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# Effects of Smoking on Oxidative Stress and Vascular Function

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.78319>

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## Abstract

Tobacco smoking is the single most preventable risk factor related to the development of cardiovascular disease. It was demonstrated that tobacco smoke contains a thousand compounds potentially harmful to human health. As tobacco use declined over time, electronic cigarettes were introduced as an alternative. E-cigarettes are a modern and technological surrogate of traditional cigarettes and use heat to convert a nicotine solution or a flavored nicotine-free solution into vapor. Even though all the ingredients contained in the liquid of E-cigarettes are approved as food additives, the harmlessness of these electronic devices is still not fully proven in humans. The general mechanisms by which smoking results in cardiovascular events include the development of atherosclerotic changes with a hypercoagulable state and an increased risk of thrombosis. Endothelial dysfunction has been recognized as a hallmark of preclinical systemic atherosclerosis and as a useful marker to stratify the risk of cardiovascular disease. Based on these considerations, in this chapter, we (1) discussed the role of endothelial dysfunction and its contributing factors, such as oxidative stress and inflammation, in the development of cardiovascular diseases and (2) reported the studies which investigated the effect of tobacco and electronic smoking on the biomarkers of endothelial dysfunction, oxidative stress, and inflammation.

**Keywords:** smoking, tobacco, electronic devices, endothelial dysfunction, inflammation, oxidative stress

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## 1. Introduction

Cardiovascular diseases (CVDs) are the main cause of death in industrialized countries. The term CVD includes all the pathologies of the heart and of the systemic circulation including stroke, ischemic and not ischemic, and peripheral arterial disease, mainly of atherosclerotic type.

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For the development of CVD, there are risk factors that can be distinguished in non-modifiable and modifiable. Non-modifiable risk factors are age (risk increase as you get older), gender (before the age of 60, men are at a greater risk than women), and family history (risk increase if relatives experienced early heart disease) [1]. Anyway, there are other factors, named modifiable, which can be changed, pharmacologically or changing lifestyle: hypercholesterolemia, hypertension, diabetes, and smoking [2]. These are atherosclerotic risk factors and are associated with endothelial dysfunction. In the diseased endothelium, all the oxidizing, inflammatory, and thrombotic molecules are not in equilibrium and therefore a pathological condition is observed. This condition is pro-inflammatory, prooxidant, and prothrombotic [3].

The mechanism of endothelial dysfunction is related to the increased vascular production of reactive oxygen species (ROS) and inflammation condition. Therefore, oxidative stress, inflammation, and endothelial dysfunction are related and together represent the risk factor for the development of atherosclerosis and subsequent clinical events such as myocardial infarction or stroke [4]. Many studies in the literature since decades demonstrated that cigarette smoking influences endothelial function, by acting on oxidative stress, inflammation, and platelet activation, and it is well known that tobacco consumption is a leading cause of death worldwide. In the last years, many alternative products, such as electronic cigarette (e-cig) and i-Quit-Ordinary-Smoking (iQOS), have entered the market. iQOS is a heat-not-burn (HNB) tobacco product that heats the tobacco just enough to release a flavorful nicotine-containing vapor but without burning the tobacco and then do not release combustion products. These devices became the sought-after product because people believe that they are safer than traditional cigarettes [5].

For these reasons, in the following paragraphs, we evaluated the impact of the traditional cigarette, e-cig, and iQOS on vascular function and CVD development.

## 2. Oxidative stress and vascular function

Human cells produce energy through aerobic respiration, a metabolic process that requires oxygen and leads to the production of ROS, small and simple molecules.

ROS play an important role in cellular function because they are implied in signal transmission and regulation, and under homeostatic conditions, cells can balance their presence through the action of antioxidant species and enzymatic defensive systems [6].

However, cellular defensive mechanisms cannot always prevent ROS accumulation. Increased levels of these species have dangerous effects, leading to serious cellular alterations. This condition is known as "oxidative stress," and it is considered to be associated with several diseases [7].

ROS are naturally produced during metabolism reactions. ROS include both free radicals, such as  $O_2^{\bullet-}$  (superoxide),  $ONOO^{\bullet-}$  (peroxynitrite), and  $OH$  (hydroxyl) and non-radicals such as  $H_2O_2$  (hydrogen peroxide) which can be generated by enzymes such as xanthine oxidase, cyclooxygenase, lipooxygenases, myeloperoxidases, cytochrome P450 monooxygenase, uncoupled nitric oxide synthase (NOS), peroxidase, and NADPH oxidase [8]. The activity of these

pro-oxidant enzymes is in opposition to the defensive role of antioxidant endogenous systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase [9]. Moreover, nonenzymatic systems can contrast the oxidant action of ROS: these systems include glutathione,  $\alpha$ -tocopherol, ascorbic acid, and many other antioxidant compounds taken with the diet.

Cellular ROS formation is a physiologic process in the vasculature and occurs in endothelial and smooth muscle cells (SMCs). Despite their high and random reactivity, they perform a fundamental role in the organism since they appear to be subtle physiologic modulators of biochemical processes involved in intracellular signaling [10].

ROS also participate in normal processes of cell growth and death and are involved in inflammatory responses, in regulation of vascular tone and in the production of erythropoietin in relation to the oxygen tension. They also play an important role in natural immunity, enhancing the effects of oxidizing agents produced by macrophages and granulocytes.

