaining (Shi et al., 2019) ● ↑ CD34 immunostaining (Shi et al., 2019) ● ↑ endothelial cell tube formation(Lee et al., 2019)	● ↑ reactive oxygen species (ROS) - H_2O_2(Biondi-Zoccai et al., 2019) - sNox2-dp (Carnevale et al., 2016) - 8-isoPGF2a (Biondi-Zoccai et al., 2019) - Plasma myeloperoxidase (Chaumont et al., 2018) - Malondialdehyde(Espinoza-Derout et al., 2019) - ↑ circulating CD40L (activates endothelial cells to release ROS) (Biondi-Zoccai et al., 2019) - ↑ serum C-reactive Protein (Chatterjee et al., 2019) ● ↓ antioxidant activity - Vitamin E levels(Biondi-Zoccai et al., 2019) - NO bioavailability - Nitric Oxide(Carnevale et al., 2016) metabolites(Chatterjee et al., 2019) - HBA%(Biondi-Zoccai et al., 2019)	● ↑ arterial stiffness - ↓ flow-mediated dilatation (Biondi-Zoccai et al., 2019) - ↑ pulse-wave velocity (Antoniewicz et al., 2019) - ↑ augmentation index x 75 (Chaumont et al., 2018) ● ↓ vasodilatory response to acetylcholine(Chaumont et al., 2018) ● ↓ vasodilatory response to methacholine(Olfert et al., 2017) ● ↑ vasoconstrictive response to phenylephrine(Olfert et al., 2017) ● ↑ endothelial progenitor cells (Antoniewicz et al., 2016) ● ↑ endothelial complement deposition(Barber et al., 2017) ● ↑ endothelial complement inhibitor expression(Barber et al., 2017) ● Endothelial barrier disruption (Schweitzer et al., 2015) ● ↑ reactive hyperaemia index (Kerr et al., 2018) ● ↑ vascular inflammatory markers - PECAM-1 (Kaisar et al., 2017) - VCAM-1 (Kaisar et al., 2017) - ICAM-1 (Chatterjee et al., 2019) ● ↑ endothelial cell: - Morphological alterations (Putzhammer et al., 2016) - DNA damage(Anderson et al., 2016) - Inhibition of migration(Lee et al., 2019) ● ↓ endothelial cell: - Proliferation(Putzhammer et al., 2016) - Cell density(Barber et al., 2017) - Metabolic activity(Barber et al., 2017) - Viability(Barber et al., 2017)	● ↑ heart rate(Antoniewicz et al., 2019) ● ↑ (exercising)(Fogt et al., 2016) systolic blood pressure(Biondi-Zoccai et al., 2019) ● ↑ (exercising)(Fogt et al., 2016) diastolic blood pressure(Biondi-Zoccai et al., 2019) ● Abnormal heart rate variability - Cardiac vagal tone(Moheimani et al., 2017a) - Sympathetic tone(Moheimani et al., 2017a) ● Peripheral vasoconstriction (Pywell et al., 2018)	● ↑ platelet: - Aggregation(Nocella et al., 2018) - Adhesion(Hom et al., 2016) - Complement deposition (Hom et al., 2016) - Alpha particle secretion (Qasim et al., 2018) - Dense particle secretion (Qasim et al., 2018) - Integrin activation(Qasim et al., 2018) - Resistance to prostacyclin inhibition(Qasim et al., 2018) - ↓ thrombomodulin(Kaisar et al., 2017) - ↓ bleeding time(Qasim et al., 2018) - ↓ occlusion time(Qasim et al., 2018)	● Altered cardiac structure: - ↑ left ventricular mass(Olfert et al., 2017) ● Altered cardiac function: - ↓ left ventricular ejection Fracture (Espinoza-Derout et al., 2019) - ↓ left ventricular fractional Shortening (Espinoza-Derout et al., 2019) - ↓ velocity of circumferential fibre Shortening(Espinoza-Derout et al., 2019) ● Cardiac mutagens: - O^6-methyldeoxyguanosines(Lee et al., 2018) - γ-Hydroxy-1,N2-propano-deoxyguanosines(Lee et al., 2018)

increase intracellular calcium in addition to affecting the autonomic nervous system and modifying heart rate variability, collectively contributing to arrhythmias (Qasim et al., 2017).

