The effects of nicotine and cigarette smoking on cardiac electrophysiology.

Affan B. Irfan

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THE EFFECTS OF NICOTINE AND CIGARETTE SMOKING ON CARDIAC ELECTROPHYSIOLOGY

By

Affan B. Irfan
M.B.B.S Aga Khan University, 2008

A Dissertation
Submitted to the Faculty of the
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THE EFFECTS OF NICOTINE AND CIGARETTE SMOKING ON CARDIAC ELECTROPHYSIOLOGY

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A Dissertation Approved on

November 27, 2019

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DEDICATION

This dissertation is dedicated to my parents, Irfan Uddin and Farzana Irfan, (without whom I would not have been here), my brother, Furqan Irfan, and sister Bismah Irfan, (without whom I would not have been the middle child), and my wife, Sommaya Naveed, and our daughter, Aiza Affan (without whom this dissertation would have been completed a year ago).
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ABSTRACT

THE EFFECTS OF NICOTINE AND CIGARETTE SMOKING ON CARDIAC ELECTROPHYSIOLOGY

Affan B. Irfan

November 27, 2019

Cigarette smoking is a leading cause of preventable disease and premature death worldwide. The adverse effects of cigarette smoking, including proarrhythmia, are related to the mixture of chemicals, including nicotine (which sustains tobacco addiction). However, it remains unclear which individual tobacco smoke constituents and biological pathways mediate this increased risk. The purpose of this research was to explore the chronic effects of cigarette smoking, as well as compare the acute effects of nicotine and cigarette smoking, and the possible role of β-adrenoreceptors, on human cardiac electrophysiology. Chapter 1 is a comprehensive literature review of (a) the ex vivo and in vivo effects of nicotine and non-nicotine constituents of cigarette smoking on cardiac ion channels, (b) the direct and indirect effects of the autonomic nervous system on cardiac electrophysiology, and (c) studies of acute and chronic effects of cigarette smoking in humans. Chapter 2 consists of two studies in which we used cotinine levels to investigate the differences in baseline cardiac electrocardiogram between chronic smokers and non-smokers, and to define smoking status and its burden. We also explored the relationship between urinary catecholamines, cotinine, and electrocardiographic changes. Chapter 3 features the 2 x 2 factorial experimental study designed to compare
the acute effects of cigarette smoking and nicotine, with and without a β-blocker (propranolol). We found that chronic cigarette smoking was associated with a shortened PR segment at baseline, and that dopamine possibly mediates this effect. There was also (corrected) QT interval shortening with increased cotinine levels. This experimental study revealed that the non-nicotine constituents in cigarette smoking were mainly responsible for PR segment shortening, through β-adrenoreceptors. Other evidence revealed that, although nicotine in cigarette smoke is primarily responsible for sympathetic activation and (corrected) QT interval shortening, it is the non-nicotine constituents that depress the ST segment. Collectively, acute and chronic exposure studies indicate that smoking may promote cardiac arrhythmia, primarily via β-adrenoreceptors, causing acceleration of dromotropy and ischemia (non-nicotine mediated), and ventricular repolarization (nicotine-mediated). This research elucidated a major physiological mechanism driving the effect of cigarette smoking and nicotine on cardiac electrophysiology. Consequently, these findings will inform U.S. Food and Drug Administration of tobacco and nicotine-containing products’ impact on the human cardiac electrical system, and potentially help regulate alternative forms of nicotine delivery and protect public health.
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CHAPTER I
INTRODUCTION

Background and context

Clinical significance of cigarette smoking, nicotine, and cardiac electrophysiology

_Cigarette smoke_

Cigarette smoking is the leading preventable cause of premature death. It is responsible for at least 480,000 premature deaths every year in the United States, with more than 41,000 of these deaths from exposure to secondhand smoke [1, 2], out of which one-third are secondary to cardiovascular disease (CVD) [3]. The mortality of current smokers among both sexes is three times that of non-smokers [4, 5]. Diseases attributable to smoking accounts for about 60% of smokers' deaths and the benefits of quitting smoking are dramatic across all age groups, with substantial gains in life expectancy, as compared to those who continue to smoke [5]. Furthermore, elevated CVD mortality has been seen even among patients smoking fewer than five cigarettes per day, suggesting a nonlinear dose-effect relationship [6]. Apart from the 21 common diseases formally attributed to cigarette smoking, there are diseases that have not been formally established as being caused by smoking (such as infections, hypertensive heart disease, renal failure, intestinal ischemia, and other respiratory diseases), and account for a significant excess in mortality [4]. The estimated annual smoking-related economic
burden in the United States is between $289–332.5 billion in direct medical care and other economic costs [1, 7]. Smoking also appears to have a multiplicative interaction with other major CVD risk factors (elevated serum lipid levels, untreated hypertension, and diabetes) [2]. In 2016, 15.5% (37.8 million) of U.S. adults were cigarette smokers. The prevalence of cigarette smoking was higher among adults who were male, 25–64 years old, of lower socio-economic status, of American Indian/Alaska Native descent or multiracial, and suffering from psychological distress [8].

**Nicotine and addiction**

Addiction to the nicotine in tobacco is the proximate cause of these diseases, because it sustains smoking behavior by acting on nicotinic cholinergic receptors in the brain to trigger the release of dopamine and other neurotransmitters [9]. Release of dopamine, glutamate, and gamma-Aminobutyric acid (GABA) [10] is particularly important in the development of nicotine dependence, and the extrahypothalamic corticotropin-releasing factor may play a key role in withdrawal [11, 12]. Nicotine addiction occurs when smokers rely on smoking to modulate mood and arousal, relieve withdrawal symptoms, or both. Therefore, the magnitude of public health harm caused by tobacco is inextricably linked to its addictive nature [9]. There is a continuum of risk for products that deliver nicotine, ranging from the most harmful combusted products (e.g., cigarettes) and electronic nicotine delivery systems (ENDS), to medicinal nicotine products.

Although significant research has been conducted to understand the pathophysiology of smoking-associated CVD, new research in this era has slowed in recent
years. This lag in research is particularly harmful, as tobacco products have continued to evolve, and new products, such as ENDS, are a popular source of nicotine, especially among children and young adults [13]. In contrast with cigarettes, however, e-cigarettes generate an aerosol by heating a liquid, usually consisting of propylene glycol or vegetable glycerin, nicotine, and flavoring agents, without any combustion [14]. Despite almost complete lack of knowledge regarding the biological effects of these new tobacco products, ENDS have been implied to help conventional cigarette smokers quit or curtail smoking. ENDS manufacturers are making claims that these products pose fewer health risks and are much safer than smoking conventional cigarettes. This postulation is concerning because such health claims are likely to lead to increased ENDS use, and may even lead to an increase in nicotine dependence in the population at large. Although e-cigarettes deliver lower levels of carcinogens than do conventional cigarettes, they still expose users to high levels of ultrafine particles and other toxins, such as volatile organic compounds (VOCs) [15], that may substantially increase risks for cardiovascular and noncancerous lung disease, which account for more than half of all smoking-related deaths, at rates similar to conventional cigarettes [16].

The primary concern for nicotine in cigarette smoking is an addiction. Tobacco combustion products cause most of the adverse health effects from smoking, but some health concerns are related to nicotine. Many of these concerns are related to the ability of nicotine to release catecholamines, including hemodynamic effects (increase in heart rate, transient increases in blood pressure, vasoconstriction of coronary and other vascular beds), adverse effects on lipids, and induction of insulin resistance [17]. Both in vitro and in vivo
animal studies suggest that nicotine may inhibit apoptosis, enhance angiogenesis, exacerbate atherosclerotic disease [18, 19], and produce endothelial dysfunction [20].

Cardiac electrophysiology

Basic cardiac electrophysiology is fundamental in understanding rhythmic cardiac function and electrical conduction, as well as changes in electrical activity associated with cardiac disease. The primary clinical tool for assessing cardiac electrical events is the electrocardiogram (ECG) [21]. The 12-lead ECG remains the most widely available, inexpensive, non-invasive, an indispensable tool for the diagnosis and prompt initiation of therapy in patients with acute coronary syndromes. It provides the most accurate means of diagnosing intraventricular conduction disturbances and arrhythmias, assessing cardiovascular risk and screening individuals in high-risk occupations and, in some cases, for participation in sports. As a research tool, it is used in long-term population-based surveillance studies and experimental trials of drugs with recognized or potential cardiac effects [22]. Therefore, ECG is used in clinical trials, as a valid, reliable, repeatable, quantitative method that is inexpensive and unbiased by clinical information.

Most ECGs used clinically are produced from digital signaling and interpreted by software using algorithms to assess cardiac rhythmicity, heart rate, heart rate variability (HRV), and intervals between conventional ECG landmarks, especially the PR, QRS, and QT intervals. These metrics provide essential insight into the autonomic function (HRV), atrioventricular conduction (PR interval and segment), ventricular depolarization (QRS), and repolarization (QT and JT). There are several major and minor ECG variables associated with cardiovascular mortality; namely P wave (duration, interatrial block, and
deep terminal negativity of the P wave in V1), QT and Tpeak-Tend (Tp-Te) intervals, QRS
duration and fragmentation, bundle branch block, ST segment depression and elevation, T
waves (inverted, T wave axes), spatial angles between QRS and T vectors, premature
ventricular contractions, and ECG hypertrophy criteria [23-25]. Apart from these
traditional ECG markers, there is evidence for several other ECG intervals that have also
been shown to be associated with total all-cause and cardiovascular-related mortality, such
as PR interval [26-30], JT interval [31, 32] and Tp-Te [33-35].

Given its low cost, ubiquity, and safety, ECG is a useful candidate tool for screening
and risk stratification of asymptomatic participants [36]. Deaths due to smoking-related
CVD are generally preceded by a subclinical cardiovascular injury that may be detected
early in the disease process [37]. To improve risk identification and stratification among
asymptomatic smokers, and to aid in preventative measures, health professionals need
more sensitive smoking-related markers of early cardiovascular damage. This research
focus is particularly imperative in the new era of ENDS, as it remains unclear how ENDS
might compare to cigarette smoke in severity and temporality of associated adverse
outcomes.

*Implications for public health practice*

Some tobacco control researchers and advocates emphasize the need for strong
policies that would protect future and current generations from new products that lead to
nicotine addiction or serve as a gateway to cigarette smoking [16]. Others emphasize the
different risks for disease associated with different tobacco and nicotine products, and
argue that policies must prioritize reducing disease risk even if that means allowing for
new products that may have high addiction potential [38]. As part of their framework announced in 2017, the Food and Drug Administration (FDA)—based on their recognition that nicotine makes tobacco products addictive, but that it does not directly cause smokers’ cancer, lung disease, or heart disease—has proposed a regulation strategy designed to limit nicotine in cigarettes to a minimal or nonaddictive level [39]. The premise is that the mode of nicotine delivery, rather than the drug nicotine itself, is the key to reducing harm at a population level. This action has the promise of helping current users quit while preventing potential future smokers—youths, in particular—from becoming addicted via escalation from experimentation to regular smoking [40]. Modeling estimates that, by appropriate nicotine regulation, about 5 million adult smokers could quit within a year, and most youths and young adults—could avoid becoming regular smokers [41]. Whether this is the right approach is unclear, as through it, the FDA Center for Drug Evaluation Research has primarily been conducted on targeting smoking cessation rather than dependence on nicotine and alternate tobacco products [42]. As smokers find it difficult to achieve desired nicotine levels from low-nicotine cigarettes, they may seek to replace cigarettes with other tobacco products that deliver nicotine. The FDA expects that making cigarettes minimally addictive or nonaddictive would reduce tobacco-related harm by promoting smoking cessation or a complete migration to alternative, uncombusted products, and by reducing initiation. Although concrete evidence is lacking, there are concerns that ENDS use may renormalize smoking behavior, sustain dual-use, and initiate or maintain nicotine addiction [13]. ENDS use also could serve as a gateway to the initiation of smoking by ex-smokers. Unregulated e-cigarette use also has the potential to erode gains in smoking cessation and smoke-free laws [13]. Furthermore, the health effects of nicotine and e-cigarettes have not
been well studied, and the potential harm incurred by long-term use of these devices remains completely unknown.

To complement Center for Tobacco Products (CTP) and CTP-funded scientific investigations to determine each product’s risks, benefits, and net public health impact, the FDA encourages submission of information from additional rigorous research (e.g., outside research institutions, or a manufacturer in an application for FDA marketing authorization of a new product [40]). Hence, extensive new research is required to assess the health effects of nicotine in order to develop appropriate regulatory policies.

Effects of cardiac autonomic system and cigarette smoke on cardiac action potential

Direct effects

Cardiac autonomic nervous system

Sympathetic and parasympathetic control: Superimposed on the intrinsic cardiac control system are the major extrinsic factors—autonomic efferent postganglionic nerve terminals— which affect the secretion of hormones into circulation, and the release of chemicals directly onto the cardiomyocyte membrane [43]. The autonomic nervous system (ANS) plays a vital role in the genesis of several cardiac arrhythmias, both in the atria and in the ventricles. Modulation of the autonomic response is a complex process, in which the final effect is the product of interactions among central, peripheral, and intracardiac components. Autonomic activation alters not only the heart rate, conduction, and hemodynamics, but also the cellular and subcellular properties of individual myocytes.

The cardiac ANS is divided into extrinsic (fibers that mediate connections between the heart and the nervous system) and intrinsic (fibers facilitating function within the
pericardial sac) components. The extrinsic cardiac ANS is divided into sympathetic and parasympathetic components. The fibers of the sympathetic nervous system (SNS) are largely derived from major autonomic ganglia along the cervical and thoracic spinal cord. The parasympathetic nervous system (PNS) originates predominantly in the nucleus ambiguus of the medulla oblongata and is carried almost entirely within the vagus nerve. In addition to the extrinsic cardiac ANS, the heart is also innervated by an exquisitely complex intrinsic cardiac ANS, with the vast majority of these ganglia organized into ganglionated plexi (GP) on the surface of the atria and ventricles, particularly at the sinus node, atrioventricular node and pulmonary vein–left atrium junction [43, 44]. There is a group of complex extracardiac and intrinsic cardiac neurons that comprise a local distributive network, process (both centripetal and centrifugal) information in cardiac control, and imply the presence of local information processing [45]. Furthermore, the influences of sympathetic and parasympathetic stimulation exert not only different effects on atrial and ventricular myocytes, but also during normal and diseased states [44]. Generally speaking, increased cardiac sympathetic efferent neuronal tone increases cardiac chronotropy, dromotropy, and inotropy; the reverse holds for the effects exerted by medullary (parasympathetic) efferent preganglionic neurons [43].

Sympathetic nerve stimulation results in well-defined changes in electrophysiological properties at the cellular and tissue levels, including enhanced conduction in working myocardium and shortening of the action potential duration and refractory periods. Sympathetic control of cardiac electrical activity is mediated by the activation of β-adrenergic receptors that regulate the activity of select ion channel proteins via cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA), or by
direct binding of cAMP to channel subunits. The activation of β-receptors regulates the function of many ion channels in the heart, including Na⁺, K⁺, and Ca²⁺ channels [46].

**Sodium and Calcium channels:** β-adrenergic receptors’ regulation of cardiac Na⁺ channels may occur via several distinct mechanisms. PKA-dependent and -independent (a more recent and poorer defined pathway) signaling pathways impact cardiac Na⁺ channel function. The indirect pathway engages canonical signaling, including PKA phosphorylation of the Na⁺ channel α-subunit. PKA-independent regulation (direct regulation) involves ion channels, such as Nav1.5, CaV1.2, and Kv1.5, which are enriched in caveolae and colocalized with Cav3. Caveolae are ready reservoirs of select cell membrane proteins; β-adrenergic receptor stimulation opens caveolae through a Gαs-involved, PKA-independent pathway, and increases membrane density of resident ion channels [47]. In addition to caveolin-associated augmentation of surface expression and channel phosphorylation, sympathetic activation increases L-type Ca²⁺ channel activity and intracellular Ca²⁺. Phosphorylation of the Nav1.5 channel results in the alteration of the voltage-dependent kinetics and whole-cell INa amplitude [48]. There is limited evidence for direct regulation of cardiac Na⁺ currents by parasympathetic activity, but the reversal of the effects of β-adrenergic receptor stimulation by acetylcholine has been described [49], likely via pre-synaptic inhibition upon muscarinic receptor activation [50]. β-adrenergic receptor stimulation appears to alter the voltage-gated calcium current via a dual mechanism, perhaps similar to that demonstrated previously for sodium channels [51]. The L-type cardiac calcium channel, CaV1.2a, is phosphorylated by PKA, which increases open channel probability, and subsequently the overall cellular calcium current [47].
Potassium channels: Sympathetic activation can directly affect all potassium currents, including Ito (responsible for transient outward current and level of the plateau in action potential). The delayed rectifier K currents IKur, IKr, and IKs are slowly activating outward currents that play major roles in the control of repolarization. β-adrenergic stimulation regulates IKr through the activation of PKA (an inhibitory effect) and elevation of c-AMP (a stimulatory effect through binding to the cyclic nucleotide binding domain of the channel), whereas α-adrenergic stimulation is inhibitory. β-adrenergic stimulation also accelerates repolarization by augmenting IKs via PKA-dependent phosphorylation of Kv7.1 (also termed KvLQT1, encoded by the Kcnq1 gene) [52-54], and β-blockers prolong transmural dispersion of repolarization and action potential duration [55]. However, there is a potential inhomogeneity of effects of β-adrenergic stimulation on potassium channels across different species, which also vary by stimulus and disease states [50]. Vagal stimulation produces the opposite effects. Vagal stimulation releases acetylcholine, which then activates a potassium current and an inward-rectifying K+ current (IKAch), following stimulation of muscarinic (M2) receptors that hyperpolarizes the membrane potential and abbreviates the action potential, slowing the Phase 4 depolarization of pacemaker cells [52, 56]. In contrast, Liang et al. tested acetylcholine shortened action potential duration in ex vivo rat ventricular tissue, and the effect was inhibited by a G-protein-coupled inward rectifier potassium (GIRK) channel blocker [57]. Furthermore, the muscarinic stimulation can also partially reduce the amplitude of the L-type Ca^{2+} current by inhibiting adenylate cyclase. Therefore, vagal stimulation’s effect on cardiac repolarization is complex and may differ by species, phenotype, or stimulus.
As illustrated in Figure 1.1, sympathetic dominance in humans produces an increase in upstroke velocity, amplitude, and conduction velocity, as well as a decrease in the effective refractory period. These effects are mainly due to stimulation of β-adrenergic receptors and the resulting augmentation of INa, ICa, IKr, and IKs. Therefore, acute sympathetic neural activation results in the shortening of RR interval, P wave, PR interval, PR segment, QRS duration, QT, and Tp-Te.
Figure 1.1 Effect of Sympathetic nervous activation on human cardiac electrophysiology