When the slight balance between ROS and cellular antioxidant defensive mechanisms fails, their physiological role quickly turns into a pathological activity. ROS demonstrate their toxic effects in various ways: for example, they can induce the oxidation of sulfhydryl groups into disulfide bonds of cysteine residues, highly present in numerous enzymes. This mechanism leads to the modification of proteins conformation that may cause alterations in the activity of enzymes or can determine DNA binding [11].

Furthermore, they can negatively interact with ion channels and transcription factors and can cause important cellular alterations such as lipid damage, an increase of cell permeability, cell apoptosis or death, alteration of growth factors, and endothelial dysfunction [7].

The vessel wall is the main molecular ROS source involved in the development of oxidative stress and associated impaired vascular function. Moreover, the activity of immune cells like polymorphonuclear lymphocytes and macrophages can enhance the severity of this stress condition [12].

Another leading factor of vessel damage is inflammation. Oxidative stress and inflammation are phenomena closely related to each other. Indeed, both result to be correlated with endothelial dysfunction and vascular damage, which have a critical role in the pathophysiology of several cardiovascular diseases [8, 13, 14].

The endothelium plays a crucial role in the maintenance of the vascular homeostasis since it can be considered an autocrine and paracrine organ which produces chemical mediators, growth factors, and vasoactive molecules with vasodilation, antiproliferative, and antithrombotic functions such as nitric oxide (NO), thromboxane (Tx) A<sub>2</sub>, prostaglandin (PG) A<sub>2</sub>, and cytokines [15].

The correct working of endothelium is fundamental for the regulation of vasal permeability, vasal tone and structure, and for the control of hemostasis, and inflammation.

The compromising of the endocrine endothelial activity, caused by typical cardiac risk factors such as hypertension, atherosclerosis, dyslipidemia, diabetes mellitus, cardiovascular disease, and smoke, can promote a pathologic condition called "endothelial dysfunction." This condition is characterized by weak vasodilatation, vascular remodeling, pro-coagulant,

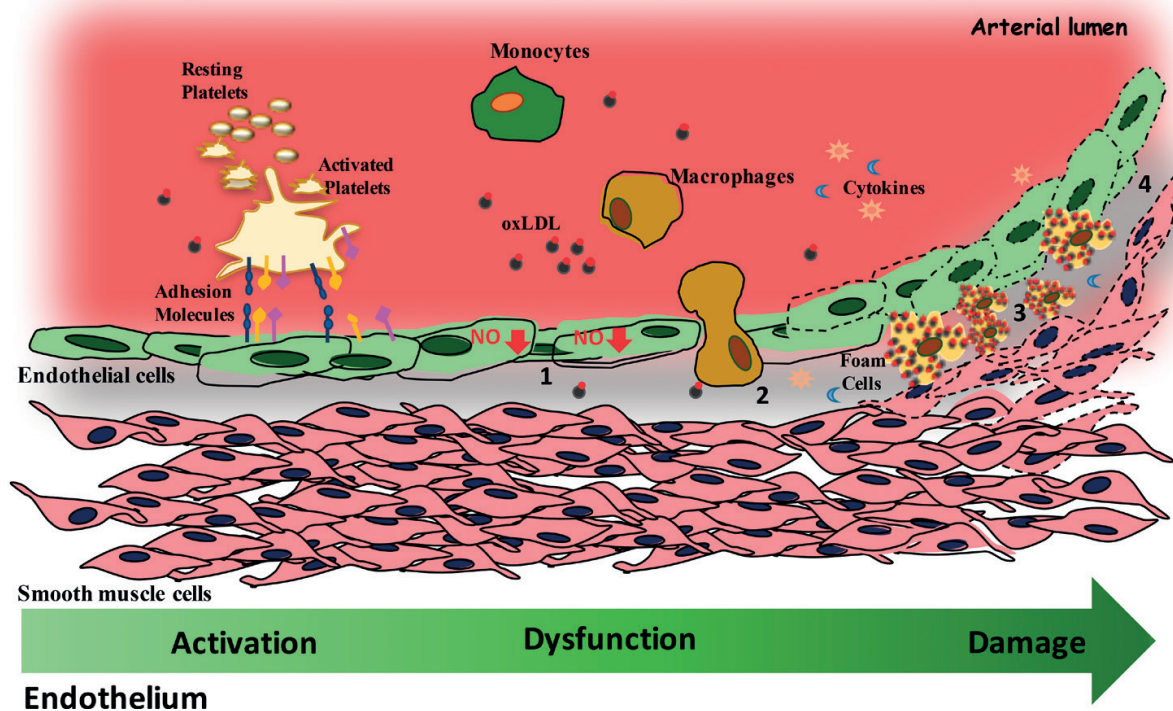
and pro-inflammatory activity that strongly expose the organism to vascular damages and cardiovascular disorders such as atherosclerosis, plaque instability, and thrombosis [16].

One of the first and most important consequences of the stimulation of the endothelium activity is the impairment of NO levels, an important vasoactive molecule that regulates vascular tone since it promotes vascular smooth cell dilatation, inhibition of platelet activity, leucocyte adhesion, and vascular smooth muscle cell proliferation [17] (**Figure 1**).

Even if NO has a protective role for the endothelium, high concentrations of this molecule can lead to the generation of a toxic compound named peroxynitrite by the reaction with superoxide anion.

Peroxynitrite and ROS as reactive intermediates have proatherogenic effects in general and are able to induce molecular changes that lead to low-density lipoprotein (LDL) oxidation [18]. This type of modification induces macrophage infiltration in the endothelium with the aim of internalizing, through scavenger receptors, and rapidly degrading modified LDL. The result is the macrophage's inability to metabolize cholesterol and the consequent formation of foam cells, a characteristic of fatty streaks [19].

