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CLINICAL REVIEW

Effects of tobacco cigarettes, e-cigarettes, and waterpipe smoking on endothelial function and clinical outcomes

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Tobacco smoking is a leading cause of non-communicable disease globally and is a major risk factor for cardiovascular disease (CVD) and lung disease. Importantly, recent data by the World Health Organizations (WHO) indicate that in the last two decades global tobacco use has significantly dropped, which was largely driven by decreased numbers of female smokers. Despite such advances, the use of e-cigarettes and waterpipes (shisha, hookah, narghile) is an emerging trend, especially among younger generations. There is growing body of evidence that e-cigarettes are not a harm-free alternative to tobacco cigarettes and there is considerable debate as to whether e-cigarettes are saving smokers or generating new addicts. Here, we provide an updated overview of the impact of tobacco/waterpipe (shisha) smoking and e-cigarette vaping on endothelial function, a biomarker for early, subclinical, atherosclerosis from human and animal studies. Also their emerging adverse effects on the proteome, transcriptome, epigenome, microbiome, and the circadian clock are summarized. We briefly discuss heat-not-burn tobacco products and their cardiovascular health effects. We discuss the impact of the toxic constituents of these products on endothelial function and subsequent CVD and we also provide an update on current recommendations, regulation and advertising with focus on the USA and Europe. As outlined by the WHO, tobacco cigarette, waterpipe, and e-cigarette smoking/vaping may contribute to an increased burden of symptoms due to coronavirus disease 2019 (COVID-19) and to severe health consequences.

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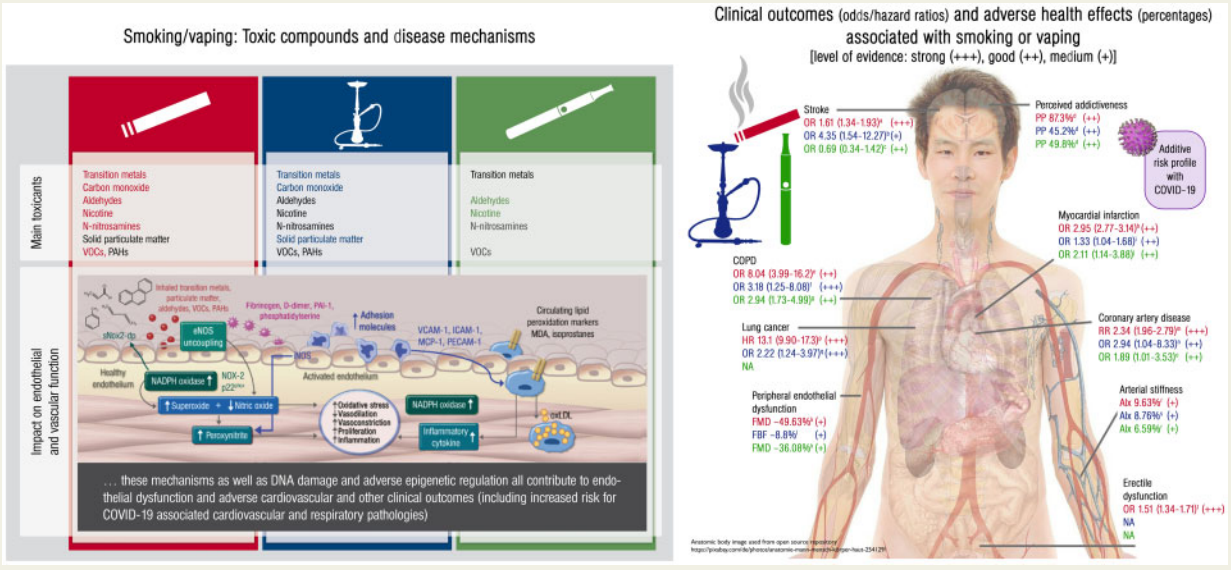
Graphical Abstract



Keywords Tobacco smoking • Shisha/waterpipe smoking • E-cigarette vaping • Endothelial function • Oxidative stress • Inflammation

Introduction

'Tobacco is a legal drug that kills many of its users when used exactly as intended by manufacturers'. That is the beginning sentence of the World Health Organizations (WHO) global report on trends in tobacco smoking from 2015.[1] Indeed, in 2019, the WHO announced that tobacco (i.e. tobacco cigarettes, pipes, cigars, waterpipes, smokeless tobacco products, and heated tobacco products, but not e-cigarettes) kills up to half of its users and more than 8 million people each year, of which 7 million are the result of direct tobacco use, while exposure to second-hand smoke accounts for 1.2 million deaths among non-smokers.[2] Thus, the tobacco epidemic represents one of the biggest threats for public health as a major but also entirely preventable cause of cardiovascular morbidity and mortality (summarized in *Figure 1*). Recent data indicate that in the last two decades global tobacco use has dropped from 1.397 billion in 2000 to 1.337 billion in 2018, which was largely driven by decreased numbers of female smokers (a decline of around 100 million).[2] The WHO projects for the first time a decline of more than 1 million male smokers by 2020 compared to 2018.[2]

Despite such advances, the use of e-cigarettes and waterpipe products (shisha, hookah, narghile) is an emerging trend, especially among younger generations. The misperception that these products are substantially less harmful than tobacco cigarettes or even represent healthier alternatives, provides an explanation for this trend.[3] The claim of being less harmful, the availability of countless 'appealing' flavours and the lack of regulations after their introduction to the broader market make e-cigarettes and waterpipe products an attractive gateway drug for adults and adolescents who have not previously smoked. Consequently, the number of e-cigarette and waterpipe users has dramatically increased, with e-cigarettes being the most commonly used smoking products in 2014 in the USA (>9-fold increase in usage from 2011 to 2015) and a projected global sales volume of US$26.84 billion by 2023.[4–6] Also the prevalence of lifetime waterpipe use is of concern, ranging from 2.1% to 44.0% in the USA, 11.6% to 40.1% in the UK, and 20.0% to 28.9% in Germany.[7] As a consequence, the USA recently announced a countrywide ban on flavoured e-cigarettes, which may also include the ban of flavoured waterpipe tobacco in the future, following the lead of other countries with a strict prohibition of the use and sale of e-cigarettes (see last sections for details).[8]

The mechanisms leading to cardiovascular diseases (CVDs) and mortality in smokers are multifactorial and not fully understood. Impaired endothelial function is an early pathophysiological biomarker in cigarette smokers as shown in 1993 by Celermajer *et al.*[9] Importantly, endothelial dysfunction has been shown to be an early critical event in the pathogenesis of most CVD.[10,11] In this review, we will discuss the current state of literature on how smoking and/or vaping induces endothelial dysfunction and atherogenesis with focus on tobacco cigarettes, e-cigarettes, and waterpipe.

