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

Ciaran D. Kennedy, May C.I. van Schalkwyk, Martin McKee, Charlotta Pisinger

PII: S0091-7435(19)30246-4
DOI: https://doi.org/10.1016/j.ypmed.2019.105770
Article Number: 105770
Reference: YPMED 105770

To appear in: *Preventive Medicine*

Received date: 14 December 2018
Revised date: 18 June 2019
Accepted date: 12 July 2019

Please cite this article as: C.D. Kennedy, M.C.I. van Schalkwyk, M. McKee, et al., The cardiovascular effects of electronic cigarettes: A systematic review of experimental studies, Preventive Medicine, https://doi.org/10.1016/j.ypmed.2019.105770

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

Ciaran D Kennedy[1], MBChB MSc, May CI van Schalkwyk[1], MPH MBBS, Martin McKee[1] MD, Charlotta Pisinger[2,3,4] MD

[1] Faculty of Public Health and Policy, London School of Hygiene and Tropical Medicine, London, UK

[2] Center for Clinical Research and Prevention Bispebjerg-Frederiksberg Universitets Hospital, Capital Region of Denmark

[3] Danish Heart Foundation, Copenhagen

[4] University of Copenhagen, Faculty of Health and Medical Science

Corresponding Author: Charlotta Pisinger charlotta.pisinger@regionh.dk

Introduction

Conventional and electronic (e-) cigarettes deliver nicotine to the bloodstream, resulting in significant production of its primary metabolite – cotinine.[1,2] Nicotine is known to affect the cardiovascular system through sympathetic nervous system activation. This increases myocardial contractility, heart rate, blood pressure and coronary vasoconstriction.[3,4] 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,[5,6] studies into smokeless tobacco use have found associations with fatal coronary artery disease;[7] with mortality rates halving in individuals who quit product use after a myocardial infarction.[8] 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.[9,10] These may trigger pathophysiological processes including vascular inflammation and platelet activation,[11–15] with chronic exposure constituting a cardiovascular risk factor.[16] Thermal degradation of e-cigarette solvent carriers glycerol and propylene glycol can also produce carbonyls, such as formaldehyde, acetaldehyde, and acrolein,[13,17] that may cause pathophysiological changes once broken down into reactive oxidant species,[18–21] potentially contributing to cardiomyopathy.[22] E-cigarettes liquids have also been manufactured with numerous flavourings, such as cinnamaldehyde, which may have cardiotoxic effects.[23] Heavy metals such as cadmium and lead have been detected in certain e-cigarette aerosols,[24] which have been associated with hypertension[25] and coronary artery disease respectively.[26] 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.[27]

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

Methods

Four researchers conducted the review applying Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.[28]

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.

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 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.

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,[29] we decided to synthesised 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* (Figure 1). [27]

Conflict of interest in studies

To assess any influence of conflict of interest (COI), influence not generally captured by traditional quality assessment tools, on appraisal of e-cigarettes 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 relationships between COI status against potentially harmful cardiovascular outcomes and conclusions supportive of e-cigarette use.

Quality assessment

Quality of studies was assessed using Cochrane Risk of Bias (RoB) tools.[30] 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).

Results

Study selection

The electronic search identified 766 records with an additional 10 from reference lists (Figure 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). 37 articles were included in the review.

Study characteristics

This review included randomised controlled-trials (n=8)[31–38]; randomised crossover studies (n=10)[39–48]; non-randomised controlled trials (n=13)[49–61] and non-randomised crossover studies (n=7)[62–68]. These articles studied human subjects (n=24)[32,33,35,37,39–48,56,57,61–68], animal subjects (n=6)[36,38,51,53,58,60] and a range of human cardiovascular cells types and platelets (n=8)[31,34,49,50,52,54,55,59]. The total duration of exposure of cells to e-cigarette aerosol extract (eCAE) in *in vitro* studies ranged from 4 hours to 72 hours (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 hour to 4 weeks (table 2).

Participant characteristics

Only 11% of human studies investigated solely non-smoking populations.[35,41,42,61] 45.8% of human studies included subjects without prior use of e-cigarettes and understanding of vaping topography (n=11),[33,35,40–42,61,63,65–68] whilst a further 33.3% of studies did not state whether subjects had previously used these devices (n=8).[37,39,45–48,57,62] Only 45% of studies

chemically verified baseline smoking abstinence (n=11).[37,39,66,40,43,45,47,48,63–65]. Mean age of human subjects, who were healthy volunteers, ranged from 22.9 to 46.6 years old.

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.[36,43,45] Only 44.7% of studies reported any electrical characteristics of devices (n=17), with voltage varying from 3.0 to 5.0 volts and resistance varying from 0.4 to 2.4 ohms. Few studies included independent chemical analyses of e-liquids (n=6)[33,40,45,50,56,67] and only *Schweitzer et al.* tested resultant vapour constituents for presence of newly formed oxidation products.[50] Only one *in vitro* study measured e-cigarette heating coil temperature[55] whilst only two considered high coil temperatures (table 3).[54,55] Reported nicotine concentration in eCAE solution varied from 0 to 36mg/mL with only 37.5% of human and animal studies estimating nicotine delivery, through plasma nicotine and/or urinary cotinine concentrations (n=9).[36,39,42,45,47,60,63,65,66] 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.[63,65]

Only 50% of human studies chemically verified abstinence (n=12), [33,35,65,66,37,39,40,43,45,47,48,64]. Most studies did not report frequency of abstinence testing, with *Farsalinos et al. (2016)* having periods up to 24 weeks without assessing abstinence.[37] 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.[68] Only *Chaumont et al.* assessed subjects' tolerance to vaping prior to investigation.[45]

Study results: *in vitro* studies

These are summarised in Table 5.

Oxidative stress

Three studies found statistically significant increases in reactive oxygen species (ROS) associated with endothelial injury.[49,52,55] *Teasdale et al.* did not however find significant upregulation in expression of genes involved in the oxidative stress pathway.[54]

Endothelial cellular function

Four studies reported statistically significant reductions in endothelial cell viability when exposed to certain eCAE,[49,52,55,59] whilst *Lee et al.* identified significant impairment in endothelial cell viability after exposure to serum from e-cigarette and cigarette smokers

compared to non-smokers.[52] *Lee et al.* also identified increased endothelial cell tube formation, reflective of increased angiogenesis.[69] Other cardiotoxic effects identified included DNA damage,[49] cell morphological changes[55] and reduced cell metabolic activity.[31] 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[31] and proliferation[55] (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,[52] 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.[59]

Schweitzer et al. identified increased endothelial cell barrier disruption[50] 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.[70]

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.[31] 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.[71,72]

Platelet function

Hom et al. reported significant increases in platelet aggregation, adhesion, activation and complement deposition after exposure to eCAE.[34] These changes have been invoked as a mechanism for increased risk of thrombosis after cigarette smoking.[73]

Study results: animal studies

These are summarised in Table 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.[38] *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.[53] *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.[36] *Espinoza-Derout et al.* however observed no significant change.[53] *Olfert et al.* observed no significant decreases in fractional shortening and ejection fraction in mice exposed to e-cigarette vapour,[36] whilst *Espinoza-Derout et al.* observed both of these findings.[53] *Shi et al.* found no significant effects of vaping on cardiac contractility, fibrosis or geometric properties.[51]

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.[58] *Espinoza-Derout et al.* identified increased expression of inflammatory and apoptotic genes[53] associated with atherosclerotic lesion formation[74] and ROS-induced heart failure.[75] *Olfert et al.* reported significant increases in pulse wave velocity (a measure of arterial stiffness associated with endothelial dysfunction[76]) 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.[77] 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.[36] *Shi et al*, identified a significant increase in angiogenesis, which could ultimately contribute to atherogenesis.[51] Most notably, *Espinoza-Derout et al.* identified statistically significant increases in atherosclerotic plaque formation in mice exposed to eCAE compared to aerosol control.[53]

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),[60] whilst *Kaisar et al.* reported statistically significant decreases in circulating thrombomodulin in mice - a molecule protective against thrombosis.[58]

Study results: human studies

Sympathetic nerve activation

18 studies measured heart rate as a biomarker of high sympathetic nerve activation – a state associated with increased cardiovascular risk.[78] Most studies reported increases (n=14),[35,39,41–46,48,56,57,64,65,67] and some decreases (n=2)[33,37] after e-cigarette use. Seven of these studies reported statistically insignificant changes,[32,37,41,44,63–65] and one reported clinically insignificant changes.[32]

17 studies investigated resting blood pressure as a proxy for sympathetic nerve activation. These found both increases (n=10)[35,39,42,43,45–47,56,57,64] and decreases (n=4)[33,37,41,67] in systolic pressure and increases (n=9)[35,39,41–43,45,47,56,57] and decreases (n=3)[33,37,64] in diastolic pressure, with differing degrees of significance. *Fogt et al.* assessed the effect of electronic cigarette use on exercising peripheral blood pressure, identifying significant increases in systolic pressure compared to nicotine-free e-cigarettes.[41] *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.[68]

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.[79–81] They identified a statistically significant decrease in cardiac vagal tone and an increase in sympathetic tone after e-cigarette use,[42] whilst *Sumartiningsih et al.* identified significant increases in exercising HRV after vaping compared to the control group.[46] Finally, *Farsalinos et al.* identified no significant effect of vaping on myocardial function after very brief exposure.[56]

Oxidative stress

Oxidative stress is an important mechanism in the development of atherosclerosis from cigarette smoking.[82] 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.[47,62] *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).[47] *Ikonomidis et al.* found significant oxidative stress after e-cigarette use, as measured by malondialdehyde (MDA);[44] 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.[42] *Chaumont et al.* identified significant increases in plasma concentrations of myeloperoxidase, an enzyme involved in oxidative stress pathways,[45] which has been associated with increased cardiovascular risk.[83] However, no significant increases were identified in the oxidative stress-associated protein-bound 3-chlorotyrosine or homocitrulline.[45] Two studies found significant increases in circulating CD40L after e-cigarette use, which leads to endothelial cell activation and the production of ROS.[47,66] Finally, *Chatterjee et al.* found significant increases in ROS generation and C-reactive protein (a biomarker of inflammatory processes including atherothrombosis)[84], as well as significant decreases in (protective) NO metabolites after vaping.[61]