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Nicotine

Cardiac autonomic nervous system: Nicotinic acetylcholinergic receptors (nAchRs) mediate the neural transmission at the ganglia of both branches of the ANS. However, different nicotinic receptors at the ganglia play distinct roles in sympathetic and parasympathetic cardiovascular responses (Figure 1.2). Specifically, activation of α4β2 nAchRs elicits a parasympathetic cardiovascular response, and activation of α7 nAchRs elicits a sympathetic cardiovascular response [67, 68]. Neff et al. used the perforated patch-clamp technique in a visualized rat brain stem slice to identify three potential sites of action by which nicotine increases the activity of cardiac vagal neurons: (a) direct activation of postsynaptic ligand-gated nicotinic channels in cardiac vagal neurons, (b) different presynaptic terminals, and (c) postsynaptic glutamatergic terminals [69]. Moreover, in various animal models and species, nicotine has been shown to evoke norepinephrine release from both the peripheral postganglionic sympathetic nerve endings and adrenal medulla [70, 71]. Another potentially sympathoexcitatory mechanism of nicotine involves the inhibition of neuronal nitric oxide synthase, which decreases central nitric oxide availability, thereby removing its tonic inhibitory effect on central sympathetic outflow [72].
Figure 1.2 Schematic diagram to illustrate the different direct sites of action for nicotine on the pathways of the cardiovascular autonomic nervous system. Nicotine acts on the Nicotinic acetylcholine receptors (nACHRs) in 1) Central nervous system 2) Pre-ganglion (at both sympathetic and parasympathetic neurotransmission) and at 3) Post ganglion and chromaffin cells in adrenal glands at sympathetic nerve transmission.

SA – Sinoatrial node. AV – Atrioventricular node.
Cardiac sodium and calcium channels: The binding of nicotine to the extracellular binding site of the nicotinic acetylcholine receptor leads to a conformational change of the central pore, which results in the influx of sodium and calcium ions [73, 74]. In addition to the increase in intracellular calcium concentration facilitated by sodium influx through the nicotine receptor [73], nicotine also evokes calcium influx by direct activation of voltage-dependent calcium channels [75-77]. More specifically, the L-type Ca2+ channels—Cav 1.2 channel, in particular—have been implicated in nicotine addiction, and are controlled by the SNS and stimulated by nicotine [78, 79]. Therefore, several studies have consistently shown that nicotine can both indirectly (catecholamine-mediated) and directly activate voltage-gated sodium and calcium ion channels, depolarize membrane potential, and increase cardiac contractility [80-83].

Cardiac potassium channels: On the other hand, nicotine directly blocks multiple types of potassium currents, (A-type K+ currents (Ito current/Kv4.3 channel), delayed rectifier K+ currents (IKr/HERG) and inward rectifier K+ currents (Ik1/Kir2.1), independent of nicotinic receptor stimulation or catecholamine release [84-86]. However, the ex vivo studies on the effects of nicotine on the duration of action potential have been inconsistent. Some found that nicotine shortens the action potential duration (particularly phase 2) [83], whereas others found that nicotine prolongs it [87]. These discrepancies could be related to the time from drug administration [82], drug concentration [80, 88] or the concomitant increase in force [89]. The shorter phase 2 could also reflect nicotine stimulation of L-type Ca2+ channel, which, together with potassium channels, dictate the duration of this phase of the action potential. Similar dose-dependent effects of nicotine
are seen in the sinoatrial node, where low doses of nicotine reduce [81, 88]—and large
doses increase [88]—the spontaneous cycle length of sinoatrial node pacemaker cells.

At physiological doses, nicotine can indirectly (through the sympathetic nervous
system) and directly (via immediate effects on cardiac ion channels) stimulate sodium and
calcium channels and block potassium channels. Per the pathways mentioned above,
nicotine is expected to induce multiple alterations in the surface ECG, including shortening
of the P wave, PR interval, PR segment, and QRS duration, and prolongation of QT
corrected (QTc) and Tp-Te. However, very few researchers have attempted to explore the
effects of nicotine on all ECG intervals in humans.

_Cigarette smoking and its non-nicotine constituents_

_Cardiac autonomic nervous system:_ Cigarette smoke-induced cardiovascular
effects are at least partly due to stimulation of sympathetic neurotransmission, and can
theoretically manifest at four different sites of the sympathetic nervous system: the brain,
pre-ganglionic and post-ganglionic sympathetic nerves, and the adrenal medulla [71].
Acute cigarette smoking increases efferent sympathetic nerve activity, primarily via the
release of the catecholamines norepinephrine, and epinephrine (Table 1.1). This
catecholamine release increases myocardial work and oxygen consumption through an
increase in blood pressure, heart rate, and myocardial contractility [19]. However, several
studies suggest that the primary effect is from direct pharmacologic stimulation of nicotinic
acetylcholine receptors, as well as the catecholamine release from localized peripheral
postganglionic sympathetic nerve endings and the adrenal medulla [90, 91]. Despite
evidence that sympathetic tone is increased during smoking, either by increased release or
decreased clearance of catecholamines at neuroeffector junctions via inhibition of monoamine oxidase (MOA) [92], Grassi et al. revealed that the central sympathetic activity is inhibited, presumably via counteracting baroreceptor reflexes [90].
Table 1.1 Summarizes the key studies on the acute effect of smoking on the circulating catecholamines. E – Epinephrine, NE – Norepinephrine. Dopa - Dopamine

<table>
<thead>
<tr>
<th>Author</th>
<th>Date</th>
<th>Journal</th>
<th>Participants</th>
<th>Cigarette Description</th>
<th>Plasma/urine Sampling Frequency</th>
<th>Catecholamines</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryer</td>
<td>1976</td>
<td>NEJM</td>
<td>10 healthy men (24-42 y)</td>
<td>Two standard non-filtered cigarettes in 10 minutes</td>
<td>Plasma -10, 0, 2.5, 5.0, 7.5, 10, 12.5, 15.0, 17.5, 20, 25, 30 (mins)</td>
<td>E, NE</td>
<td>Max at 10 and 12.5 min for E and NE</td>
<td>E significantly higher even at end.</td>
</tr>
<tr>
<td>Siess</td>
<td>1982</td>
<td>Circulation</td>
<td>6 healthy men, mean age 30 y</td>
<td>Two cigarettes of 1.6 mg nicotine each</td>
<td>Plasma End of smoking</td>
<td>E, NE</td>
<td>Increased from baseline</td>
<td></td>
</tr>
<tr>
<td>Mundal</td>
<td>1988</td>
<td>Am J Hypertens</td>
<td>18 normotensive, 13 hypertensive white men (mean age 42 y)</td>
<td>Two cigarettes, 1.7 mg nicotine</td>
<td>Plasma Baseline, after each cigarette and after 60 minutes from start of smoking</td>
<td>E, NE</td>
<td>E increased after second cigarette (not after 60 min) only among hypertensive group</td>
<td>15 ng/ml after 1st cigarette and 23 ng/ml after second cigarette</td>
</tr>
<tr>
<td>Niedermaier</td>
<td>1993</td>
<td>Circulation</td>
<td>8 healthy, 5 men (19 – 44 y)</td>
<td>Low and medium-nicotine research cigarette (30-45 mins apart)</td>
<td>Plasma 10 mins after each smoking period (to coincide with max catecholamines)</td>
<td>E, NE</td>
<td>No difference</td>
<td>7 and 16 ng/ml after low and medium cigarette</td>
</tr>
<tr>
<td>Grassi</td>
<td>1994</td>
<td>Circulation</td>
<td>8 men, 1 woman, (21 – 48 y)</td>
<td>One filtered cigarette 1.1 mg nicotine (within 5 mins)</td>
<td>Plasma End of smoking</td>
<td>E, NE</td>
<td>Significant increase</td>
<td>After 1st cigarette 44 ng/ml</td>
</tr>
<tr>
<td>Krzysztof</td>
<td>1998</td>
<td>Circulation</td>
<td>14 healthy smokers (13 men). Mean age 23 y</td>
<td>Two cigarettes containing 1.1 mg nicotine (5 mins apart). 45 mins later smoked third cigarette</td>
<td>Plasma Outset of study and 3 mins after second and third cigarette</td>
<td>NE</td>
<td>Increased from 171 (baseline) to 189 (after 2 cigarettes) and 214* (3rd cigarette)</td>
<td>17 ng/ml and 20 ng/ml of nicotine, after 2nd and 3rd cig. Smoked third</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Group</td>
<td>Description</td>
<td>Method</td>
<td>Monitored</td>
<td>Outcome</td>
<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Gourlay 1997</td>
<td>Clin Pharmacol Ther</td>
<td>12 healthy male smokers, mean age 38 y</td>
<td>6 got nicotine nasal spray (1 x 0.5 mg in each nostril) and other 6 did smoking (usual brand)</td>
<td>Plasma</td>
<td>0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, and 60 mins</td>
<td>E, NE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bragg 1956</td>
<td>J Appl Physi</td>
<td>11 healthy young men</td>
<td>Their choice of standard cigarettes during 2 / 8 hour (4 and 10 cigs) cigarette smoking (own choice), transdermal nicotine (21 mg/d) and placebo</td>
<td>Urine</td>
<td>8-hour urine collection</td>
<td>E, NE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benowitz 1993</td>
<td>JACC</td>
<td>12 healthy male smokers, 31 – 65 y</td>
<td></td>
<td>Urine</td>
<td>24 hr Urine collected day 5</td>
<td>Dopamine, E, NE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benowitz 1989</td>
<td>Ann Intern Med</td>
<td>8 healthy male smokers</td>
<td>oral snuff, chewing tobacco, and cigarettes</td>
<td>Urine</td>
<td>24-hour urine after 3- or 4-day blocks</td>
<td>Dopamine, E, NE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Venous E and NE remain unchanged

E increased, NE unchanged

Significantly higher with smoking

Significantly higher with smoking
Non-nicotine constituents:

In addition to nicotine, several other cigarette components have been implicated in inducing proarrhythmia, such as VOCs (aldehydes [acrolein, formaldehyde, acetaldehyde, crotonaldehyde], benzene, toluene), particulate matter, gases (carbon monoxide), and polycyclic aromatic hydrocarbons. It is estimated that about 90% of non-cancer mortality from tobacco smoke is due to aldehydes (acrolein, formaldehyde, and acetaldehyde) [93]. In animal models, a single exposure to acrolein significantly increased HRV and arrhythmia independent of heart rate, possibly through activation of the transient-receptor potential ankyrin-1 channel (TRPA1), an irritant receptor channel found in the airways [94, 95]. Treatment with atenolol reduced this response, whereas atropine enhanced it, suggesting parasympathetic dominance and sympathetic modulation [95]. Non-nicotinic alkaloids, such as nornicotine or anabasine (which represent 8–12% of total alkaloid content in tobacco), also exert an agonistic activity on nicotinic receptors [96]. Acute exposure to toluene, the most abundant aromatic compound in mainstream smoke from full-flavored cigarettes, enhances heart rate and blood pressure at baseline conditions, primarily due to systemic increases in circulating catecholamines [97, 98], and the enhanced protein expression of β1 adrenergic receptors [97]. Additionally, intravenous acetaldehyde in anesthetized cats has been found to increase systemic blood pressure and heart rate (presumably from release of endogenous catecholamines from cardiac tissue) [99]. Formaldehyde has been shown to induce significant bradycardia and negative inotropic responses in both in situ preparations of guinea pig and rabbit hearts, and in vitro cardiac preparations [100]. Notably, the negative chronotropic effect of formaldehyde in
animals seems to be caused mainly by the inhibition of sympathetic nervous activity through the central nervous system [100].

Several non-nicotine cigarette ingredients have long been recognized as cardiotoxic and linked with cardiac electrical activity disturbance and arrhythmias, such as aromatic compounds [101], carbon monoxide [102], and aldehydes [99]. However, literature on their individual drug concentration effects on cardiac ion channels and the action potential is scarce.

**Cardiac sodium and calcium channels:** Toluene inhibits activated currents through ligand and voltage-gated sodium and calcium channels [97, 103-106]. Phenol has been shown to exert a dose-dependent negative inotropic effect in an isolated mammalian cardiac muscle, possibly via blocking calcium channels [107]. In a patch-clamp electrophysiology and confocal imaging experiment with isolated ventricular myocytes, carbon monoxide activated nitric oxide synthase. This led to the nitric-oxide-mediated nitrosylation of Nav1.5, as well as increased the sustained (late) component of the inward Na(+) current, and inhibited peak Nav1.5 current amplitude, ultimately resulting in prolonged action potential and associated intracellular Ca(2+) transient [102]. In addition, carbon monoxide inhibits native rat cardiomyocyte L-type Ca2+ currents and the recombinant α1C subunit of the human cardiac L-type Ca2+ channel [108, 109]. Formaldehyde and other aldehydes have previously been shown to cause dramatic deceleration of sodium inactivation, depress Ina, and prolong action potential [110-113]. On the other hand, acetaldehyde has also been shown to increase the ICa, and thereby increase the contractile force [114], Its effects can be potentiated by the additional stimulation of α-adrenergic receptors [115]. Several other cigarette constituents have also
been found to, at minute quantities, significantly inhibit sodium and calcium channels in isolated cardiac cells, such as cyanide [116, 117], lead [118], and cadmium [119]. Particulate matter, encountered during cigarette smoking, is also a significant cause of cardiovascular morbidity and mortality. Particulate matter has been shown to dysregulate prominent Na\(^+\) and K\(^+\) channel pathway genes [120], and carotid body sensitivity [120].

**Cardiac potassium channels:** Aldehydes have been proven to considerably inhibit IK1 and Ito in animal atrial and ventricular myocytes [121-123]. In one study, ethanol and acetaldehyde inhibited the (Na\(^+\) + K\(^+\))-activated ATPase activity of plasma membranes prepared from a guinea-pig heart in a dose-dependent manner [124]. In addition, several volatile agents found in cigarette smoke, commonly used as anesthetics, have been shown to inhibit G-protein-coupled inwardly rectifying potassium channels [125]. Benzene derivatives inhibit delayed rectifier K\(^+\) currents [126], specifically IKr2.1, in a voltage-independent manner [127]. Carbon monoxide also inhibits inward-rectifying potassium (Kir) channels, and prolongs the action potential duration [128, 129]. In their study, Ficker et al. demonstrated that, despite there being an increase in cardiac calcium current, there is reduced trafficking of cardiac potassium channel (hERG channels) to the cell surface among patients treated with arsenic trioxide, resulting in QT prolongation [130]. Another study revealed that arsenic trioxide blocks both IKr and IKs at clinically relevant concentrations. However, it also activates IK-ATP [131]. Graff et al. discovered that exposing rat cardiac myocytes to noncytotoxic concentrations of zinc and vanadium slowed the spontaneous beating rate [132].

In summary, the studies on the several components of cigarette smoke with known or suspected cardiotoxicity consistently show that they depress sodium and potassium
channels, with some mixed effects on calcium channels, and a range of effects on ECG (Figure 1.3). Overall, the constituents appear to result in prolonged action potential duration from inhibition of sodium and potassium channels. Apart from formaldehyde, most other cigarette non-nicotine constituents have been shown to stimulate the cardiac sympathetic nervous system. With >4000 non-nicotine chemicals in cigarette smoke, and a complex mixture of effects on the sympathetic and parasympathetic pathways, the non-nicotine constituents can have a varied effect on surface ECG. Furthermore, most of the studies on the effects of non-nicotine constituents of cigarette smoke are derived from ex vivo or in vitro animal studies, with concentrations typically higher than those possibly encountered during cigarette and nicotine use. For instance, carbon monoxide causes tissue hypoxia, and, in addition to nicotine, has been implicated in promoting cardiac arrhythmias [133]; however, Benowitz et al. found that when carbon monoxide was administered under conditions similar to those of cigarette smoking, it had no significant effect on blood pressure, heart rate, plasma catecholamines, platelet aggregation, or C-reactive protein [134]. These results suggest that the short-term chronotropic, pro-thrombotic, and pro-inflammatory effects of smoking are probably due to components of cigarette smoke other than carbon monoxide.
Figure 1.3 Direct effects of nicotine and non-nicotine constituents on cardiac ion channels and action potential. SNS – Sympathetic Nervous Activation
Indirect (systemic) effects

There are several short- and long-term homeostatic mechanisms to ensure adequate blood flow, pressure, distribution, and perfusion, and are categorized into three groups: neural, humoral, and autoregulatory mechanisms. These mechanisms can also indirectly alter cardiac autonomic output and electrophysiology. Table 1.2 summarizes the stimuli, receptors, and physiologic effects most pertinent to cigarette smoking and nicotine-related changes in human autonomic reflexes.