Another possible source of ROS that may act as a potential stimulus for ox-LDL generation is represented by platelets [20]. It was demonstrated that these circulating cells express four



**Figure 1.** Schematic representation of signaling pathways involved in endothelial dysfunction. Oxidative stress activates the endothelium by inducing the expression of adhesion molecules and the activation of macrophages and platelets. Endothelial dysfunction is characterized by: (1) the reduction of NO levels; (2) platelet adhesion and macrophages transendothelial migration; (3) oxidative modification of LDL and their scavenger receptor-mediated uptake by macrophages inducing the formation of the so-called foam cells; and (4) the loss of function of smooth muscle cells (SMCs) in the vessel media and of endothelial cells.

subunits of NADPH oxidase: the most important enzymatic source of  $O_2^{\bullet-}$  [21]. Under particular conditions, platelets may produce superoxide, which in turn is able to stimulate their functions and to help their recruitment [22, 23].

Furthermore, endothelial dysfunction contributes to the expression of the adhesive molecules and to the production of chemotactic signals that may induce macrophages and T lymphocytes adhesion and migration starting inflammatory state [24].

Increased ROS intracellular levels lead to the activation of the nuclear factor NF- $\kappa$ B, whose activity is normally inhibited by NO. NF- $\kappa$ B is a nuclear factor able to enhance the transcription of pro-inflammatory genes and the expression of the adhesive molecule (E-selectin, VCAM1, and ICAM1). The inflammatory response induced by this mechanism is therefore implicated in the immune cells recruitment to the vessel wall, in the release of constrictor agents such as angiotensin-II and in the loss of endothelium antithrombotic function through a reduced production of prostacyclin and fibrinolytic factors [25].

Oxidative stress and endothelial dysfunction represent the most important pathophysiological mechanism of serious growing conditions and diseases.

### **3. Traditional smoking and oxidative stress-mediated vascular dysfunction**

#### **3.1. Functional, cellular, and molecular implication of the cigarette tobacco smoke on cardiovascular disease**

Cigarette smoking is a risk factor for the development and progression of CVD. This is demonstrated by many epidemiological studies, which showed a significant correlation between morbidity, mortality, and cardiovascular disease. Indeed, tobacco smoke is associated with pathological conditions such as endothelial dysfunction, inflammation, insulin resistance, dyslipidemia, hemodynamic alterations, and hypercoagulability that act together to furthering the progression of atheromatous plaque in tobacco users [26].

Cigarette smoke is represented by a mixture of different toxic chemicals. Among the constituents of the cigarette, which are considered the major participants in the development of CVD, there are nicotine, carbon monoxide, and oxidizing gases [27].

Nicotine is a potent stimulant of the ganglial nervous system and the central nervous system. Nicotine increases heart rate, blood pressure, and cardiac output, all of which translates into an increased myocardial oxygen demand. It is not yet clear whether nicotine has a direct role in the development of CVD. The effects reported are, mainly, on NO, contributing to endothelial dysfunction in cigarette users [28].

Carbon monoxide (CO), another constituent of cigarette smoke, does not appear to be involved in the processes of atherosclerosis or thrombosis associated with cigarette smoking. In unhealthy people with similar concentrations to tobacco users, CO does not affect blood pressure, plasma catecholamines, and platelet aggregation or serum reactive protein [29].

Oxidizing gases could contribute to the occurrence of a physiopathological state called oxidative stress. Oxidative stress is mediated by ROS overproduction and plays an important role in the development of CVD. In the context of cigarette smoking, free radicals could derive from the following:

1. vapor or particulate phases of cigarette smoke;
2. macrophages or neutrophils circulating or activated in situ;
3. platelets activation;
4. ROS production deriving from endogenous systems such as uncoupled endothelial nitric oxide synthase, xanthine oxidase, and the mitochondrial electron transport chain.

Oxidizing chemicals, including NO and many free radicals, are present at high concentrations in cigarette smoke and over time mediate endothelial dysfunction [30].

Cigarette smoke also contains a large number of metals, including aluminum, cadmium, copper, lead, mercury, nickel, and zinc, which are involved in the oxidation of cellular proteins causing structural damage and endothelial dysfunction [31].

As previously mentioned, tobacco influences various pathophysiological pathways that lead to the development and progression of atherosclerosis. Endothelial dysfunction, oxidative stress, inflammation, and prothrombotic state seem to be the main mechanisms involved.

Cigarette smoke could cause damage to the vascular wall, with a consequent reduction of prostacyclin production and an increased activation of platelets that interact with the injured vessel. The effect on endothelial function is mainly associated with the action of oxidative chemicals, which oxidize low-density lipoproteins (LDLs) and reduce the generation of NO [32].

It has been demonstrated that cigarette smoke-induced oxidative stress is responsible for endothelium activation through the expression of adhesion molecules and the activation of macrophages and platelets. Endothelial activation is characterized by a reduction of NO levels in endothelial cells (ECs) and consequently a loss of cellular function, in particular of the smooth muscle cells (SMC) of the vessel. After exposure to smoking, endothelial cells release inflammatory and proatherogenic cytokines. These events contribute to endothelial dysfunction. The direct effect of smoke compounds is the ROS overproduction that induces endothelial cell loss through apoptosis or necrosis processes. In addition to endothelial cells, also macrophages are activated. The expression of receptors promotes the recognition of adhesion molecules on the endothelium. After adhesion and migration through the endothelium, macrophages capture the oxidized lipids. These oxidative changes are promoted by the increase of smoking-induced ROS production. The receptor-mediated uptake induces the formation of the foaming cells inside the vessel wall and thus leads to the generation of lipid-induced plaques. Furthermore, it has been suggested that smoke induces an increase of SMC's proliferation and migration; this process leads to a thickening of the intima and plaque formation. Another consequence of exposure to cigarette smoke is the death of SMC by necrosis resulting in the activation of inflammatory signals, the release of proteolytic enzymes, and damage to the extracellular matrix [33].