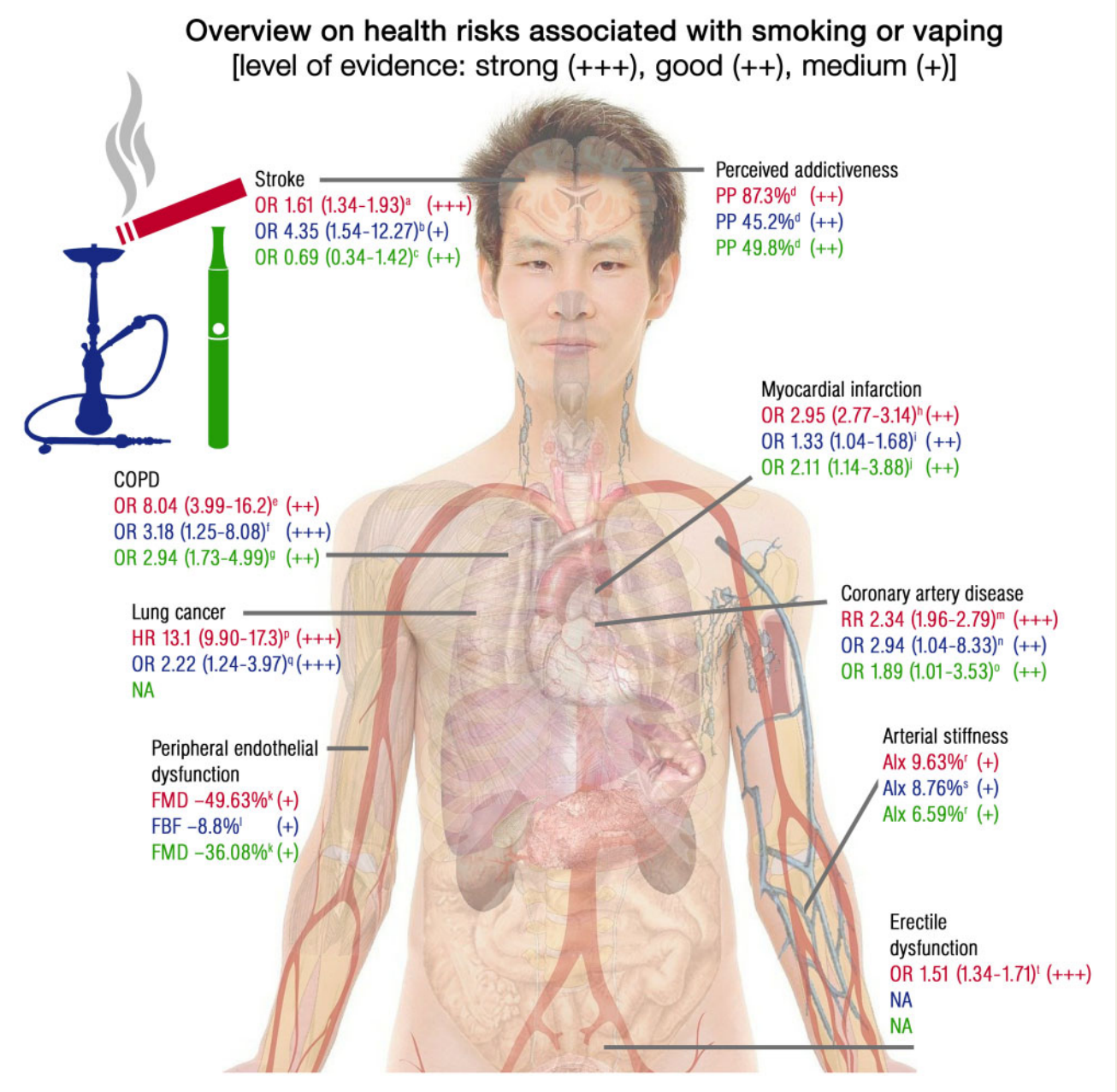


Figure 1 Hazard/odds ratio (HR/OR) or adverse effects (percentages) for smoking or vaping associated health risks or complications based on selected representative studies. All alphabetical cross references in this figure ([a, b, c, ...]) are linked to literature reference numbers in the figure legend that can be found in the Supplementary material online with exactly identical numbering. *Stroke* source reports: a[165] (+++), b[166] (+), and c[167] (++). *Perceived addictiveness* source reports: d[168] (++). Percentages refer to the proportion of participants (PP) who believe that smoking/vaping is addictive. Chronic obstructive pulmonary disease (COPD) source reports: e[169] (++), f[170a] (+++), and g[170b] (++) *Myocardial infarction* source reports: h,[171] i,[172] and j[173] (++). *Peripheral endothelial dysfunction* source reports: k[94] (+) and l[143] (+). Percentages denote (k) relative difference in flow-mediated dilation (FMD) of the brachial artery in response to acute tobacco cigarette and e-cigarette use and (l) relative decrease in arterial forearm blood flow (FBF) in response to acute waterpipe smoking. *Coronary artery disease* source reports: m[174] (+++), n[175] (++), and o[173] (++). *Lung cancer* source reports: p[176] (+++) and q[177] (+++). *Arterial stiffness* source reports: r[178a] (+) and s[178b] (+). Percentages denote relative increase in augmentation index (AIx) measured by tonometry in response to chronic tobacco cigarette and e-cigarette use or acute waterpipe smoking. *Erectile dysfunction* source reports: t[179] (+++). NA means not available. + means medium (single cohort study <1000 subjects), ++ means good (single cohort study >1000 subjects) and +++ means strong (large scale meta-analysis) clinical evidence. Open access source for body image can be found at Pixabay (https://pixabay.com/de/photos/anatomie-mann-mensch-körper-haut-254129/). Note of caution: The presented values are based on selected representative studies with different quality levels of evidence highlighting the need for more research.

Table 1 Toxic compounds in smoke from tobacco cigarettes and waterpipe, and vapour from e-cigarettes (all references in this table can be found in the Supplementary material online with exactly identical numbering)

Toxic compound type	Toxic compound	Concentration range cigarette	Concentration range e-cigarette	Concentration range waterpipe
Carbonyls	Formaldehyde	7–10 μg/puff[2,32,180]	0.12–82 μg/puff[181–184]	0.21–0.65 μg/puff[185,186]
	Acetaldehyde	50–140 μg/puff[2,32,180]	0.2–53 μg/puff[181–184]	2.0–5.5 μg/puff[185,186]
	Acrolein	6–14 μg/puff[2,32,180]	0.12–3.3 μg/puff[181–184,187]	0.06–1.19 μg/puff[185,186]
	Propionaldehyde	0.4–5.9 μg/puff[2,32,180]	0.057–1.79 μg/puff[181,182]	0.05–1.06 μg/puff[185,186]
	Crotonaldehyde	1–2 μg/puff[2,32,180]	ND–0.04 μg/puff[188]	0.78–1.39 μg/puff[189]
N-Nitrosamines	N′-Nitrosonornicotine (NNN)	0.5–370 ng/puff[2,32,180]	ND–0.029 ng/puff[22,183,190]	0.2 ng/puff[185,191]
	N′-Nitrosoanabasine (NAB)	ND–15 ng/puff[2,32,180]	ND–0.01 ng/puff[22,190]	0.05 ng/puff[185,191]
	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	1.2–77 ng/puff[2,32,180]	ND–0.019 ng/puff[22,183,190]	0.27 ng/puff[185,191]
	N′-nitrosoanatabine (NAT)	0.8–16 ng/puff[2,32,180]	ND–0.085 ng/puff[22,190]	0.6 ng/puff[185,191]
VOCs	Toluene	0.8–6.9 μg/puff[2,32,180]	ND–1.53 μg/puff[192]	0.058 μg/puff[185,193]
	Benzene	0.6–4.5 μg/puff[2,32,180]	ND–0.41 μg/puff[192]	1.58 μg/puff[185,193]
Inorganic compounds	Nickel	ND–60 ng/puff[2,32,180]	0.1–6.4 ng/puff[194]	9.9 ng/puff[185,195]
	Cobalt	0.013–0.02 ng/puff[2,32,180]	0.05–0.58 ng/puff[196]	0.7 ng/puff[185,195]
	Chromium	0.4–7 ng/puff[2,32,180]	0.05–9 ng/puff[194]	13.4 ng/puff[185,195]
	Lead	3.4–8.5 ng/puff[2,32,180]	0.16–3.8 ng/puff[197]	68.7 ng/puff[185,195]
	Carbon monoxide (CO)	1–2.3 mg puff[2,32,180]	not applicable	1.15–1.67 mg/puff[40,185,186]
PAHs	Benz[a]anthracene	2-7 ng/puff[2,32,180]	Not applicable[a]	1.3–1.6 ng/puff[40,185,191]
	Benzo[b + k]fluoranthene	1–3.4 ng/puff[2,32,180]	Not applicable[a]	0.13–2.16 ng/puff[40,185,191]
	Benzo[a]pyrene	2–4 ng/puff[2,32,180]	Not applicable[a]	0.78–1.79 ng/puff[40,185,191]
	Dibenzo[a, h]anthracene	0.06–0.4 ng/puff[2,32,180]	Not applicable[a]	0.86–ng/puff[40,185,191]
Nicotine		0.1–0.3 mg/puff[2,32,180]	0–0.142 mg/puff[196]	0–0.058 mg/puff[185,186,198]
Particulate matter	TPM	0.1–1.7 mg/puff[32]	0.87–5.8 mg/puff[199,200,b]	1.8–9.3 mg/puff[40,198,201]

PAHs, polycyclic aromatic hydrocarbons and heterocyclic aromatic hydrocarbons; VOCs, volatile organic compounds.
[a]Only a few studies find concentrations of PAHs in e-cigarette vapour that are close to the limit of quantification.
[b]Liquid aerosol droplets.

Physiology and prognostic implications of endothelial function

The endothelium is fundamental in the regulation of vascular tone, inflammation, vascular growth, platelet aggregation, and coagulation.[10,11] The endothelium produces important vasodilators with anti-atherosclerotic and antiaggregatory properties such as nitric oxide (•NO) and prostacyclin. Endothelial dysfunction (impaired biochemical pathways in endothelial cells) is a characteristic feature of coronary artery disease,[12] and a predictor of atherosclerosis[13] and future cardiovascular events.[14] Endothelial dysfunction is associated with traditional cardiovascular risk factors such as age, smoking, diabetes mellitus, hyperlipidaemia, genetic predisposition, and arterial hypertension.[10,11] Endothelial dysfunction in humans is mostly measured by flow-mediated dilation (FMD) in the forearm, a technique where the reinitiated blood flow after occlusion (ischaemia) for some minutes causes endothelial •NO formation and vasodilation by mechanical and reoxygenation-mediated stimuli (called hyperaemia).[15] Although the underlying pathophysiological mechanisms of endothelial dysfunction are likely to be multifactorial, an increased production of reactive oxygen species (ROS) within the vascular wall by systems such as the nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, the mitochondrial electron transport chain, and uncoupled endothelial nitric oxide synthase (eNOS) are thought to contribute to this phenomenon.[10,11,16] The extremely fast, diffusion-limited reaction of •NO with superoxide leads to formation of the highly reactive oxidant peroxynitrite ($ONOO^-$), that has vasoconstrictive and cytotoxic effects, causing oxidative damage to proteins, lipids, and DNA. Oxidative degradation of •NO by superoxide, uncoupling of eNOS and tyrosine nitration/inactivation of prostacyclin synthase are three particular examples of how oxidative stress impairs endothelial function.