Endothelial function

Endothelial dysfunction is prognostic of atherosclerosis.[85] Studies reported various measures of arterial stiffness, indicative of endothelial dysfunction. *Carnevale et al.* measured arterial flow-mediated dilatation (FMD) finding significant impairment.[62] However, *Szołtysek-Bołdys et al.* reported insignificant changes in arterial stiffness index (SI) and reflection index (RI) after e-cigarette use.[64] Four studies identified significant increases in augmentation index normalised to a heart rate of 75 beats per minute (AIx75),[43–45,48] whilst five studies reported significant increases in pulse wave velocity (PWV) after e-cigarette use.[43–45,48,57]

Kerr et al. found an increase in reactive hyperaemia index,[67] an indicator of endothelial dysfunction,[86] whilst *Antoniewicz et al.* found significant increases in circulating reparative endothelial progenitor cells (EPCs) - suggesting vascular injury from vaping.[40] *Chatterjee et al.* found significant increases in soluble and endothelial ICAM-1 (an adhesion molecule involved in endothelial activation and dysfunction),[61] however *Kerr et al.* did not.[67] 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.[45]

Platelet activation

Smoking can induce pathophysiological platelet activation, resulting in thrombosis and in turn ischaemia and potentially infarction.[82] *Kerr et al.* found significant increases in platelet microparticle secretion,[67] whilst *Nocella et al.* found significant increases in platelet aggregation after e-cigarette use.[66] Two studies found significant increases in soluble Platelet (P-) selectin [47,66] whilst *Kerr et al.* found a significant decrease in P-selectin.[67]

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).

Conflicts of interest in studies

21.1% of studies included in this review were deemed to have a potential COI (n=8)[32,33,37,39,49,56,59,64] 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.[54,58] 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.[63,65]

Seven of the eight studies with a potential COI had conclusions that were supportive of electronic cigarette use[32,33,37,39,49,56,59] in addition to one study with no apparent COI.[54] 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,[41,46,51,64,65,68] of which one had a COI.[64]

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 are opinions on e-cigarettes, with 6 (54.5%) having conclusions that were supportive of e-cigarette use (appendix 6).

Discussion

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 heart-rate variability, suggestive of sympathetic nerve activation. Both *in vitro* and *in vivo* studies showed an increase in reactive oxygen species production and a reduction in anti-oxidants after e-cigarette exposure, constituting an atherosclerotic risk. This was evidenced in one murine studying 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.

Consistency of findings with previous reviews

Benowitz undertook two literature reviews of the cardiovascular effects of nicotine[3] and e-cigarettes[88] 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 increase intracellular calcium in addition to affecting the autonomic nervous system and modifying heart rate variability, collectively contributing to arrhythmias.[89]

Two further reviews were published while this one was under review. One is a narrative review which addresses a series of practical questions.[90] 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.[29] No previous review focused on the cardiovascular system examined potential conflicts of interest.

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 a huge 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.

Limitations of this review

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.[91–93] 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.[94–96] Numerous conference abstracts lacked matching full-text papers.

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, reporting significant increases in circulating cholesterol and triglycerides[97] and hyperglycaemia[98], following exposure to e-cigarette vapour while one study found that nicotine impaired transfer of glucose across the blood brain barrier in ischaemic conditions, with implications for recovery from ischaemic stroke.[99]

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,[100] which in turn may be predictive of smoking initiation in young people.[101–104] 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 fibromuscular dysplasia .[105]

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,[106] Studies have also shown higher voltage devices to produce more carbonyls.[107] Notably, all three studies which reported to utilise newer generation devices identified potentially harmful cardiovascular outcomes.[36,43,45]

Most of the primary literature compares cardiovascular consequences of electronic 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.[108]

There may be few if any cardiovascular benefits for those who only reduce cigarette consumption,[109] given the non-linear dose-response relationship between number of cigarettes smoked per day and cardiovascular disease.[4,110] 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 electronic cigarette vapour.[111] Finally, the studies included have mostly examined specific mechanisms whereas, in practice, what will matter is their combined effects: This will require long-term follow up studies.

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.[112,113] 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.[114,115] In the light of such findings, the British Medical Journal, American Thoracic Society, Tobacco Control and PLOS Medicine have already decided they will not publish tobacco industry–funded research.[116]

Conclusion

Primary studies suggest potentially harmful cardiovascular effects from electronic 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 electronic cigarettes in subjects with primary cardiovascular disease, and to distinguish effects of nicotine-containing and nicotine-free e-cigarettes.

List of references

1. Etter J-F. A longitudinal study of cotinine in long-term daily users of e-cigarettes. *Drug Alcohol Depend*. 2016;160:218–221.
2. Etter J-F, Bullen C. Saliva cotinine levels in users of electronic cigarettes. Eur. Respir. J. 2011;38:1219–1220.
3. Benowitz NL, Burbank AD. Cardiovascular toxicity of nicotine: Implications for electronic cigarette use. *Trends Cardiovasc Med* [Internet]. 2017;26:515–523. Available from: http://dx.doi.org/10.1016/j.tcm.2016.03.001
4. United States Department of Health and Human Services. The Health Consequences of Smoking—50 Years of Progress A Report of the Surgeon General. Atlanta (GA): 2014.
5. Ford CL, Zlabek JA. Nicotine replacement therapy and cardiovascular disease. *Mayo Clin Proc*. 2005;80:652–656.
6. Lindson-Hawley N, Hartmann-Boyce J, Fanshawe TR, Begh R, Farley A, Lancaster T. Interventions to reduce harm from continued tobacco use. *Cochrane database Syst Rev*. 2016;10:CD005231.
7. Gupta R, Gupta S, Sharma S, Sinha DN, Mehrotra R. Risk of coronary heart disease among smokeless tobacco users: results of systematic review and meta-analysis of global data. *Nicotine Tob Res*. 2018;
8. Arefalk G, Hambraeus K, Lind L, Michaelsson K, Lindahl B, Sundstrom J. Discontinuation of smokeless tobacco and mortality risk after myocardial infarction. *Circulation*. 2014;130:325–332.
9. Fuoco FC, Buonanno G, Stabile L, Vigo P. Influential parameters on particle concentration and size distribution in the mainstream of e-cigarettes. *Environ Pollut*. 2014;184:523–529.
10. Zhang Y, Sumner W, Chen D-R. In vitro particle size distributions in electronic and conventional cigarette aerosols suggest comparable deposition patterns. *Nicotine Tob Res*. 2013;15:501–508.
11. Fernandez E, Ballbe M, Sureda X, Fu M, Salto E, Martinez-Sanchez JM. Particulate Matter from Electronic Cigarettes and Conventional Cigarettes: a Systematic Review and Observational Study. *Curr Environ Heal reports*. 2015;2:423–429.
12. Geiss O, Bianchi I, Barahona F, Barrero-Moreno J. Characterisation of mainstream and passive vapours emitted by selected electronic cigarettes. *Int J Hyg Environ Health*. 2015;218:169–180.
13. Tianrong C. Chemical evaluation of electronic cigarettes. *Tob Control*. 2013;23:ii11–ii17.
14. Soule EK, Maloney SF, Spindle TR, Rudy AK, Hiler MM, Cobb CO. Electronic cigarette use and indoor air quality in a natural setting. *Tob Control* [Internet]. 2017;26:109 LP – 112. Available from: http://tobaccocontrol.bmj.com/content/26/1/109.abstract
15. Pellegrino R, Tinghino B, Mangiaracina G, Marani A, Vitali M, Protano C, Osborn J, Cattaruzza M. Electronic cigarettes: an evaluation of exposure to chemicals and fine particulate matter (PM). *Ann Ig*. 2012;24:279–288.
16. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux A V, Holguin F, Hong Y, Luepker R V, Mittleman MA, Peters A, Siscovick D, Smith SC, Whitsel L, Kaufman JD. Particulate Matter Air Pollution and Cardiovascular Disease. *Circulation* [Internet]. 2010;121:2331 LP – 2378. Available from: http://circ.ahajournals.org/content/121/21/2331.abstract