Cigarette smoke induces an alteration of the fibrinolytic system by inhibiting the tissue plasminogen activator release from the vascular endothelium. Furthermore, it is responsible for activation of different pathways, involved in platelets activation and thrombus formation. In fact, cigarette smoke leads to a greater expression of the adhesion molecules on the platelet surface and therefore to a greater activation of the platelets [34].

Cigarette smoking is a risk factor for the development of type 2 diabetes, and the risk decreases after cessation [35]. It is documented that smokers show an increase of insulin resistance, central obesity, dyslipidemia, and a greater risk of metabolic syndrome onset. The link between smoking and insulin resistance could be attributed to the role of nicotine on the activation of the sympathetic nervous system and to the release of corticosteroids [36].

However, the mechanism is unclear. Nevertheless, it has been demonstrated that insulin resistance negatively affects the lipid profile, induces endothelial dysfunction and oxidative stress, driving to the formation of atheromatous plaque, and the development of cardiovascular diseases [37]. Moreover, a recent study compared plasma glucagon concentrations between 11 smokers and 12 nonsmokers diabetic patients before and after meal intake. The authors demonstrated that diabetic smokers had higher levels of glucagon compared to nonsmokers diabetic patients. In particular, they showed that nicotine smoke-derived and subsequent activation of nicotinic cholinergic receptors in the gastrointestinal tract and in the autonomic nervous system has an injurious effect on postprandial glucose metabolism. For this reason, a link between cigarette smoking and the development of type 2 diabetes could be hypothesized [38].

### **3.2. Cigarette smoke and biomarkers of oxidative stress**

Free radicals are a product of cigarette smoke and are considered to have negative effects producing oxidative stress.

The imbalance between ROS and antioxidant systems inside the cell is called oxidative stress. The cell, under physiological conditions, produces ROS by oxygen metabolism, which plays an important role in cellular signaling and safe. The presence of oxidative stress, a pathophysiological condition, causes an excessive production of ROS that causes lipid peroxidation, DNA strand breaks, and other damages that are injurious to structure and functionality cell [39].

ROS that accumulate inside cells can be exogenous and endogenous. Exogenous ROS derive mainly from inhaled toxic gases (e.g., environmental pollutants, car exhaust fumes, and cigarette smoke). Endogenous ROS come from the processes of mitochondrial respiration, from peroxisomes, from the NADPH oxidase system, and from inflammatory cells [40]. However, the process of oxidative phosphorylation in mitochondria is one of the main endogenous sources of ROS [41].

Tobacco smoke contains about 5000 compounds of harmful chemicals, which include polycyclic aromatic hydrocarbons, free radicals, and oxidative gases [42].

Therefore, together with the induction of ROS intracellular production, the components of cigarette smoke reduce intracellular antioxidant mechanisms, leading to oxidation stress [43]. Many studies showed the involvement of cigarette smoke in the oxidative stress. Recently, Karademirci et al. in a case-control analytical study, conducted on 78 smoking and



82 nonsmoking men, demonstrated that the total antioxidant status (TAS), vitamin C, and vitamin E parameters were significantly higher in the nonsmoker group than in the smoker group. The total oxidant status (TOS) and oxidative stress index (OSI) levels were higher in the smoker group [44].

In another study on 32 healthy volunteers, 16 active smokers and 16 nonsmokers, the markers of oxidative stress in the blood were analyzed before and after exposure to cigarette smoke. The results obtained showed that acute exposure to cigarette smoking affects hematological indexes and oxidative stress biomarkers negatively. The same results are found in both groups [45]. Carnevale and collaborators demonstrated the acute impact of tobacco cigarette on oxidative stress by measuring important biomarkers. Their data showed that cigarette smoke leads to a significant increase in the levels of soluble NOX2-derived peptide (sNOX2-dp) and 8-iso-prostaglandin F<sub>2</sub>α (8-isoPGF<sub>2</sub>α), while vitamin E levels were reduced [46].

Other studies, on animal models, showed that a reduced and defective antioxidant defense after cigarette smoke exposure (a decreased glutathione peroxidase and superoxide dismutase activity, an increased lipid peroxidation, and mitochondrial dysfunction) [47] leads to the increase of H<sub>2</sub>O<sub>2</sub> generation [48]. This unbalance results in an increased cardiac damage caused by oxidative stress induced by cigarette smoking.

### 3.3. Cigarette smoke and biomarkers inflammation

Inflammation is strongly implicated in the pathogenesis of atherosclerosis, and there are numerous lines of evidence that associate cigarette smoking with conditions of chronic inflammation [26].

Oxidative stress and inflammation are two closely related conditions. Indeed, the overproduction of ROS triggers NF-κB and histone acetyltransferase activation, promoting the expression of inflammatory genes and consequently the production of inflammatory cytokines [49].