(Toxic) compounds in tobacco cigarette smoke, e-cigarette vapour, and waterpipe smoke

The literature on toxic compounds found in tobacco cigarette smoke, e-cigarette vapour, and waterpipe smoke is vast. Here, we present only the most important toxic components (summarized in *Table 1*), while a detailed overview can be found in the Supplementary material online. Some basic facts on heat-not-burn tobacco products (e.g. iQOS), their regulation and data on their toxicity were reviewed previously[17] and more details can be found in the Supplementary material online.

Nicotine is the main reason why humans consume tobacco cigarettes and their alternatives. This alkaloid is known to increase blood pressure and heart rate, but it can also cause FMD due to the increased cardiac output.[18] Effects of nicotine have been controversial since its consumption has been associated with lower incidence of Parkinson's disease and Alzheimer's disease,[19] and also with protection against ulcerative colitis.[20]

Since classical tobacco cigarettes and waterpipe both burn tobacco, similar toxic compounds are produced. Most important classes of toxic compounds in tobacco-derived smoke are nitrosamines and polycyclic aromatic hydrocarbons. These two groups of compounds are known carcinogens and have been associated with many other complications including CVD.[21,22] Tobacco specific nitrosamines have also been found in nicotine containing e-cigarette vapour.

E-cigarette liquid contains only nicotine, propylene glycol, and glycerine. Accordingly, most of the toxic compounds found in e-cigarette vapour are derived from the three ingredients. The main group of toxic compounds responsible for the observed health effects of e-cigarettes are carbonyl compounds. Toxic aldehydes and ketones that are generated as degradation products during heating of e-cigarette liquid have known negative health effects. Most notably, formaldehyde and acrolein exhibit their toxicity through adduct formation with proteins and DNA, causing oxidative stress and endoplasmic reticulum stress, mitochondrial dysfunction and inflammation.[23]

Other groups of toxic compounds found in both tobacco cigarette/waterpipe smoke and e-cigarette vapour are volatile organic compounds and inorganic compounds such as metals and carbon monoxide (CO). Carbon monoxide is a gaseous compound that is produced as a result of incomplete combustion of organic matter. Carbon monoxide is toxic to humans since it can irreversibly bind to haemoglobin and inhibit oxygen delivery and causes hypoxia.[24] Although CO is toxic to humans, even at low concentrations, it is also an intrinsic signalling molecule produced by mammalian cells, with roles in vasodilation, regulation of leucocyte aggregation (anti-atherosclerotic effect), and reduction of ischaemia/reperfusion injury.[25] Carbon monoxide can also play a role in ischaemic preconditioning, which could be one of the explanation for the 'smoker's paradox'.[26] Since the effects of CO on the cardiovascular system are numerous and contradictory and have been explored before,[27] we only summarized the main facts here and provide some more details in the Supplementary material online.

All of these compounds have known toxic effects on the human body including, but not limited to, inflammation, cancer, CVD, and impairment of cognitive ability.[27–30]

Details on the legal regulations of these toxic compounds as well as the selection criteria for the inclusion of literature on toxic compounds in tobacco smoke and e-cigarette vapour (Supplementary material online, *Tables S1* and *S2*) can be found in the Supplementary material online.

Comparison of smoking/vaping patterns for tobacco cigarette, e-cigarette, and waterpipe

Smoking (puffing) topography is a set of parameters that describes how frequently and with what volume a person inhales during smoking. Smoking topographies of tobacco cigarette, e-cigarette, and waterpipe users are described in *Table 2*. When consuming their preferred device, users exhibit very different smoking topographies. Since puffing topography is an indirect method of determining exposure to smoke or vapour, the higher puff volumes and more frequent puffs observed in e-cigarette and waterpipe user could potentially increase the exposure to toxicants.[33]

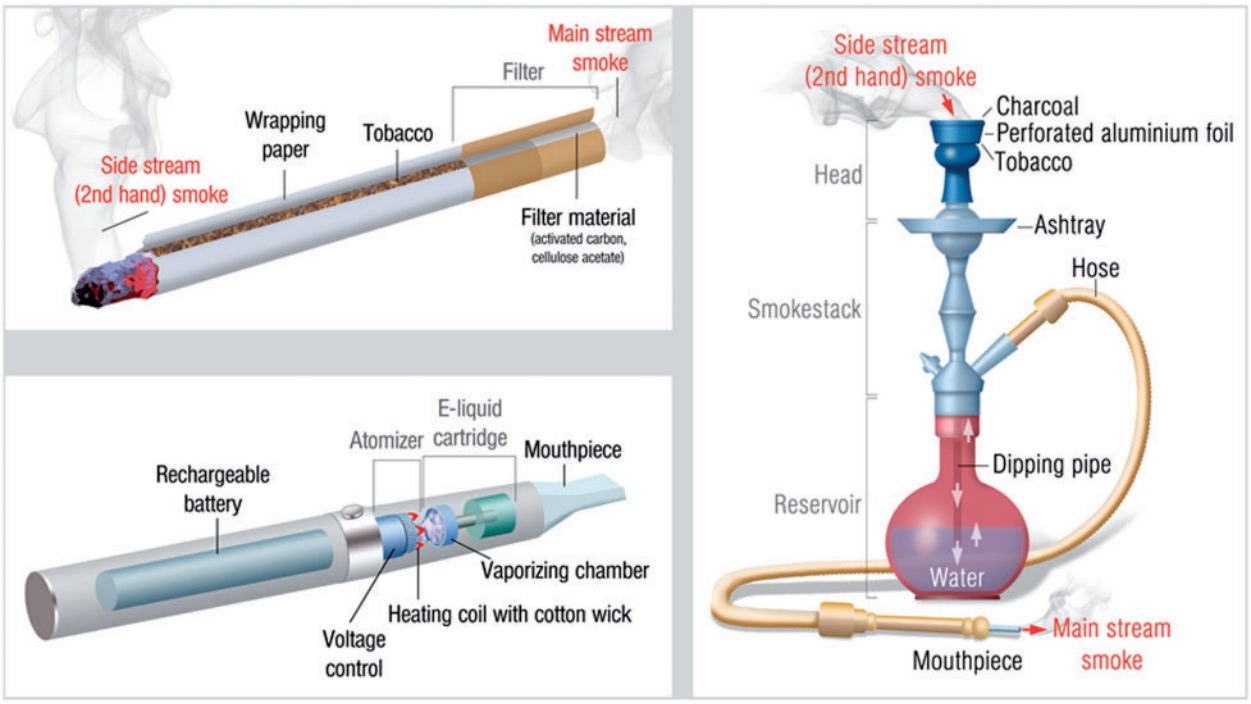
Smoking/vaping and cardiovascular events

Over the last several decades, an undeniable body of evidence has emerged demonstrating the causal association between tobacco cigarette smoking and cardiovascular events including coronary heart disease, myocardial infarction, stroke, heart failure, and cardiovascular mortality.[35] The relationship between tobacco cigarette smoking and CVD was first established in large epidemiological studies, in particular, the British Doctors Study[36] and the Framingham Heart Study.[37] These studies and the following examples provide evidence of a significant contribution of cigarette smoking on major atherosclerotic CVD outcomes and mortality. In the INTERHEART multicentre case–control study with 27 089 participants from 52 countries current smoking was associated with a higher risk of non-fatal acute myocardial infarction [odds ratio (OR) 2.95, 95% confidence interval (CI) 2.77–3-14] when compared with never smoking.[38] The ARIC study showed ($N = 13\,355$) over a median follow-up of 26 years a dose–response relationship between pack-years of smoking and the incidence of peripheral artery disease, coronary heart disease, and stroke.[39] A meta-analysis of the association of cigarette smoking with cardiovascular mortality, including prospective studies from 25 cohorts ($N = 503\,905$), found a hazard ratio of 2.07 (95% CI 1.82–2.36) and 1.37 (95% CI 1.25–1.49) for current and former smokers, respectively.[40] Risk estimates in this study for acute coronary and stroke events followed a similar pattern. Also epigenetic changes may represent valuable risk markers in smokers as lower F2RL3 methylation was strongly related to higher mortality among a cohort of smokers with stable coronary heart disease[41] (for more details see Supplementary material online).