Neural reflexes to tobacco exposure: The baroreflex feedback loop is one of the most important mechanisms controlling arterial pressure on a beat-to-beat basis. It achieves this through arterial baroreceptors located in the carotid sinus and aortic arch. These receptors are mechano-sensitive, and the distension of the vessels that occurs at each heart beat leads to action potential generation on peripheral nerves that transmit to the central nervous system, buffering arterial pressure fluctuations through changes in sympathetic and parasympathetic activity. Therefore, when blood pressure rises, the baroreceptor afferent tone increases, leading to increased vagal efferent activity and diminished sympathetic outflow. These effects will lead to a decrease in cardiac output by decreasing heart rate and cardiac contractility. Additionally, the fall in sympathetic tone to blood vessels, as well as increased vagal effect activity (through increased guanylyl cyclase and cGMP activity) leads to vasodilation and diminished vascular resistance. There is strong evidence that this crucial inhibitory role of the baroreflex arc is blunted in habitual smokers, and also impaired during acute exposure to smoking [72, 135, 136]. Grassi et al.’s study showed that there is sympathetic activation induced by smoking via increased release or a reduced clearance of catecholamines at the neuroeffector junctions. However, the central
sympathetic activity is inhibited by smoking, presumably via baroreceptor stimulation triggered by a pressor response to smoking [90]. There is also evidence that nicotine possibly decreases the baroreceptor sensitivity [137]. Besides nicotine, there are several other cigarette components, mainly PM 2.5, that directly alter baroreflex responsiveness in smokers [72, 138]. In one study, a one-time exposure to acrolein caused a decrease in the sensitivity of baroreflex and increased incidence of arrhythmia in rats [139].

The peripheral arterial chemoreceptors in the carotid and aortic bodies are stimulated by decreased arterial PO2, increased PCO2, and increased H+ concentration. Their stimulation causes hyperventilation, as well as increases in sympathetic neural activity and the rate and volume of breathing; chronic arterial chemoreflex sensitization in smokers could also lead to sustained sympathetic activation. Arterial chemoreceptors are activated by hypoxia, and chronic smokers may be at risk to toxic effects of carbon monoxide in tobacco smoke. Perez et al. showed that acrolein-exposure-induced cardiovascular effects in rats (i.e., an increase in systolic, diastolic and mean arterial blood pressure during exposure, and a decrease in cardiac contractility one day after exposure) were prevented after a blockade of carotid body signal transduction. This suggests that acrolein-induced cardiovascular responses may be mediated by carotid body-triggered changes in autonomic tone [140]. However, to date, there is no evidence for augmented arterial chemoreceptor sensitivity in habitual smokers. Instead, nicotine does not affect chemoreflex sensitivity, as evidenced by unchanged minute ventilation, apnea duration, and oxygen saturation after nicotine and placebo in normoxia [141, 142].

Airways are lined with vagal afferent nerve fibers, including non-myelinated afferent C-fibers sensitive to noxious chemicals. A subset of these vagal C-fibers expresses
the TRP channels, which sense a variety of mechanical and chemical stimuli, such as mechanical stretch, shear stress, oxidative stress, inflammation, and endogenous and exogenous chemicals. When exposed to irritants, TRP channels induce the local release of neuropeptides from cells, resulting in a local inflammatory response that reflexively increases efferent sympathetic nerve activity. This may cause further airway irritation and reflex responses, such as cough and reactive airway dysfunction [50, 143, 144]. However, there is little evidence to support whether smoking and its constituents directly activate TRP channels to modulate the human cardiac autonomic nervous system.

There are also respiratory-related changes in heart rate, specifically termed respiratory sinus arrhythmia, to help match pulmonary blood flow to lung inflation, and to maintain an appropriate diffusion gradient for oxygen in the lungs (heart rate increases during inspiration and decreases during the post-inspiration/expiration period) [145]. Consequently, different respiratory patterns during or after cigarette smoking may differentially affect cardiac autonomic function [146]. Cardiopulmonary receptors are found in low-pressure portions of the circulation, such as walls of the atria and pulmonary arteries. These mechano-sensitive receptors are activated by the distension of the vessel walls, responding to changes in central blood volume. The cardiopulmonary baroreflexes normally exert a tonic inhibitory influence on the SNS. However, cigarette smoking and nicotine product use in humans does not typically produce challenges that are extreme enough to affect these cardiopulmonary receptors or other similar innate reflexes that also alter cardiac electrophysiology (such as temperature, hypoxia, acidosis etc).

**Autoregulation:** Local, self-regulatory mechanisms, including chemical and myogenic controls, allow each region of tissue to adjust its blood flow, and thus its
perfusion. Chemosensitive nerve endings are also found throughout the cardiovascular and respiratory systems, and are stimulated by several exogenous chemicals and endogenous chemicals formed and released in response to conditions such as hypoxia, ischemia, certain mechanical demands, inflammation, or toxin exposures. The efferent pathways of the reflex involve inhibition of sympathetic outflow to peripheral vessels and increased activity in efferent vagal fibers to the heart [50]. The myogenic response is a reflex to the stretching of the smooth muscle of the arteriolar walls as changes in blood flow occur through the vessel (e.g., vasoconstriction in response to increased intraluminal pressure). Increased peripheral vascular resistance, cigarette smoking, and nicotine also have detrimental effects on coronary microvascular function (e.g., increases in coronary flow velocity and resistance, and decreases in flow reserve) and can cause vascular dysfunction [147-151], possibly via β-adrenergic receptor [152].

**Humoral to tobacco exposure:** Several studies have suggested that humoral systems play a vital role in maintaining cardiac electric activity, and changes in their production or action pathways may contribute to various cardiac diseases. Beyond the neurotransmitters acetylcholine and norepinephrine, there is a local presence of peptidergic and nitrergic neurons along with their associated neurotransmitters, such as neuropeptide-Y, vasostatin, galactin, vasoactive intestinal peptide, nitric oxide synthase, and angiotensin-II [153, 154]. Neuropeptide-Y, coreleased by prolonged sympathetic activation, reduces acetylcholine release from the nearby vagal nerve ending, and it is an excellent example of sympathovagal cross-talk. These non-cholinergic, non-adrenergic neurotransmitters often exert effects similar to cholinergic or adrenergic agonists or antagonists [153]. The release of these neurotransmitters/modulators is often highly dependent on the level of neuronal
stimulation, and they tend to be slowly diffusing molecules that often function as neuromodulators, rather than classical neurotransmitters.

Smoking has multiple effects on hormone secretion, some of which bear crucial clinical implications, and are mainly mediated by nicotine. Most acute data are from nicotine administration, whereas chronic data are predominantly from studies on cigarette smokers. Smoking affects pituitary, thyroid, adrenal, testicular and ovarian function, calcium metabolism, and the action of insulin differently in acute and chronic conditions [155, 156]. In particular, the activation of nicotinic acetylcholine receptors in the adrenal medulla leads to increased circulating catecholamine levels with corresponding cardiovascular and metabolic responses. The renin-angiotensin-aldosterone system (RAAS) and adrenal gland is also activated by the hypothalamic-pituitary-adrenal axis [155, 156].

Other mechanisms by which smoking, and nicotine can influence cardiac autonomic function are inflammation and oxidative stress. The role of autonomic function in regulating oxidative stress is supported by previous evidence that the increase in adrenergic drive may result in catecholamine excitotoxicity, increased oxidative stress, and free-radical myocardium injury. There are two main phases in cigarette smoke; particulate phase and gas phase [157]. The two phases are rich in free radicals, and non-radical oxidants. Therefore, acute and chronic exposure to smoking causes increased oxidative stress from direct damage by radical species and the inflammatory response, as well as through sympathetic neural activation [72, 158-160]. In one study, smoking decreased the overall α- and β-adrenergic receptor concentration almost immediately after tobacco smoke
exposure in rats—perhaps through receptor desensitization resulting from a release of catecholamines—but was rapidly reversible after the termination of the exposure [161].
<table>
<thead>
<tr>
<th>System</th>
<th>Receptors</th>
<th>Stimulus</th>
<th>Effect on SNS</th>
<th>Effect on PNS</th>
<th>Studies on the effects of smoking / nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baroreflex</td>
<td>Carotid artery / Aorta</td>
<td>High Blood Pressure</td>
<td>Decrease</td>
<td>Increase</td>
<td>Activates the system by increasing blood pressure through release of catecholamines from end terminals. Smoking also impairs the baroreflex system</td>
</tr>
<tr>
<td>Chemoreflex</td>
<td>Carotid / aortic (peripheral), medulla (central)</td>
<td>Low PaO2, High PaCO2/pH</td>
<td>Increase</td>
<td>-</td>
<td>Nicotine does not increase chemoreflex sensitivity to hypoxia.</td>
</tr>
<tr>
<td>Temperature</td>
<td>CNS</td>
<td>High Temp</td>
<td>Increases</td>
<td>-</td>
<td>Temperature increases in lungs; decreases in skin temp</td>
</tr>
<tr>
<td>Inflammation / Oxidative stress</td>
<td>Lung afferent C fibers, vascular, myocardial hypophysalamic–pituitary axis</td>
<td>Direct / Reactive Oxygen Species</td>
<td>Increase</td>
<td>Decrease</td>
<td>Increases inflammation and oxidative stress during acute and chronic exposure</td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
<td>Increase</td>
<td>-</td>
<td>Chronic: increases thyroid hormones, cortisol, possibly testosterone and estradiol. Decreases prolactin and growth hormone Acute: Increases prolactin, cortisol, growth hormone, vasopressin, endorphin, neuropeptide Y</td>
</tr>
<tr>
<td>Cardiopulmonary</td>
<td>Ventricles</td>
<td>High SNS (low vol LV)</td>
<td>Decrease</td>
<td>Increase</td>
<td>-</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Pulmonary</td>
<td>chemical stimuli, inflammation</td>
<td>?</td>
<td>Increase</td>
<td>-</td>
</tr>
<tr>
<td>Bainbridge</td>
<td>Atria</td>
<td>High blood vol</td>
<td>Increase</td>
<td>Decrease</td>
<td>-</td>
</tr>
</tbody>
</table>
Literature review of effects of autonomic nervous system and smoking on electrocardiogram

Autonomic nervous system

Heart rate: The sinoatrial node, also referred to as the pacemaker of the heart, coordinates heart contractions. Located in the upper right atria wall, it generates impulses that result in cardiac contraction and determines the heart rate. The ANS predominantly determines the actual heart rate, but nonautonomic contributions (e.g., hypoxia and temperature via chemoreflex and baroreceptors pathways) also affect the intrinsic heart rate. The heart rate provides a static index of the net effects of autonomic input to the sinus node, but it does not reflect direct information about individual sympathetic or parasympathetic input. However, this simple measure has prognostic value, as reflected in population-based studies, in which high resting heart rate (net predominance of sympathetic influence) is associated with increased all-cause mortality, death from CVD, and sudden death [162-164]. The elevated heart rate may not only predict the outcome, but may also be an actual causal determinant of CVD through several different mechanisms [165].

Heart rate variability: Efferent sympathetic and vagal activities directed to the sinus node are characterized by a synchronous discharge with each cardiac cycle that can be modulated by central (vasomotor and respiratory centers) and peripheral (oscillation in arterial pressure and respiratory movements) oscillators. These oscillators generate rhythmic fluctuations in efferent neural discharge that manifest as short- and long-term oscillation in the heart period. Analysis of these rhythms may permit inferences on the state and function of (a) the central oscillators, (b) the sympathetic and vagal efferent activity,
(c) humoral factors, and (d) the sinus node [166]. The oscillation in the intervals between consecutive heartbeats can be measured for indices of HRV, using either time domain approaches (based on statistical analysis of R-R intervals) or frequency domain approaches (spectral analysis of a sequence of R-R intervals) [166]. Large population studies have shown a higher risk of coronary artery disease, death, and cardiac mortality in individuals with decreased HRV (in both healthy populations and patients with cardiac disease) [167].

P wave: The P wave is the expression of atrial depolarization and intraatrial conduction. Electrocardiographic P wave indices consist of the P wave duration, morphology, and amplitude, and provide information about the atrial structure and function. A prolonged P-wave duration (>120 ms) is considered a marker of atrial cardiopathy, which, in chronic cardiac disease, is usually reflective if reduced atrial conduction related to architectural changes of atrial walls. P-wave duration is affected by autonomic tone. In general, both sympathetic stimulation and parasympathetic blockade shorten P-wave duration, whereas sympathetic blockade prolongs it [58]. P-wave terminal force in lead V1 (PTFV1) is defined as the value of the amplitude multiplied by the duration of the terminal’s negative deflection of the P wave in lead V1 of a standard 12-lead ECG. P-wave area (PWA) is the total geometric area under the P wave in the 12-lead ECG. It is usually represented by the product of the duration and peak amplitude of the P wave, and is measured in microvolt × milliseconds. Both of these P-wave indices, together with P-wave axis (net direction of electrical forces within the atria), are also markers of atrial cardiopathy [168, 169]. Therefore, acute sympathetic activation may shorten P-wave duration, whereas chronic sympathetic activation may prolong P-wave duration from long-term structural atrial remodeling. The P amplitude, which is mostly governed by atrial
pressures, may be increased in height (and in depth of PTFV1) in acute and chronic sympathetic stimulation.

PR interval: PR interval is the period of time from the start of the P wave (atrial depolarization) until the start of the QRS complex (ventricular depolarization). Therefore, the determinants of PR interval are atrial depolarization and the conduction time from the sinus node to the atrioventricular node, His bundle, and Purkinje fibers [170]. The duration of PR interval is normally between 120 and 200 ms. In most cases, a prolonged PR interval (>200 ms) is determined by conduction delay in the atrioventricular node. The ANS’s acute effects on PR intervals are well known, considering that autonomic innervation influences the conduction through the atrioventricular node junction by modulating the refractory period [61, 171]. However, in the chronic state, autonomic-imbalance-induced atrial fibrosis may also cause PR interval prolongation by slowing atrial depolarization and dromotropy. Both short [26, 27, 29, 30] and prolonged [30, 172-174] PR intervals have been associated with adverse clinical outcomes (stroke, atrial fibrillation, and all-cause mortality).

QRS complex: The QRS complex represents the electrical impulse as it spreads through the ventricles, and depicts ventricular depolarization. Ventricular depolarization is also influenced by autonomic modulation of the heart. In humans, increased sympathetic tone by β-adrenergic stimulation shortens overall QRS duration [175], heterogeneity of ventricular activation during disease states, and bundle branch blocks (mechanical and electrical dyssynchrony) [176]. The QRS complex voltage reflects the viable left ventricular mass and can be increased (e.g., athlete’s heart, hypertensive heart disease [177]) or decreased (post-myocardial infarction, infiltrative cardiomyopathies, etc. [178])
during different disease states by several mechanisms [179], and both carry critical clinical implications [180-182]. The QRS amplitudes can also potentially be affected by the cardiac ANS [183-185]. However, stimulation of the left stellate ganglion produces little or no change in conduction velocity in the Purkinje system, or in the pattern of epicardial depolarization, suggesting that cardiac sympathetic nerve stimulation does not result in significant changes in the sequence of ventricular excitation [61].

QT interval: The QT interval is a marker of ventricular depolarization (QRS duration) and repolarization (JT interval), and autonomic tone is the primary determinant of their duration [65, 186]. Changes in autonomic tone may alter QT intervals both indirectly, by modulating basal heart rate, and directly, by affecting ventricular repolarization kinetics of myocardial cells through neural and receptor-mediated mechanisms [53, 54, 57]. The effect of sympathetic nervous activity on the QT has been demonstrated [187-190]; however, in their study, Cappato et al. did not find that sympathetic tone influenced QT significantly, but rather that the cholinergic system appeared to slow down ventricular refractoriness and QT interval [191].

T wave: The T wave is the asymmetrical wave in the ECG that reflects ventricular repolarization; it comes after the QRS complex and typically lasts approximately 150 ms. The difference between the peak of the T wave and the isoelectric level during the same heart cycle is defined as T-wave amplitude (TWA). Several studies have suggested that TWA decreases, and even may invert, with sympathetic stimulation; conversely, the additional role of the cholinergic system remains unclear [192]. The Tp-Te interval is an index of transmural dispersion of repolarization, a marker of ventricular arrhythmia vulnerability [193]. An increase in Tp–Te reflects increased sympathetic nerve activity
[194], rather than the release of circulating norepinephrine [194]. However, more recently, the influence of the ANS on the TWA and Tp-Te interval has fallen under heavy scrutiny, and appears to be an unreliable index of myocardial sympathetic activity [195-197].

In summary, acute and chronic cardiac autonomic imbalance varies by phenotype (normal and diseased states), and influences several ECG parameters differently.

Role of β-adrenergic receptors

Physiologic doses of epinephrine alter electrophysiology through β-receptor activation, and manifest as an acceleration of atrioventricular nodal conduction and shortening of refractoriness in the atrium and ventricle [198]. In contrast, norepinephrine slows atrioventricular nodal conduction and lengthens the atrial and ventricular effective refractory periods [199]. Therefore, norepinephrine may counteract several of the electrophysiological effects of circulating epinephrine during physiologic degrees of stress. Furthermore, α-adrenergic stimulation by epinephrine in the presence of propranolol was found to prolong atrial and ventricular refractoriness [199].