Different studies demonstrated that the inflammatory process is reflected by numerous markers expression, which play an important role in the atherosclerosis process [50]. Furthermore, it is well known and accepted that inflammatory processes are strongly induced by exposure to cigarette smoke [51]. Between inflammatory markers, it is possible to analyze leukocyte count that increases in a dose-related manner with the number of cigarettes smoked daily. Indeed, many crossover and cross-sectional studies have observed an increase in the number of leukocytes following exposure to cigarette smoke [52].

Another marker analyzed in association with smoking is the plasma level of high-sensitivity C-reactive protein (hs-CRP). This marker of inflammation is significantly increased in long-term smoking and is correlated to the number of pack-years of cigarettes. Inflammation markers were found significantly increased in another study where levels of circulating total white blood cells, lymphocytes, monocytes, neutrophils, basophils, and C-reactive protein (CRP) were higher in 31 current smokers than in 33 never-users of tobacco products [53].

Important inflammatory markers are inflammatory and anti-inflammatory cytokines. IL-6 is a pro-inflammatory cytokine that in the acute phase of inflammation is able to induce other cytokines and growth factors as well as activating the platelets and the coagulation cascade [54].

Many studies conducted on large populations of smokers showed an increase in the expression of IL-6 compared to nonsmokers. A recent case-control study showed that smoking increases inflammatory marker such as IL-6 and VEGF levels, while IL-10, an anti-inflammatory marker, was lower in smokers group [55].

These data are important because high levels of IL-6 are related with an increased risk of cardiovascular disease, in particular, with myocardial infarction and severe heart failure [56].

### **3.4. Cigarette smoke and biomarkers of endothelial dysfunction**

Endothelial cells are the main protagonists in vascular function control. The lack of regulatory mechanisms activated by these cells leads to inflammation, vascular remodeling, and development of endothelial dysfunction. Endothelial dysfunction is an early event in atherosclerosis and is characterized by an imbalance between vasodilatation and vasoconstriction, a pro-inflammatory endothelial cell status, an increased monocyte adhesion, and a reduced bioavailability of NO [57]. There is considerable evidence that cigarettes smoke induces functional, biochemical, and morphological changes of the endothelium [58].

Wiest et al. demonstrated that flow-mediated dilation (FMD) as a clinical evaluation of endothelial dysfunction is compromised in smokers. FMD can be considered a predictor marker for future cardiovascular events and that smoking cessation can improve this parameter [59].

Furthermore, in a crossover study, performed on 40 healthy subjects, FMD and NO bioavailability levels were measured. The authors documented a significant decrease of these parameters, proving the negative effect of smoking on endothelial function [46].

Moreover, the serum nitrate and nitrite concentration, the final metabolic products of NO, are significantly decreased in smokers than in nonsmokers. Furthermore, in smoker subjects, low-density lipoprotein (LDL) is more susceptible to oxidation by excessive ROS and NOS presence. Oxidized LDL (ox-LDL) causes less bioactivity of NO deriving from the endothelium; this loss of bioavailability is associated with an increase of inflammatory cells that cross the vascular wall. Finally, the uptake of ox-LDL by macrophages via recognition by receptors results in foam cells formation [60].

Another mechanism involved in endothelial dysfunction is the increased ability of endothelial cells to adhere to effector immunity cells (monocytes, macrophages, T lymphocytes, platelets). In fact, the level of adhesion molecules is higher in plasma of smokers. Many studies reported significantly higher levels of soluble intracellular adhesion (ICAM-1), P-selectin, and E-selectin in smokers than in nonsmokers [56, 61]. Generally, endothelial dysfunction caused by cigarette smoking can lead to an increased possibility of atheromatous plaque formation and progression [62].

### **3.5. Cigarette smoke and biomarkers of platelet activation**

Platelet activation and enhanced coagulation are two events related to cardiovascular disease and atheromatous plaque formation. Platelet activation and aggregation are involved in both physiological hemostasis and pathological thrombus formation [63]. Smoking has been reported to enhance platelet aggregability [64]; in fact, it has long been demonstrated that

platelets isolated from tobacco cigarette users showed an increase of aggregation [65]. In an *in vitro* study, traditional smoking extracts from cigarettes significantly increased oxidative stress-induced platelet activation [66].

Platelet activation can be evaluated by various markers. An important platelet activation pathway is the synthesis of thromboxane A<sub>2</sub> via COX-1. The evaluation of urinary levels 11-dehydrothromboxane B<sub>2</sub>, a stable metabolite of thromboxane A<sub>2</sub>, reflects this type of platelet activation. 11-dehydrothromboxane B<sub>2</sub> levels in the urines of 13 healthy smokers were significantly elevated as compared to 10 healthy nonsmokers [67]. Numerous studies arrived at the same conclusions. In fact, data that correlate the influence of cigarette smoke and platelet activation, considering various markers, are present in the study since the early 1980s [68–70].