17. Hecht SS, Carmella SG, Kotandeniya D, Pillsbury ME, Chen M, Ransom BWS, Vogel RI, Thompson E, Murphy SE, Hatsukami DK. Evaluation of toxicant and carcinogen metabolites in the urine of e-cigarette users versus cigarette smokers. *Nicotine Tob Res*. 2015;17:704–709.
18. Lerner CA, Sundar IK, Watson RM, Elder A, Jones R, Done D, Kurtzman R, Ossip DJ, Robinson R, McIntosh S, Rahman I. Environmental health hazards of e-cigarettes and their components: Oxidants and copper in e-cigarette aerosols. *Environ Pollut*. 2015;198:100–107.
19. Goel R, Durand E, Trushin N, Prokopczyk B, Foulds J, Elias RJ, Richie JPJ. Highly reactive free radicals in electronic cigarette aerosols. *Chem Res Toxicol*. 2015;28:1675–1677.
20. Zhao J, Zhang Y, Sisler JD, Shaffer J, Leonard SS, Morris AM, Qian Y, Bello D, Demokritou P. Assessment of reactive oxygen species generated by electronic cigarettes using acellular and cellular approaches. *J Hazard Mater*. 2017;344:549–557.
21. Lerner CA, Sundar IK, Yao H, Gerloff J, Ossip DJ, McIntosh S, Robinson R, Rahman I. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. *PLoS One* [Internet]. 2015;10:e0116732. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med8&NEWS=N&AN=25658421
22. Henning RJ, Johnson GT, Coyle JP, Harbison RD. Acrolein Can Cause Cardiovascular Disease: A Review. *Cardiovasc Toxicol* [Internet]. 2017;17:227–236. Available from: https://doi.org/10.1007/s12012-016-9396-5
23. Farsalinos K, Romagna G, Allifranchini E, Ripamonti E, Bocchietto E, Todeschi S, Tsiapras D, Kyrzopoulos S. Comparison of the cytotoxic potential of cigarette smoke and electronic cigarette vapour extract on cultured myocardial cells. Int. J. Environ. Res. Public Health. 2013;10:5146–5162.
24. Hess CA, Olmedo P, Navas-Acien A, Goessler W, Cohen JE, Rule AM. E-cigarettes as a source of toxic and potentially carcinogenic metals. *Environ Res*. 2017;152:221–225.
25. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. Lead exposure and cardiovascular disease--a systematic review. *Environ Health Perspect* [Internet]. 2007;115:472–482. Available from: https://www.ncbi.nlm.nih.gov/pubmed/17431501
26. Tellez-Plaza M, Jones MR, Dominguez-Lucas A, Guallar E, Navas-Acien A. Cadmium exposure and clinical cardiovascular disease: a systematic review. *Curr Atheroscler Rep* [Internet]. 2013;15:356. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23955722
27. Middlekauff HR. Cardiovascular impact of electronic-cigarette use. Trends Cardiovasc. Med. 2019;
28. Moher D, Liberati A TJ and AD. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Ann Intern Med*. 2009;151:264–9.
29. Skotsimara G, Antonopoulos AS, Oikonomou E, Siasos G, Ioakeimidis N, Tsalamandris S, Charalambous G, Galiatsatos N, Vlachopoulos C, Tousoulis D. Cardiovascular effects of electronic cigarettes: A systematic review and meta-analysis. *Eur J Prev Cardiol*. 2019;2047487319832975.
30. Higgins J, Sterne J, Savović J, Page M, Hróbjartsson A, Boutron I, Reeves B, Eldridge S.

A revised tool for assessing risk of bias in randomized trials In: *Cochrane Database Syst Rev*. 2016;

31. Barber K, Ghebrehiwet B, Yin W, Rubenstein D. Endothelial Cell Inflammatory Reactions Are Altered in the Presence of E-Cigarette Extracts of Variable Nicotine. Cell. Mol. Bioeng. 2017;10:124–133.
32. Cravo AS, Bush J, Sharma G, Savioz R, Martin C, Craige S, Walele T. A randomised, parallel group study to evaluate the safety profile of an electronic vapour product over 12 weeks. *Regul Toxicol Pharmacol* [Internet]. 2016;81:S1–S14. Available from: http://dx.doi.org/10.1016/j.yrtph.2016.10.003
33. D'Ruiz CD, O'Connell G, Graff DW, Yan XS. Measurement of cardiovascular and pulmonary function endpoints and other physiological effects following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers. *Regul Toxicol Pharmacol*. 2017;87:36–53.
34. Hom S, Chen L, Wang T, Ghebrehiwet B, Yin W. Platelet activation, adhesion, inflammation, and aggregation potential are altered in the presence of electronic cigarette extracts of variable nicotine concentrations. Platelets. 2016;27:694–702.
35. Cooke W, Pokhrel A, Dowling C, Fogt D. Acute inhalation of vaporized nicotine increases arterial pressure in young non-smokers: a pilot study. Clin. Auton. Res. 2015;25:267–270.
36. Olfert IM, DeVallance E, Hoskinson H, Branyan KW, Clayton S, Pitzer CR, Sullivan DP, Breit MJ, Wu Z-X, Klinkhachorn P, Mandler WK, Erdreich BH, Ducatman BS, Bryner RW, Dasgupta P, Chantler PD. Chronic exposure to electronic cigarette (E-cig) results in impaired cardiovascular function in mice. *J Appl Physiol*. 2017;jap.00713.2017.
37. Farsalinos K, Cibella F, Caponnetto P, Campagna D, Morjaria J, Battaglia E, Caruso M, Russo C. Effect of continuous smoking reduction and abstinence on blood pressure and heart rate in smokers switching to electronic cigarettes. Intern. Emerg. Med. 2016;11:85–94.
38. Lee H, Park S, Weng M, Wang H, Huang WC, Lepor H. E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. 2018;
39. Yan X, D'Ruiz C. Effects of using electronic cigarettes on nicotine delivery and cardiovascular function in comparison with regular cigarettes. Regul. Toxicol. Pharmacol. 2015;71:24–34.
40. Antoniewicz L, Bosson JA, Kuhl J, Abdel-Halim SM, Kiessling A, Mobarrez F, Lundback M. Electronic cigarettes increase endothelial progenitor cells in the blood of healthy volunteers. *Atherosclerosis*. 2016;255:179–185.
41. Fogt DL, Levi MA, Rickards CA, Stelly SP, Cooke WH. Effects of Acute Vaporized Nicotine in Non-Tobacco Users at Rest and during Exercise. *Int J Exerc Sci* [Internet]. 2016;9:607–615. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5154719/
42. Moheimani RS, Bhetraratana M, Peters KM, Yang BK, Yin F, Gornbein J, Araujo JA, Middlekauff HR. Sympathomimetic Effects of Acute E-Cigarette Use: Role of Nicotine and Non-Nicotine Constituents. *J Am Heart Assoc*. 2017;6.
43. Franzen KF, Willig J, Talavera SC, Meusel M, Sayk F, Reppel M, Dalhoff K, Mortensen K, Droemann D. E-cigarettes and cigarettes worsen peripheral and central hemodynamics as well as arterial stiffness : A randomized, double-blinded pilot study. 2018;

44. Ikonomidis I, Vlastos D, Kourea K, Kostelli G, Varoudi M, Pavlidis G, Efentakis P, Triantafyllidi H, Parissis J, Andreadou I, Iliodromitis E, Lekakis J. Electronic Cigarette Smoking Increases Arterial Stiffness and Oxidative Stress to a Lesser Extent Than a Single Conventional Cigarette: An Acute and Chronic Study. Circulation. 2018;137:303–306.
45. Chaumont M, Becker B De, Zaher W, Culié A, Delporte C, Debbas N, Boudjeltia KZ, Borne P Van De. Differential Effects of E-Cigarette on Microvascular Endothelial Function , Arterial Stiffness and Oxidative Stress : A Randomized Crossover Trial. *Sci Rep*. 2018;8:1–9.
46. Sumartiningsih S, Lin H-F, Lin J-C. Cigarette Smoking Blunts Exercise-Induced Heart Rate Response among Young Adult Male Smokers. *Int J Environ Res Public Health*. 2019;16:1032.
47. Biondi-Zoccai G, Sciarretta S, Bullen C, Nocella C, Violi F, Loffredo L, Pignatelli P, Perri L, Peruzzi M, Marullo AGM, De Falco E, Chimenti I, Cammisotto V, Valenti V, Coluzzi F, Cavarretta E, Carrizzo A, Prati F, Carnevale R, Frati G. Acute Effects of Heat-Not-Burn, Electronic Vaping, and Traditional Tobacco Combustion Cigarettes: The Sapienza University of Rome-Vascular Assessment of Proatherosclerotic Effects of Smoking (SUR-VAPES) 2 Randomized Trial. *J Am Heart Assoc*. 2019;8.
48. Antoniewicz L, Brynedal A, Hedman L, Lundbäck M, Bosson JA. Acute Effects of Electronic Cigarette Inhalation on the Vasculature and the Conducting Airways. *Cardiovasc Toxicol* [Internet]. 2019;Available from: https://doi.org/10.1007/s12012-019-09516-x
49. Anderson C, Majeste A, Hanus J, Wang S. E-Cigarette Aerosol Exposure Induces Reactive Oxygen Species, DNA Damage, and Cell Death in Vascular Endothelial Cells. *Toxicol Sci* [Internet]. 2016;154:332–340. Available from: http://dx.doi.org/10.1093/toxsci/kfw166
50. Schweitzer KS, Chen SX, Law S, Van Demark M, Poirier C, Justice MJ, Hubbard WC, Kim ES, Lai X, Wang M, Kranz WD, Carroll CJ, Ray BD, Bittman R, Goodpaster J, Petrache I. Endothelial disruptive proinflammatory effects of nicotine and e-cigarette vapor exposures. *Am J Physiol Lung Cell Mol Physiol* [Internet]. 2015;309:L175-87. Available from: http://europepmc.org/abstract/MED/25979079
51. Shi H, Fan X, Horton A, Haller ST, Kennedy DJ, Schiefer IT, Dworkin L, Cooper CJ, Tian J. The Effect of Electronic-Cigarette Vaping on Cardiac Function and Angiogenesis in Mice. *Sci Rep*. 2019;9:1–9.
52. Lee WH, Ong S-G, Zhou Y, Tian L, Bae HR, Baker N, Whitlatch A, Mohammadi L, Guo H, Nadeau KC, Springer ML, Schick SF, Bhatnagar A, Wu JC. Modeling Cardiovascular Risks of E-Cigarettes With Human-Induced Pluripotent Stem Cell–Derived Endothelial Cells. *J Am Coll Cardiol* [Internet]. 2019;73:2722 LP – 2737. Available from: http://www.onlinejacc.org/content/73/21/2722.abstract
53. Espinoza-Derout J, Hasan KM, Shao XM, Jordan MC, Sims C, Lee DL, Sinha S, Simmons Z, Mtume N, Liu Y, Roos KP, Sinha-Hikim AP, Friedman TC. Chronic Intermittent Electronic Cigarette Exposure Induces Cardiac Dysfunction and Atherosclerosis in Apolipoprotein E (ApoE) Knockout Mice. *Am J Physiol Heart Circ Physiol*. 2019;
54. Teasdale JE, Newby AC, Timpson NJ, Munafo MR, White SJ. Cigarette smoke but not electronic cigarette aerosol activates a stress response in human coronary artery endothelial cells in culture. *Drug Alcohol Depend*. 2016;163:256–260.
55. Putzhammer R, Doppler C, Jakschitz T, Heinz K, Förste J, Danzl K, Messner B, Bernhard