Two further reviews were published while this one was under review. One is a narrative review which addresses a series of practical questions (Middlekauff, 2019) (. The other conducted meta-analyses of the associations between e-cigarette use and haemodynamic effects, identifying significant acute increases in heart rate, systolic blood pressure and diastolic blood pressure (Skotsimara et al., 2019). No previous review focused on the cardiovascular system examined potential conflicts of interest.

4.3. Limitations of the primary literature.

Overall, there were many methodological weaknesses in the studies included. Their utility was further compromised by the number of papers with potential COI. A comprehensive exploration of limitations is in Appendix 7 but some of the most important are as follows. First, there is marked product variation. Liquids tested represent only a very small proportion of the seemingly innumerable variants available on the market. Second, most studies utilised conventional cigarettes, one of the most harmful legal products, as a study comparator. This may have resulted in the neglect of other potential harms, not associated with cigarette smoking, such as those arising from the aerosol (solvent carriers and flavours) or the solvent. Few investigated nicotine-free e-cigarettes. Third, some *in vitro* studies exposed cells to extracts with nicotine concentrations which might be greater than those delivered to the bloodstream from vaping. Fourth, many human experimental studies had small sample sizes and lacked blinding or randomisation, whilst certain cross-over studies utilised short washout periods. There are distinct differences in electronic and conventional cigarette topography, with e-cigarettes requiring longer puff length and vaping duration to attain comparable nicotine levels. As participants in experiments often were e-cigarette-naïve smokers, this implied a risk of under-exposure. Some exposures were extremely small, with two exposing subjects to only 9 puffs. Variations in device voltage, vaporizers, e-liquid levels and pH may also influence nicotine, and other compound, delivery. Failure to assess plasma nicotine made it difficult to ascertain whether there was enough time after vaping to reach peak delivery, or whether it was too long and effects were waning. Fifth, few studies assessed abstinence but those that did found some subjects self-reporting as abstinent were current smokers. Sixth, heating coil pre-activation time, puff length, inter-puff intervals and total fluid consumption varied between intervention and comparator groups within studies, and between intervention groups across studies. Most human studies exposed participants to vaping for only a few minutes.

4.4. Limitations of this review

4.4.1. Appropriateness of search strategy

Non-experimental studies were excluded on methodological grounds but most also point to potentially harmful cardiovascular effects, with three cross-sectional studies associating daily e-cigarette use (adjusted for conventional cigarette use) with increased risk of myocardial infarction (Osei et al., 2019; Bhatta and Glantz, 2019; Alzahrani et al., 2018). Longitudinal studies will be essential to elicit the long-term effects of vaping but the cohort studies we identified were small, with important methodological limitations. Several studies with seeming experimental designs were also excluded as lacking either control/comparator groups or cross-over methodologies (van Staden et al., 2013; Vansickel, 2013; St Helen et al., 2016). Numerous conference abstracts lacked matching full-text papers.

4.4.2. Limited range of outcome measures

Cardiovascular disease results from many complex processes acting on different metabolic pathways and physiological mechanisms. Three metabolic studies in animals did not meet inclusion criteria but may have long-term cardiovascular implications. These reported significant increases in circulating cholesterol and triglycerides, (Canistro et al., 2017) and hyperglycaemia (El Golli et al., 2016) following exposure to e-cigarette vapour; and found that nicotine impaired transfer of glucose across the blood brain barrier in ischaemic conditions, with implications for recovery from ischaemic stroke (Sifat et al., 2018).