## 4. New electronic devices and their effect on vascular function

### 4.1. The rise of electronic cigarette: the impact on public health

The first reference to the electronic cigarette (e-cig) has been documented since 1927 but it was in 2003 that a Chinese pharmacist, Hon Lik, invented the modern version of the e-cig. Afterwards, this device was patented internationally in 2007 and was subsequently introduced into the global market. Today, the e-cig represents an alternative to traditional cigarettes and has gained popularity particularly among young adults. Structurally, these products are designed in order to closely resemble traditional tobacco cigarettes [71]. There are four types of e-cig: disposable; first-generation e-cigs that are tobacco cigarette-shaped; the second generation that looks like pens, larger than a cigarette, with a refillable cartridge; third generation considerably larger than the first- or second-generation e-cig [72]. Functionally, e-cigs are battery-operated devices with a heating element that heats the e-liquid, a solution of nicotine, and other additives including propylene glycol, vegetable glycerine, and flavoring agents, to a temperature of about 200–300°C to form an aerosol which is inhaled into the lungs. As tobacco cigarette, nicotine is the primary addictive substance in e-cig with levels, efficacy, and consistency that vary considerably among different brands and models. Schroeder et al. [73] revised the study related to the clinical pharmacology of nicotine contained in the e-cig. They reported studies that analyzed nicotine yield in the aerosol. For example, according to the analysis conducted by Goniewicz and colleagues [74] on the levels of nicotine vaporization of 16 cig brands, 20 series of 15 puffs generate a level of nicotine in the vapor that vary from 0.5 to 15.4 mg. By an HPLC method, Trehy and colleagues [75] evaluated nicotine content, ranging from 11 to 24 mg/cartridge. In 100-mL puffs from different e-cig brands, nicotine yield was highly variable, ranging from 0 to 43.2 µg nicotine. Unlike the traditional cigarette, the e-liquid of e-cig contains different flavoring agents including butyry minty, cherry or almond cinnamon, and chocolate flavors. Several studies suggest that some flavorings will promote e-cig use among youth [76, 77] so that we have observed the exponential spread of e-cig use in high school student in the last few years [78]. However, up to 250 different compounds have been identified in the inhaled E-liquid vapor [79, 80], suggesting that e-cig discharges a range of compounds capable of physiological damage to users.

Indeed, there is increasing evidence showing the short-term negative effect of e-cig. The results of these studies documented the effect on the respiratory tract, which is the primary system exposed

to vapors from e-cig. In healthy smokers, using an e-cig for 5 min has an immediate adverse effect on pulmonary function evaluated as exhaled nitric oxide measurements, lung volumes, and total respiratory resistance [81]. However, Flouris et al. [82] evaluated the lung function in 15 cigarette smokers after a brief session of active e-cigarette smoking and after a 1-h passive e-cigarette smoking and they found that e-cig generates smaller changes in lung function.

Other studies demonstrated the deleterious effects of e-cig use on multiple biological systems, such as central nervous system, immune system, and cardiovascular system. Regarding this latter, as previously discussed, there is definitive evidence that cardiovascular disease is the major cause of death among smokers. On the contrary, few and contrasting data regarding the effect of e-cig on the cardiovascular system have been obtained. There are several studies that evaluated the detrimental effect on cardiovascular function such as the increase in heart rate [83] or an increase in both diastolic blood pressure and heart rate in e-cig smokers, but to a lesser extent when compared with tobacco smokers [84]. These data were also supported by a recent study demonstrating that habitual e-cigarette use was associated with the imbalance of cardiac autonomic tone toward sympathetic predominance [85]. On the other hand, some studies have shown that short-term exposure to e-cig has no effect on cardiovascular system. The use of electronic cigarettes causes no changes in arterial stiffness [86], in myocardial function [87], and in smokers heart rate [88].

Here, in this paragraph, we report the study analyzing the effect of e-cig on cardiovascular risk factor, in particular, endothelial dysfunction, oxidative stress, and inflammation that are interrelated factors in the etiology of cardiovascular disease.

#### *4.1.1. E-cig smoking and biomarkers of endothelial dysfunction*

The assessment of endothelial function consists of endothelial cells responsiveness to different stimuli. The methods include (1) *in vivo* analysis performed by the flow-mediated dilation (FMD) [46], which is a noninvasive technique, in use in clinical practice; (2) the determination of endothelial cell functions by *in vitro* analysis, such as cultures of endothelial cells [89–91]; (3) the evaluation of circulating biomarkers, such as endothelial progenitor cells (EPCs) and microvesicles (MVs) [92].

Specifically, in 40 healthy subjects, 20 smokers and 20 nonsmokers, FMD was significantly affected by cigarette smoking, both tobacco and electronic, coincidentally with a significant decrease in NO bioavailability, although e-cig seemed to have a lesser impact [46]. Because of the limited availability of human vascular endothelial tissue, for *in vitro* studies, human umbilical vein endothelial cells (HUVEC) represent a good and useful model to understand endothelial physiology. Indeed, this cellular line is used to study the interaction of endothelial cells (ECs) with blood cells and different mediators [93].

Exposition of cells to extracts produced from e-cig vapor alters endothelial cell functions as suggested by a significant decrease in metabolic activity for HUVEC exposed, a significant increase in complement deposition, and in the expression of the receptors for C1q [89]. Moreover, the exposition of HUVEC to e-cig vapor extracts causes high cytotoxicity, inhibition of cell proliferation, and significant morphological alterations in endothelial cells disrupting the functional endothelial monolayer [90]. Anderson et al. supported e-cig-induced

cytotoxicity by demonstrating that cigarette aerosol extract (EAE) triggers both apoptosis and programmed necrosis pathways in HUVEC model [91]. Finally, Antoniewicz and colleagues evaluated in healthy young volunteers two circulating biomarkers, that is, endothelial progenitor cells (EPCs) and microvesicles (MVs). Acute endothelial dysfunction and inflammation may generate both circulating EPCs and MVs. Short-term exposure to e-cig vapor significantly increased the number of circulating EPCs without affecting MVs release [92].