D. Vapours of US and EU market leader electronic cigarette brands and liquids are cytotoxic for human vascular endothelial cells. *PLoS One* [Internet]. 2016;11:1–15. Available from: http://dx.doi.org/10.1371/journal.pone.0157337

56. Farsalinos KE, Tsiapras D, Kyrzopoulos S, Savvopoulou M, Voudris V. Acute effects of using an electronic nicotine-delivery device (electronic cigarette) on myocardial function: comparison with the effects of regular cigarettes. *BMC Cardiovasc Disord* [Internet]. 2014;14:78. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=medl&NEWS=N&AN=24958250
57. Vlachopoulos C, Ioakeimidis N, Abdelrasoul M, Terentes-Printzios D, Georgakopoulos C, Pietri P, Stefanadis C. Electronic Cigarette Smoking Increases Aortic Stiffness and Blood Pressure in Young Smokers. J. Am. Coll. Cardiol. 2016;67:2802–2803.
58. Kaisar MA, Villalba H, Prasad S, Liles T, Sifat AE, Sajja RK, Abbruscato TJ, Cucullo L. Offsetting the impact of smoking and e-cigarette vaping on the cerebrovascular system and stroke injury: Is Metformin a viable countermeasure?. *Redox Biol*. 2017;13:353–362.
59. Taylor M, Jaunky T, Hewitt K, Breheny D, Lowe F, Fearon I. A comparative assessment of e-cigarette aerosols and cigarette smoke on in vitro endothelial cell migration. Toxicol. Lett. 2017;
60. Qasim H, Karim ZA, Silva-espinoza JC, Khasawneh FT, Ellis CC, Bauer SL, Almeida IC, Alshbool FZ. Short-Term E-Cigarette Exposure Increases the Risk of Thrombogenesis and Enhances Platelet Function in Mice. 2018;13–15.
61. Chatterjee S, Tao J-Q, Johncola A, Guo W, Caporale A, Langham MC, Wehrli FW. Acute exposure to e-cigarettes causes inflammation and endothelial oxidative stress in non-smoking healthy young subjects. *Am J Physiol Lung Cell Mol Physiol* [Internet]. 2019;Available from: http://www.ncbi.nlm.nih.gov/pubmed/31042077
62. Carnevale R, Sciarretta S, Violi F, Nocella C, Loffredo L, Perri L, Peruzzi M, Marullo AGM, De Falco E, Chimenti I, Valenti V, Biondi-Zoccai G, Frati G. Acute Impact of Tobacco vs Electronic Cigarette Smoking on Oxidative Stress and Vascular Function. *Chest*. 2016;150:606–612.
63. Eissenberg T. Electronic nicotine delivery devices: ineffective nicotine delivery and craving suppression after acute administration. *Tob Control*. 2010;19:87 LP – 88.
64. Szoltysek-Boldys I, Sobczak A, Zielinska-Danch W, Barton A, Koszowski B, Kosmider L. Influence of inhaled nicotine source on arterial stiffness. *Przegl Lek* [Internet]. 2014;71:572–575. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=medl&NEWS=N&AN=25799846
65. Vansickel A, Cobb C, Weaver M, Eissenberg T. A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": Nicotine delivery profile and cardiovascular and subjective effects. Cancer Epidemiol. Biomarkers Prev. 2010;19:1945–1953.
66. Nocella C, Biondi-Zoccai G, Sciarretta S, Peruzzi M, Pagano F, Loffredo L, Pignatelli P, Bullen C, Frati G, Carnevale R. Impact of Tobacco Versus Electronic Cigarette Smoking on Platelet Function. *Am J Cardiol*. 2018;122:1477–1481.
67. Kerr DMI, Brooksbank KJM, Taylor RG, Pinel K, Rios FJ, Touyz RM, Delles C. Acute effects of electronic and tobacco cigarettes on vascular and respiratory function in healthy volunteers: a cross-over study. *J Hypertens*. 2018;

68. Pywell MJ, Wordsworth M, Kwasnicki RM, Chadha P, Hettiaratchy S, Halsey T. The Effect of Electronic Cigarettes on Hand Microcirculation. *J Hand Surg Am*. 2018;43:432–438.
69. DeCicco-Skinner KL, Henry GH, Cataisson C, Tabib T, Gwilliam JC, Watson NJ, Bullwinkle EM, Falkenburg L, O'Neill RC, Morin A, Wiest JS. Endothelial cell tube formation assay for the in vitro study of angiogenesis. *J Vis Exp* [Internet]. 2014;e51312–e51312. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25225985
70. Chistiakov DA, Orekhov AN, Bobryshev Y V. Endothelial Barrier and Its Abnormalities in Cardiovascular Disease. *Front Physiol*. 2015;6:365.
71. Yin W, Ghebrehiwet B, Weksler B, Peerschke EIB. Regulated complement deposition on the surface of human endothelial cells: effect of tobacco smoke and shear stress. *Thromb Res*. 2008;122:221–228.
72. Yin W, Ngwe EC, Ghebrehiwet B, Rubenstein DA. The combined effect of sidestream smoke and dynamic shear stress on endothelial cell inflammatory responses. *Thromb Res*. 2015;135:362–367.
73. Hung J, Lam JY, Lacoste L, Letchacovski G. Cigarette smoking acutely increases platelet thrombus formation in patients with coronary artery disease taking aspirin. *Circulation*. 1995;92:2432–2436.
74. Morton LF, Barnes MJ. Collagen polymorphism in the normal and diseased blood vessel wall: Investigation of collagens types I, III and V. *Atherosclerosis* [Internet]. 1982;42:41–51. Available from: http://www.sciencedirect.com/science/article/pii/0021915082901241
75. Nojiri H, Shimizu T, Funakoshi M, Yamaguchi O, Zhou H, Kawakami S, Ohta Y, Sami M, Tachibana T, Ishikawa H, Kurosawa H, Kahn RC, Otsu K, Shirasawa T. Oxidative stress causes heart failure with impaired mitochondrial respiration. *J Biol Chem*. 2006;281:33789–33801.
76. Ohkuma T, Ninomiya T, Tomiyama H, Kario K, Hoshide S, Kita Y, Inoguchi T, Maeda Y, Kohara K, Tabara Y, Nakamura M, Ohkubo T, Watada H, Munakata M, Ohishi M, Ito N, Nakamura M, Shoji T, Vlachopoulos C, Yamashina A. Brachial-Ankle Pulse Wave Velocity and the Risk Prediction of Cardiovascular Disease: An Individual Participant Data Meta-Analysis. *Hypertens (Dallas, Tex 1979)*. 2017;69:1045–1052.
77. Tzemos N, Lim PO, Mackenzie IS, MacDonald TM. Exaggerated Exercise Blood Pressure Response and Future Cardiovascular Disease. *J Clin Hypertens (Greenwich)*. 2015;17:837–844.
78. Middlekauff HR, Park J, Moheimani RS. Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: Mechanisms and implications for cardiovascular risk. *J Am Coll Cardiol* [Internet]. 2014;64:1740–1750. Available from: http://dx.doi.org/10.1016/j.jacc.2014.06.1201
79. Kleiger RE, Miller JP, Bigger JTJ, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol*. 1987;59:256–262.
80. Hillebrand S, Gast KB, De Mutsert R, Swenne CA, Jukema JW, Middeldorp S, Rosendaal FR, Dekkers OM. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: Meta-analysis and dose-response meta-regression. *Europace*. 2013;15:742–749.
81. Tsuji H, Larson MG, Venditti FJJ, Manders ES, Evans JC, Feldman CL, Levy D. Impact of

reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation*. 1996;94:2850–2855.

82. Csordas A, Bernhard D. The biology behind the atherothrombotic effects of cigarette smoke. *Nat Rev Cardiol*. 2013;10:219–230.
83. Rudolph TK, Wipper S, Reiter B, Rudolph V, Coym A, Detter C, Lau D, Klinke A, Friedrichs K, Rau T, Pekarova M, Russ D, Knoll K, Kolk M, Schroeder B, Wegscheider K, Andresen H, Schwedhelm E, Boeger R, Ehmke H, Baldus S. Myeloperoxidase deficiency preserves vasomotor function in humans. *Eur Heart J*. 2012;33:1625–1634.
84. Ridker PM. Clinical Application of C-Reactive Protein for Cardiovascular Disease Detection and Prevention. *Circulation* [Internet]. 2003;107:363–369. Available from: https://www.ahajournals.org/doi/10.1161/01.CIR.0000053730.47739.3C
85. Shechter M, Shechter A, Koren-Morag N, Feinberg MS, Hiersch L. Usefulness of brachial artery flow-mediated dilation to predict long-term cardiovascular events in subjects without heart disease. *Am J Cardiol*. 2014;113:162–167.
86. Bonetti PO, Pumper GM, Higano ST, Holmes DRJ, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol*. 2004;44:2137–2141.
87. Farsalinos K, Romagna G, Allifranchini E, Bocchietto E, Esposito M, Kyrzopoulos S, Tsiapras D, Todeschi S. Effects of electronic cigarette vapor and tobacco cigarette smoke on cultured cardiomyoblasts [Internet]. Eur. J. Heart Fail. 2014;16:202–203. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed16&NEWS=N&AN=71486926
88. Benowitz NL, Fraiman JB. Cardiovascular effects of electronic cigarettes. *Nat Rev Cardiol* [Internet]. 2017;14:447–456. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=prem&NEWS=N&AN=28332500
89. Qasim H, Karim ZA, Rivera JO, Khasawneh FT, Alshbool FZ. Impact of Electronic Cigarettes on the Cardiovascular System. *J Am Heart Assoc* [Internet]. 2017;6. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=prem&NEWS=N&AN=28855171
90. Middlekauff HR, Araujo JA. Increased Cardiovascular Risk Associated With E-Cigarette Use-Reply. *JAMA Cardiol* [Internet]. 2017;Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=medp&NEWS=N&AN=28593271
91. Osei AD, Mirbolouk M, Orimoloye OA, Dzaye O, Uddin SMI, Benjamin EJ, Hall ME, DeFilippis AP, Stokes A, Bhatnagar A, Nasir K, Blaha MJ. Association Between E-Cigarette Use and Cardiovascular Disease Among Never and Current Combustible-Cigarette Smokers. *Am J Med*. 2019;
92. Bhatta DN, Glantz SA. Electronic Cigarette Use and Myocardial Infarction Among Adults in the US Population Assessment of Tobacco and Health. *J Am Heart Assoc*. 2019;8:e012317.
93. Alzahrani T, Pena I, Temesgen N, Glantz SA. Association Between Electronic Cigarette Use and Myocardial Infarction. *Am J Prev Med*. 2018;55:455–461.
94. van Staden SR, Groenewald M, Engelbrecht R, Becker PJ, Hazelhurst LT. Carboxyhaemoglobin levels, health and lifestyle perceptions in smokers converting