Most studies on the effect of β-adrenergic receptors on cardiac electrophysiology have been performed with either propranolol, a non-selective β-blocker, or isoproterenol, a non-selective β-adrenoreceptor agonist. Propranolol decreases heart rate and prolongs atrioventricular nodal conduction [200], and has been shown to have no significant effect on intra-ventricular conduction. Shortening of action potential plateau was also not evident after treatment with propranolol in normal cat ventricular muscles [89]. In the sinoatrial node and left atrial appendage cells of the guinea-pig heart, propranolol antagonized the positive chronotropism of nicotine and norepinephrine [88]. Studies conducted on five
human volunteers showed that intravenous propranolol followed by smoking significantly decreased cardiac output, and significantly increased blood pressure and systemic vascular resistance compared to smoking alone [201]. Similar effects were observed in another study among 10 participants [202]. Smoking alone increased cardiac output, mean arterial blood pressure, and decreased the calculated systemic vascular resistance [201]. The cardiac output increases were due to a fall in systemic vascular resistance. The effect is exaggerated after propranolol, potentially via the inhibition of β-adrenergic receptors, either by preventing the vasodilatory effect of β-2 adrenergic stimulation, or as increased availability of α-receptors may result in unopposed pronounced effects of vasoconstriction from epinephrine. However, another study was conducted using 80 mg of oral propranolol on six volunteers, and there was no significant difference from the placebo in blood pressure or forearm hemodynamics, and no prevention of the acute vascular effects of cigarette smoking with β-blocker pre-treatment [203]. β-blockers are first line therapy for patients with Long QT syndrome (LQTS)—a genetic disorder that can potentially cause life-threatening cardiac arrhythmia, is characterized by delayed myocardial repolarization, and manifests as QT prolongation [204]. The response to β-blockers and epinephrine depends on the genotype [204, 205]. Therefore, the differences in β-adrenergic receptor-mediated effects on ventricular repolarization are likely related to the stimulus (e.g., frequency of cigarette smoking), dose (route) of propranolol use, and the underlying genotype. Furthermore, the plasma propranolol steady-state concentration is lower among smokers compared to non-smokers, possibly via an increased rate of drug metabolism [206].
Besides β-adrenergic receptors and muscarinic receptors from the cholinergic system, there are several other receptors (α-adrenergic receptors, dopamine receptors, and adenosine receptors) found throughout the myocardium, with critical clinical effects on ECG (Table 1.3). However, the impacts of smoking and nicotine on the ECG via receptors other than β-adrenergic receptors are not well defined.

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Receptors</th>
<th>Chronotropy (SA node)</th>
<th>Dromotropy (AV node)</th>
<th>Inotropy (Ventricle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>α1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>β1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>β2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dopamine</td>
<td>D1, D2, D3, D5</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>M2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1.3 Effects of cardiac receptors on chronotropy, dromotropy and inotropy

Acute and chronic exposure from smoking

Chronic exposure to cigarette smoke (nicotine and non-nicotine constituents)

Due to the injurious and addictive nature of smoking, it is not ethical to perform an experimental study to investigate the chronic effects of smoking and nicotine-containing products in humans. Therefore, studies can best describe associations of outcomes (biomarkers of CVD and risk) from occupational exposures or chronic smoking. Occupational exposure to polycyclic aromatic hydrocarbons results in decreased HRV [207]. A similar decrease in parasympathetic activity has been observed in workers exposed to organic solvents (n-hexane, xylene, and toluene) [208]. Sub-chronic and long-term exposure to ambient fine particulate matter (PM_{2.5}) at concentrations several orders of magnitude lower than those in cigarette smoke has been associated with increased QTc
duration in an elderly cohort [209]. Among 34 middle-aged individuals with metabolic syndrome, QRS and QTc intervals increased and HRV decreased 2 h after exposure to concentrated ambient ultrafine particles [210].

Table 1.4 A (atrial electrocardiographic indices) and B (ventricular electrocardiographic indices) summarizes the major human studies on the chronic effects of smoking. Overall, most large studies (sample size of greater than 100 smokers) consistently show that PR interval is decreased among chronic smokers compared to non-smokers. We were not able to find any study that directly investigated the effect of smoking on the PR segment. In a prospective study of 60 smokers, Varenicline (partial agonist at the α4 β2 nicotinic acetylcholine receptor) treatment was associated with a near-significant attenuation in PR interval (varenicline: 163.5±18.3 ms, vs. placebo: 168.2±17.9 ms; \( P = .053 \)). However, RR interval, QT interval, and QTd were not significantly altered [211]. Two studies showed shorter P duration but increased P amplitude among smokers [212, 213]. The effects of smoking on QRS duration were the most varied. Most studies consistently showed that there was no significant difference in QT interval between smokers and non-smokers. Two studies found that the ST segment was decreased in chronic smokers versus non-smokers [214, 215]. Few studies have found that the Tp-Te interval, Tpe/QT ratio, and Tpe/QTc ratio were higher among smokers [216, 217], even after varenicline administration [218].

Apart from the Zhang et al. study [219], none of these studies featured a quantitative method of smoking burden, such as cotinine, or an adjustment for clinically important covariates known to affect the baseline ECG (such as age, gender, body mass index, diabetes, coronary artery disease, etc).
Table 1.4 Major human studies on the chronic effects of smoking on atrial (A) and ventricular (B) electrocardiographic indices

The effect mentioned of the ECG parameters is among smokers compared to non-smokers are mentioned.

**A) Atrial electrocardiographic indices**

<table>
<thead>
<tr>
<th>Ref</th>
<th>Author, Year</th>
<th>Chronic Smokers</th>
<th>Non-smokers</th>
<th>P wave duration</th>
<th>PR interval</th>
<th>PR segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>[220]</td>
<td>Goldenberg, 2006</td>
<td>98</td>
<td>619</td>
<td>-</td>
<td>Shorter</td>
<td>-</td>
</tr>
<tr>
<td>[221]</td>
<td>Chatterjee, 1989</td>
<td>224</td>
<td>232</td>
<td>-</td>
<td>Shorter</td>
<td>-</td>
</tr>
<tr>
<td>[212]</td>
<td>Sharma, 2017</td>
<td>150</td>
<td>50</td>
<td><strong>Shorter</strong></td>
<td><strong>Shorter</strong></td>
<td>-</td>
</tr>
<tr>
<td>[222]</td>
<td>Baden, 1982</td>
<td>208</td>
<td>291</td>
<td>-</td>
<td><strong>Shorter</strong></td>
<td>-</td>
</tr>
<tr>
<td>[223]</td>
<td>Khan, 2011</td>
<td>75</td>
<td>30</td>
<td>-</td>
<td>Shorter</td>
<td>-</td>
</tr>
<tr>
<td>[213]</td>
<td>Swathi, 2015*</td>
<td>200</td>
<td>200</td>
<td><strong>Shorter</strong></td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[214]</td>
<td>Sandhya, 2015</td>
<td>64</td>
<td>63</td>
<td>-</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[224]</td>
<td>Karapinar, 2010</td>
<td>30</td>
<td>30</td>
<td>No difference</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[225]</td>
<td>Goette, 2007</td>
<td>46</td>
<td>49</td>
<td>No difference</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[226]</td>
<td>Siddiqui, 2013</td>
<td>30</td>
<td>30</td>
<td>No difference</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[227]</td>
<td>Venkatesh, 2010</td>
<td>50</td>
<td>50</td>
<td>No difference</td>
<td>No difference</td>
<td>-</td>
</tr>
</tbody>
</table>
### B) Ventricular electrocardiographic indices

<table>
<thead>
<tr>
<th>Ref</th>
<th>Author, Year</th>
<th>Chronic Smokers</th>
<th>Non-smokers</th>
<th>QRS duration</th>
<th>cQT / QT interval</th>
<th>cJT interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>[226]</td>
<td>Siddiqui, 2013</td>
<td>30</td>
<td>30</td>
<td>Shorter</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[213]</td>
<td>Swathi, 2015*</td>
<td>200</td>
<td>200</td>
<td>Shorter</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[227]</td>
<td>Vanketash, 2010</td>
<td>50</td>
<td>50</td>
<td>Shorter</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[214]</td>
<td>Sandhya, 2015</td>
<td>64</td>
<td>63</td>
<td>Shorter</td>
<td>No difference</td>
<td>Shorter ST</td>
</tr>
<tr>
<td>[223]</td>
<td>Khan, 2011</td>
<td>75</td>
<td>30</td>
<td>No difference</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[222]</td>
<td>Baden, 1982</td>
<td>291</td>
<td>208</td>
<td>No difference</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[215]</td>
<td>Devi, 2013</td>
<td>44</td>
<td>44</td>
<td>No difference</td>
<td>No difference</td>
<td>Shorter ST</td>
</tr>
<tr>
<td>[219]</td>
<td>Zhang, 2011</td>
<td>3306</td>
<td>2242</td>
<td>-</td>
<td>No difference (adjusted and used cotinine)</td>
<td>-</td>
</tr>
<tr>
<td>[216]</td>
<td>İlgenli, 2015</td>
<td>24</td>
<td>23</td>
<td>-</td>
<td>No difference</td>
<td>-</td>
</tr>
<tr>
<td>[228]</td>
<td>Karjalainen, 1997</td>
<td>10,717</td>
<td>-</td>
<td>Decreased</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[229]</td>
<td>İleri, 2011</td>
<td>30</td>
<td>30</td>
<td>-</td>
<td>Increased</td>
<td>-</td>
</tr>
<tr>
<td>[212]</td>
<td>Sharma, 2017</td>
<td>150</td>
<td>50</td>
<td>No difference</td>
<td>Increased</td>
<td>-</td>
</tr>
</tbody>
</table>

* There were no statistical analyses performed.
Acute exposure to smoking (nicotine and non-nicotine constituents)

Apart from the case reports, the scarce literature on the acute effects of individual constituents of cigarette smoking on human \textit{in vivo} ECG has mainly investigated nicotine and carbon monoxide. Acute carbon monoxide intoxication in children impaired ventricular repolarization with higher mean heart rate, QT dispersion, QTc dispersion, P dispersion, Tp-Te, QTc durations, Tp-Te dispersion, and Tp-Te/QT ratio compared to controls [230, 231]. However, it increased heart rate, ST-T wave changes, QTc dispersion, and TpTe dispersion in adults [232, 233]. There is also long-term increased risk of acute myocardial infarction in carbon monoxide poisoning [234]. Among 16 smokers undergoing electrophysiological study, exposure to cigarette smoke produced a statistically significant shortening of the sinus cycle length after chemical denervation of the sinus node with β-blockers and atropine [235]. In a single case report of mild nicotine toxicity, a young smoker presented with a typ1 Brugada pattern, raising the possibility of sodium channel blockade [236]. Nicotine has proved to be arrhythmogenic in animals and cell preparations; however, despite some case reports linking nicotine to atrial fibrillation in humans, these findings have not been confirmed in pharmacological studies of nicotine administered to human subjects [133].

There are very few human experimental studies that have investigated the acute effects of smoking on the ECG. In their analysis of 40 healthy male participants (20 smokers), Akbarzadeh et al. discovered that heart rate, mean QT, and QTc dispersion increased 10 minutes after smoking a single cigarette [237]. In another study, 31 male smokers with atypical chest pain were screened with a treadmill exercise test, which revealed that the heart rate increased within 10 minutes and returned to baseline after 30
minutes. Smoking was also associated with increased ectopic beats in these patients on the 24-hour Holter monitor [238]. On a signal averaged ECG, there was a minor lengthening of the filtered QRS duration among 15 long-term smokers after smoking two cigarettes [239].

To date, the most comprehensive assessment of acute smoking’s impact on myocardial conduction was performed among 28 habitual smokers by Soad Bekheit and Evan Fletcher in 1976 using His bundle electrograms [240]. The following measurements were made from His bundle recordings in the control and “nicotine tracings” (immediately after the first puff, sequential atrial pacing at identical rates to the control tracings): intraventricular conduction time (P1A), A-V nodal conduction time (AH), His-Purkinje-system conduction time (HV), and total intraventricular conduction time (HS). They found that a few puffs of a cigarette increase the velocity of conduction and shortens the effective refractory period of the A-V node, whereas the conduction velocity in the His-Purkinje system is not affected [240]. However, these effects were not studied against those from nicotine exposure.

Acute cigarette smoking increases efferent sympathetic nerve activity, primarily via the release of the catecholamines norepinephrine and epinephrine (Table 4). This catecholamine release increases myocardial work and oxygen consumption through an increase in blood pressure, heart rate, and myocardial contractility [19]. Overall, the vast majority of published evidence suggests that acute and chronic active and passive exposure to cigarette smoke generates marked disruptions in normal autonomic function characterized by increased SNS drive, reduced PNS modulation, and overall HRV. This phenomenon is partly attributable to a nicotine-induced up-regulation of catecholamine
release, generating potent acute and chronic effects on cardiovascular regulation through SNS activation [241].

**Hypothesis**

**Research Hypothesis:**

Nicotine in cigarette smoke alters myocardial conduction through β-adrenergic receptors

**Alternate Hypothesis:**

Non-nicotine constituents in cigarette smoke alter myocardial conduction though β-adrenergic receptors and non-β-adrenergic pathways

**Central hypothesis:**

**Aim 1** Explore the relationship between cigarette smoking and properties of myocardial conduction.

**Aim 2** Test the influence of catecholamines in the association between smoking and myocardial conduction.

**Aim 3** Acute effects of cigarette smoking and nicotine with and without β-blocker on electrocardiogram.
Ethical considerations

The primary therapeutic use of nicotine is in treating nicotine dependence. Controlled levels of nicotine are given to patients through gum, dermal patches, lozenges, inhalers, or nasal and oral sprays to wean them off their dependence, increasing quitting success by 50%–70% [242]. In contrast to recreational nicotine products, which have been designed to maximize the likelihood of addiction, nicotine replacement products are designed to minimize addictiveness. Hence, physiological studies on the effects of chronic use of pure nicotine on humans are accompanied by the concern for potential addiction among non-smokers, or the tolerance or modulation of neural plasticity among habitual smokers. It is for this reason that there are no robust human physiological studies investigating the cause–effect relationship of prolonged exposure to pure nicotine. There are some studies on prolonged nicotine replacement therapies in smokers who have quit smoking, and in these studies, no adverse effects have been found when nicotine medication was administered for months to several years. Given the highly addictive nature of nicotine, these studies are more appropriately termed as acute or chronic exposure of nicotine in chronic tobacco (nicotine) users. Therefore, to investigate the chronic effects of tobacco use on the electrocardiogram, we analyzed plasma/urine cotinine, a highly sensitive and specific marker of chronic nicotine exposure, as well as urinary catecholamines, in order to investigate the relationship between the sympathetic nervous system, smoking, and the electrocardiogram. Furthermore, to study the acute effects of smoking and nicotine with and without a β-blocker, we designed an open-label 2 x 2 factorial experimental trial.
CHAPTER II

CHRONIC EFFECTS OF CIGARETTE SMOKING ON ELECTROCARDIOGRAM

**Cigarette smoking, ECG and interaction with Atrioventricular nodal blockers**

**Aim 1** Explore the relationship between cigarette smoking and properties of myocardial conduction

Participants from NHANES database

The availability of serum cotinine levels and digital ECG in the Third National Health and Nutrition Examination Survey (NHANES-III) provides a unique opportunity to examine the association between tobacco exposure and ECG parameters, in a large nationally representative human population. The NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States. The survey is unique in that it combines interviews and physical examinations. The survey examines a nationally representative sample of about 5,000 persons each year. These persons are located in counties across the country, 15 of which are visited each year. The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. The examination component consists of medical, dental, and physiological measurements, as well as laboratory tests administered by highly trained medical personnel. To produce reliable statistics, NHANES over-samples persons 60 and older, African Americans, and Hispanics. All participants visit the physician and all (but the very young) have a blood sample taken. Health interviews are conducted in respondents’ homes. Health measurements are performed in specially-designed and equipped mobile centers, which travel to locations throughout the country [243].
Complete details about NHANES survey components, survey methodology, and sampling procedures are available from the Centers for Disease Control NHANES website [244]. Briefly, NHANES data are not obtained using a simple random sample. Rather, a complex, multistage, probability sampling design is used to select participants representative of the civilian, non-institutionalized US population. The sample does not include persons residing in nursing homes, members of the armed forces, institutionalized persons, or U.S. nationals living abroad. A sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, nonresponse adjustment, and adjustment to independent population controls. NHANES is designed to sample larger numbers of certain subgroups of particular public health interest. Oversampling is done to increase the reliability and precision of estimates of health status indicators for these population subgroups.

**Measures**

During the NHANES, individuals are asked questions related to smoking status, duration, and smoking-related behaviors. Smoking status was assessed in the home, by trained interviewers using the Computer-Assisted Personal Interviewing System (CAPI). Participants responded to whether they currently smoke cigarettes daily, some days, or not at all. Participants were categorized as never-smokers (individuals who have smoked <100cig/lifetime), former smokers (having smoked >100cig/lifetime but do not currently smoke), and current smokers. Current smokers are further classified as daily smokers
(smoking cigarettes every day) and nondaily smokers (identifies as a smoker, but does not
smoke cigarettes every day).

Cotinine

Measures such as cigarettes per day are imprecise indicators of tobacco smoke exposure because of variability in how smokers smoke their cigarettes. There is considerable individual variability in smoke intake, even by people smoking the same brand of cigarettes, and the cigarette design and how the cigarette is smoked influence toxic exposures. Therefore, the optimal assessment of exposure to tobacco smoke is the analysis of biomarkers for quantifying the systemic exposure of smokers to toxic constituents of smoke derived from tobacco use [245]. Nicotine measurement is highly specific for tobacco use or exposure (in the absence of nicotine medication use), but because of nicotine’s short half-life (2 h) the method is not recommended for general use. Nicotine is extensively metabolized to a number of metabolites by the liver, of which quantitatively, the most important metabolite of nicotine is the lactam derivative, cotinine. In humans, about 70–80% of nicotine is converted to cotinine. Cotinine is a highly specific and sensitive marker for tobacco use (in the absence nicotine medication use) and has the advantages of a fairly long half-life (16 h) [245]. Measuring cotinine in people’s blood is the most reliable way to determine exposure to nicotine for a marker for both active smoking, and as an index to Environmental Tobacco Smoke (ETS) exposure, or "passive smoking". There is a high correlation among cotinine concentrations measured in plasma, saliva, and urine, and measurements in any one of these fluids can be used as a marker of nicotine intake. Cotinine concentrations tend to be higher (3–8x) in urine than in serum;
however, for studies requiring a quantitative assessment of exposure, plasma or serum is regarded as the fluid of choice. Therefore, serum is used for cotinine measurement in NHANES [243].