#### 4.1.2. E-cig smoking and biomarkers of oxidative stress

As previously reported, the ability by tobacco smoke to generate ROS and to induce oxidative stress is well documented so that it is considered as a driving factor in smoking-related diseases [94, 95]. Regarding e-cig, in a recent study, Zhao et al. [96] performed ROS characterization of e-cig emissions using acellular and cellular approaches. Findings of this study confirm total ROS and H<sub>2</sub>O<sub>2</sub> generation in e-cig emissions that can contain a comparable level of ROS compared to tobacco cigarette. The role of e-cig smoking in oxidative stress generation is also supported by other studies. As oxidative stress can be defined as an alteration of the balance between pro-oxidants and antioxidants, oxidative status is studied by (1) the evaluation of intracellular oxidant species generation by fluorescent dye, that is, 2',7'-dichlorofluorescein diacetate (DCFDA) [97, 98] and (2) changes in antioxidant defense, that is, nonenzymatic  $\alpha$ -tocopherol [91], enzymatic glutathione ratio [98], or total antioxidant defense [97].

Specifically, the treatment of HUVEC with e-cig aerosol extract (EAE) induces an unbalance between pro- and antioxidant molecules. Indeed, there is an increase of ROS production in a concentration-dependent manner and a decrease of antioxidant molecules such as  $\alpha$ -tocopherol and n-acetyl-l-cysteine. This alteration induces cell death in vascular endothelial cells providing evidence about the role of ROS in e-cigarette-induced cytotoxicity [91]. The role of e-cig aerosol extract in oxidative was also supported by Ganapathy et al. [97]. By using human oral and lung epithelial cells, they found that e-cig aerosol extracts exposure significantly increased ROS production with a concomitant reduction in antioxidant defense, evaluated by total antioxidant capacity (TAC).

Taylor and colleagues [98], using an *in vitro* model of the airway epithelium (human bronchial epithelial cells), evaluated the effect of aqueous aerosol extracts (AqE) on oxidative stress. They evaluated the cellular ratios of reduced glutathione (GSH) to GSSG, intracellular generation of ROS, and the activation of the transcription factor nuclear factor erythroid-related factor 2 (Nrf2) that activates systems implicated in the neutralization of ROS and repair oxidative damage. After exposure to AqE, they did not find significant responses in the *in vitro* assays of oxidative stress or cytotoxicity [98].

*In vivo*, the effect of e-cig on systemic oxidative stress is evaluated in two studies. In healthy subjects, short-term e-cig smoking is associated with an increased oxidative stress, as demonstrated by an increased activation of NADPH oxidase (Nox) 2, which is an essential enzyme producing ROS by phagocytes [99] and platelets [100]. Moreover, there is an increase in 8-iso-prostaglandin F<sub>2</sub> $\alpha$  production coincidentally with a decreased antioxidant vitamin E [46]. In healthy individuals, the habitual e-cig use is associated with an increase in low-density

lipoprotein (LDL) oxidizability, indicative of the susceptibility of apolipoprotein B-containing lipoproteins to oxidation, compared to nonusers. Conversely, no difference was observed for high-density antioxidant/anti-inflammatory capacity and paraoxonase-1 activity [85], which exerts its physiological function in the degradation of specific oxidized cholesteryl esters and oxidized phospholipids in lipoproteins and cell membranes [101].

#### *4.1.3. E-cig smoking and biomarkers of inflammation*

As documented in the previous paragraph, tobacco products can lead to cardiovascular disease development by activating pro-inflammatory and/or prothrombotic function within the vasculature. The absorbed or dissolved fine-particulate matter, toxic chemicals, and nicotine act to produce oxidative stress factors that could induce the release of pro-inflammatory cytokines. Therefore, oxidative stress and inflammation are closely interrelated. Regarding the role of e-cig in inflammation, its effect was evaluated by using different model of in vitro cells and by measuring in particular the complement activation [102, 103] and interleukins release [102, 104].

Specifically, Rubenstein and colleagues used, as in vitro model, the Kupffer cells that are macrophages resident in liver sinusoids [105]. These cells interact with platelets and leukocytes to mediate inflammatory response [106]. In this study, the treatment of Kupffer cells with e-cig vapor extracts induces (1) the deposition of complement products C1q, C3b, C4d, and C5b-9, (2) the expression of gC1qR and cC1qR complement receptors, and (3) the release of pro-inflammatory interleukins 2, 4, 6, and 13 [102]. The deposition of complement was also analyzed onto the platelet surface [103]. After exposure to e-vapor extracts, the deposition of C1q and C5b-9 was not altered after exposure, whereas the deposition of C3b and C4d was significantly enhanced. Moreover, using differentiated THP-1 macrophages, the effect of e-cigarette components such as e-liquid flavors, nicotine, vegetable glycerine, and propylene glycol has been evaluated. Results demonstrated that IL-8 secretion increased with flavor and nicotine, while TNF $\alpha$ , IL-1b, IL-6, MIP-1a, MIP-1b, and MCP-1 decreased after exposure to most flavors and nicotine [104].