Moreover, there is extensive evidence on the association between chronic waterpipe smoking and cardiovascular health.[42] A recent systematic review and meta-analysis revealed an OR of 1.67 (95% CI 1.25–2.24) for the association of prevalent cardiovascular conditions such as ischaemic heart disease and heart failure with waterpipe smoking.[43] In general, adverse cardiovascular effects of long-term waterpipe smoking are comparable to those associated with tobacco cigarette smoking, showing that chronic use exceeding 40 waterpipe-years is associated with a three-fold increase in the odds of coronary artery stenosis.[44] Moreover, in a large prospective study ($N = 20\,033$) from Bangladesh waterpipe smoking was shown to be associated with increased risk of death due to ischaemic heart disease in both men and women.[45] More evidence for the adverse effects of waterpipe smoke on the heart was summarized in ref.46

Although there is a broad range of evidence for the adverse acute effects of e-cigarettes and their toxic properties on the cardiovascular system including oxidative stress and endothelial dysfunction,[47] studies concerning the long-term use of e-cigarettes and CVD risk are limited and controversial. A large study based on cross-sectional

Table 2 Schematic construction of tobacco cigarette, e-cigarette, and waterpipe with their corresponding puffing topographies



Tobacco cigarette	Waterpipe	E-cigarette
Tobacco cigarette users display very little deviation in their smoking topography.[31] This allowed for a standardized smoking protocol to be implemented in the form of an ISO 3308 standard (2 s puff duration, 35 mL puff volume, and 60 s duration between puffs). An average smoker consumes roughly between 10 and 20 cigarettes per day.	Waterpipe users usually attend only one smoking session per day which lasts for approximately 1 h. Puff volume, duration, and puffing frequency are all higher in waterpipe users in comparison to tobacco cigarette users. There is no ISO standard for waterpipe smoking, but most of the research groups use the 'Beirut' protocol (2.6 s puff duration, 530 mL puff volume, and 17 s duration between puffs).[32]	E-cigarette users show a much more pronounced deviation in puffing parameters, making a standard vaping protocol hard to design.[33,34] In 2019, an ISO 20768 standard was developed for routine analytical vaping experiments (3 s puff duration, 55 mL puff volume, and 30 s duration between puffs).

NHIS data from 2016 ($N = 33\,028$) and 2017 ($N = 26\,742$) observed that some days use of e-cigarettes was associated with increased risk of myocardial infarction (OR 2.11, 95% CI 1.14–3.88) after adjustment for other cardiovascular risk factors.[48] However, no statistically significant association was observed in the pooled analysis (daily e-cigarette use: OR 1.35, 95% CI 0.80–2.27, $P = 0.267$). Likewise, another study found daily e-cigarette use to be independently associated with increased odds of myocardial infarction (OR 1.79, 95% CI 1.20–2.66), as it was the case for daily tobacco cigarette smoking (OR 2.72, 95% CI 2.29–3.24).[49] In contrast, former and some days use of e-cigarettes were not significantly associated with higher probability of myocardial infarction. In addition, a recent longitudinal study established an independent association between former and current e-cigarette use and incident respiratory disease after adjustment for tobacco cigarette smoking, demographic, and clinical variables.[50] However, a recent systematic review and meta-analysis concluded that despite the adverse acute effects of e-cigarettes on heart rate, systolic, and diastolic blood pressure, benefits may occur concerning blood pressure regulation when switching from tobacco to chronic e-cigarette use.[51]

In line with this, a previous experimental study demonstrated that there was a significant improvement of endothelial function and vascular stiffness within 1 month of switching from tobacco to e-cigarettes in healthy smokers, whereas no clear trend in systolic blood pressure and heart rate was observed.[52] This finding indicates that switching to e-cigarettes may reduce the CVD burden in former tobacco cigarette smokers. It is important to note that the majority of

e-cigarette users are former smokers or dual users and that the availability of e-cigarettes may also promote the risk of smoking initiation in never smokers.[53] Moreover, the potential efficacy of e-cigarettes as a cessation tool remains unclear. In a recent randomized trial ($N = 886$) evaluating different smoking cessation approaches, the 1-year abstinence rate in the e-cigarette group was 18.0% when compared with 9.9% in the nicotine replacement group.[54] Among successful quitters, 80% of subjects in the e-cigarette group continued to use them a year later, compared to only 9% in the nicotine replacement group indicating that although the use of e-cigarettes was superior in terms of smoking cessation, quitting nicotine products was largely achieved in the nicotine replacement group.

Evidence of the association between smoking/vaping tobacco cigarette, e-cigarette, and waterpipe on vascular/endothelial function

A variety of different techniques have been employed to study the vascular consequences of smoking. Evidence for tobacco cigarette smoking-induced endothelial dysfunction and vascular damage stems from an array of studies, whereas data on the consequences of waterpipe and e-cigarette use are rather limited. Between and within these studies large differences in results may occur due to a variety of reasons such as differences in methods of measurements, time of assessment post-exposure, analysed populations, used tobacco/vaping products, and the amount and duration of inhaled smoke/vapour. Thus, difficulties in drawing absolute conclusions may arise, demanding caution in interpreting results. Here, a brief overview of the findings shall be provided. Supplementary material online, *Table S3* summarizes selected studies to date that have provided insights on vascular/endothelial effects of smoking/vaping exposure.

Tobacco cigarettes

As mentioned before, Celermajer *et al.*[9] provided early evidence that pack-years of smoking were dose-dependently associated with impaired FMD of the brachial artery. Likewise, long-term smoking was also associated with impaired endothelium-dependent coronary vasodilation.[55] Importantly, decreased FMD of the brachial artery in chronic smokers was shown to increase by 1% after 1 year of smoking cessation.[56] Impaired FMD was even measured in chronic smokers of light cigarettes (5.59%), showing no significant difference compared to chronic regular cigarette smokers (6.26%).[57] Furthermore, smoking a single cigarette, irrespective of type, impaired FMD in chronic smokers, indicating analogous chronic, and acute effects. The association between exposure to second-hand smoke and FMD was assessed in further clinical studies revealing strong effects. Dose-dependent impairment of FMD in healthy young subjects in response to second-hand smoke exposure was reported,[58] that may be reversible as demonstrated by a subsequent cross-sectional study.[59] In that study, FMD in former and current passive smokers was significantly impaired when compared to non-smoking controls, while former passive smokers (5.1%) displayed better FMD than current passive smokers (2.3%).

Heitzer *et al.*[60] provided first evidence for the contribution of ROS formation and dysfunctional uncoupled eNOS in the pathogenesis of smoking-induced atherosclerosis. The authors showed that smoking strongly enhances endothelial dysfunction in patients with the cardiovascular risk factor hypercholesterolaemia and that the antioxidant vitamin C almost completely reversed endothelial dysfunction in chronic smokers, pointing to a crucial role of ROS in the decreased vascular NO bioavailability of endothelial dysfunction. The eNOS co-factor tetrahydrobiopterin (BH4, cofactor of eNOS), but not NH4 (tetrahydroneopterin)[61] was associated with improved vasodilation in response to acetylcholine (*Figure 2A*), pointing to eNOS as a significant superoxide source in chronic smokers. In the ALSPAC study ($n = 1266$ teenagers) cigarette and alcohol use during adolescence showed additive cardiovascular health risk.[64]

Waterpipe smoke

Several harmful substances present in tobacco cigarette smoke are also present in waterpipe smoke, even exceeding those established in cigarette smoke. Importantly, a recent statement from the American Heart Association (AHA) concluded that the acute cardiorespiratory toxicity of a single session of waterpipe smoking is worse than smoking a single tobacco cigarette due to the significantly higher levels of cardiorespiratory toxicants such as heavy metals and particulate matter.[65] This is particularly concerning as there are general misperceptions about waterpipe smoke's addictive potential and adverse health effects including that a majority of users believe it is less harmful and addictive than tobacco cigarette smoking.[65] In addition to changes in cardiac function and blood pressure regulation as seen for tobacco cigarette smoking, a 30-min waterpipe smoking session in healthy young smokers was associated with increased vascular resistance and decreased forearm blood flow.[66] These results were later extended to subjects with lower physical activity and fitness level exhibiting heightened vascular responses, probably due to the lack of beneficial effects of physical activity on endothelial function.[67] In contrast, 30-min acute exposure to waterpipe smoke was not associated with changes in microvascular endothelial function using the Endo-PAT technique, which may indicate differences in the pathophysiological characteristics of endothelial dysfunction between circulatory beds.[68]