Serum cotinine is measured by an isotope dilution-high performance liquid chromatography / atmospheric pressure chemical ionization tandem mass spectrometry (ID HPLC-APCI MS/MS). Briefly, the serum sample is spiked with methyl-D3 cotinine as an internal standard, and after an equilibration period, the sample is applied to a basified solid-phase extraction column. Cotinine is extracted off the column with methylene chloride, the organic extract is concentrated, and the residue is injected onto a short, C18 HPLC column. The eluant from these injections is monitored by APCI-MS/MS, and the m/z 80 daughter ion from the m/z 177 quasi-molecular ion is quantitated, along with additional ions for the internal standard, external standard, and for confirmation. Cotinine concentrations are derived from the ratio of native to labeled cotinine in the sample, by comparisons to a standard curve [243].

**Electrocardiogram:** Participants aged \( \geq 40 \) years who attended the medical examination received a resting 12-lead ECG with a Marquette MAC 12-unit (Marquette Electronics, Inc., USA), and analyzed using the NOVACODE ECG program, which classified the ECGs as per the Minnesota Coding (MC) System. Details of the ECG examination have been published previously [244]. Computerized automated analysis of the electrocardiographic data was performed with visual inspection of outlier values by a trained technician in a central ECG core laboratory (EPICARE Center at the Wake Forest School of Medicine, Winston Salem, NC). PR interval and P duration in lead II and global QRS duration and QT interval were automatically measured. PR segment was calculated
as the difference between PR interval and P duration. QT interval was Heart rate corrected using the Framingham formula (QTc), calculated as $QT + 154 \times (1 - \frac{60}{HR})$ [246]. To measure the association between cotinine and the ECG intervals (PR interval, P duration, PR segment, QRS, QTc and JT) we divided them into three groups; >95th percentile (long), 5 – 95th percentile (reference) and <5th percentile (short). For the purpose of this analysis, we only included NHANES III participants who had good quality ECG recording and with no major ECG abnormalities including electrocardiographic evidence of myocardial infarction or ischemia as defined by Minnesota Electrocardiogram Classification, and available serum cotinine data, medical history, medication use, and anthropometric measurements.

**Covariates definitions:** Since several ECG characteristics significantly differ by demographics, anthropometric features and cardiovascular risk factors and cardiac medications [247-252], we identified clinically important covariates to adjust for their effect on ECG parameters. Diabetes was defined as a fasting plasma glucose level of ≥126 mg/dl, glycosylated hemoglobin A1C values ≥6.5, or a history of glucose-lowering medications. Hypertension was defined as systolic blood pressure of ≥130 mm Hg, diastolic blood pressure of ≥80 mm Hg, or use of blood pressure-lowering medications. Body mass index was computed as the weight in kilogram divided by the square of the height in meter, and obesity was defined as a body mass index of >30 kg/m2. Age, gender, race/ethnicity, and smoking status were self-reported. Chronic Obstructive Pulmonary Disease (COPD) was defined as patients with a combination of asthma and emphysema. Alcohol consumption was assessed by the food frequency questionnaire. Participants reported the number of times that they drank beer, wine, and hard liquor in the past month,
and we categorized total alcohol consumption into 4 groups (0, 1–4, 5–13, ≥14 drinks/month). Serum cotinine levels >15 ng/ml were used to categorize the participants as smokers, while those ≤15 ng/ml were categorized as non-smokers.

**Statistical analyses**

The ECG sampling weights were used in the analysis to account for the complex sampling design [253]. Categorical variables were reported as frequency and population percents, whereas continuous variables were recorded as geometric mean ± standard error for all demographic tables. Statistical significance in demographic tables was tested using survey weighted analysis; for continuous variables, t test or ANOVA, whereas Rao-Scott chi-square was used for categorical variables. Survey weighted Multinomial regression was used to calculate the odds ratios and 95% confidence intervals for the association between serum cotinine levels (> 15 ng/ml) and ECG intervals using the group 5 – 95th percentile as reference. A sensitivity analyses was carried out, and the overall results did not differ after excluding those participants who had serum cotinine > 15 ng/ml, and identified themselves as never-smokers (n=134) or ex-smokers (n=216). Multivariable adjusted models were constructed with incremental adjustments as follows: model 1 adjusted for age, sex and race-ethnicity; and model 2 adjusted for model 1 covariates and heart rate, obesity, diabetes, hypertension, dyslipidemia, previous CVD, congestive heart failure, COPD and alcohol intake; and model 3 adjusted for model 2 covariates and β-blockers, calcium channel blockers and anti-arrhythmic drugs. Model 4 was created based only on the baseline characteristics associated with the ECG intervals with p<0.10 (Supplementary 2.1.2-2.1.7). The heart rate was not included in the model for QTc. We
also performed fully adjusted survey weighted linear regression between continuous cotinine levels and ECG intervals as continuous variables. We conducted subgroup analyses stratified by age (cut-off point by median - 59 years) and gender. A 2-sided p value of ≤0.05 was considered significant for main effects and for interactions. Data were analyzed using the survey procedures in SAS, version 9.4 (SAS Institute, North Carolina).

Results

A total of 5,633 study participants (mean age 59±13 years, 53% women, 48% non-Hispanic white) were included in this analysis. A total of 1,580 (28%) participants were identified as smokers (serum cotinine levels > 15 ng/ml). Smokers were more likely to be younger in age, men, with lower prevalence of dyslipidemia and β-blocker use and higher prevalence of COPD, higher resting heart rate and higher alcohol intake (Table 2.1.1). The 5th and 95th percentiles of ECG variables are shown in Supplementary Table 2.1.1. and Supplementary Tables 2.1.2-2.1.7 represent the geometric mean and standard error for ECG variables, and represent the population percentages for categorical baseline characteristics.
**Table 2.1.1** Baseline Participants Characteristics (Total N=5,653)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Smoker</th>
<th>Smoker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No ≤ 15 ng/ml n=4073, 72%</td>
<td>Yes &gt; 15 ng/ml n=1580, 28%</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>56.4 ± 0.44</td>
<td>53.4 ± 0.45</td>
</tr>
<tr>
<td>Women</td>
<td>2371 (59.1%)</td>
<td>629 (43.7%)</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>2122 (82.2%)</td>
<td>738 (80.8%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>2381 (56.1%)</td>
<td>134 (5.8%)</td>
</tr>
<tr>
<td>Current</td>
<td>67 (1.6%)</td>
<td>1230 (78.4%)</td>
</tr>
<tr>
<td>Past</td>
<td>1625 (42.3%)</td>
<td>216 (15.8%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>479 (7.5%)</td>
<td>143 (7.0%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1427 (31.8%)</td>
<td>489 (28.8%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>1066 (29.3%)</td>
<td>308 (23.1%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>853 (18.0%)</td>
<td>233 (15.3%)</td>
</tr>
<tr>
<td>COPD</td>
<td>263 (6.8%)</td>
<td>160 (10.8%)</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>67.6 ± 0.3</td>
<td>68.9 ± .04</td>
</tr>
<tr>
<td>Prior cardiovascular disease</td>
<td>172 (3.2%)</td>
<td>67 (3.5%)</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>131 (1.8%)</td>
<td>54 (1.7%)</td>
</tr>
<tr>
<td>Alcohol drinks per month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2415 (50.7%)</td>
<td>762 (45.1%)</td>
</tr>
<tr>
<td>1-4</td>
<td>670 (18.0%)</td>
<td>253 (17.2%)</td>
</tr>
<tr>
<td>5-13</td>
<td>440 (13.9%)</td>
<td>200 (13.3%)</td>
</tr>
<tr>
<td>&gt;13</td>
<td>539 (17.4%)</td>
<td>359 (24.4%)</td>
</tr>
<tr>
<td>B blockers</td>
<td>320 (8.2%)</td>
<td>87 (5.9%)</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>333 (6.7%)</td>
<td>103 (6.0%)</td>
</tr>
<tr>
<td>Antiarrhythmic drugs</td>
<td>34 (0.6%)</td>
<td>17 (1.5%)</td>
</tr>
</tbody>
</table>

*Except for age (which is represented by geometric mean and standard deviation), all other variables are represented as frequency and column percentages.
**PR interval, P wave and PR segment**

In multinomial logistic regression models adjusted for demographics, smoking was associated with increased odds of short PR interval and short PR segment (p-value < 0.01) ([Table 1.2](#)). This association was not attenuated after further adjustment for CVD risk factors and AV nodal blockers, and was consistent in subgroups stratified by age and sex ([Supplementary Table 2.1.8](#)). No significant association was observed with smoking status and P wave duration. Follow-up linear regression analyses revealed a significant negative association between continuous cotinine levels and PR segment, but not with P wave and PR interval. According to model 4, the PR segment shortened by 0.554 ms per 100 ng/ml increase in cotinine levels ([Table 2.1.3](#)).

**QTC interval, JT interval and QRS duration**

In fully adjusted multinomial regression models, serum cotinine was associated with short QRS and long JT (p-value <0.01), but not with abnormal QTc ([Table 2.1.4](#)). The association between smoking status and short QRS duration (<5<sup>th</sup> percentile) and long JT interval (>95<sup>th</sup> percentile) was consistent in subgroups stratified by age and sex ([Supplementary Table 2.1.8](#)). However, linear regression showed a significant association overall between continuous cotinine levels and QTc, but not with QRS duration and JT interval ([Table 2.1.5](#)). According to model 4, QTc shortened by -1.345 ms with every 100 ng/ml increase in cotinine.

The effect of smoking status on baseline ECG (results from logistic regression) is shown in [Figure 2.1.1](#), and the relationship between cotinine and ECG (results from linear regression) is shown in [Figure 2.1.2](#).
**Table 2.1.2** Association between smoking status and abnormal PR interval and its components

ECG variables groups: Short (<5th percentile), reference (5-95th percentile) and Long (>95th percentile). N=5,653

<table>
<thead>
<tr>
<th></th>
<th>PR interval</th>
<th>P duration</th>
<th>PR segment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>p-value</td>
<td>Odds ratio</td>
</tr>
<tr>
<td></td>
<td>(95% CI)</td>
<td></td>
<td>(95% CI)</td>
</tr>
<tr>
<td>Short</td>
<td>1.63</td>
<td><strong>0.024</strong></td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>(1.07, 2.48)</td>
<td></td>
<td>(0.72, 1.57)</td>
</tr>
<tr>
<td>Model 1*</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>0.73</td>
<td></td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>(0.46, 1.16)</td>
<td></td>
<td>(0.55, 1.40)</td>
</tr>
<tr>
<td>Short</td>
<td>1.57</td>
<td><strong>0.042</strong></td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>(1.02, 2.43)</td>
<td></td>
<td>(0.75, 1.60)</td>
</tr>
<tr>
<td>Model 2†</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>0.76</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>(0.49, 1.19)</td>
<td></td>
<td>(0.57, 1.48)</td>
</tr>
<tr>
<td>Short</td>
<td>1.50</td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>(0.95, 2.36)</td>
<td></td>
<td>(0.67, 1.43)</td>
</tr>
<tr>
<td>Model 3^</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>0.73</td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>(0.47, 1.13)</td>
<td></td>
<td>(0.57, 1.47)</td>
</tr>
<tr>
<td>Short</td>
<td>1.58</td>
<td><strong>0.047</strong></td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>(1.01, 2.48)</td>
<td></td>
<td>(0.70, 1.49)</td>
</tr>
<tr>
<td>Model 4Δ</td>
<td>Reference</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>0.74</td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>(0.47, 1.15)</td>
<td></td>
<td>(0.56, 1.41)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and race-ethnicity
†Adjusted for variables in model 1 plus heart rate, obesity, diabetes, hypertension, dyslipidemia, previous cardiovascular disease, congestive heart failure, chronic obstructive pulmonary disease and alcohol intake
^Adjusted for variables in model 2 plus β-blockers, calcium channel blockers and anti-arrhythmic drugs
ΔAdjusted for variables found significantly associated with the ECG intervals:
- PR interval: age, sex, previous cardiovascular disease, β-blockers, calcium channel blockers, heart rate
- P duration: age, sex, race-ethnicity, congestive heart failure, alcohol intake, β-blockers, calcium channel blockers, heart rate
- PR segment: age, sex, alcohol intake, calcium channel blockers, heart rate
Table 2.1.3 Adjusted survey weighted linear regression between serum cotinine levels and PR interval, P duration and PR segment

N=5,653

<table>
<thead>
<tr>
<th>Model</th>
<th>PR interval (ms)</th>
<th>P duration (ms)</th>
<th>PR segment (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-(95% CI)</td>
<td>P-value</td>
<td>β-(95% CI)</td>
</tr>
<tr>
<td>Model 1*</td>
<td>-0.832 (-1.549, -0.116)</td>
<td>0.0237</td>
<td>-0.078 (-0.444, 0.288)</td>
</tr>
<tr>
<td>Model 2†</td>
<td>-0.593 (-1.320, 0.134)</td>
<td>0.1074</td>
<td>-0.036 (-0.415, 0.343)</td>
</tr>
<tr>
<td>Model 3^</td>
<td>-0.542 (-1.262, 0.179)</td>
<td>0.1374</td>
<td>0.036 (-0.362, 0.434)</td>
</tr>
<tr>
<td>Model 4Δ</td>
<td>-0.603 (-1.301, 0.094)</td>
<td>0.0885</td>
<td>-0.008 (-0.391, 0.374)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and race-ethnicity
†Adjusted for variables in model 1 plus heart rate, obesity, diabetes, hypertension dyslipidemia, previous cardiovascular disease, congestive heart failure, chronic obstructive pulmonary disease and alcohol intake
^Adjusted for variables in model 2 plus β-blockers, calcium channel blockers and anti-arrhythmic drugs
ΔAdjusted for variables found significantly associated with the ECG intervals:
- PR interval: age, sex, previous cardiovascular disease, β-blockers, calcium channel blockers, heart rate
- P duration: age, sex, race-ethnicity, congestive heart failure, alcohol intake, β-blockers, calcium channel blockers, heart rate
- PR segment: age, sex, alcohol intake, calcium channel blockers, heart rate
Table 2.1.4 Association between smoking status and abnormal corrected QT interval and its components

ECG variables groups: Short (<5th percentile), reference (5-95th percentile) and Long (>95th percentile). N=5,653

<table>
<thead>
<tr>
<th>ECG variables groups</th>
<th>Corrected QT interval (ms)</th>
<th>QRS duration (ms)</th>
<th>JT interval (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>p-value</td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>Short</td>
<td>1.23 (0.87, 1.74)</td>
<td>0.243</td>
<td>1.46 (1.07, 1.99)</td>
</tr>
<tr>
<td>Model 1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>1.17 (0.71, 1.92)</td>
<td>0.544</td>
<td>1.04 (0.69, 1.57)</td>
</tr>
<tr>
<td>Short</td>
<td>1.17 (0.84, 1.63)</td>
<td>0.364</td>
<td>1.43 (1.04, 1.98)</td>
</tr>
<tr>
<td>Model 2†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>1.15 (0.68, 1.95)</td>
<td>0.603</td>
<td>1.05 (0.71, 1.57)</td>
</tr>
<tr>
<td>Short</td>
<td>1.01 (0.71, 1.46)</td>
<td>0.946</td>
<td>1.60 (1.13, 2.26)</td>
</tr>
<tr>
<td>Model 3^</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>1.29 (0.79, 2.11)</td>
<td>0.301</td>
<td>1.11 (0.71, 1.73)</td>
</tr>
<tr>
<td>Short</td>
<td>1.05 (0.71, 1.54)</td>
<td>0.813</td>
<td>1.39 (1.02, 1.88)</td>
</tr>
<tr>
<td>Model 4Δ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>1.26 (0.79, 2.01)</td>
<td>0.328</td>
<td>1.09 (0.73, 1.63)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and race-ethnicity
†Adjusted for variables in model 1 plus heart rate (not for QTc), obesity, diabetes, hypertension, dyslipidemia, previous cardiovascular disease, congestive heart failure, chronic obstructive pulmonary disease and alcohol intake
^Adjusted for variables in model 2 plus β-blockers, calcium channel blockers and anti-arrhythmic drugs
ΔAdjusted for variables found significantly associated with the ECG intervals:
- Corrected QT interval: age, chronic obstructive pulmonary disease, β-blockers, calcium channel blockers
- QRS duration: sex, obese, chronic obstructive pulmonary disease, heart rate
- Uncorrected JT interval: age, sex, dyslipidemia, alcohol intake, heart rate, anti-arrhythmic drugs
Table 2.1.5 Adjusted survey weighted linear regression between serum cotinine levels and corrected QT interval, QRS duration and JT interval

N=5,653

<table>
<thead>
<tr>
<th></th>
<th>Corrected QT</th>
<th>QRS duration</th>
<th>JT interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-(95% CI)</td>
<td>p-value</td>
<td>β-(95% CI)</td>
</tr>
<tr>
<td>Model 1*</td>
<td>-1.448</td>
<td>0.0029</td>
<td>-0.251</td>
</tr>
<tr>
<td></td>
<td>(-2.375, -0.521)</td>
<td></td>
<td>(-0.489, -0.013)</td>
</tr>
<tr>
<td>Model 2†</td>
<td>-1.402</td>
<td>0.0045</td>
<td>-0.184</td>
</tr>
<tr>
<td></td>
<td>(-2.347, -0.456)</td>
<td></td>
<td>(-0.414, 0.045)</td>
</tr>
<tr>
<td>Model 3^</td>
<td>-1.112</td>
<td>0.0218</td>
<td>-0.194</td>
</tr>
<tr>
<td></td>
<td>(-2.056, -0.169)</td>
<td></td>
<td>(-0.433, 0.044)</td>
</tr>
<tr>
<td>Model 4Δ</td>
<td>-1.345</td>
<td>0.0052</td>
<td>-0.139</td>
</tr>
<tr>
<td></td>
<td>(-2.269, -0.421)</td>
<td></td>
<td>(-0.363, 0.086)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex and race-ethnicity
†Adjusted for variables in model 1 plus heart rate (not for QTc), obesity, diabetes, hypertension, dyslipidemia, previous cardiovascular disease, congestive heart failure, chronic obstructive pulmonary disease and alcohol intake
^Adjusted for variables in model 2 plus β-blockers, calcium channel blockers and anti-arrhythmic drugs
ΔAdjusted for variables found significantly associated with the ECG intervals:
• Corrected QT interval: age, chronic obstructive pulmonary disease, β-blockers, calcium channel blockers
• QRS duration: sex, obese, chronic obstructive pulmonary disease, heart rate
• Uncorrected JT interval: age, sex, dyslipidemia, alcohol intake, heart rate, anti-arrhythmic drugs
Figure 2.1.1 The effect of smoking status on baseline ECG
Figure 2.1.2 Panel A shows the distribution of PR segment (ms) and QTc interval (ms) with cotinine levels (N=5,653). Panel B depicts the visual representation of corresponding progressive shortening of PR segment and QTc with increasing cotinine levels.
Discussion

In a large sample representative of the general US population, we found that higher levels of serum cotinine, a measure of tobacco exposure, was associated with extremely shortened PR interval, PR segment, QRS duration and extremely prolonged JT interval. We also found that, although cotinine-confirmed smoking did not associate with extreme changes in QTc, smoking severity as measured by serum cotinine had a significant negative linear relationship with QTc in the overall cohort.