from tobacco cigarettes to electronic cigarettes. *S Afr Med J*. 2013;103:865–868.
95. Vansickel A. Electronic cigarettes: Effective nicotine delivery after acute administration [Internet]. Nicotine Tob. Res. 2013;15:267–270. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed15&NEWS=N&AN=366344572
96. St Helen G, Havel C, Dempsey D, Jacob P. Nicotine delivery, retention and pharmacokinetics from various electronic cigarettes [Internet]. Addiction. 2016;111:535–544. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emex&NEWS=N&AN=614820450
97. Canistro D, Vivarelli F, Cirillo S, Babot Marquillas C, Buschini A, Lazzaretti M, Marchi L, Cardenia V, Rodriguez-Estrada MT, Lodovici M, Cipriani C, Lorenzini A, Croco E, Marchionni S, Franchi P, Lucarini M, Longo V, Della Croce CM, Vornoli A, Colacci A, Vaccari M, Sapone A, Paolini M. E-cigarettes induce toxicological effects that can raise the cancer risk. *Sci Rep* [Internet]. 2017;7:2028. Available from: https://doi.org/10.1038/s41598-017-02317-8
98. El.Golli N, Dkhili H, Dallagi Y, Rahali D, Lasram M, Bini-Dhouib I, Lebret M, Rosa J-P, El Fazaa S, Asmi MA-E. Comparison between electronic cigarette refill liquid and nicotine on metabolic parameters in rats. *Life Sci* [Internet]. 2016;146:131–138. Available from: http://www.sciencedirect.com/science/article/pii/S0024320515301429
99. Sifat AE, Vaidya B, Kaisar MA, Cucullo L, Abbruscato TJ. Nicotine and electronic cigarette (E-Cig) exposure decreases brain glucose utilization in ischemic stroke. *J Neurochem*. 2018;147:204–221.
100. Neff LJ, Arrazola RA, Caraballo RS, Corey CG, Cox S, King BA, Choiniere CJ, Husten CG. Frequency of Tobacco Use Among Middle and High School Students — United States, 2014. *CDC Morb Mortal Wkly Rep*. 2015;64:1078–1082.
101. Soneji S, Barrington-Trimis JL, Wills TA, Leventhal AM, Unger JB, Gibson LA, Yang J, Primack BA, Andrews JA, Miech RA, Spindle TR, Dick DM, Eissenberg T, Hornik RC, Dang R, Sargent JD. Association Between Initial Use of e-Cigarettes and Subsequent Cigarette Smoking Among Adolescents and Young Adults: A Systematic Review and Meta-analysis. *JAMA Pediatr*. 2017;171:788–797.
102. Loukas A, Marti CN, Cooper M, Pasch KE, Perry CL. Exclusive e-cigarette use predicts cigarette initiation among college students. *Addict Behav*. 2018;76:343–347.
103. Goldenson NI, Leventhal AM, Stone MD, McConnell RS, Barrington-Trimis JL. Associations of Electronic Cigarette Nicotine Concentration With Subsequent Cigarette Smoking and Vaping Levels in Adolescents. *JAMA Pediatr*. 2017;171:1192–1199.
104. Bold KW, Kong G, Camenga DR, Simon P, Cavallo DA, Morean ME, Krishnan-Sarin S. Trajectories of E-Cigarette and Conventional Cigarette Use Among Youth. *Pediatrics*. 2017;
105. Mastrangeli S, Carnevale R, Cavarretta E, Sciarretta S, Peruzzi M, Marullo AGM, De Falco E, Chimenti I, Valenti V, Bullen C, Roever L, Frati G, Biondi-Zoccai G. Predictors of oxidative stress and vascular function in an experimental study of tobacco versus electronic cigarettes: A post hoc analysis of the SUR-VAPES 1 Study. *Tob Induc Dis* [Internet]. 2018;16. Available from: http://dx.doi.org/10.18332/tid/89975
106. McRobbie H, McEwen A. E-cigarette briefing. *Natl Cent Smok Cessat Train* [Internet].

2014;1-16. (accessed on: 22 July 2017). Available from: http://www.ncsct.co.uk/usr/pub/e-cigarette_briefing.pdf
107. Kosmider L, Sobczak A, Fik M, Knysak J, Zaciera M, Kurek J, Goniewicz ML. Carbonyl compounds in electronic cigarette vapors: effects of nicotine solvent and battery output voltage. *Nicotine Tob Res*. 2014;16:1319–1326.
108. Goniewicz ML, Leigh NJ, Gawron M, Nadolska J, Balwicki L, McGuire C, Sobczak A. Dual use of electronic and tobacco cigarettes among adolescents: a cross-sectional study in Poland. *Int J Public Health*. 2016;61:189–197.
109. Godtfredsen NS, Holst C, Prescott E, Vestbo J, Osler M. Smoking reduction, smoking cessation, and mortality: a 16-year follow-up of 19,732 men and women from The Copenhagen Centre for Prospective Population Studies. *Am J Epidemiol*. 2002;156:994–1001.
110. Barnoya J, Glantz SA. Cardiovascular Effects of Secondhand Smoke. *Circulation* [Internet]. 2005;111:2684 LP – 2698. Available from: http://circ.ahajournals.org/content/111/20/2684.abstract
111. Hess I, Lachireddy K, Capon A. A systematic review of the health risks from passive exposure to electronic cigarette vapour. *Public Heal Res Pract*. 2016;26:1–9.
112. McCambridge J, Daube M, McKee M. Brussels Declaration: A vehicle for the advancement of tobacco and alcohol industry interests at the science/policy interface? *Tob Control*. 2019;28:7–12.
113. Stuckler D, Ruskin G, McKee M. Complexity and conflicts of interest statements: A casestudy of emails exchanged between Coca-Cola and the principal investigators of the International Study of Childhood Obesity, Lifestyle and the Environment (ISCOLE). *J Public Health Policy* [Internet]. 2018;39:49–56. Available from: https://doi.org/10.1057/s41271-017-0095-7
114. Pisinger C, Godtfredsen N, Bender AM. A conflict of interest is strongly associated with tobacco industry–favourable results, indicating no harm of e-cigarettes. *Prev Med (Baltim)* [Internet]. 2019;119:124–131. Available from: https://doi.org/10.1016/j.ypmed.2018.12.011
115. Hendlin YH, Vora M, Elias J, Ling PM. Financial Conflicts of Interest and Stance on Tobacco Harm Reduction: A Systematic Review. *Am J Public Health*. 2019;e1–e8.
116. Godlee F, Malone R, Timmis A, Otto C, Bush A, Pavord I, Groves T. Journal policy on research funded by the tobacco industry. *BMJ* [Internet]. 2013;347. Available from: https://www.bmj.com/content/347/bmj.f5193
117. Buettner-Schmidt K, Miller DR, Balasubramanian N. Electronic Cigarette Refill Liquids: Child-Resistant Packaging, Nicotine Content, and Sales to Minors. *J Pediatr Nurs*. 2016;31:373–379.
118. Han S, Chen H, Zhang X, Liu T, Fu Y. Levels of Selected Groups of Compounds in Refill Solutions for Electronic Cigarettes. *Nicotine Tob Res*. 2016;18:708–714.
119. Kucharska M, Wesolowski W, Czerczak S, Socko R. Testing of the composition of e-cigarette liquids - Manufacturer-declared vs. true contents in a selected series of products. *Med Pr*. 2016;67:239–253.
120. Raymond BH, Collette-Merrill K, Harrison RG, Jarvis S, Rasmussen RJ. The Nicotine Content of a Sample of E-cigarette Liquid Manufactured in the United States. *J Addict Med*. 2017;
121. Visser W, Geraets L, Klerx W, Hernandez L, Stephens E, Croes E, Schwillens P, Cremers H, Bos P, Talhout R. The health risks of using e-cigarettes. *RIVM Rep*. 2015;