Smoking electrically heated waterpipe, similar to tobacco cigarettes, acutely impaired FMD (-27%), whereas smoking charcoal heated waterpipe led to increased FMD (*Figure 2B*).[62] The authors concluded that the acute endothelial dysfunction was masked by the potent vasodilator CO, generated by the combustion of charcoal. A case–control study of healthy subjects comparing endothelial function in users of chronic tobacco cigarettes vs. waterpipes vs. non-users showed that chronic waterpipe use (7.9%) was more detrimental to FMD than cigarette use (12%) compared to non-smoking (21.5%). These findings may be explained by differences in the amount of exposure since most waterpipe users smoked three to five sessions per day, while the number of cigarettes smoked per day was 10–20 for more than 5 years.[69] These findings are to be contrasted to a subsequent study that observed that chronic waterpipe and cigarette smoking caused a similar worsening of FMD as well as similar alterations of C-reactive protein levels.[70]

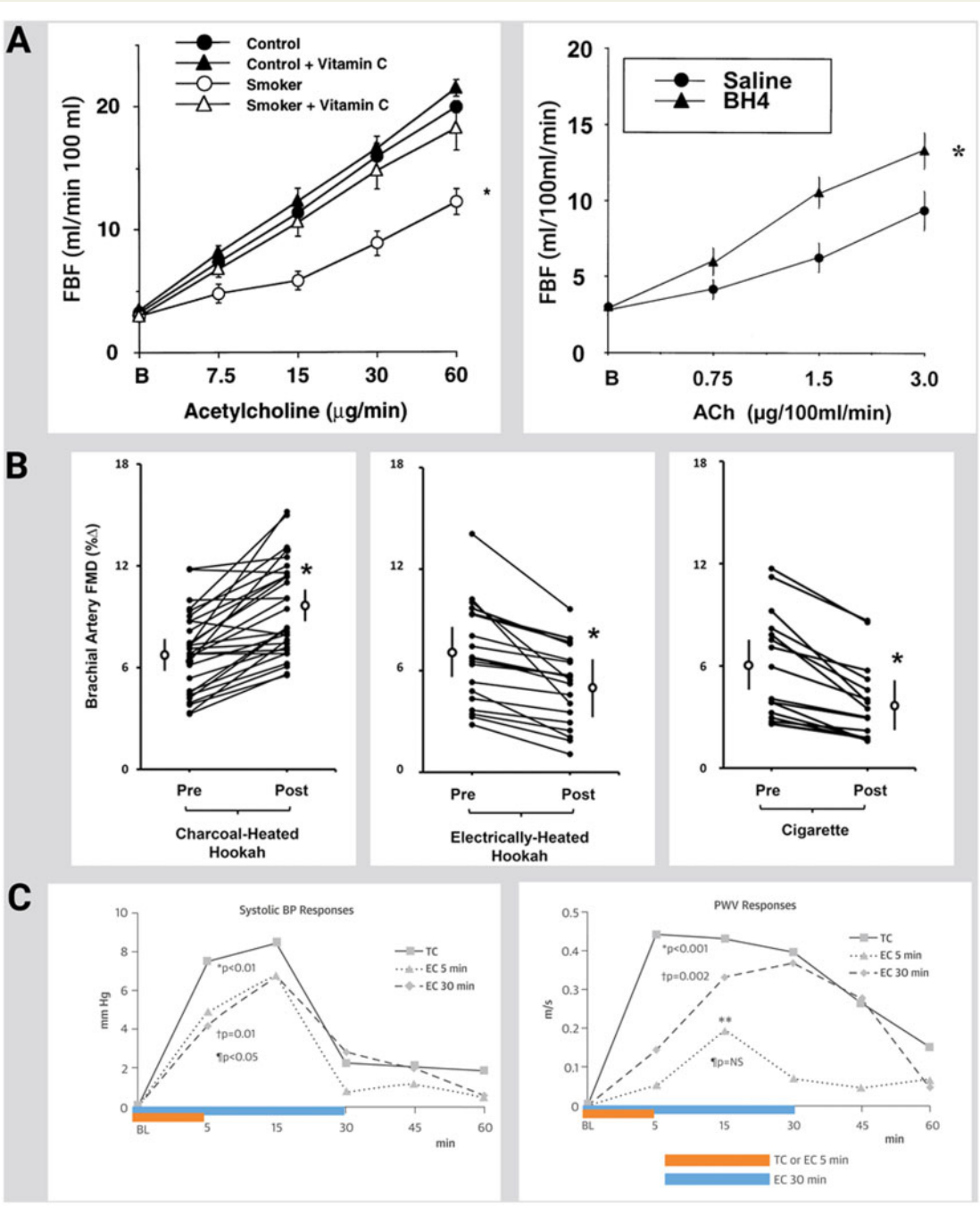


Figure 2 Effects of different forms of smoking and vaping on endothelial function in human subjects and antioxidant interventions. (*A*) *Left*, Vitamin C treatment markedly improves endothelium-dependent vasodilation to acetylcholine in chronic smokers while having no significant effect on the dose–response curve of control subjects. Mean ± SEM responses of forearm blood flow to intra-arterial acetylcholine in control subjects ($n = 10$) and chronic smokers ($n = 10$) with and without concomitant administration of vitamin C. *Significant difference in the overall dose–response relationship compared with control subjects with and without vitamin C and with smokers with vitamin C. Adopted from ref.[60] with permission of the publisher. Copyright © 2000, Wolters Kluwer Health. *Right*, Effect of BH4 on ACh-induced vasodilation in chronic smokers. BH4 significantly improved ACh-mediated vasodilation in chronic smokers but failed to change the ACh dose–response relationship in control subjects (not shown). Data are presented as mean ± SEM. *Significant differences in the overall dose–response vs. saline treatment. Adopted from Ref.[61] with permission of the publisher. Copyright © 2000, Wolters Kluwer Health. (*B*) *Left*, Individual and mean percentage changes before and after 30 minutes of charcoal-heated hookah smoking. *Middle*, Individual and mean percentage changes before and after 30 min of electrically heated hookah smoking. *Right*, Individual and mean percentage changes before and after smoking 1 cigarette. The circles with bars reflect the overall mean ± SEM. *$P < 0.05$ (pre- vs post-exposure). FMD indicates flow-mediated dilation. Adopted from ref.[62] with permission of the publisher. Copyright © 2000, Wolters Kluwer Health. (*C*) Systolic BP (*Left*) and PWV (*Right*) responses. Each line represents response defined as net TC/EC smoking effect minus sham procedure effect at each time point. BL means baseline; NS means non-significant. The *P*-values refer to the composite effect of TC/EC at 5 and 30 min vs. sham during the whole study duration. The composite effect of TC/EC vs. sham was determined by using mean pressure as covariate. *TC vs. sham, ¶EC at 5 min vs. sham, †EC at 30 min vs. sham, **$P < 0.001$, PWV change between EC 5 min session and sham session after 15 min smoking using the Student's *t*-test for paired measures. Adopted from ref.[63] with permission of the publisher. © 2016 by the American College of Cardiology Foundation.

E-cigarettes

While studies providing evidence for effects of long-term e-cigarette use on endothelial function are generally limited, more studies evaluating their short-term consequences have been conducted. Importantly, difficulties in drawing absolute conclusions based on current evidence arise from a variety of different approaches used in studies including differences in methods of endothelial function measurement, study population, device voltage, liquid composition (nicotine content and flavouring), and the amount and duration of vapour inhaled. However, the potential harmful effects of e-cigarette use are widely acknowledged as outlined in a recent editorial by Eissenberg *et al.*[3] that also called for adequate evaluation of the current evidence on the harms of e-cigarettes. In agreement with this, a recent systematic review and meta-analysis concluded that e-cigarettes should not be labelled as a safe product with respect to the cardiovascular system, in part due to the evidence linking e-cigarettes to impaired endothelial function.[51]

One study compared the acute impact of tobacco vs. e-cigarettes with same nominal nicotine content in healthy smokers and non-smokers on endothelial function, $^{\bullet}$NO bioavailability, markers of oxidative stress, and vitamin E levels. Both tobacco and e-cigarettes were shown to increase markers of oxidative stress and to decrease FMD, $^{\bullet}$NO bioavailability, and vitamin E levels in smokers and non-smokers, displaying no significant difference between tobacco and e-cigarettes.[71] Our previous work demonstrated that short-term e-cigarette use in healthy smokers caused marked impairment of endothelial function and an increase in arterial stiffness.[72] Others demonstrated acute microvascular endothelial dysfunction measured by acetylcholine-mediated vasodilation in smokers along with increased markers of oxidative stress and arterial stiffness after exposure to e-cigarettes with nicotine, but not after e-cigarettes without nicotine.[73] A similar impairment of vascular compliance was observed following acute tobacco or e-cigarette use in smokers (*Figure 2C*),[63] while a study of women smokers found a significant difference in stiffness after smoking just one tobacco cigarette, but not after use of e-cigarettes.[74] In healthy seldom smokers, 10 puffs of e-cigarette vapour inhalation caused an increase in circulating endothelial progenitor cells measured by flow cytometry, qualitatively analogous to the changes seen after smoking one tobacco cigarette.[75] A recent study comparing the acute effects of heat-not-burn devices, e-cigarettes, and tobacco cigarettes in healthy smokers revealed that single use of any product was associated with impaired FMD where heat-not-burn and e-cigarettes were equally less harmful than tobacco cigarettes.[76]