**PR interval, P wave and PR segment**

There is a wide variation in the results of prior studies on the effects of tobacco exposure and PR interval and its components: PR segment and P duration [227, 254, 255]. However, studies with greater sample size consistently show smoking associated with shorter PR interval [212, 221, 223, 256-258]. Few studies showed increased P wave duration among smokers [222, 259] while other studies did not show any significant difference, [225, 227] or found a trend towards decreasing P wave duration with smoking [212]. We did not find any significant association between P wave duration and cotinine levels, suggesting less overt effects of tobacco exposure on atrial activity and atrial conduction. In a smaller cohort we recently revealed an inverse association between cotinine and PR interval, with evidence that this relationship was mediated via increased circulating dopamine[260]. In the present study, our observations spanned a large nationally representative cohort and further corroborate these findings that smoking accelerates atrioventricular conduction.
These findings are particularly important in light of emerging data on the prognostic value of short PR interval to predict atrial fibrillation [27] and cardiovascular mortality [26, 29, 261]. Therefore, exposure to cigarette smoke (and perhaps nicotine specifically) accelerates atrioventricular conduction (as exhibited by short PR interval) and may possibly be a mechanism for increased risk of stroke and atrial fibrillation.

\textit{QTc interval, JT interval and QRS duration}

Similar to the results of studies on PR interval and its components, there are mixed results on the effects of smoking on QTc and QRS duration [212, 228, 229, 262-265]. We were not able to find any study that explored the relationship between JT interval and smoking exposure. Only one study has investigated the effect of smoking, measured specifically by cotinine, on JT interval. Like us, Zhang et al [219] used the NHANES III database, and among 7795 men and women found that in fully adjusted models QTc was not associated with smoking and cotinine levels. At first this appears to conflict with the results of our study, but ancillary analyses (not shown) revealed that this discrepancy stems from their use of quartiles instead of continuous QTc, and their restricting analyses to current smokers only. In addition, we also studied the association of cotinine with the components of QTc interval, and found that higher cotinine levels are associated with abnormally short QRS duration and long JT interval, but without any significant linear relationship with either subcomponents.

The QT interval encompasses the time from the beginning of ventricular depolarization (QRS duration) and ventricular repolarization (JT interval) [170]. Prolonged QRS duration [182] and JT interval [31, 32] are well known predictors of mortality in the
general population, but the clinical implications for short QRS duration are not known. Sympathetic neural stimulation and systemic catecholamine release may underlie smoking-induced acceleration of AV nodal conduction, while also explaining the shortening of QRS (ventricular depolarization)[63, 266, 267] and prolongation of JT (ventricular repolarization) [194, 268, 269]. Nicotine has been found to directly inhibit IKr and Ito repolarizing currents [270]. Notably, nicotine also directly stimulates release of catecholamines from nerve terminals and the adrenal medulla [71, 271], effects that acutely augment IKs current and directly cause phosphorylation of its corresponding potassium channel to acutely accelerate repolarization via β-adrenergic receptors [272]. In addition to increased sympathetic tone and direct effects on ion channels, nicotine may also cause a pro-fibrotic state [273] and endothelial cell injury [274, 275]. Thus, nicotine is a particularly plausible culprit of these effects. Nevertheless, particulate matter and aldehydes within tobacco smoke may be key constituents and irritant reflexes or ischemia may be critical pathways through which smoking disrupts electrophysiologic homeostasis [276]

Limitations

It has not escaped our attention that a single measurement of serum cotinine may not fully reflect chronic smoking intensity. Moreover, because cotinine only reflects nicotine exposure, and by an imperfect proxy, smoking intensity, we are unable to assess relationships between other cigarette smoke constituents and cardiac electrophysiology. Although we have adjusted for several potential confounders, we recognize the possibility of residual confounding that is similar to other studies with a cross-sectional design. Also,
though the automated measurement of ECG intervals and segments, are routinely reported and can easily be calculated by the ECG systems, we did not perform manual measurements. Despite these limitations, this is the first study examining the association between serum cotinine, an objective measure of tobacco exposure, with PR and QT interval and their components.

Conclusions

We found in a large racially diverse sample of the US population, that elevated serum cotinine levels are independently associated with abnormally short PR interval, short PR segment, short QRS duration, and long JT interval. Additionally, increases in cotinine were associated with progressive shortening of QTc across all individuals. Collectively, our findings indicate that exposure to cigarette smoke increases risk for abnormally fast atrioventricular conduction and ventricular depolarization and abnormally long ventricular repolarization. However, smoking induces a progressive prolongation of repolarization when also evaluating individuals within normal QTc ranges. These observations enhance our understanding of the relationship between smoking and cardiac arrhythmias and implicate specific pathophysiological mechanisms by which smoking increases cardiovascular morbidity and mortality, including for stroke and sudden cardiac death. More research is warranted to examine the specificity and selectivity of these effects and to delineate the direct contribution of specific tobacco constituents such as nicotine.
Effects of cigarette smoking on electrocardiogram mediated via catecholamines

Aim 2 Test the influence of catecholamines in the association between smoking and myocardial conduction.

This study was designed to study the effect of nicotine and cigarette smoke exposure on PR interval and its components in a cohort of patients with intermediate-to-high CVD risk and to discern the role of sympatho-adrenal activity in these effects on atrioventricular conduction through analyzing urinary metabolites of nicotine and catecholamines (dopamine, norepinephrine, and epinephrine).

Participants from Louisville Healthy Heart Study

The study was approved by the Institutional Review Board at the University of Louisville. Individuals (>18 years of age) with intermediate to high CVD risk were recruited from the University of Louisville Hospital and affiliated clinic system between October 2009 and March 2011 as described previously [277]. All accessible patients visiting the clinics during this time period were pre-screened through a review of medical records prior to recruitment in order to exclude individuals that did not meet the enrollment criteria. In addition, persons unwilling or unable to provide informed consent or with significant and/or severe comorbidities were excluded. Exclusion criteria included: significant chronic lung, liver, kidney, hematological, or neoplastic disease, chronic neurological or psychiatric illness, chronic infectious disease such as HIV or hepatitis, severe coagulopathies, drug/substance abuse, and chronic cachexia. Pregnant women, prisoners, and other vulnerable populations were also excluded from the study. Patients who met the enrollment criteria and gave written consent were consented and administered
a questionnaire to provide demographic information and baseline characteristics. Medical records were reviewed for past medical history, vital signs and medication history. To reduce selection bias, all consecutive participants who were eligible for this study were recruited. For our analyses, only those patients with complete urinary biomarkers and ECG with normal sinus rhythm were included (Supplementary Figure 2.2.1).

**Electrocardiogram (ECG) measurement protocol**

Standard 12-lead ECGs with 2.5 seconds of each lead and 10 seconds of rhythm strip (lead II) from medical records were used for ECG analyses. The following ECG intervals were measured: P wave duration (from beginning to end of P) and PR interval from lead II (from beginning of P to beginning of Q) [26], QRS duration from lead V6 (from beginning of Q to end of S) [278], and QT interval from V5 (from beginning of Q to end of T) [279]. The PR segment was calculated as the difference between PR interval and P wave duration. QT was also corrected using the Framingham formula [246] (Appendix A). All intervals were measured with electronic calipers and adjusted to scale by reported automated measures of RR (or heart rate). Two trained analysts (CA and AI) independently and manually measured each ECG interval (except RR) from the first 3 sinus beats. HRV parameters were derived from digital caliper measurements of all RRs in the rhythm strip. When average of any ECG interval for a given patient differed between the two analysts by >10%, both investigators re-measured that ECG interval independently. If the parameter remained >10% different between analysts, the analysts reviewed the ECG together and reached consensus on the appropriate measure.

**Urinary measurements**
A spot urine sample was collected on the day of study enrollment. Urinary cotinine is a well-established metabolite for cigarette smoke exposure [245], and was measured by Ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS) using D3-cotinine as an internal standard [280]. For UPLC-MS/MS analysis of dopamine, norepinephrine, epinephrine, and their metabolites (metanephrine, normetanephrine, vanillylmandelic acid, 3-methoxytyramine, homovanillic acid), urine samples were thawed on ice, vortexed and diluted 1:50 with 0.2% formic acid containing isotopic labeled internal standards. 1 µL of the mixture was analyzed on an UPLC-MS/MS instrument (ACQUITY UPLC H-Class system and Xevo TQ-S micro triple quadrupole mass spectrometer, all from Waters Inc., MA). Separation was performed on an Acquity UPLC HSS PFP (150 mm × 2.1 mm, 1.8 μm) column (Waters Inc., MA) with a binary gradient comprised of 0.2% formic acid (Solvent A) and methanol (Solvent B). Three multiple reaction monitoring (MRM) transitions were set up for each sample: one for quantification, one for confirmation, and one for labeled internal standard. At least 12 data points were collected for each peak. Analytes were quantified using peak area ratio based on 8 point-standard curves run before and after the urine samples. The concentration values of analytes were normalized to creatinine level which was measured on a COBAS MIRA-plus analyzer (Roche, NJ) with Infinity Creatinine Reagent (Thermo Fisher Scientific, MA).
Statistical Analysis

Baseline subject characteristics were summarized by smoking status. Categorical characteristics, frequencies and percentages are reported along with Chi-square test p-values, which were used to compare distributions across study groups. In addition to visual inspection of histograms, Shapiro-Wilk tests were conducted for continuous characteristics to determine if the characteristics were approximately normally distributed. Mean and standard deviation are reported for continuous characteristics with a normal distribution, whereas median and interquartile range are reported for continuous characteristics with a skewed distribution, and the study groups were compared by the appropriate statistical test based on normality. P-values were derived from Student’s t-tests for study group comparisons of normally-distributed variables, whereas Mann-Whitney tests were used for variables lacking a normal distribution. The urinary metabolites (cotinine, dopamine, norepinephrine and epinephrine and their daughter metabolites) were log-transformed because their distribution was skewed. We tested the associations between ECG parameters and urinary metabolites by linear regression. ECG parameters were dichotomized by their median levels into high- and low-value groups. Baseline characteristics associated with ECG variables in bivariate analyses (with p<0.01) were used to build fully adjusted models using linear regression analyses. Smoking status was determined as reported by the participant (active smoker, former smoker or non-smoker) and by urinary cotinine levels of 50 ng/ml [245]. Finally, mediation was assessed by the bootstrapping technique and macro put forth by Preacher and Hayes [281]. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 24, SPSS, Inc, Chicago, IL, USA).
Results

Baseline characteristics

A total of 136 participants were in normal sinus rhythm and had ECGs and urinary metabolites available. The participants were approximately evenly split by gender (male n=72, 53%), about half of all participants were Caucasians (n=77, 57%), and mean age was 52 years. Participants had a high prevalence of CVD risk factors; with a majority diagnosed with hypertension (n=119, 87%) and/or on β-blockers (n=98, 73%) (Table 2.2.1). Several of the participants were diagnosed with diabetes (n=43, 32%) or prior myocardial infarction (n=62, 46%), and/or were taking calcium channel blockers (n=32, 24%) or β-blockers (n=98, 73%). The relationship of baseline characteristics with dichotomized PR interval, P duration, and PR segment are shown in Supplementary Table 2.2.1. Mean age and proportion of females were significantly higher among individuals in the upper stratum for PR interval, whereas mean BMI, or proportion of participants who were female, hypertensive, or taking calcium channel, ACE, or angiotensin receptor inhibitors were higher among those with longer P duration. Conversely, only the number of participants on diuretics were higher among those with longer PR segment.
## Table 2.2.1 Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Current smoker</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=136</td>
<td>Yes (N=53, 39%)</td>
<td>No (N=83, 61%)</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>52, 10</td>
<td>53, 10</td>
<td>50, 9</td>
</tr>
<tr>
<td>BMI (kg/m2)*</td>
<td>33, 8</td>
<td>34, 8</td>
<td>31, 7</td>
</tr>
<tr>
<td>SBP (mm Hg)*</td>
<td>133, 23</td>
<td>132, 22</td>
<td>136, 24</td>
</tr>
<tr>
<td>DBP (mm Hg)*</td>
<td>81, 13</td>
<td>80, 11</td>
<td>81, 16</td>
</tr>
<tr>
<td>Heart rate (beats/min)*</td>
<td>73, 15</td>
<td>74, 15</td>
<td>73, 15</td>
</tr>
<tr>
<td>Male gender</td>
<td>72, 53%</td>
<td>43, 52%</td>
<td>30, 57%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>77, 57%</td>
<td>46, 55%</td>
<td>31, 59%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>118, 87%</td>
<td>74, 89%</td>
<td>45, 85%</td>
</tr>
<tr>
<td>Prior MI</td>
<td>62, 46%</td>
<td>40, 48%</td>
<td>23, 43%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>43, 32%</td>
<td>28, 34%</td>
<td>15, 28%</td>
</tr>
<tr>
<td>Stroke</td>
<td>23, 17%</td>
<td>14, 17%</td>
<td>10, 19%</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>45, 33%</td>
<td>28, 34%</td>
<td>17, 32%</td>
</tr>
<tr>
<td>B blocker</td>
<td>98, 73%</td>
<td>65, 78%</td>
<td>34, 64%</td>
</tr>
<tr>
<td>CCB</td>
<td>32, 24%</td>
<td>24, 29%</td>
<td>8, 15%</td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>89, 66%</td>
<td>54, 65%</td>
<td>35, 66%</td>
</tr>
<tr>
<td>Statin</td>
<td>84, 62%</td>
<td>55, 66%</td>
<td>30, 57%</td>
</tr>
<tr>
<td>Aspirin</td>
<td>81, 60%</td>
<td>49, 59%</td>
<td>33, 62%</td>
</tr>
<tr>
<td>Diuretics</td>
<td>58, 43%</td>
<td>38, 46%</td>
<td>20, 38%</td>
</tr>
</tbody>
</table>

Abbreviations: BMI - Body mass index, SBP - Systolic Blood Pressure, DBP – Diastolic Blood Pressure, MI – Myocardial Infarction, CCB – Calcium Channel Blocker, ACEI - Angiotensin-Converting Enzyme Inhibitor, ARB - Angiotensin II Receptor Blocker

For each variable counts and column percentage has been reported except for where indicated * where the mean and standard deviation (SD) are reported.
Smoking status and catecholamine levels

Overall smoking prevalence was 61% (n=83) by self-report and 46% when defined by urinary cotinine >50 ng/ml (Cohen’s κ=0.79, p<0.001 for agreement between these measures). Three participants self-reported as never smokers had urinary cotinine levels >50 ng/ml and two who self-reported as active smokers had urinary cotinine levels of ≤50 ng/ml (Supplementary Table 2.2.2). None of the cohort characteristics were significantly different between active smokers and the rest of patients; although trends of higher age, BMI, and use of calcium channel and/or β-blockers was observed among smokers. All urinary metabolites (cotinine, dopamine, epinephrine and norepinephrine) were higher among current smokers vs never-smokers. None of the catecholamines were different between current smokers and ex-smokers (p>0.05); however, catecholamines were higher in the high-cotinine group relative to the low-cotinine group (Supplementary Table 2.2.3 A).

Supplementary Table 2.2.4 shows that cotinine was significantly associated with dopamine and norepinephrine, but not with epinephrine. We also explored the possible effect of cotinine on catecholamine metabolism by the association of cotinine and the daughter metabolites and their ratios. Each catecholamine’s intermediate metabolite, and none of the final metabolites, had a significant positive association with cotinine. Among the ratio of intermediate/parent metabolites, cotinine associated positively with 3-methoxytyramine/Dopamine (an index of catechol-o-methyltransferase activity) and inversely with vanillyl mandelic acid/normetanephrine and vanillyl mandelic acid/metanephrine (both indices of MOA activity).
Association of cotinine with P- and PR-parameters

Smokers did not differ from non-smokers in proportion of participants within strata of long or short P duration, PR interval, or PR segment. Distribution of participants into high- or low-PR or P-duration parameters also did not differ by cotinine dichotomization (Supplementary Table 2.3 B). When analyzed as a continuous variable, cotinine was significantly higher among participants with short PR segment compared to those with long PR segment (p=0.03). PR interval also showed a similar trend (p=0.06), whereas cotinine did not differ between participants with long vs. short P duration (p=0.25) (Table 2.2.2). Among all participants, cotinine had a significant negative association with PR interval and PR segment (but not with P duration) in linear regressions, even after adjusting for age, gender and heart rate (Table 2.3). Figure 2.1 shows the scatterplot distributions of PR interval, P duration, and PR segment across log-transformed urinary cotinine levels normalized by creatinine. Cotinine was not significantly associated with other ECG parameters, including QRS duration, QT interval and corrected QT, in unadjusted and adjusted models (Supplementary Table 2.5).