4.4.3. Generalisability of findings

Samples of aerosols tested may not be generalisable to other products. The short duration of most interventions also limits insights on long-term outcomes. Additionally, *in vitro*/animal studies may not be generalisable to human populations.

The absence of never-smoking subjects in most studies prevents generalisation of findings to never-smokers using e-cigarettes. This is a significant limitation as adolescents represent a potential at-risk group, with proportionally the highest uptake of e-cigarettes (Neff et al., 2015), which in turn may be predictive of smoking initiation in young people (Soneji et al., 2017; Loukas et al., 2018; Goldenson et al., 2017; Bold et al., 2017). Interestingly, a recent post-hoc analysis by *Carnevale* et al. found that never-smokers had greater adverse oxidative and vascular reactions to vaping (comparable to those of smoking a cigarette) than experienced by smokers. Additionally, women taking the oral contraceptive (a common potential at-risk group that has not yet been considered) have significantly more unfavourable changes in vitamin E levels and FMD(Mastrangeli et al., 2018).

The prominence of 1st and 2nd generation e-cigarettes tested in these studies should be noted, as this is not reflective of current e-cigarette use - with many users owning 3rd and 4th generation devices. These have different nicotine delivery profiles and electrical characteristics, including controls over both wattage and voltage, which enables users to increase device power and consequently liquid consumption per puff (McRobbie and McEwen, 2014). Studies have also shown higher voltage devices to produce more carbonyls (Kosmider et al., 2014). Notably, all three studies which reported to utilise newer generation devices identified potentially harmful cardiovascular outcomes (Olfert et al., 2017; Franzen et al., 2018; Chaumont et al., 2018).

Most of the primary literature compares cardiovascular consequences of e-cigarette use with cigarettes or non-smoking. Whilst this makes it easier to elucidate the cardiovascular effects attributable to these devices, it is not generalisable to the vaping population, most of whom are dual users (Goniewicz et al., 2016).

There may be few if any cardiovascular benefits for those who only reduce cigarette consumption (Godtfredsen et al., 2002), given the non-linear dose-response relationship between number of cigarettes smoked per day and cardiovascular disease (United States Department of Health and Human Services, 2014; Barnoya and Glantz, 2005). Exposure to even low levels of harmful constituents from e-cigarettes might have a pronounced effect on the cardiovascular system. A recent systematic review highlighted the potential for harmful health effects of passive exposure to e-cigarette vapour (Hess et al., 2016). Finally, the studies included have mostly examined specific, isolated mechanisms, whereas in practice what will matter is their combined effects. This will require long-term follow up studies.

4.4.4. Conflict of interest

We were only able to identify potential COI where it was reported, either in the papers included or others by the authors. However, there is growing evidence that conflicts can be concealed or nuanced, with a new area of scholarship emerging on this subject (McCambridge et al., 2019; Stuckler et al., 2018). The COIs identified in this paper were revealed by the authors themselves, as required by the journals. Whilst disclosing conflicts of interest is good practice, it does not negate the influence of said conflict, as even acknowledged financial support appears to influence outcomes (Pisinger et al., 2019; Hendlin et al., 2019). In the light of such findings, the British Medical Journal, American

Thoracic Society, Tobacco Control and PLOS Medicine have decided they will not publish tobacco industry–funded research (Godlee et al., 2013).

5. Conclusion

Primary studies suggest potentially harmful cardiovascular effects from e-cigarettes, through inducing sympathetic nerve activation, oxidative stress, endothelial dysfunction and platelet activation. Notably, one murine study found e-cigarette aerosol accelerated atherosclerotic plaque formation. It is concerning that COI status and median-high risk of bias were both significantly associated with the identification of no harmful cardiovascular effects. Further research is required to assess effects of e-cigarettes in subjects with primary cardiovascular disease, and to distinguish effects of nicotine-containing and nicotine-free e-cigarettes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ypmed.2019.105770.

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