122. Khlystov A, Samburova V. Flavoring Compounds Dominate Toxic Aldehyde Production during E-Cigarette Vaping. *Environ Sci Technol*. 2016;50:13080–13085.
123. Behar RZ, Davis B, Wang Y, Bahl V, Lin S, Talbot P. Identification of toxicants in cinnamon-flavored electronic cigarette refill fluids. *Toxicol Vitr*. 2014;28:198–208.
124. Behar RZ, Luo W, Lin SC, Wang Y, Valle J, Pankow JF, Talbot P. Distribution, quantification and toxicity of cinnamaldehyde in electronic cigarette refill fluids and aerosols. *Tob Control*. 2016;25:ii94–ii102.
125. Clapp PW, Pawlak EA, Lackey JT, Keating JE, Reeber SL, Glish GL, Jaspers I. Flavored e-cigarette liquids and cinnamaldehyde impair respiratory innate immune cell function. *Am J Physiol Lung Cell Mol Physiol*. 2017;313:L278–L292.
126. Haddad C, Salman R, El-Hellani A, Talih S, Shihadeh A, Saliba NA. Reactive Oxygen Species Emissions from Supra- and Sub-Ohm Electronic Cigarettes. *J Anal Toxicol*. 2018;
127. Fetterman JL, Weisbrod RM, Feng B, Bastin R, Tuttle ST, Holbrook M, Baker G, Robertson RM, Conklin DJ, Bhatnagar A, Hamburg NM. Flavorings in Tobacco Products Induce Endothelial Cell Dysfunction. *Arterioscler Thromb Vasc Biol*. 2018;38:1607–1615.
128. Kaisar MA, Prasad S, Liles T, Cucullo L. A decade of e-cigarettes: Limited research & unresolved safety concerns. *Toxicology*. 2016;365:67–75.
129. Evans SE, Hoffman AC. Electronic cigarettes: abuse liability, topography and subjective effects. *Tob Control* [Internet]. 2014;23:ii23 LP-ii29. Available from: http://tobaccocontrol.bmj.com/content/23/suppl_2/ii23.abstract
130. Hua M, Alfi M, Talbot P. Health-related effects reported by electronic cigarette users in online forums. *J Med Internet Res*. 2013;15:e59.
131. Kosmider L, Kimber CF, Kurek J, Corcoran O, Dawkins LE. Compensatory Puffing With Lower Nicotine Concentration E-liquids Increases Carbonyl Exposure in E-cigarette Aerosols. *Nicotine Tob Res*. 2017;
132. Lee YO, Nonnemaker JM, Bradfield B, Hensel EC, Robinson RJ. Examining Daily Electronic Cigarette Puff Topography Among Established and Non-established Cigarette Smokers in their Natural Environment. *Nicotine Tob Res*. 2017;
133. Hutzler C, Paschke M, Kruschinski S, Henkler F, Hahn J, Luch A. Chemical hazards present in liquids and vapors of electronic cigarettes. *Arch Toxicol*. 2014;88:1295–1308.
134. Stepanov I, Fujioka N. Bringing attention to e-cigarette pH as an important element for research and regulation. Tob. Control. 2015;24:413–414.
135. Farsalinos KE, Voudris V, Poulas K. E-cigarettes generate high levels of aldehydes only in “dry puff” conditions. *Addiction*. 2015;110:1352–1356.
136. Shihadeh A, Talih S, Eissenberg T. Commentary on Farsalinos et al. (2015): E-cigarettes generate high levels of aldehydes only in “dry puff” conditions. Addiction. 2015;110:1861–1862.
137. Pankow JF, Strongin RM, Peyton DH. Formaldehyde from e-cigarettes--it’s not as simple as some suggest. Addiction. 2015;110:1687–1688.
138. Khlystov A, Samburova V. Comment on “Do flavouring compounds contribute to aldehyde emissions in e-cigarettes?” by Farsalinos and Voudris. Food Chem. Toxicol. 2018;120:724–725.
139. Salamanca JC, Meehan-Atrash J, Vreeke S, Escobedo JO, Peyton DH, Strongin RM. E-cigarettes can emit formaldehyde at high levels under conditions that have been

reported to be non-averse to users. *Sci Rep*. 2018;8:7559.
140. Vlachopoulos C, Hirata K, O'Rourke MF. Effect of caffeine on aortic elastic properties and wave reflection. *J Hypertens*. 2003;21:563–570.
141. Moheimani RS, Bhetraratana M, Yin F, Peters KM, Gornbein J, Araujo JA, Middlekauff HR. Increased Cardiac Sympathetic Activity and Oxidative Stress in Habitual Electronic Cigarette Users: Implications for Cardiovascular Risk. *JAMA Cardiol* [Internet]. 2017;2:278–284. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=prem&NEWS=N&AN=28146259
142. Kasama S, Toyama T, Kurabayashi M. Usefulness of Cardiac Sympathetic Nerve Imaging Using (123)Iodine-Metaiodobenzylguanidine Scintigraphy for Predicting Sudden Cardiac Death in Patients With Heart Failure. *Int Heart J*. 2016;57:140–144.
143. Czogala J, Cholewinski M, Kutek A. [Evaluation of changes in hemodynamic parameters after the use of electronic nicotine delivery systems among regular cigarette smokers]. Przegl. Lek. 2012;69:841–845.
144. Kim CS, Kim MK. Influence of Electronic-cigarette Smoke on Cardiac Autonomic Nerve Responses in Comparison with Conventional-cigarette Smoke. *J Life Sci*. 2018;28:587–596.
145. Chaumont M, Becker B De, Zaher W, Culié A, Delporte C, Debbas N, Boudjeltia KZ, Borne P Van De. Supplement to: Differential Effects of E-Cigarette on Microvascular Endothelial Function, Arterial Stiffness and Oxidative Stress: A Randomized Crossover Trial. :1–18. Available from: https://doi.org/10.1038/s41598-018-28723-0.
146. Hobkirk AL, Nichols TT, Foulds J, Yingst JM, Veldheer S, Hrabovsky S, Richie J, Eissenberg T, Wilson SJ. Changes in resting state functional brain connectivity and withdrawal symptoms are associated with acute electronic cigarette use. *Brain Res Bull*. 2017;

Figure 1 Potential pathways by which e-cigarettes might affect the cardiovascular system