Catecholamines and PR interval

All three catecholamines were significantly elevated among participants with short P duration relative to those with long P duration (Table 2.2). Dopamine was also elevated among those with shorter PR-interval relative to those with longer PR-interval (p=0.01). Dopamine showed a trend of being higher among patients with short vs. those with long PR-segment. The adjusted linear regression showed all three catecholamines inversely associated with PR interval (p<0.05), none of the catecholamines associated with P
duration, and only dopamine inversely associated with PR segment (Supplementary Table 2.2.6).
Table 2.2.2 Comparison of creatinine-normalized urinary biomarkers (median [interquartile range]) among participants dichotomized into high and low atrial and atrioventricular conduction parameters

N=136

<table>
<thead>
<tr>
<th>biomarker</th>
<th>All patients</th>
<th>PR interval</th>
<th></th>
<th>P wave duration</th>
<th></th>
<th>PR segment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 163.32 ms</td>
<td>&gt; 163.33 ms</td>
<td>p value</td>
<td>≤ 104.73 ms</td>
<td>&gt; 104.74 ms</td>
<td>p value</td>
</tr>
<tr>
<td>Cotinine (ng/g)</td>
<td>23.1 [1.6 - 794.1]</td>
<td>30.6 [1.5 - 948.4]</td>
<td>23.0 [1.8 - 647.7]</td>
<td>0.45</td>
<td>238.3 [2.3 - 102.5]</td>
<td>5.8 [1.4 - 627.1]</td>
</tr>
<tr>
<td>Dopamine (μg/g)</td>
<td>165.4 [127.6 - 214.1]</td>
<td>179.8 [142.3 - 228.5]</td>
<td>150.6 [119.7 - 189.5]</td>
<td>0.01</td>
<td>180.1 [142.0 - 234.7]</td>
<td>111.6 [180.3 - 168.8]</td>
</tr>
<tr>
<td>Epinephrine (μg/g)</td>
<td>4.7 [2.4 - 7.9]</td>
<td>5.6 [2.9 - 8.6]</td>
<td>8.6 [4.1 - 7.5]</td>
<td>0.23</td>
<td>5.9 [3.5 - 9.8]</td>
<td>3.9 [1.7 - 6.4]</td>
</tr>
<tr>
<td>Norepinephrine (μg/g)</td>
<td>37.2 [26.0 - 53.3]</td>
<td>36.7 [26.9 - 52.6]</td>
<td>38.0 [22.9 - 56.4]</td>
<td>0.86</td>
<td>41.2 [30.7 - 54.5]</td>
<td>31.9 [22.1 - 52.3]</td>
</tr>
</tbody>
</table>
Table 2.2.3 Estimated effects (β-coefficients) of an increase in cotinine on PR interval, P wave, and P segment, with corresponding P-values, from unadjusted and adjusted linear regressions.

Urinary cotinine was log-transformed. N=136

<table>
<thead>
<tr>
<th></th>
<th>PR interval</th>
<th>P wave</th>
<th>PR segment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p value</td>
<td>β</td>
</tr>
<tr>
<td>Unadjusted Cotinine</td>
<td>-2.38</td>
<td><strong>0.04</strong></td>
<td>0.33</td>
</tr>
<tr>
<td>*Adjusted Cotinine</td>
<td>-2.67</td>
<td>0.05</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* model adjusted for age, body mass index, and gender (PR interval); BMI, SBP, DBP, gender, hypertension, CCB, and ACEI, and ARB (P duration); or BMI, β-blocker, and diuretics (PR segment).
Figure 2.2.1 Scatterplot and linear relationships of (A) PR interval, (B) P wave and (C) PR segment with log transformed urinary cotinine levels.

P values represent unadjusted linear regression. N=136
Mediation analyses

In linear regression analyses cotinine was inversely associated with PR segment and PR interval, and dopamine was the only catecholamine significantly associated with both PR segment and PR interval (inversely in both cases). We therefore conducted mediation analyses to determine whether dopamine mediated the association between cotinine and PR segment and also between cotinine and PR interval. Figure 2 shows the relationship between cotinine and dopamine (path a); the relationship between dopamine and PR interval/segment (path b); and the total effect of cotinine on PR interval/segment (path c). The total effect (c) is the sum of direct (c’) and indirect (ab) effects. The direct effect is the relationship between cotinine and PR interval/segment while controlling for mediators. The indirect effect represents the mediated effect. Mediation analysis showed that dopamine completely mediated the association of cotinine with PR interval (c’, p=0.46) and PR segment (c’, p=0.09). Specifically, the indirect effect (path a × b) of cotinine on PR interval through dopamine had a point estimate of -1.23 and an upper and lower 95% CI of -2.90 and -0.26; and the indirect effect (path a × b) of cotinine on PR segment through dopamine had a point estimate of -0.52 and an upper and lower 95% CI of -1.57 and -0.08 (Figure 2.2.2).
Figure 2.2.2 Mediation analyses of cotinine, dopamine, and PR interval and PR segment

N=136
Discussion

The effects of smoking on atrial and atrioventricular nodal conduction velocity and their underlying mechanisms are currently unknown. To address this gap in knowledge, we measured PR interval, P wave duration, and PR segment from lead II of a 12-lead EKG, urinary catecholamines, and nicotine and its metabolites in a cohort of 136 participants with intermediate-high cardiovascular risk. We report three major findings from this study. First, cotinine had a significant inverse association with PR interval and PR segment, and not with P wave duration in adjusted linear regression models. Second, urinary dopamine was inversely associated with PR interval and both its components (PR segment and P duration), while epinephrine and norepinephrine did not associate with PR segment. Third, mediation analyses indicate that dopamine may primarily account for the association between cotinine and shortening of both PR interval and segment. Together, these findings suggest smoking may increase cardiovascular morbidity and mortality through nicotine-associated increases in catecholamine release and downstream modulation of atrioventricular conduction.

Nicotine and PR interval and its components

Previous work on the chronic effects of smoking on PR interval has produced mixed results. Some studies found short PR interval at baseline among chronic smokers vs non-smokers [212, 221, 257], while others found no difference [254, 282]. Similarly, a few studies showed increased P wave duration among smokers [222, 259], while other studies did not show any significant difference [225, 227] or found a trend towards decreasing P wave duration with smoking [212]. There are several reasons for the wide variation in the
results of prior studies; 1) small sample size, 2) insufficient consideration for the role of the components of PR interval, and 3) inadequate assessment of nicotine exposure and its potential impact. To the best of our knowledge, to date, no previous study investigated the effects of nicotine exposure on PR interval and its components in humans. This is particularly important in light of recent findings that short PR interval associates with increased risk for atrial fibrillation and cardiovascular mortality [26, 29, 261, 283]. Beyond overall cardiovascular mortality, smoking also associates with increased risk of atrial fibrillation through unknown mechanisms [284]. Thus, by demonstrating that exposure to cigarette smoke (and perhaps nicotine specifically) accelerates atrioventricular conduction, our findings provide further insight into how smoking may confer cardiovascular risk, including risk for atrial fibrillation.

In the present study, cotinine inversely associated with PR interval and PR segment, indicating that increased cigarette smoke exposure accelerates atrioventricular conduction. PR interval is mainly influenced by the sum of atrial activity and atrioventricular nodal conduction [170]. Atrial pathology usually results in prolonging (rather than shortening) of PR interval, whereas shortening of PR interval is likely a result of accelerated atrioventricular nodal conduction. Hence, it is plausible from our results that chronic nicotine exposure expedites atrioventricular nodal conduction. Moreover, because P wave duration was not associated with cotinine, our findings suggest nicotine has limited effects on atrial conduction. Using our linear regression coefficient, we found that each 100 ng/ml cotinine is associated with a 12.3-ms decrease in PR interval and 9.7-ms decrease in PR segment.
Cotinine and catecholamines

Cigarette smoking and nicotine result in increased central and peripheral sympathoadrenal activation. The activation of nicotinic acetylcholine receptors in the adrenal medulla leads to increased catecholamine levels [285]. We found urinary dopamine and norepinephrine (but not epinephrine) were significantly higher among smokers and those with higher cotinine, suggesting smokers have chronically increased sympathetic neuronal activity (norepinephrine), but similar adrenal medullary hormone secretion (epinephrine). Interestingly, we also found that the intermediate metabolites of all three catecholamines significantly associated with cotinine, but not with the final product, and there was a negative association between cotinine and the ratio of the final and intermediate metabolite. This suggests cigarette smoking is associated with increased synthesis of dopamine and norepinephrine and at the same time decreases the catabolism of the intermediate metabolites of all three catecholamines. This corroborates the findings of several other studies that showed cigarette smoking inhibits the activity of MOA [286, 287]; an enzyme primarily responsible for catabolism of the intermediate metabolites of norepinephrine and epinephrine to their final metabolites.

There are several mechanisms through which nicotine can possibly increase atrioventricular nodal conduction velocity. Nicotine stimulates sympathetic neurotransmission via activation of nicotinic acetylcholine receptors localized on peripheral postganglionic sympathetic nerve endings and the adrenal medulla which in turn cause catecholamine release [91, 285]. Catecholamines mediate positive chronotropic, inotropic, dromotropic, and bathmotropic effects (i.e., increased rate, force conductivity and excitability) [288]. Furthermore, evidence from animal models and ex-vivo studies
suggests nicotine may also directly cause endothelial cell injury [275], a pro-fibrotic state [273], and inhibition of cardiac A type potassium channels [85].

The mediation analysis is a novel component to our study that reveals, among catecholamines, dopamine fully mediates the effect of cotinine and shortening PR interval/segment. The positive dromotropic effects of dopamine accelerate atroventricular nodal conduction [289, 290], thereby expected to shorten the PR interval/segment without affecting P wave duration, as seen in our study.

Cotinine was even associated with altered metabolism of the three catecholamines (ratio of metabolite/parent). The positive association of cotinine with 3-methoxytyramine/Dopamine ratio suggests smoking increases dopamine synthesis and/or systemic secretion and, in compensation, also increases catechol-O-methyltransferase (COMT) activity. Also, cotinine’s inverse association with Vanillylmandelic acid/Normetanephrine and Vanillylmandelic acid/Metanephrine suggest that smoking decreases MAO activity, consistent with observations of decreased MAO-B in the amygdalae of smokers and the antidepressant effects of nicotine [291, 292]

_Catecholamines and PR interval_

Interestingly, in our study only dopamine and not epinephrine and norepinephrine were associated with PR interval and PR segment. As all catecholamines are known to exert a dromotropic effect, plausible reasons for our finding include: 1) short and long term stress (smoking) disproportionately affect circulating dopamine relative to norepinephrine and epinephrine [293], perhaps secondary to nicotine-mediated declines in dopamine uptake [294]; 2) cigarette smoking and/or nicotine cause an immediate surge in dopamine
from neuronal [9], that may cross the blood brain barrier to increase circulating dopamine; 3) as the precursor of both epinephrine and norepinephrine, dopamine may have less temporal variability, making it a more stable marker of chronic SNS activation; and 4) at low pathophysiological stress levels, dopamine may have higher affinity than epinephrine and norepinephrine for β-1- and β-2- adrenergic receptors, which modulate dromotropy in the atrioventricular node [295].

Other ECG parameters

We did not observe any association of smoking status or cotinine with QRS, QT or cQT and. Previous studies in this area have yielded mixed results, including QT prolongation [229, 262, 263], QT shortening [228], or no relationship [219] with smoking.

Limitations

While this is the first known investigation of the influence of catecholamines in nicotine-mediated alterations in cardiac conduction, the sample size is limited and participants were drawn at random from the outpatient clinic setting with intermediate to high cardiovascular risk. Thus, we might have missed significant associations between cotinine and other parameters (e.g., P-wave duration) due to insufficient statistical power. Additionally, ECGs were obtained retrospectively from medical records and not on the day of enrollment. The median and interquartile range of number of days from study enrollment (urine sample collection) and ECG was 79 [20 – 320] days. We did not collect data on time of last cigarette/nicotine exposure and states that may affect catecholamines (stress, noise, discomfort, body position, consumption of food, caffeinated beverages and drugs).
Nevertheless, we used urinary analytes (cotinine and catecholamines) that are established markers for chronic nicotine and sympatho-adrenal activation and are unlikely to exhibit large acute variation in the outpatient clinic setting from which this cohort was derived. We did not collect 24-hour urine to account for diurnal and intra-individual variation in catecholamines [296]; however, summative analysis of 24-h catecholamine production may mask elevations due to dilution. Importantly, a HRV parameter (RMSSD: square root of the mean of squared differences of successive NN intervals), when dichotomized, tended to inversely associate with urinary norepinephrine and dopamine (r= -0.13, p=0.10 for both), suggesting concordance between measures of sympathetic activity in both ECG and subsequent urine samples. Although most participants were on β-blockers, prevalence did not significantly differ by cotinine strata, and PR and P wave durations did not differ by β-blocker use. This latter point accords with observations that β-blockers do not alter resting PR interval whereas they partially attenuate PR and RR interval shortening during exercise-induced sympatho-excitation [297, 298]. Additionally, we were unable to assess associations between other cigarette components or smoking habits (e.g., frequency) and cardiac electrophysiology. Other constituents within tobacco smoke (e.g., particulate matter and aldehydes) have been shown to alter autonomic balance and may thus plausibly alter catecholamine synthesis, secretion, and metabolism. Finally, smoking and nicotine can affect myocardial conduction velocity through induction of cardiac remodeling, oxidative stress, and/or ion channel dysfunction, which were not assessed in this study and may occur independent of sympathetic activation. Nevertheless, sympathetic activation can induce all three of these pathogenic processes [299] and may thereby indirectly mediate atrioventricular conduction defects.
Conclusions

Collectively, our findings suggest that exposure to cigarette smoke accelerates atrioventricular conduction and that dopamine mediates these effects. More research is warranted to examine the specificity and selectivity of these effects and to delineate the direct contribution of nicotine. These observations identify a pathway by which smoking may increase risk for cardiovascular morbidity and mortality.
CHAPTER III  
ACUTE EFFECTS OF NICOTINE AND CIGARETTE SMOKING ON ELECTROCARDIOGRAM

Aim 3 Acute effects of cigarette smoking and nicotine with and without β-blocker on electrocardiogram.

Cigarette smoking has acute deleterious effects on the cardiovascular system, resulting in increased incidence of coronary artery disease, atrial and ventricular arrhythmias and sudden cardiac death among smokers [300, 301]. Despite, a strong dose-response relationship between cigarette smoking and CVD, it remains unclear which tobacco smoke constituents and biological pathways mediate this increased risk. The main addictive component in cigarette smoke, nicotine, has been implicated as possibly the major mediator of acute cigarette smoke induced ANS dysregulation of the heart via increased sympathetic nerve activity and multiple downstream mechanisms [133]. Imbalance within the ANS is increasingly recognized as a major culprit of CVD. Indeed, both acute and chronic activation of the sympatho-adrenal system, promotes cardiovascular dysfunction and disease, including arrhythmia, hypertension, heart failure, myocardial infarction, and ischemic stroke [302, 303]. The rising popularity of ENDS, and the enhanced nicotine dose of some ENDS devices relative to conventional smoking [304], have created an imperative to determine the cardiovascular effects of nicotine and the role of autonomic imbalance within them.
The ECG is a widely available, inexpensive, non-invasive, routine method of measuring electrical activity of the heart, and can be used to screen high risk populations and identify and predict CVD. Several ECG parameters have also been used clinically to assess cardiovascular autonomic dysfunction as a diagnostic and prognostic factor. There is now abundant evidence that acute ANS imbalance has profound effects on electrophysiology [305, 306]. Further, acute variations in hemodynamics [307] and conventional ECG parameters, including dispersion of ECG intervals and amplitudes, may result from acute ANS imbalance and, when frequent, carry worse long term prognosis [308-310]. Additional parameters have recently been added to the repertoire of ECG indices that reliably predict cardiovascular morbidity and mortality (i.e., P wave amplitude, PR interval, and JT interval) [26, 30, 283, 311-313].

Despite known cigarette induced acute ECG changes and powerfully predictive value of ECG changes for future CVD events, no prior study has systematically 1) evaluated the acute temporality of the acute electrocardiographic effects of smoking, and 2) compared these with nicotine alone, and 3) explored the potential underlying mechanisms. To the best of our knowledge, this is the first report on the immediate effects of cigarette smoking and nicotine on ECG morphologic endpoints. We therefore conducted an open label 2 x 2 factorial experimental trial to study the acute effects of smoking and nicotine, with and without β-blocker.
Participants and setting of the experimental trial

Study design

Participants were recruited via online and flyer advertisements in the public areas of Huntington, WV. The study enrolled healthy adult male and female smokers, aged 18–65 years, who had smoked at least 10 cigarettes/day for at least one year prior to the trial. Eligible participants had to weigh at least 55 kg and have a body mass index (BMI) within the range 17.5–30.0 kg/m2. Females who were pregnant or breast feeding, vulnerable population (such as patients with mental illness, prisoners etc), and participants with any medical illness requiring routine medications were excluded from the trial. Participants were screened over the phone call before entering the trial. The study design is shown in Supplementary Figure 3.1 A.