Evidence on adverse effects of smoking/vaping on endothelial function from animal studies *Table S4*

Prolonged exposure to tobacco cigarette smoke impaired acetylcholine-dependent vascular relaxation and coronary blood flow in rats that was associated with increased plasma cholesterol levels. Two weeks of tobacco cigarette smoke exposure caused mild hypertension and endothelial dysfunction in mice by down-regulation of sirtuin-3 and increased mitochondrial ROS formation that was improved by overexpression of mitochondria-targeted catalase.[77] Chronic cigarette smoking (up to 32 weeks) also decreased NO$^{\bullet}$ bioavailability and caused cardiac remodelling in mice that was also associated with structural damage of the endothelium. Genetic deletion of the detoxifying enzyme glutathione-S-transferase in mice aggravated tobacco smoking-induced endothelial dysfunction and increased acrolein-protein-adduct formation. Exposure of rabbits to chronic passive smoking for several weeks impaired endothelium-dependent relaxation of isolated rabbit arteries and caused left ventricular hypertrophy, but did not affect neurogenic contractions. Also mainstream smoke exposure of rabbits for several weeks impaired endothelial function of vascular tissues that was either corrected by ascorbic acid therapy or aggravated by a hypercholesterolaemic diet.

As indicated by our own results, e-cigarette vapour exposure (up to 5 days) caused endothelial dysfunction of isolated aortic segments of exposed mice that was associated with cardiovascular and cerebral oxidative stress, eNOS uncoupling, NOX-2 activation, endothelin-1 expression, as well as acrolein-adduct formation.[72] These adverse effects were corrected by the endothelin-receptor blocker macitentan and genetic *Nox2* deletion, as well as pharmacological NOX-2 inhibition. Studies on endothelial function in various animal models suggest comparable effects on smoking- or vaping-induced endothelial dysfunction. Also, chronic exposure of mice to e-cigarette vapour or tobacco smoke for 8 months induced a similar degree of arterial stiffness or endothelial dysfunction in isolated vessels (*Figure 3A and B*).[78] Acute exposure of rats to the vapour of high nicotine containing e-cigarette liquid (JUUL), normal nicotine containing e-cigarette liquid, or traditional tobacco smoke (Marlboro red) resulted in similar decrease in endothelial function (*Figure 3C and D*).[79,80] The nicotine uptake was measured by serum cotinine levels and correlated with FMD.

In summary, animal data suggest very similar extent of endothelial dysfunction by e-cigarette vaping or tobacco smoking, with oxidative stress and inflammation as central players of this process (*Figure 4*). These data are also in accordance with previous reviews on tobacco cigarette smoking,[82] waterpipe smoking,[42,83] and e-cigarette vaping.[47,84,85] More details on the molecular mechanisms (e.g. the interplay of toxic compounds, oxidative stress and inflammation as well as epigenetic/circadian regulations) of the induction of endothelial dysfunction by e-cigarette vaping or tobacco smoking can be found in the Supplementary material online (summarized in Supplementary Table S4). Epigenetic regulations (e.g. via sirtuin-1) may also negatively affect endothelial function and life span of smokers, e.g. by activation of the mitochondrial adaptor protein p66^{Shc} and subsequent mitochondrial dysfunction and oxidative stress.[86]

Recommendations/regulatory updates for tobacco, e-cigarette, and waterpipe smoking

Most of the countries in the world have implemented some form of tobacco products regulation. In the USA, the FDA regulates all aspects of tobacco production and sales. In 2016, FDA has included waterpipe and e-cigarettes into the list of tobacco products, so they are regulated the same way as tobacco cigarettes. Although FDA allows sales of e-cigarettes, some organizations within the USA have called for their total ban. The Center for Disease Control (CDC)

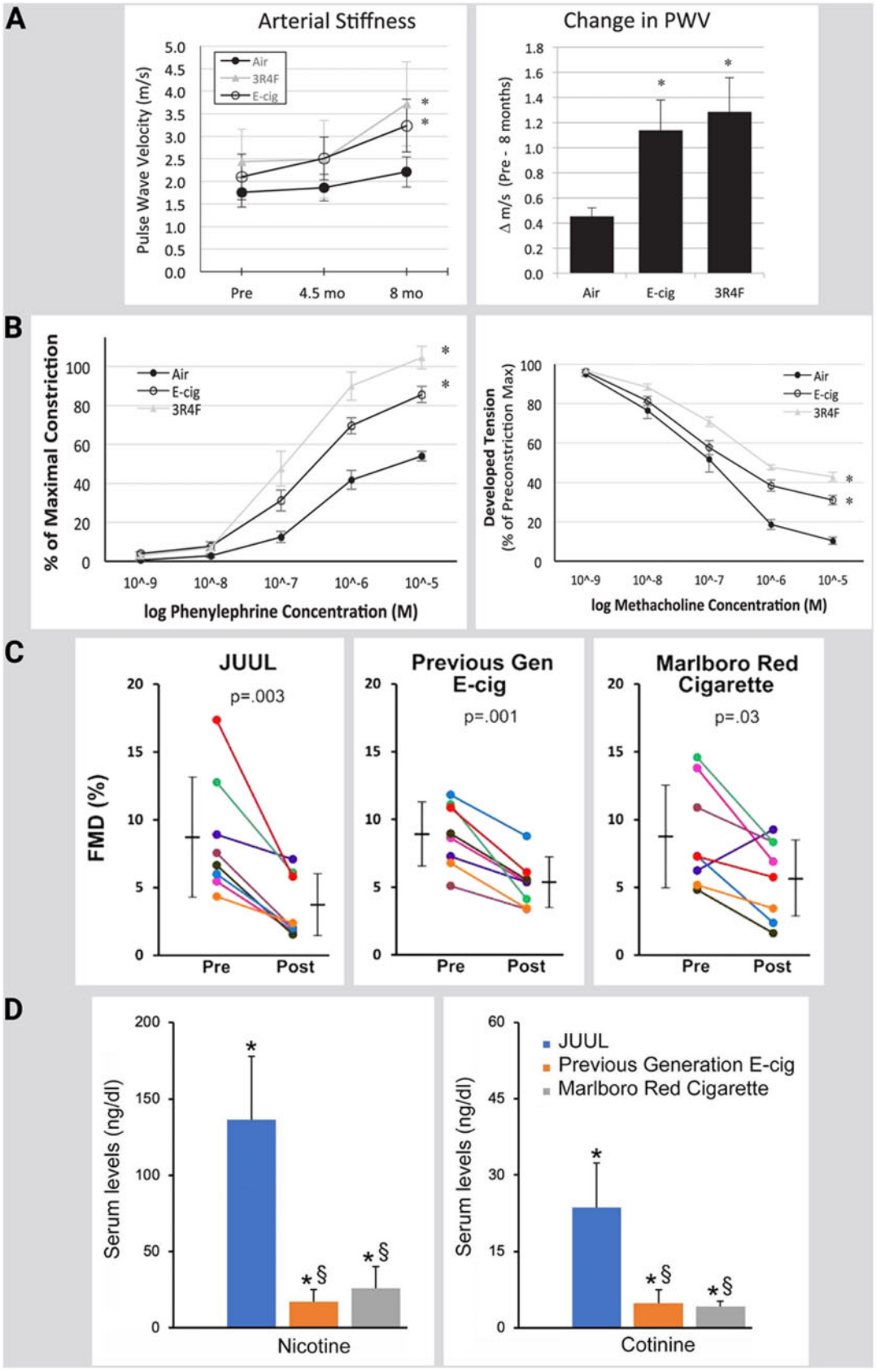


Figure 3 Chronic and acute effects of different forms of smoking and vaping on endothelial function in animals. (*A*) B-mode Doppler ultrasound *in vivo* data from the carotid artery of mice under anaesthesia (inhaled isoflurane) before, during (at ~4.5 months), and after 8 months of chronic

(continued)

recommends that e-cigarettes of all kinds 'never be used by youths'[87] and the WHO expressed reservations about the value of e-cigarettes and grave concerns about their risks.[88] The AHA has started a campaign to ban all e-cigarette sales.[89] The AHA claims that there is enough evidence associating e-cigarettes with teenager's addiction to nicotine and with seduction of non-smokers to smoking. A recent 'epidemic' of lung injury, and even deaths, resulting from use of e-cigarettes has caught attention of many health organizations.[90] Although the majority of the reported cases of lung injury have been associated with use of e-cigarettes for THC consumption as well as vitamin E additives, the CDC has responded by recommending that people should avoid vaping altogether.[91]