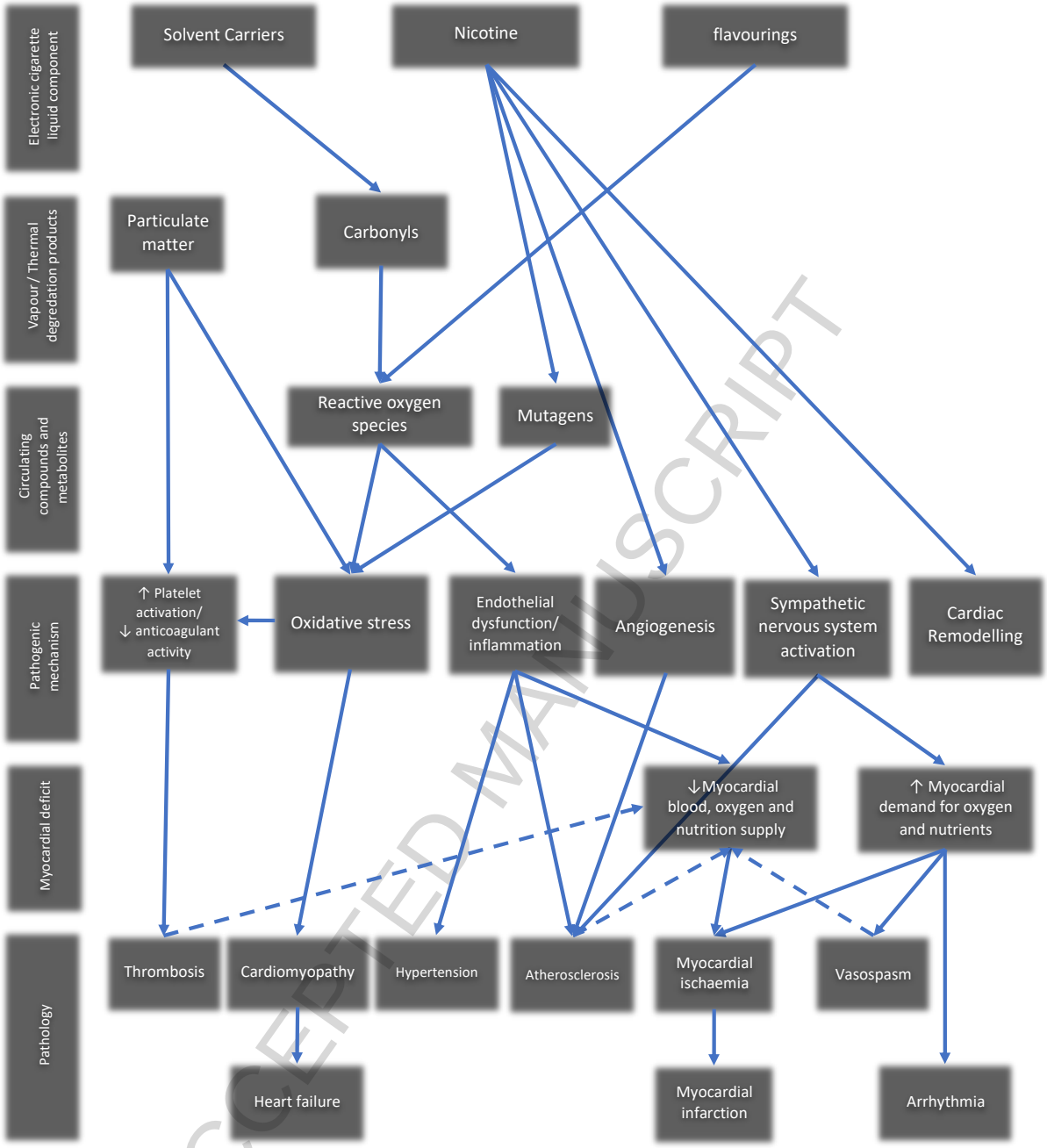

Figure 2 PRISMA study selection process

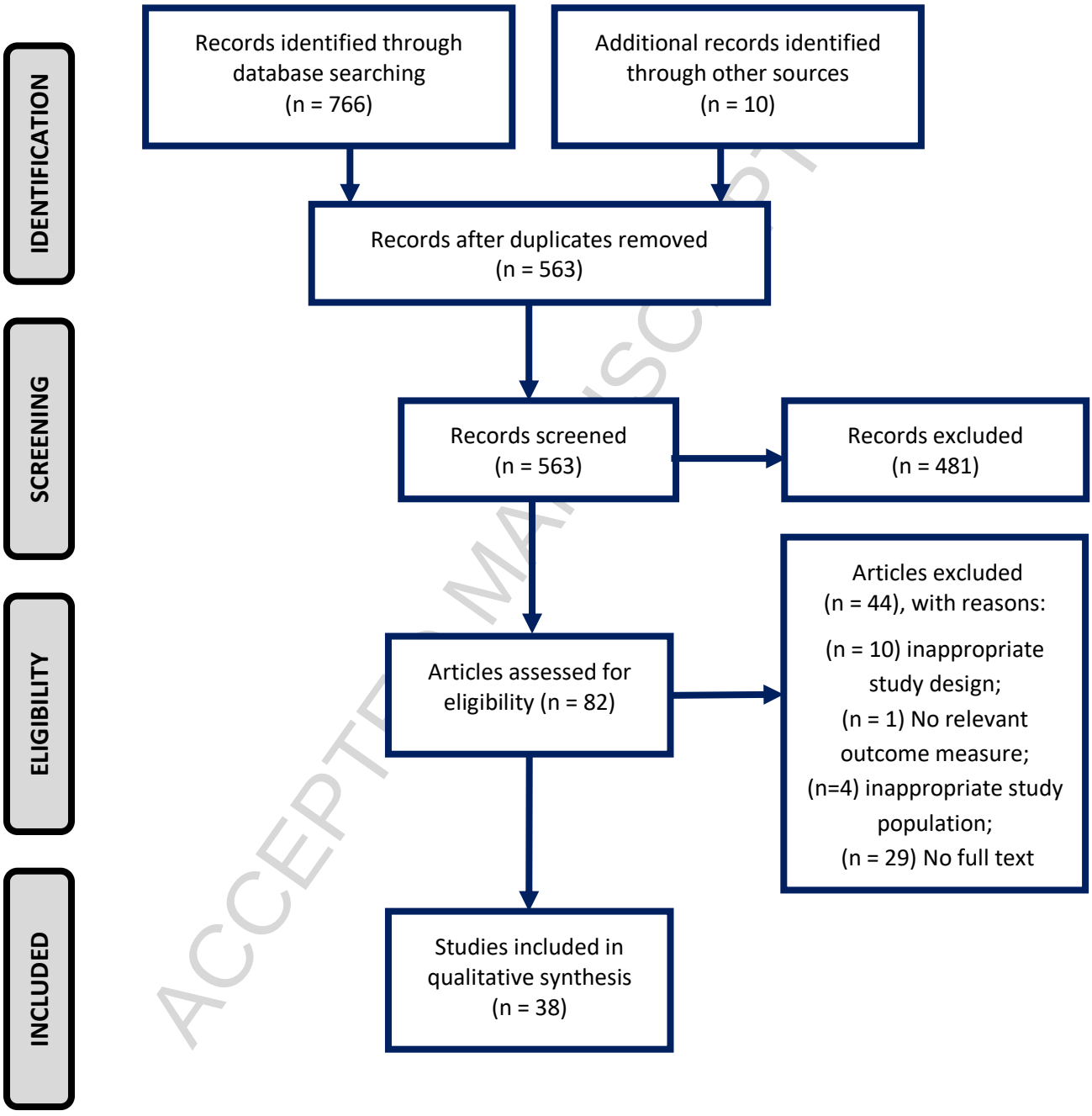

Table 1 Characteristics of in vitro studies (n = 8)

Author (year)	Conflict of interest	Type of study	Cell type	Length of exposure	Outcome measures
Anderson et al. (2016)[49]	✓	Non-randomised, controlled trial	umbilical vein endothelial cells	72 hours	Cell viability, Reactive oxygen species, DNA damage
Barber et al. (2016)[31]	×	Randomised, controlled trial	umbilical vein endothelial cells	48 hours	Cell viability, Cell metabolic activity, Complement deposition, gC1qR & cC1qR expression, Complement inhibitor expression
Hom et al. (2016)[34]	×	Randomised, controlled trial	platelets	4 hours	Platelet aggregation, Platelet adhesion, Complement deposition, C1q receptor expression
Lee et al. (2019)[52]	×	Non-randomised, controlled trial	pluripotent stem cell–derived endothelial cells	48 hours, (16 hours)[&]	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. (2015)[55]	×	Non-randomised, controlled trial	umbilical vein endothelial cells	48 hours	Cell viability, Inhibition of cell proliferation, Reactive oxidative species, morphological alterations
Schweitzer et al. (2015)[50]	×	Non-randomised, controlled trial	pulmonary microvascular cells	10 hours	Endothelial barrier disruption*
Taylor et al. (2017)[59]	✓	Non-randomised, controlled trial	umbilical vein endothelial	20 hours	Inhibition of cell migration

			cells		
Teasdale et al. (2016)[54]	✗	Non-randomised, controlled trial	coronary artery endothelial cells	64 hours	Genetic markers of oxidative stress#

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

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	16	0.5%	cigarette / sham	48 hours	Heart rate

		Crossover			smoking		
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)
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	40	0%	cigarette	1 week	Platelet aggregation,

		crossover					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
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.

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)[49]	Green smoke, Blu, Njoy, Vuse	3R4F*	1st	Not measured	Not measured	Not measured	+	500 µM	2 x (2 s puff / min), total vol = 55ml	0.22 µm
Barber et al. (2016)[31]	NJoy OneJoy, eGO OKC	Marlboro (1.2% nicotine)	1st	Not measured	Not measured	Not measured	+	1.2%, 1.8%, 0mg, 12mg, 18mg	2 x (5 s puff / min) 5 min total length	No
Hom et al. (2016)[34]	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)[52]	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. (2015)[55]	Unknown brand high-nicotine	NS	1st & non-1st?	Not measured	116 +/- 3.9°C in 10 seconds 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)[50]	iClear v6 Filtered	2R4F*	NS	Not measured	Not measured	Propylene glycol, glycerol, flavours, acrolein#	NS	2.5mM, 3.5mM, 5mM, 10 mM	1 cigarette / min, Similar rate for e-cigarette	0.2 µm

Taylor et al. (2017)[59]	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: 2s puff /30s vol = 55mL Cigarette: 3s puff /30s vol = 55mL	No
Teasdale et al. (2016)[54]	iStick, Aerotank, Haven fluid	Marlboro gold (0.6mg nicotine)	NS	4.2v, 10.8w, 1.8 ohms	Not measured (constant flow rate to minimise temperature spikes)	Not measured	-	18mg/mL	5 x (5s / 15s) 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.

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 glycerin38i buse38e (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 39i 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 Week 13 –	eCO ≤ 7 ppm

								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
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	Not assessed

								switched to ab libitum	
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. Insignificant increase in e-	e-cigarette: 50 x (5 s puffs / 30 s) & ad lib use / 1 hr 1 cigarette:	eCO ≤ 12 ppm+

							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
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)[34]	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. (2016)[31]	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)[49]	Cell viability	Reduced	P < 0.001	Reduced	P <0.001
Barber et al. (2016)[31]	Cell viability	Reduced	(4/5) P < 0.05	Reduced	P = NS
Lee et al. (2019)[52]	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	Increased	p = ???	-	-

	and lipid uptake)				
	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. (2015)[55]	Cell viability	Reduced	(5 / 11) P < 0.05	Reduced	P < 0.001
Anderson et al. (2016)[49]	DNA Damage	Detected	-	Detected	-
Putzhammer et al. (2015)[55]	Cell morphological alterations	Detected	-	Detected	-
Barber et al. (2016)[31]	Cell density	Reduced	P < 0.05	Reduced	P < 0.05
Putzhammer et al. (2015)[55]	Cell proliferation	Reduced	(4 / 11) P < 0.001	Reduced	P < 0.001
Taylor et al. (2017)[59]	Cell migration	Reduced	P = NS	Reduced	P < 0.05
Anderson et al. (2016)[49]	Reactive oxygen species	Increased	P < 0.001	Increased	P < 0.001
Putzhammer et al. (2015)[55]	Reactive oxygen species	Increased	(1 / 11) P < 0.001	Increased	P < 0.001
Teasdale et al. (2016)[54]	Upregulation of genetic markers of oxidative stress*	No	P = NS	Yes	P < 0.05
Barber et al. (2016)[31]	Cellular metabolic activity	Reduced	P < 0.05	Reduced	P < 0.05
Schweitzer et al (2015)[50]	Endothelial Barrier Disruption#	Yes	P < 0.0001	Yes	P < 0.0001

* (HMOX1, GCLM, OSGIN1, PAR4, CYP1A1, CYP1B1, IL8 and NTPX1) # Only for 5mM and 10mM not 2.5mM or 3.5mM 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
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	Systolic pressure	Increased	P = NS	Increa	P = NS	-

al. (2014)[64]				sed		
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	
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	Increased	P = NS	-	-	Increas

	Microvesicles					ed
	E-selectin positive microvesicles	Increased	P = 0.038	-	-	Increased
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	-
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
Espinoza-Derout et al. (2019)[53]	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
	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+	-	-	-
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+	-	-	-
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.

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[51] • ↑ CD34 immunostaining[51] • ↑ Endothelial cell tube formation[52]	• ↑ Reactive Oxygen species (ROS) - H_2O_2[47] - sNox2-dp[62] - 8-iso-PGF2a[47] - Plasma myeloperoxidase[45] - Malondialdehyde[53] - ↑ Circulating CD40L (activates endothelial cells to release ROS)[47] - ↑ Serum C-Reactive Protein[61] • ↓ Antioxidant activity - Vitamin E levels[47] - NO bioavailability - Nitric Oxide[62] metabolites[61] - HBA%[47]	• ↑ Arterial stiffness - ↓ Flow-mediated dilatation[47] - ↑ Pulse-wave velocity[48] - ↑ Augmentation index x 75[45] • ↓ vasodilatory response to acetylcholine[45] • ↓ vasodilatory response to methacholine[36] • ↑ vasoconstrictive response to phenylephrine[36] • ↑ Endothelial progenitor cells[40] • ↑ Endothelial complement deposition[31] • ↑ Endothelial complement inhibitor expression[31] • Endothelial barrier disruption[50] • ↑ Reactive hyperaemia index[67] • ↑ Vascular inflammatory markers - PECAM-1[58] - VCAM-1[58] - ICAM-1[61]	• ↑ Heart rate[48] • ↑ (Exercising)[41] systolic blood pressure[47] • ↑ (Exercising)[41] diastolic blood pressure[47] • Abnormal heart rate variability - Cardiac vagal tone[42] - Sympathetic tone[42] • Peripheral vasoconstriction[68]	• ↑ Platelet: - Aggregation[66] - Adhesion[34] - Complement deposition[34] - Alpha particle secretion[60] - Dense particle secretion[60] - Integrin activation[60] - Resistance to prostacyclin inhibition[60] - ↓ thrombomodulin[58] - ↓ Bleeding time[60] - ↓ Occlusion time[60]	• Altered cardiac structure: - ↑ Left ventricular mass[36] • Altered cardiac function: - ↓ Left Ventricular Ejection Fracture[53] - ↓ Left Ventricular Fractional Shortening[53] - ↓ Velocity of Circumferential Fibre Shortening[53] • Cardiac mutagens: - O^6-methyldeoxyguanosines[38] - γ-hydroxy-1,N2-propano-deoxyguanosines[38]

			• ↑ Endothelial cell: - Morphological alterations[55] - DNA damage[49] - Inhibition of migration[52] • ↓ Endothelial cell: - Proliferation[55] - Cell density[31] - Metabolic activity[31] - Viability[31]			

Highlights

- Electronic cigarettes appear to be harmful to the cardiovascular system.
- Numerous studies have substantial bias or a conflict of interest.
- Studies with a conflict of interest appraise e-cigarettes more favourably.
- Studies with substantial bias tend to appraise e-cigarettes favourably.

