

**STATE-OF-THE-ART PAPER**

# The Pathophysiology of Cigarette Smoking and Cardiovascular Disease

## An Update

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Cigarette smoking (CS) continues to be a major health hazard, and it contributes significantly to cardiovascular morbidity and mortality. Cigarette smoking impacts all phases of atherosclerosis from endothelial dysfunction to acute clinical events, the latter being largely thrombotic. Both active and passive (environmental) cigarette smoke exposure predispose to cardiovascular events. Whether there is a distinct direct dose-dependent correlation between cigarette smoke exposure and risk is debatable, as some recent experimental clinical studies have shown a non-linear relation to cigarette smoke exposure. The exact toxic components of cigarette smoke and the mechanisms involved in CS-related cardiovascular dysfunction are largely unknown, but CS increases inflammation, thrombosis, and oxidation of low-density lipoprotein cholesterol. Recent experimental and clinical data support the hypothesis that cigarette smoke exposure increases oxidative stress as a potential mechanism for initiating cardiovascular dysfunction. (J Am Coll Cardiol 2004;43:1731-7) © 2004 by the American College of Cardiology Foundation

Epidemiologic studies strongly support the assertion that cigarette smoking (CS) in both men and women increases the incidence of myocardial infarction (MI) and fatal coronary artery disease (CAD) (1-11). Even low-tar cigarettes and smokeless tobacco have been shown to increase the risk of cardiovascular events in comparison to non-smokers (12,13). Furthermore, passive smoking (environmental tobacco exposure) with a smoke exposure about one-hundredth that of active CS is associated with approximately a 30% increase in risk of CAD, compared with an 80% increase in active smokers (14,15). Thus, the evidence linking cigarette smoke exposure with cardiovascular disease is clearly present, yet the exact components of cigarette smoke and the mechanisms responsible for this association have not been clearly elucidated. This article updates the present clinical and experimental observations on the potential pathobiology and mechanisms involved in smoking-related cardiovascular disease (Fig. 1).

### PHYSICAL AND BIOCHEMICAL PROPERTIES OF CIGARETTE SMOKE

Conventionally, cigarette smoke is divided into two phases: a tar phase and a gas phase. The tar or particulate phase is defined as the material that is trapped when the smoke stream is passed through the Cambridge glass-fiber filter that retains 99.9% of all particulate material with a size

>0.1  $\mu\text{m}$  (16). The gas phase is the material that passes through the filter. The particulate (tar) phase of cigarette smoke contains  $>10^{17}$  free radicals/g, and the gas phase contains  $>10^{15}$  free radicals/puff (16). The radicals associated with the tar phase are long-lived (hours to months), whereas the radicals associated with the gas phase have a shorter life span (seconds) (16-18).

Cigarette smoke that is drawn through the tobacco into an active smoker's mouth is known as mainstream smoke. Sidestream cigarette smoke is the smoke emitted from the burning ends of a cigarette. Mainstream cigarette smoke comprises 8% of tar and 92% of gaseous components (16). Environmental tobacco smoke results from the combination of sidestream smoke (85%) and a small fraction of exhaled mainstream smoke (15%) from smokers (19). Sidestream cigarette smoke contains a relatively higher concentration of the toxic gaseous component than mainstream cigarette smoke (14). Of all the known constituents, nicotine, a component of the tar phase, is the addictive substance of cigarette smoke (20).

### CS AND ATHEROSCLEROSIS: CLINICAL AND EXPERIMENTAL OBSERVATIONS

Cigarette smoking predisposes the individual to several different clinical atherosclerotic syndromes, including stable angina, acute coronary syndromes, sudden death, and stroke. Aortic and peripheral atherosclerosis are also increased, leading to intermittent claudication and abdominal aortic aneurysms (1).

Various clinical imaging techniques have been utilized to directly ascertain the relationship between CS and atherosclerosis. Early studies associated pack years of smoking

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**Abbreviations and Acronyms**

CAD	= coronary artery disease
CO	= carbon monoxide
CS	= cigarette smoking
CSE	= cigarette smoke extract
EDV	= endothelium-dependent vasodilation
HUVECs	= human umbilical vein endothelial cells
LDL	= low-density lipoprotein
MI	= myocardial infarction
NO	= nitric oxide
TF	= tissue factor
TFPI-1	= tissue factor pathway inhibitor-1

with the severity of angiographically determined atherosclerosis (21–23). Cigarette smoking was also found to be an independent predictor of new coronary lesion formation in the Canadian Coronary Atherosclerosis Intervention trial (24). As angiography is an insensitive indicator of the amount or progression of atherosclerosis, other techniques have been utilized to assess atherosclerotic changes associated with cigarette smoke exposure. Thoracic aortic atherosclerosis as assessed by transesophageal echocardiography was increased in cigarette smokers (25). It has also been reported that both active and passive smoking are associated with a consistent increase in intimal-medial thickness of the carotid artery as assessed by carotid ultrasound (26,27).