Countries belonging to the WHO Framework Convention on Tobacco Control (FCTC—181 countries, excluding USA) have all agreed that they will implement the recommended guidelines on protection of public health policies, protection from exposure to tobacco smoke, regulation of tobacco products, advertising, sales, and also for reduction measures concerning tobacco dependence and cessation.[92] The WHO does not have the power to create laws, but most of its member countries have implemented their recommendations to some extent. In the European Union (EU), tobacco products and smoking are heavily regulated. In most countries, there is a ban on smoking in the workplace and enclosed public spaces. Some countries (such as Hungary, UK, and Ireland) have a total ban on smoking in bars and hotels, while other countries (such as France, Germany, and Italy) allow for a designated smoking area in bars and hotels. Some countries have even banned smoking in outdoor areas near to hospitals, children's playgrounds, and bus stops.[93]

Nevertheless, the position of Europe in particular with respect to e-cigarettes is not consistent at all. In the UK, the National Health Service (NHS) and the British Heart Foundation (BHF) strongly support the use of e-cigarettes in order to quit tobacco smoking. According to a 2019 YouGov survey,[94] more than 3.6 million adults in Great Britain use e-cigarettes ~7.1% of the adult population. Of these users, 54% are ex-smokers, suggesting they are trying to stop smoking. It is proposed that it might be easier to quit with an e-cigarette. They also recommend that any smoker with a heart condition should try e-cigarettes in case he has tried to quit in the past, and failed. They propose that it might be a lot easier to quit smoking with an e-cigarette although they are probably not completely safe.

Waterpipe is considered a tobacco product in most countries and it also has to follow the no-smoking laws and regulations. Waterpipe bars are prohibited in some countries but can operate in others. Some countries have specific regulations for smoking establishments (usually no food or drinks can be served), and some treat them as regular smoking areas within a bar. E-cigarettes have been legally recognized as tobacco products in most countries, although individual countries regulate them differently. In some countries that ban indoor smoking (such as UK and Ireland), e-cigarettes could be used to circumvent no-smoking laws, since the ban does not apply to them. According to the 2019 report from the Global Center for Good Governance in Tobacco Control (GGTC), e-cigarette sales are banned in 42 countries and regulated in 56 countries.[95] The sales regulation by country is shown in *Figure 5A*. The 42 countries that have banned e-cigarette sales, represent 35% of the world population with another 35% living in the countries where sales are regulated. Some countries, such as Turkey, have issued a statement that they will completely ban all e-cigarette sales, but haven't done so at the present.

Advertising

In the EU, TV and radio advertising of cigarettes, and tobacco products is not permitted. Some countries (such as Slovenia and Norway) have strict laws that ban all types of advertising, even at the place of sale. Germany is unfortunately the only EU country where tobacco products, including e-cigarettes, can be advertised in public spaces via billboards. Also in the US advertising of both tobacco products and e-cigarettes through the medium of billboards is allowed. E-cigarettes are mostly considered as tobacco products, but the regulations on their advertising are not always clear. The map of Europe shows in which country advertising of e-cigarettes is legal (*Figure 5B*).

Impact of coronavirus disease 2019 (COVID-19) on cardiovascular risk in smokers and vapers

Recently, evidence has emerged indicating that tobacco use may increase the risk of adverse health outcomes in coronavirus disease

Figure 3 Continued
exposure to electronic cigarette (E-cig) vapour and reference tobacco (3R4F) cigarette smoke. *Left*: significant increase in arterial stiffness [measured as pulse wave velocity (PWV)] for E-cig and 3R4F groups following 8-month exposure. *Right*: significantly greater change in PWV (translating to greater arterial stiffness) after 8 months in E-cig- and 3R4F-exposed than control (air-exposed) mice. Slight, non-significant, rise in PWV in control mice following 8 months is consistent with the normal aging effect. $n = 5$–8 mice/group. $^{*}P < 0.05$ vs. air. (*B*) *Ex vivo* dose–response curves for phenylephrine (*Left*) and methacholine (*Right*), obtained from thoracic aorta ring segments following 8 months of exposure to E-cig vapour, reference tobacco (3R4F) cigarette smoke, and filtered air. α-Adrenergic vasoconstrictor response was greater (*Left*), while the endothelium-mediated vasodilatory response was impaired (*Right*), following 8 months of exposure to E-cig vapour and 3R4F cigarette smoke. Response to sodium nitroprusside was not altered or different between groups (not shown). $n = 5$ mice/group. $^{*}P < 0.05$ vs. air. Adopted from ref.[78] with permission of the publisher. Copyright © 2018, The American Physiological Society. (*C*) FMD was impaired by acute exposure to JUUL aerosol, previous generation e-cig aerosol, and Marlboro Red cigarette smoke. FMD after 5 min of exposure is shown. Coloured lines denote individual rats. Horizontal black bars denote the mean of the respective groups. *P*-values are derived from paired two-tailed *t*-tests. (*D*) Serum levels (ng/mL) of nicotine and cotinine from sera collected after 20 min of exposure. $^{*}P < 0.001$ compared to air group. $^{\S}P < 0.001$ compared to JUUL. 'Previous Gen' means previous generation. Adopted from ref.[79] Permission granted by Tobacco Regulatory Science Group to use figures. Rao P, Liu J, Springer ML. JUUL and combusted cigarettes comparably impair endothelial function. Tob Regul Sci 2020;6:30-37.

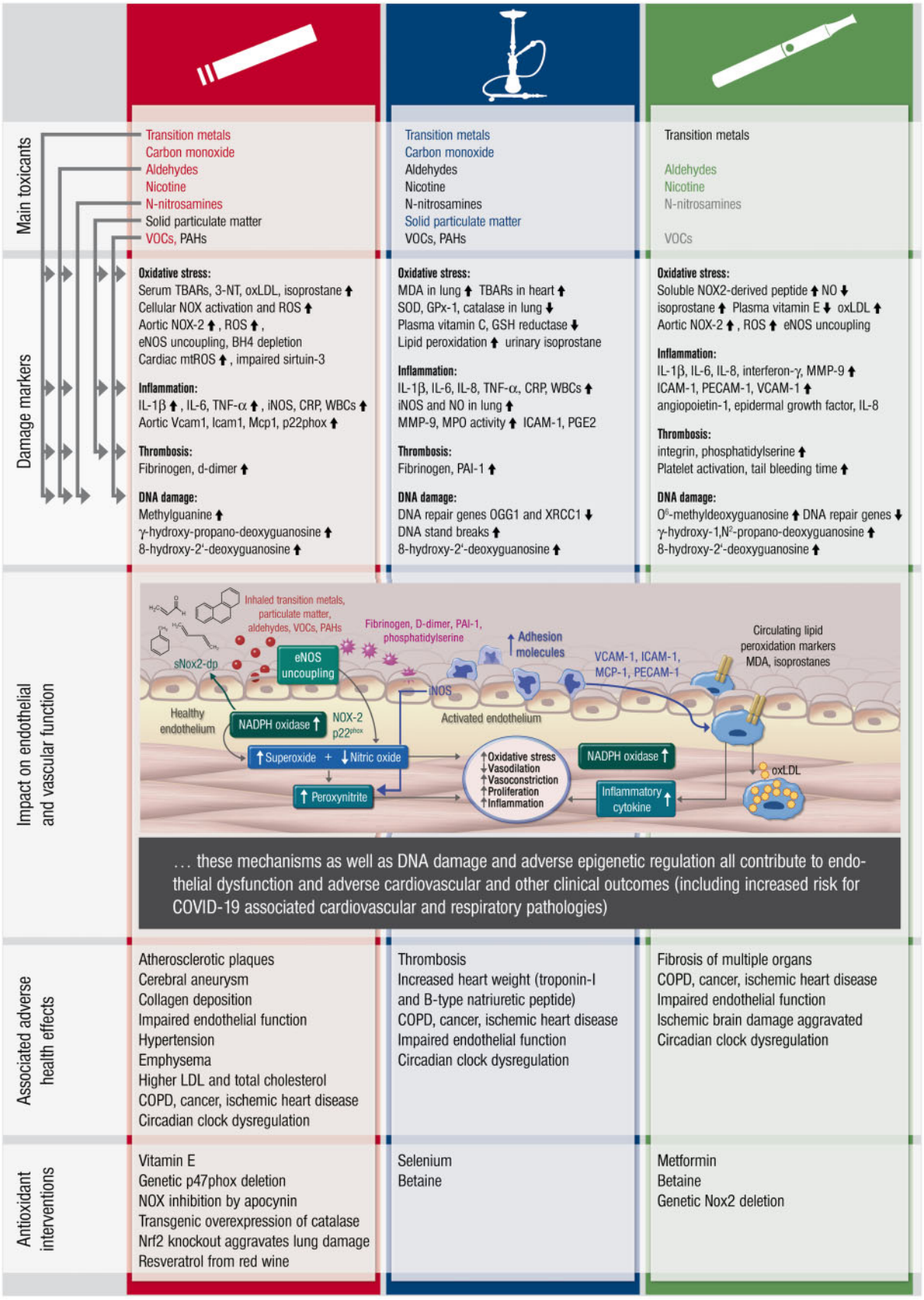


Figure 4 Effects of different forms of smoking and vaping as summarized from human and animal studies. The major toxicants (red, blue and green = high quantity, black = intermediate quantity, grey = trace amounts) for tobacco cigarette and waterpipe smoking as well as e-cigarette vaping are listed. The molecular link of these toxicants on major damage markers reported for the different forms of smoking and vaping with respect to oxidative stress is shown on the left side. The effects of these toxicants on endothelial (vascular) function are summarized in the inserted scheme (modified from ref.[81] with permission). The associated adverse health effects as well as antioxidant interventions are also shown.