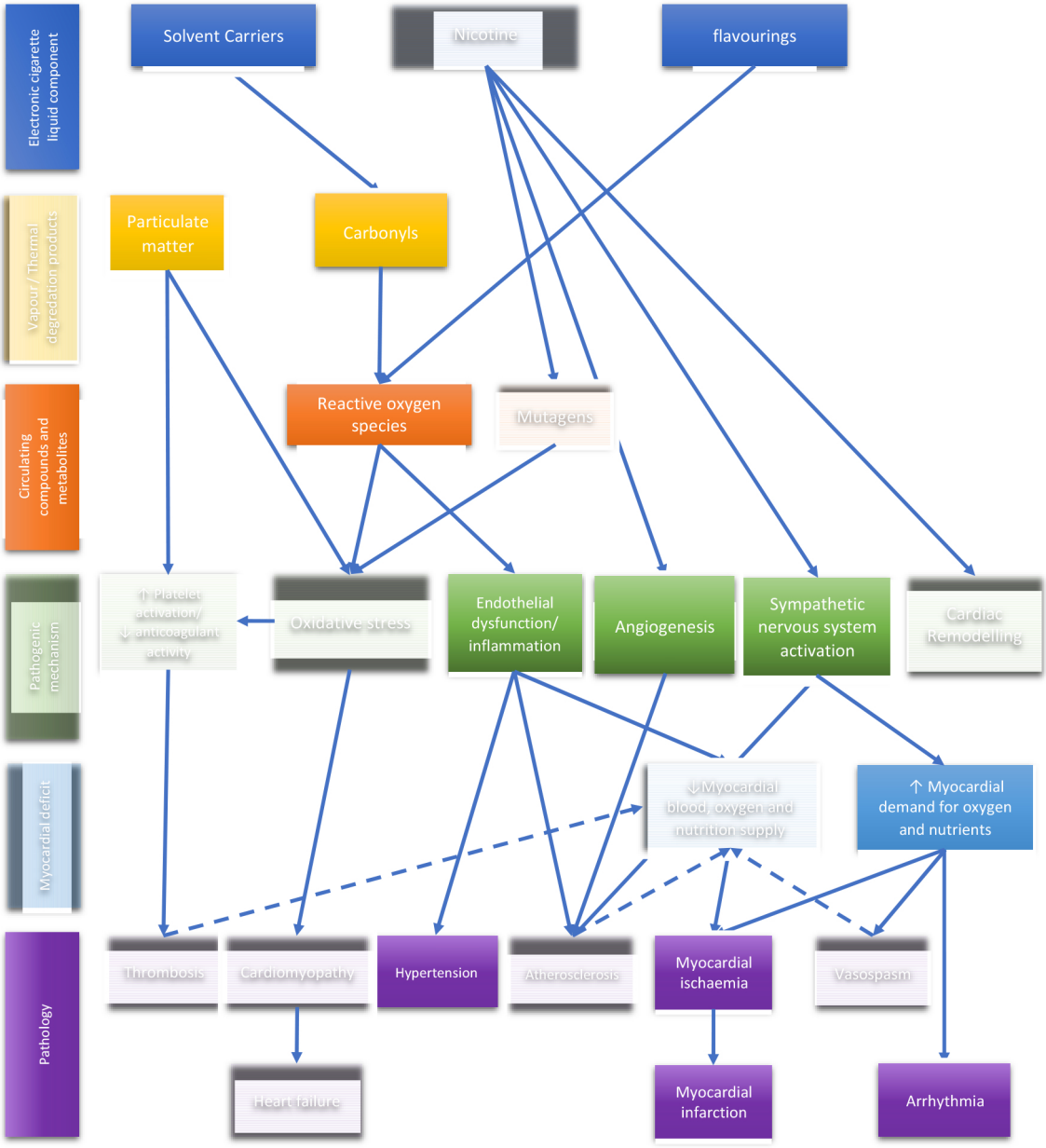
Electronic cigarette liquid component
Solvent Carriers
Nicotine
flavourings
Vapour / Thermal degredation products
Particulate matter
Carbonyls
Circulating compounds and metabolites
Reactive oxygen species
Mutagens
Pathogenic mechanism
↑ Platelet activation/ ↓ anticoagulant activity
Oxidative stress
Endothelial dysfunction/ inflammation
Angiogenesis
Sympathetic nervous system activation
Cardiac Remodelling
Myocardial deficit
↓Myocardial blood, oxygen and nutrition supply
↑ Myocardial demand for oxygen and nutrients
Pathology
Thrombosis
Cardiomyopathy
Hypertension
Atherosclerosis
Myocardial ischaemia
Vasospasm
Heart failure
Myocardial infarction
Arrhythmia

Figure 1

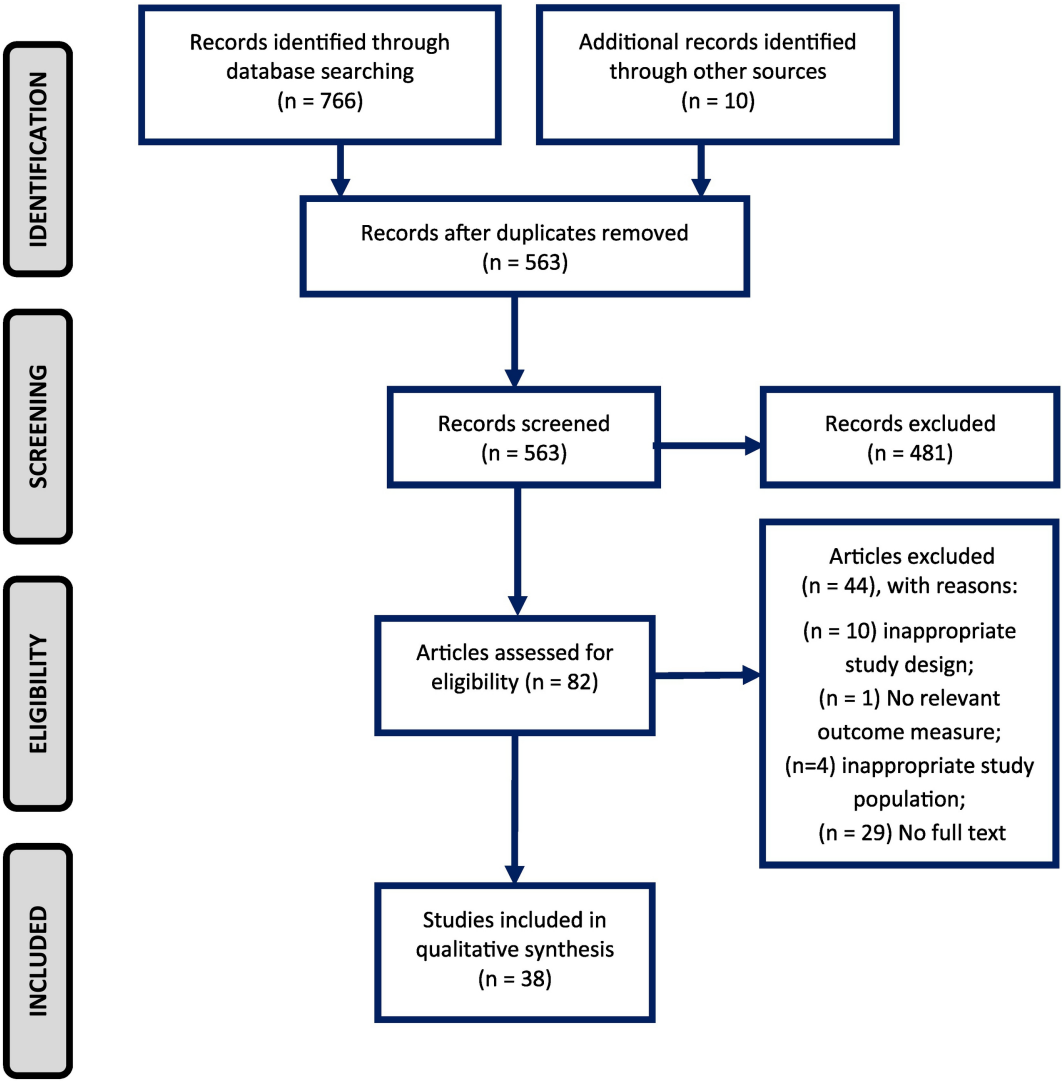
IDENTIFICATION
SCREENING
ELIGIBILITY
INCLUDED
Records identified through database searching (n = 766)
Additional records identified through other sources (n = 10)
Records after duplicates removed (n = 563)
Records screened (n = 563)
Records excluded (n = 481)
Articles assessed for eligibility (n = 82)
Articles excluded (n = 44), with reasons:
(n = 10) inappropriate study design;
(n = 1) No relevant outcome measure;
(n=4) inappropriate study population;
(n = 29) No full text
Studies included in qualitative synthesis (n = 38)

Figure 2