Vasomotor dysfunction, inflammation, and modification of lipids are integral components for the initiation and progression of atherosclerosis. These components precede the apparent structural and clinicopathologic manifestations of atherosclerosis (28,29). The following sections address the present knowledge regarding the effects of CS on these components of atherogenesis.

**Vasomotor dysfunction.** Impairment of vasodilatory function is one of the earliest manifestations of atherosclerotic changes in a vessel. In both animal and human models, several studies have demonstrated that both active and passive cigarette smoke exposure were associated with a decrease in vasodilatory function (30–40). In humans, cigarette smoke exposure impaired endothelium-dependent vasodilation (EDV) in macrovascular beds such as coronary and brachial arteries and in microvascular beds (30–35).

Nitric oxide (NO), a free radical, is primarily responsible for the vasodilatory function of the endothelium (41). Using cigarette smoke extract (CSE) or isolated components such as nicotine, multiple *in vitro* studies have found that CS was associated with decreased NO availability (36–38). Because there are numerous known and unknown components of cigarette smoke whose metabolic fate in the human body is unknown, an appropriate *in vitro* model of CS exposure remains to be established. In an attempt to produce a more physiologic *in vitro* model, our group has incubated endothelial cells with sera from smokers. Utilizing this model, Barua et al. (31,39) demonstrated that exposure to smokers' sera decreased NO availability from both human umbilical vein endothelial cells (HUVECs) and human coronary

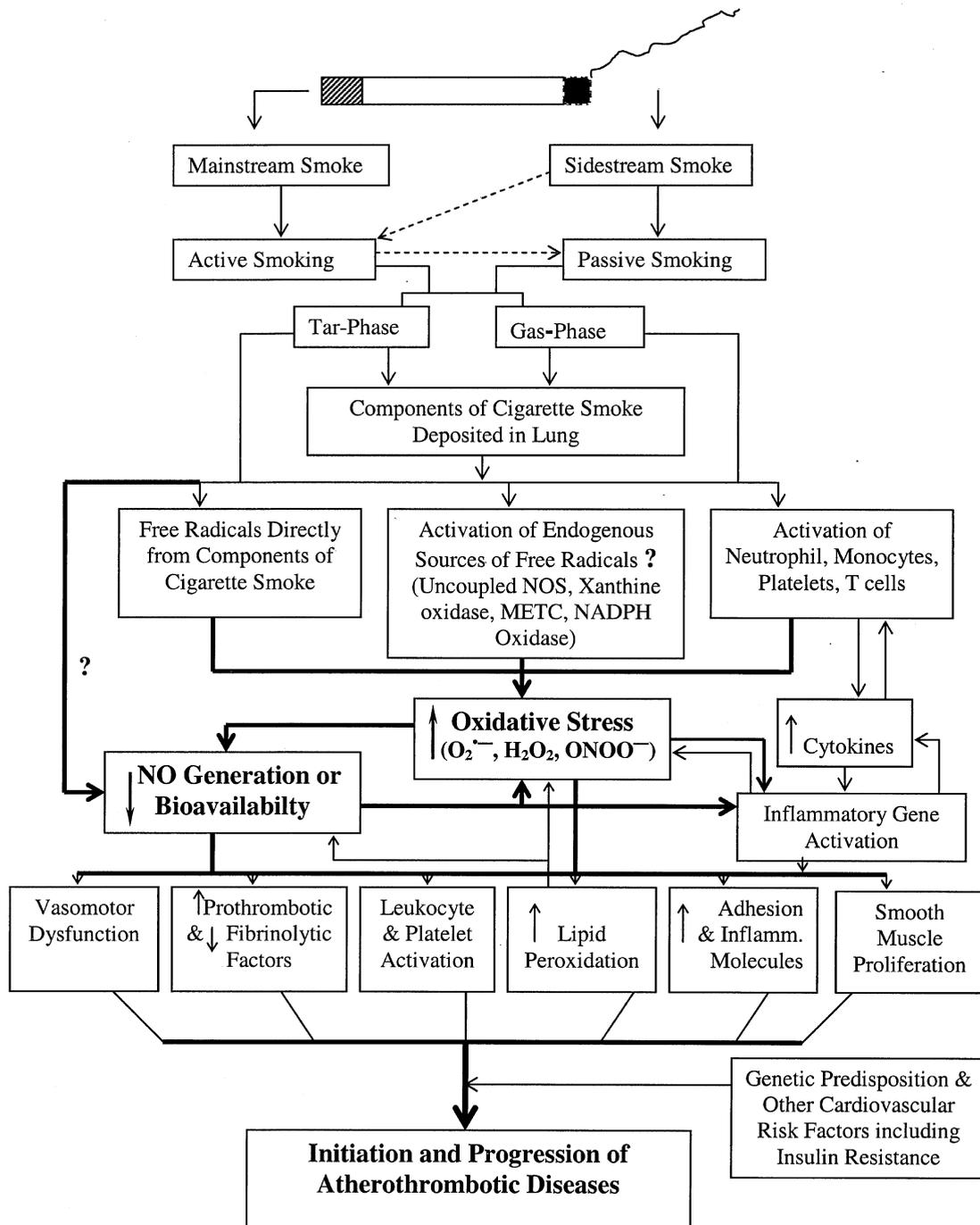
artery endothelial cells by altering the expression and activity of the endothelial NO synthase enzyme. A significant correlation existed between flow-mediated brachial artery EDV and NO availability from cultured HUVECs exposed to serum from the same individuals (31). Similarly, other studies utilizing an *in vivo* infusion of L-NMMA have indirectly demonstrated that the reduced EDV associated with smoking was attributable to a decreased NO availability (33,40).