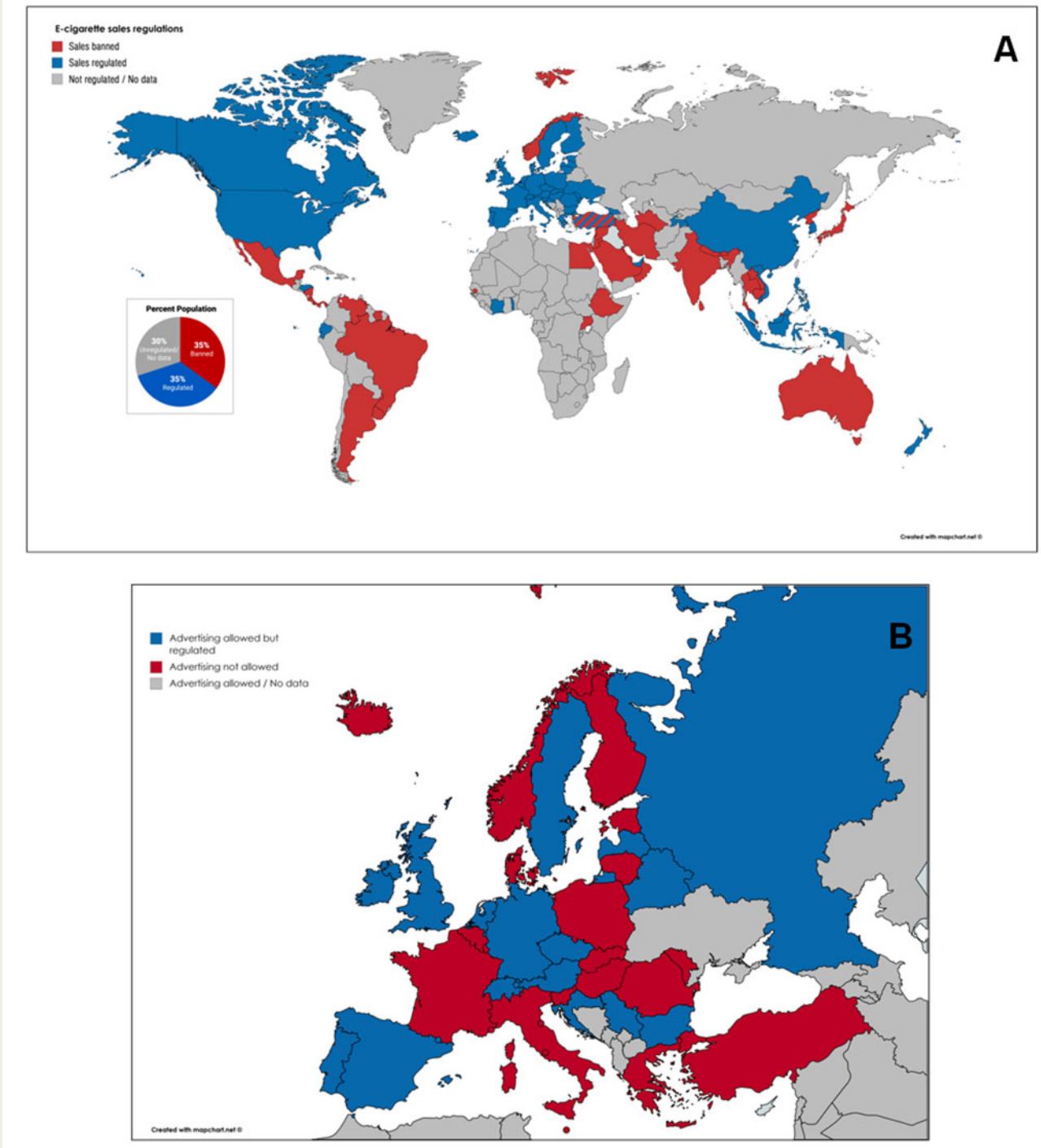


Figure 5 Overview on global regulations on e-cigarette products sales (*A*) and on European regulations on tobacco product advertising (*B*). Maps were created using data from www.globaltobaccocontrol.org, www.tobaccocontrollaws.org and https://ggtc.world (last accessed 31/05/2020).

2019 (COVID-19) patients. As outlined by the WHO, tobacco cigarette and waterpipe smoking may contribute to increased burden of symptoms due to COVID-19 compared to non-smoking, including being admitted to intensive care, requiring mechanical ventilation, and suffering severe health consequences.[96] Since smoking *per se* is a well-established risk factor for respiratory infections and increases the probability of having pre-existing conditions such as CVD, it could make COVID-19 patients more susceptible to severe symptoms, thus leading to increased mortality. Accordingly, considering the potential acute pulmonary and cardiovascular toxicity of e-cigarettes, the use of these products may put patients at higher risk of severe illness from COVID-19.[97] As a potential mechanistic basis for COVID-

19 associated cardiovascular complications, recent data by electron microscopy revealed viral inclusion structures in endothelial cells leading to endotheliitis that was associated with an accumulation of inflammatory cells in the endothelium as well as apoptotic bodies in the heart,[98] with the consequence of impaired microcirculatory function. The authors explain that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects the host using the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed in several organs, including the lung, heart, kidney, and the intestine. Of note, ACE2 is also expressed by endothelial cells.[99] Thus, the endothelium should be targeted with antiviral substances and cardiovascular drugs with anti-inflammatory pleiotropic effects such as angiotensin-converting enzyme inhibitors and statins. In addition, this therapeutic strategy may be implemented mainly in subjects with already existing endothelial dysfunction due to male sex, smoking, hypertension, diabetes, obesity, and established CVD, all of which are associated with adverse outcomes in COVID-19.[98]

Conclusions, clinical implications and gaps in current knowledge

Taken together, there is no doubt tobacco cigarette smoke has severe cardiovascular side effects leading to endothelial dysfunction, increased oxidative stress, and increased cardiovascular morbidity and mortality. There is also evidence that e-cigarette vapour is less toxic than tobacco smoke. Nevertheless, acute e-cigarette smoking increases blood pressure, causes endothelial dysfunction and increases vascular and cerebral oxidative stress. Despite being less toxic, the proposed 95% less harmful assertion by the Public Health England and the Royal College of Physician should be rapidly re-evaluated due to the growing body of hard evidence regarding harm caused by e-cigarettes. Waterpipe smoking is not less harmful than tobacco smoking and thus cannot be considered a healthy alternative. Hookah smoke has significant cardiovascular side effects, causes endothelial dysfunction, oxidative stress within the vasculature and arterial hypertension. The greater smoke volumes expelled from waterpipe sessions may lead to even higher exposure to toxicants as compared to tobacco cigarette smoking. In general, the increased use of e-cigarettes and waterpipe is concerning and as recently recommended broader tobacco control efforts by raising tobacco taxes, adopting smoke-free laws, conducting mass media campaigns, and restricting tobacco marketing should be implemented for better health protection of the general population.[100] Future research should focus in particular on the long-term adverse effects of e-cigarette and waterpipe smoking on the cardiovascular system or respiratory diseases and cancer, as strong evidence is still missing (summarized in *Figure 1*). In particular, individuals exposed to second-hand smoke will benefit from any increased knowledge concerning the impact on CVD from tobacco/waterpipe (shisha) and e-cigarette smoking/vaping. There is no doubt, however, that smoking cessation is and will remain the most powerful approach to prevent smoking-induced cardiovascular and respiratory disease.[101] This may be even more important in light of the actual COVID-19 pandemic as it increases the risk for COVID-19 associated cardiovascular and other severe complications in smokers and vapers.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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