#### **4.2. The new heat-not-burn smoking device: the little we know**

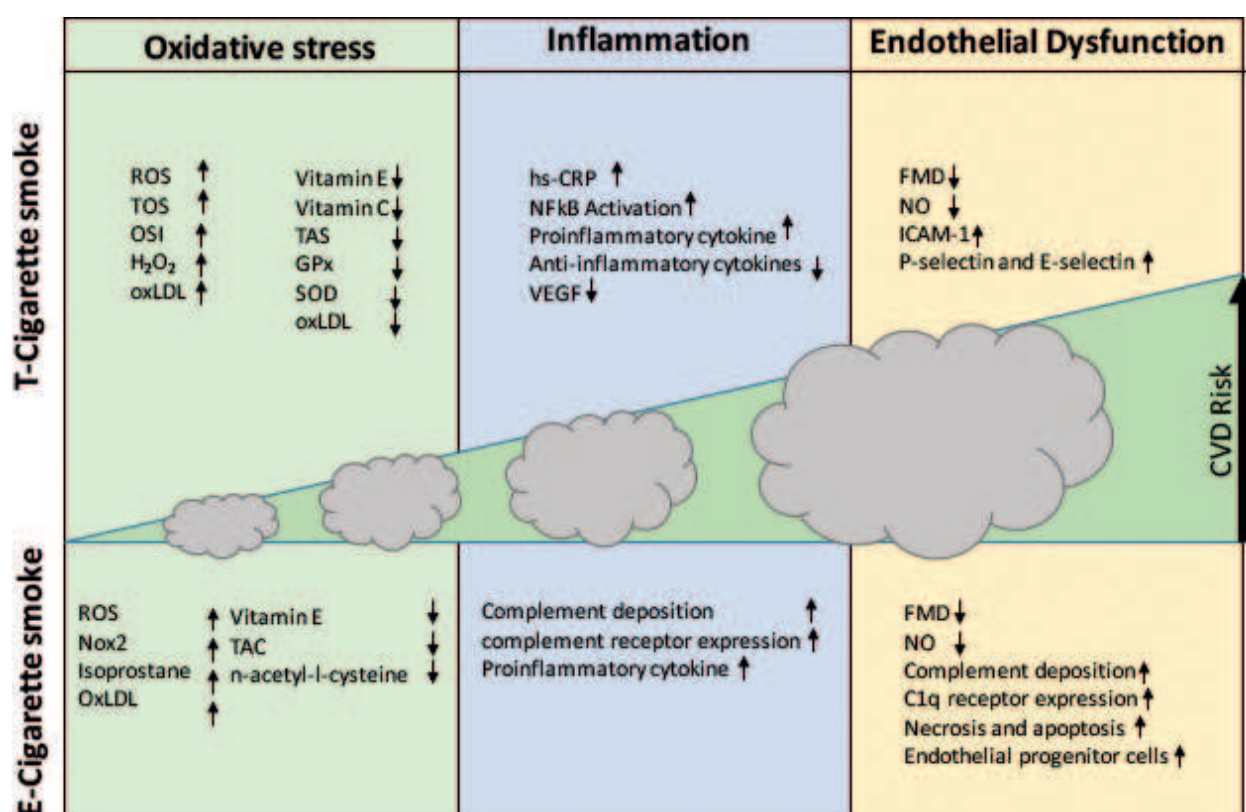
The heat-not-burn (HnB) tobacco device iQOS was introduced in 2014 in Japan and Italy and currently is marketed in 30 countries. Unlike the e-cig, which heats the e-liquid containing different substances but not tobacco, the iQOS device allows heating a cigarette composed of tobacco without activating a combustion process. Farsalinos and colleagues [107] evaluated the content and nicotine delivery to the aerosol of iQOS compared to e-cig and tobacco cigarette. First, the authors concluded that HnB devices contain nicotine in concentration similar to that found in tobacco cigarettes and that deliver nicotine less than tobacco but higher than e-cig. Another study [108] confirmed the presence of nicotine levels in tobacco fillers and in the mainstream smoke, same as those of conventional cigarettes. Regarding the effect of iQOS on health, there are no scientific data. For this reason, it is necessary to invest in the research aimed at defining the impact of these new smoking devices on health.

## 5. Conclusions

The evidence of the deleterious effects of smoking standard cigarette on cardiovascular system dates to the first half of 1900, and since then, the risk of smoking on health has been confirmed by numerous studies. Years of research into the effects of smoking have led to the conclusion that smoking represents an important risk factor for many diseases affecting numerous biological systems. Here, we described the effect of smoking on cardiovascular diseases, with attention to the pathways implicated in endothelial dysfunction. These data support the effect of cigarette smoking on oxidative stress and inflammation with a generally detrimental effect on cardiovascular system (**Figure 2**).

According to the World Health Organization, by 2030, tobacco will be responsible for more than 10 million deaths per year. From these data, the need to quit smoking considering the general and almost immediate benefits in the cardiovascular health is evident [109].

In the program of smoking cessation, e-cig represents an alternative to the traditional cigarette. Despite the increase in use especially among younger, the e-cig is not safer than traditional. As reported in the dedicated paragraph, e-cig contains potentially harmful substances that can affect health in general and the cardiovascular system in particular (**Figure 2**). Several studies report the deleterious effects of e-cigs even if to a lesser extent than the traditional cigarette. At present, the data in the literature concerning this topic are derived from short-term



**Figure 2.** The impact of tobacco cigarette and E-cigarette exposure on CVD risk. The increased exposure to the smoke of tobacco cigarette and e-cig has an important impact on health. Smoking affects biomarkers of oxidative stress, inflammation, and endothelial dysfunction, thereby increasing the risk of cardiovascular disease. A schematic summary of the effect of smoking on the biomarkers considered in the chapter is reported.

exposure studies. To have a complete view of the impact of e-cig on health, it is important to evaluate the long-term effects. These researches would be important not only because it is crucial to clarify the effects of e-cig on health but also for its social impact. Indeed, while e-cig could help tobacco smokers to quit smoking, they could encourage nonsmokers, especially teenagers, initially to use e-cigs and later traditional ones [110].

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