Not only is NO a vasoregulatory molecule, it helps regulate inflammation, leukocyte adhesion, platelet activation, and thrombosis (41). Therefore, an alteration in NO biosynthesis could have both primary and secondary effects on the initiation and progression of atherosclerosis and on thrombotic events.

**Inflammation.** The inflammatory response is an essential component in the initiation and evolution of atherosclerosis. Several studies have indicated that CS causes about a 20% to 25% increase in the peripheral blood leukocyte count (17). *In vivo*, CS is associated with an increased level of multiple inflammatory markers including C-reactive protein, interleukin-6, and tumor necrosis factor alpha in both male and female smokers (42–45).

Local recruitment of leukocytes on the surface of endothelial cells is an early event in atherosclerosis. Elevations of various proinflammatory cytokines increase leukocyte-endothelial cell interaction leading to leukocyte recruitment. Indeed, soluble VCAM-1, ICAM-1, E-selectin levels are higher in smokers (43,46). Cigarette smoking also causes activation of proatherogenic molecules leading to alteration in cell-cell interactions. Cigarette smoking extract exposure was associated with a 70% to 90% increase in adherence between human monocytes and HUVECs in culture attributable to the increased expression of adhesion molecules on the surface of both monocytes and HUVECs (47). Exposure to CSE increased by 200% the rate of transendothelial migration of monocyte-like cells across a HUVEC monolayer (48). Monocytes isolated from smokers increased expression of the integrin CD 11b/CD 18, which augmented the adhesiveness of the monocytes to HUVECs in culture (49). Similarly, Adams et al. (50), exposing human monocytes and HUVECs to smokers' serum, found a significant increase in adhesion between these cells, which was associated with increased expression of ICAM-1 on HUVECs. Thus, CS fuels the fire of inflammation in the blood and at the vessel wall.

**Modification of lipid profile.** Cigarette smoking could promote atherosclerosis, in part, by its effects on lipid profile. Smokers have significantly higher serum cholesterol, triglyceride, and low-density lipoprotein (LDL) levels, but high-density lipoprotein is lower in smokers than in non-smokers (51). The mechanisms responsible are not clearly elucidated, and the role of dietary differences between smokers and non-smokers is unknown. The triglyceride/high-density lipoprotein abnormalities have recently been suggested to be related to insulin resistance. In fact, it has



**Figure 1.** Potential pathways and mechanisms for cigarette smoking-mediated cardiovascular dysfunction. The **bold boxes and arrows** in the flow diagram represent the probable central mechanisms in the complex pathophysiology of cigarette-smoking-mediated athero-thrombotic disease. H<sub>2</sub>O<sub>2</sub> = hydrogen peroxide; METC = mitochondrial electron transport chain; NADPH = nicotinamide adenine dinucleotide phosphate reduced form; NOS = nitric oxide synthase; ONOO<sup>-</sup> = peroxynitrite; O<sub>2</sub><sup>-</sup> = superoxide.

been proposed that insulin resistance is a potential key link between CS and cardiovascular disease (52).

Cigarette smoking also increases oxidative modification of LDL. Circulating products of lipid peroxidation and autoantibody titers to oxidized LDL are significantly increased in smokers (53). In 1988, Yakode et al. (54) reported that exposure to CSE caused a modification of LDL, which was actively taken up by the macrophages to

form foam-cells in culture. Frei et al. (55) observed that exposure of human plasma to the gas phase of cigarette smoke caused oxidative modification of plasma LDL. Furthermore, HUVECs isolated from smokers significantly increased oxidative modification of LDL compared with HUVECs isolated from non-smokers (56). Cigarette smoke extract exposure may also decrease the plasma activity of paraoxonase, an enzyme that protects against LDL oxida-

tion (57). More recently, in a hyperlipidemic rabbit model, injection of CSE accelerated atherosclerosis through oxidative modification of LDL (58).

**Genetic predisposition.** Recently, genetic predisposition was found to influence the development of atherogenesis in individuals exposed to cigarette smoke. The intersubject variability in the atherosclerotic process in smokers may be partially mediated by genetic variants. Either CYP1A1 *MSP* polymorphism or certain endothelial NO synthase intron 4 polymorphisms increased the susceptibility to cigarette smoke exposure-related atherosclerotic diseases including multi-vessel CAD and MI (59,60). However, at present, the importance of these genetic variants is unknown, as their prevalence in the entire population of cigarette smokers has not been determined.

### CS AND THROMBOSIS—CLINICAL AND EXPERIMENTAL OBSERVATIONS

Cigarette smoking is associated with an increased incidence of acute MI. Cessation of smoking significantly reduces this risk over a one- to three-year period with an exponential decline approaching the risk in ex-smokers within five years of cessation (61,62). Recent data indicate an immediate reduction in thrombotic events with smoking cessation. A preliminary, oral presentation study (presented by Sargent, Shepard, and Glantz at the 52nd Annual American College of Cardiology Conference in March 2003) reported that a citywide smoking ban in public places over a six-month period in Helena, Montana, reduced the incidence of acute MI by 60% during that time period. Furthermore, pathologic studies of sudden coronary death indicate that CS increased the risk of plaque rupture and acute thrombosis of a lipid-rich, thin-capped atheroma in men; in female smokers, the prevailing mechanism was plaque erosion with superimposed thrombosis (63,64). Acute cigarette smoke exposure may also increase coronary artery vascular resistance reducing coronary blood flow (65). Smoking may also be a risk factor for coronary vasospasm (66).

The prothrombotic effects of exposure to cigarette smoke have been repeatedly demonstrated to cause alterations in platelet function, antithrombotic/prothrombotic factors, and fibrinolytic factors. The following sections address the present knowledge regarding these effects.

**Platelet dysfunction.** Platelets isolated from smokers exhibited an increased stimulated as well as spontaneous aggregation (67,68). After exposure to smokers' serum, platelets isolated from non-smokers demonstrated hyperaggregability (69). Cigarette smoking may decrease availability of platelet-derived NO and decrease platelet sensitivity to exogenous NO, leading to increased activation and adhesion (70,71).

**Alteration of antithrombotic and prothrombotic factors.** Current smokers have higher fibrinogen levels that correlate with the number of cigarettes smoked. Ex-smokers have fibrinogen levels similar to non-smokers (72,73). Alter-

ations of tissue factor (TF) and TF pathway inhibitor-1 (TFPI-1) and a consequent increase in thrombotic potential have also been documented. Human umbilical vein endothelial cells exposed to serum from chronic smokers showed a significantly decreased TFPI-1 level and relatively higher but nonsignificant increase in TF level in culture (74). An increased TF immunoreactivity and an increase in TF activity were observed in atherosclerotic plaques isolated from apoE<sup>-/-</sup> mice exposed to half of a non-filtered research cigarette five days a week, for eight weeks (75). In smokers 2 h after smoking two cigarettes, an increase in circulating TF activity has also been reported in human plasma (76). Furthermore, higher red blood cell counts, hematocrits, blood viscosity, and an ongoing inflammatory process potentiate the prothrombotic process associated with smoke exposure (14,17,20).

**Alteration in fibrinolysis.** Human umbilical vein endothelial cells exposed to chronic smoker's serum have significant decreases in both basal and substance-P-stimulated t-PA release in culture with a significant alteration in t-PA/PAI-1 molar ratio (74). Similarly, decreased plasma t-PA antigen and activity were observed in smokers in samples isolated from brachial and coronary arteries after pharmacologic stimulation (77,78).

Therefore, CS is associated with dysfunctional thrombohemostatic mechanism(s) that promote the initiation and/or propagation of thrombus formation and limit its effective dissolution.

### FACTORS AND MECHANISMS RESPONSIBLE FOR SMOKING-MEDIATED VASCULAR DYSFUNCTION

Cigarette smoke contains over 4,000 known components, of which only a few components have been examined in isolation. Carbon monoxide (CO) is one such component, but its effects on athero-thrombotic disease have been equivocal. An earlier study suggested that CO could be responsible for smoking-related cardiovascular alterations (79). However, more recent data suggest that CO from cigarette smoke was an unlikely cause for atherosclerosis or thrombus (80–82). Polycyclic aromatic hydrocarbons found in the tar fraction of cigarette smoke have also been studied, and these components, at least in experimental models, accelerate atherosclerosis (83).

Nicotine in cigarette smoke is probably the most studied component. Although nicotine plays a major role in smoking-related increases in cardiac output, heart rate, and blood pressure, its role in CS-related athero-thrombotic disease remains controversial (17,84–89). Nicotine exposure alone had been reported to cause no change, a decrease, or an increase in EDV or NO availability (85–88). In various models, although high doses of nicotine favor atherogenic changes, the majority of current evidence suggests that nicotine, at concentrations similar to a smoker's blood level, has a minor effect on the initiation or propagation of atherosclerosis (17,85). Similarly, the effect of

nicotine on thrombo-hemostatic factors such as platelets, fibrinogen, or t-PA, PAI-1 appears to be insignificant in the setting of smoking (17,84,89). As mentioned earlier, nicotine is the known addictive substance in cigarette smoke, and its addictive qualities likely perpetuate exposure to the other more detrimental components.

Currently, free radical-mediated oxidative stress is emerging as the pivotal step for the development of atherosclerosis (90-92). In a setting of CS, free radicals could arise from: 1) the gas or tar phase of cigarette smoke; 2) circulating or in situ-activated macrophages and neutrophils; and 3) endogenous sources of reactive oxygen species such as uncoupled eNOS, xanthine oxidase, and the mitochondrial electron transport chain (16-20,39,93-96). A reaction between free radicals such as superoxide and NO not only decreases NO availability but also generates peroxynitrite, which further enhances the cellular oxidative stress (91). Increased oxidative stress with the loss of the protective effect of NO tips the cellular balance towards a proatherogenic and prothrombotic milieu (92,97). Many of the abnormalities described above, including endothelial dysfunction, proinflammatory effects on the vessel wall, prothrombotic effects such as increased platelet reactivity, reduced endogenous fibrinolysis, and lipid peroxidation, can largely be explained by the effects of increased oxidative stress (16-20,94-96). Furthermore, antioxidants or agents that reduced the oxidative stress or increased NO availability have been shown to either improve or reverse the proatherogenic, proinflammatory, and prothrombotic attributes associated with CS (39,98-100).

## NONLINEAR DOSE EFFECT OF SMOKING ON CARDIOVASCULAR FUNCTION

Although the association between CS and cardiovascular risk has clearly been demonstrated, an unanswered question is whether or not there is a linear dose effect. Several recent large epidemiologic studies showing a trend for more cardiovascular events in heavier active smokers have failed to find a significant dose-dependent correlation between cardiovascular risk and the number of cigarettes smoked or the pack-years of exposure (9,14). More recently, heavy and light active smokers had a similar decrease in brachial artery EDV and similar abnormalities of NO biosynthesis (101). Similarly, even with passive smoking, certain atherothrombotic markers such as a reduced EDV and increased platelet activation were similar to that of active smoking (32,34,102). The data presented above suggest that the underlying biochemical and cellular processes may become saturated with small doses of toxic components from cigarette smoke causing a nonlinear dose-response on cardiovascular function. The exact mechanisms involved require further study.

**Conclusions.** Epidemiologic studies have established worldwide that cigarette smoke exposure is an important cause of cardiovascular morbidity and mortality. Clinical

and experimental studies indicate that either active or passive exposure promotes vasomotor dysfunction, atherogenesis, and thrombosis in multiple vascular beds. Although the precise mechanisms responsible remain undetermined, free radical-mediated oxidative stress appears to play a central role in CS-mediated athero-thrombotic diseases. These free radicals could potentially arise directly from cigarette smoke and indirectly from endogenous sources as well. Furthermore, potentiated by multiple prothrombotic and antifibrinolytic effects, intravascular thrombosis is the predominant cause of acute cardiovascular events. An increasing body of epidemiologic, clinical, and experimental data also suggest that the pathophysiologic effects of cigarette smoke exposure on cardiovascular function may be nonlinear. Future studies investigating the potential cigarette smoke-inducible endogenous cellular mechanisms could further our understanding of the complex pathobiology of cigarette smoke and cardiovascular dysfunction.

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