Participants

The study was approved by the Institutional Review Board at Marshall University. Each participant signed an informed consent before inclusion in the study. Twenty healthy smokers (65% men; 37±13 years of age; body mass index, 28±5 kg/m2) and ten healthy non-smokers smokers (70% men; 28±5 years of age; body mass index, 26±3 kg/m2) were included in the study. All of the smokers were regular habitual cigarette smokers (22±8 cigarettes per day for 21±12 years). The baseline characteristics along with details of smoking history and cigarette are provided in Supplementary Table 3.1. Except for one smoker (who used rescue inhaler occasionally for well controlled mild asthma), no other participant had any known medical illness nor routine medication use (including birth control pills). All subjects were at least high school graduates.
Study visits and exposures

All participants had abstained from food, coffee, and tobacco overnight before each study visit. Each smoker completed two-day visits, where on their first day they smoked single preferred brand of Combustible Cigarette (Cig) in their usual manner, and second day they were administered 4 mg of nicotine from Nicotine mouth Spray device (NicS) designed to mimic the acute nicotine delivery of cigarettes. Ten of the twenty smokers were randomly invited for two additional visits, where the exposures remained the same (i.e. preferred single combustible cigarette smoke on day 3 and 4 mg nicotine spray on day 4), but were pre-treated with 80 mg oral propranolol (non-selective β-blocker) for two hours prior to the start of the study (BB-Cig and BB-NicS). The oral propranolol was administered by physician, and participant’s vitals were closely monitored during the day 3 and 4. Ten of the twenty smokers also completed a fifth visit to serve as controls, where they simulated smoking by inhaling through their unlit cigarette (“Sham”), for the similar duration of smoking period on Cig.

The nicotine mouth spray device (Nicorette oromucosal nicotine spray, 1 mg/spray, McNeil AB) consists of a plastic bottle with 13.2 ml of a clear to slightly opalescent liquid with a mint flavor. After priming, one depression of the spray nozzle delivers a metered dose of 1 mg of nicotine in 0.073 ml of a 10% ethanol-in-water solution. The spray liquid also contains very small amounts of levomenthol (0.7 mg/spray dose) and other flavorings (less than 0.3 mg/spray dose) [314]. The 4 mg of nicotine was administered by study personnel by pressing the nozzle four times in rapid succession, directed straight into the participant’s mouth. Participants were advised to avoid respiring during, and swallowing
immediately after, administration of the spray. The dose-concentration curve from the oral spray (Cmax 9.1 ng/ml and Tmax, 10 minutes, T½ 2.6 hr) [314], mimics that of smoking cigarette with about 1 mg of nicotine content (Cmax 11.9 ng/ml and Tmax, 8 minutes, T½ 2.5 hr) [315].

**Procedures**

All study visits were separated by washout periods of at least 24 hours, and were started between 8 AM and 10:00 AM. After arrival to the research clinic, and obtaining informed consent, each participant completed a detailed health and tobacco use questionnaire. Height and weight were measured. There was a rest of 5 minutes before each blood pressure measurement and ECG recording session. Pre and post exposure blood pressure were measured in sitting position using a validated automated monitor, in accordance with the guidelines [316]. Pre and post exposure serum and plasma were also collected, and stored at −20 °C until analysis (except for on Sham smoking day). A standard continuous 12 lead ECG was recorded (Cardio Card, Nasiff Associates, Central Square, NY), using as per guidelines [317], for 5 mins indoor while supine (pre-exposure), 20 mins outside while seated (during exposure) and 5 mins indoors while supine (post-exposure). The exposures were always performed while seated in the same spot; which was outside in an open public area, (200 feet away from the research building); which was not a designated public smoking place and away from main road traffic area. **Supplementary Figure 3.1 B** and **Appendix B** show the schematic timeline of the study day protocol.
**ECG and HRV analyses**

Data from Lead II was extracted and each beat was analyzed using the commercially available software LabChart/ECG and HRV Analysis Add-On (version 8.0; ADInstruments, Colorado Springs, CO, USA) (Appendix C). The artifacts were identified and removed from the analysis using the ECG Beat Classifier; which measures the Activity (sample-to-sample voltage differences), isoelectric noise, form factor (shape and time course of QRS complex) and RR interval. Supplementary Figure 3.2 depicts a typical ECG waveform, with the intervals and amplitudes that were measured automatically by the software. The data was further cleaned by excluding variables with > 20% difference from the median of 6 surrounding beats. To decrease variance, the ECG waveforms were averaged over three-minute time intervals during the 20 minutes exposure, and were averaged over 5 minutes during the pre- and post-exposure. For HRV, Time and frequency domain analysis were performed, at 5 min intervals and the entire 20 minutes during exposure, to record root mean square of successive differences (RMSSD) and standard deviation of normal beat intervals (SDNN) and LF/HF (ratio of LF to HF power), using the spectral settings as per the international guidelines [166].

**Plasma measurements**

The collected blood samples were immediately centrifuged and the plasma fraction were frozen until the end of study for batch analysis. The plasma Nicotine, Cotinine and Trans-3’-Hydroxy Cotinine in plasma samples were measured by UPLC-MS/MS using a Xevo TQ-S micro quadrupole mass spectrometer with an ESI ionization source, interfaced
with Waters Acquity Class-H UPLC equipped with a quaternary pump system (Waters, MA). Details about the procedure is given in Appendix D.

Statistical analyses

Each participant’s absolute change ($\Delta$) for ECG waveforms (intervals and amplitudes), and HRV variables were calculated from the pre-exposure (baseline). The QTc was calculated by using Bazett formula (QT Interval / $\sqrt{\text{RR interval}}$). The Area Under the Curve (AUC) for the ECG parameter changes from baseline during exposure (20 minutes) were calculated using the trapezoidal rule [318] for each participant and as average for all participants per study visit. Time series physiologic data are presented as change from baseline to allow consistent comparison to reported effects of cigarette, nicotine, with and without propranolol. We analyzed time-series deltas (each participant’s change during exposure from their own value at baseline) with linear mixed effects models (PROC MIXED) for different exposure effects using SAS 9.3 (SAS Systems; Cary, NC). A trendline function was also derived for ECG parameter that had significant change from Sham, and utilized LINEST function without ‘forced-intercept’ [319], along with six different regression types, to find the best fit curve, while maximizing the R-square and minimizing the variance to avoid under- and over-fitting. Spearman correlation was used to assess the relationship between the $\Delta$ in nicotine and its metabolites with the change in ECG parameters and Blood Pressure. The potential mediation of effects on ECG morphology by acute changes in autonomic balance (measured by RMSSD) was assessed by the bootstrapping technique and a macro put forth by Preacher and Hayes [281].
A paired t-test was used to calculate the difference in blood pressure pre and post exposure. All statistics with P < 0.05 were considered significant, and were performed in SAS 9.3 and Statistical Package for Social Sciences (SPSS) software (version 24, SPSS, Inc, Chicago, IL, USA). We also created a typical one-minute ECG waveforms, from pre and during exposure, to represent the ECG changes in a Figure, between different exposures, using ecgAuto, v3.3 (Emka Technologies, Paris, France).

Results

On Cig, NicS and Sham days, the baseline (pre-exposure) ECG parameters, except for QTc and JTc, were similar. Pre-treatment with propranolol, decreased the Heart Rate, and increased the RMSSD and SDNN and the ST height. There was no significant difference in any baseline ECG parameter between BB-Cig and BB-NicS days (Supplementary Figure 3.3).

The AUC for each exposure and study visit along with Sham is shown in Figure 3.1, while the raw values are shown in Supplementary Figure 3.4.

Heart Rate and HRV: Cig and NicS both immediately increased heart rate, which peaked at minutes 3 to 6 (+20 beats/minute and +14 beats/minute, respectively). After both exposures, the heart rate remained elevated up-to 20 minutes and returned to baseline post exposure. However, the AUC of heart rate for Cig was higher than that for NicS (p<0.05). Pre-treatment with propranolol had a significant impact on HR, with much lower AUC observed with BB-Cig vs Cig alone; while there was a non-significant decrease in AUC for BB-NicS vs NicS day. Cig immediately decreased RMSSD (within first 5 minutes and lasting for 10 minutes), while NicS had delayed albeit significant and similar decrease in
HRV measurements at 10 minutes. The overall significant decrease (vs Sham) in AUC for RMSSD was similar between Cig and NicS (P>0.05). Despite RMSSD increased after treatment with propranolol on both days, cigarette smoke decreased the RMSSD (-50 ms) vs Cig day (-20 ms) (p<0.05); while the AUC for NicS and BB-NicS was similar. Similar patterns were observed with SDNN and LF/HF.

**Atrial indices:** The smoking and NicS related changes from baseline in P duration were not different from those of Sham. However, cigarette use induced immediate and significant shortening of PR interval (-14 ms at 9th minute) and PR segment (-10 ms at 9th minute) (P<0.05 vs Sham), and this effect was abolished with pre-treatment with propranolol (P > 0.05 vs. Sham), while NicS had no significant impact on PR segment. Both, Cig and NicS significantly and similarly increased the P amplitude at 3-6 minutes of exposure compared to Sham (+21 µV and +28 µV, respectively), and interestingly this effect remained unaltered by propranolol. The AUC of P amplitude for Cig was small because, this effect reversed directionality to become negative (though not significantly) at 9-12 minutes of exposure (i.e. the above zero baseline AUC was similar between Cig and NicS).

**Ventricular indices:** Cig caused greater shortening of QTc (-11 ms) relative to NicS (-4 ms), and both Cig and Nic significantly shortened QTc relative to Sham (P < 0.05 vs. Sham). On BB days, this effect was abolished and the AUC for BB-Cig and BB-NicS were similar to Sham, and higher than that of Cig and NicS, respectively. Cig immediately shortened QTc in the first 3 min, with a subsequent plateau effect for the remainder of the exposure and returning to baseline in the post-exposure period. Cig and NicS similarly deepened Q wave amplitude (-59 µV and -72 µV, respectively, P < 0.05 vs. BL or Sham),
with propranolol blocking that effect. There was no significant effect of Cig or NicS on QRS and Tp-Te durations. The ST height gradually decreased after Cig and significantly differed from Sham minutes 3-6 ( -27 µV), with maximum effect at 9-12 minutes ( -41 µV) and slowly subsiding thereafter (remaining significantly decreased at minute 18-21) to baseline levels in the post exposure period. NicS had no such effect, and propranolol abolished the Cig-induced ST depression.

The typical 1-minute average ECG morphology during six exposures are shown in Figure 3.2. Figure 3.3 shows the best fitting equation for the trendline of the changes in Heart Rate, P amplitude, QTc from Cig and NicS and PR segment and ST height from Cig. Supplementary Figure 3.4 show the raw and relative of the important ECG parameters on different days among smokes.

Nicotine and its metabolites: The raw and relative changes in nicotine, cotinine and 3OH were similar among different visit days of smokers; and higher compared to non-smokers (Supplementary Figure 3.5; p<0.05 for each biomarker). There was significant correlation between change in nicotine levels with that of heart rate, ST-height, PR interval, PR segment, Q amplitude, SDNN and RMSSD among smokers (Cig and NicS days) and non-smokers (Table 3.1).
Table 3.1 Correlation between Area under the Curve of ECG parameters with change in Nicotine, Cotinine and 3-hyroycotinine (3-OH) among smokers on Cig-Day and NicS-Day and Non-smokers

<table>
<thead>
<tr>
<th></th>
<th>Δ Nicotine</th>
<th>Pre-Cotinine</th>
<th>Pre-3HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>0.443**</td>
<td>0.165</td>
<td>0.099</td>
</tr>
<tr>
<td>ST height</td>
<td>-0.299*</td>
<td>-0.246</td>
<td>-0.155</td>
</tr>
<tr>
<td>PR interval</td>
<td>-0.303*</td>
<td>-0.015</td>
<td>0.077</td>
</tr>
<tr>
<td>PR segment</td>
<td>-0.318*</td>
<td>0.081</td>
<td>0.135</td>
</tr>
<tr>
<td>P duration</td>
<td>0.015</td>
<td>-0.021</td>
<td>-0.062</td>
</tr>
<tr>
<td>P amplitude</td>
<td>0.123</td>
<td>0.304*</td>
<td>0.219</td>
</tr>
<tr>
<td>QTc interval</td>
<td>-0.140</td>
<td>-0.180</td>
<td>-0.084</td>
</tr>
<tr>
<td>QRS duration</td>
<td>0.191</td>
<td>0.128</td>
<td>-0.039</td>
</tr>
<tr>
<td>Q amplitude</td>
<td>-0.364**</td>
<td>-0.149</td>
<td>0.004</td>
</tr>
<tr>
<td>SDNN</td>
<td>-0.344*</td>
<td>-0.126</td>
<td>-0.157</td>
</tr>
<tr>
<td>RMSSD</td>
<td>-0.446**</td>
<td>-0.147</td>
<td>-0.126</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>0.438**</td>
<td>0.162</td>
<td>0.148</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>0.374**</td>
<td>0.003</td>
<td>-0.061</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

ANS and other ECG parameters: To test for statistical mediation of the electrophysiologic effects of Cig and NicS exposures by ANS imbalance, we also evaluated the correlation of RMSSD with the other ECG parameters (Supplementary Table 3.2) where only Heart Rate and ST-height correlated with the nicotine levels among smokers on Cig-day and non-smokers. Mediation analysis showed that RMSSD completely mediated the association of nicotine with ST-height; specifically, the indirect effect (path
a x b) of Δ nicotine on ST-height through RMSSD had a point effect estimate (β) of -26.57 and an upper and lower 95% CI of -82.80 and -0.90. While change in nicotine directly affected Heart Rate (c’ path; p=0.036), and this effect was independent of changes in RMSSD (Figure 3.4).

There was no effect of baseline cotinine on the relationship between changes in nicotine and that of ECG parameters (Heart Rate, PR segment, ST height and root mean squared of successive differences (RMSSD)), among smokers on Cig-Day and NicS-Day and Non-smokers (Supplementary Figure 3.6).

**Blood pressure:** Both Cig and NicS similarly and significantly increased post-exposure systolic and diastolic blood pressures relative to Sham and non-smokers (Supplementary Figure 3.7). At pre-exposure, blood pressures were similar between Cig, NicS and Sham. There was no significant change in blood pressure on the BB days post Cig and post NicS. The change in nicotine levels also significantly correlated with the changes in blood pressure (Table 3.1), which were not observed on BB days (p>0.05).
Heart Rate

- Without BB
- With BB

RMSSD

ST Height

PR interval

P duration

PR segment

P amplitude

Stars and hashtags indicate statistical significance:

★: <0.05 vs Sham
#: <0.05 vs Nicotine
β: <0.05 vs non-β-blocker
Figure 3.1 The Area Under the Curve for the raw changes (Δ) in ECG parameters from baseline for each visit day among smokers

N=20 on Cigarette and NicSpray days. N=10 on sham days
Figure 3.2 ECG morphology in a typical participant during exposure on different days

Each ECG individual waveform is a 1-minute average before or during exposure to unlit cigarette (sham), cigarette, or nicotine spray.
### Cigarette

- **Heart rate**
  - Equation: $y = -0.0014x^4 + 0.0799x^3 - 1.5878x^2 + 12.109x - 12.945$
  - $R^2 = 0.9066$

- **P amplitude**
  - Equation: $y = -0.0119x^4 + 0.6146x^3 - 11.139x^2 + 79.524x - 143.64$
  - $R^2 = 0.9565$

- **QT c**
  - Equation: $y = 3E-05x^3 - 0.001x^2 + 0.0104x - 0.0475$
  - $R^2 = 0.4694$

### Nicotine Spray

- **Heart rate**
  - Equation: $y = -0.0013x^4 + 0.0676x^3 - 1.2402x^2 + 8.7277x - 8.5387$
  - $R^2 = 0.8437$

- **P amplitude**
  - Equation: $y = -0.0137x^4 + 0.6326x^3 - 9.8718x^2 + 58.53x - 70.331$
  - $R^2 = 0.9050$

- **QT c**
  - Equation: $y = 4E-05x^3 - 0.0014x^2 + 0.0158x - 0.0605$
  - $R^2 = 0.7192$
Figure 3.3 The best fitting line to predict changes in Heart Rate, P amplitude, and QTc upon Cigarette and Nicotine exposures, and additional lines for PR segment and ST height upon Cigarette exposure

N=20 on Cigarette and NicSpray days. N=10 on sham days
Figure 3.4 Mediation analyses of changes in Nicotine, root mean squared of successive differences (RMSSD) and Heart Rate and ST-height among smokers on Cig-Day and NicS-Day and Non-smokers

Nicotine directly and linearly increases the heart rate, independent of pathway involving RMSSD / β-adrenoreceptors. RMSSD/ β-adrenoreceptors significantly and fully mediates the effect of smoking on ST height.
Discussion

We have demonstrated in healthy habitual smokers that either smoking one cigarette or self-administering 4 mg oral nicotine acutely alters ECG morphology, with some key differences between the effects of the two. Moreover, non-selective β-adrenergic blockade inhibited many but not all of these effects. These transient ECG changes provide a plausible mechanism underlying smoking-induced cardiovascular events, with important clinical implications related to recreational use of nicotine delivery products as well. Table 3.2 summarizes the major findings of the study.

Table 3.2 Major findings of the experimental study

<table>
<thead>
<tr>
<th></th>
<th>Cigarette vs Sham</th>
<th>NicSpray vs Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>Increased 🔻</td>
<td>Increased 🔻</td>
</tr>
<tr>
<td>RMSSD</td>
<td>Decreased ○</td>
<td>Decreased ○</td>
</tr>
<tr>
<td>ST height</td>
<td>Decreased ✗</td>
<td>No effect</td>
</tr>
<tr>
<td>PR segment</td>
<td>Decreased ✗</td>
<td>No effect</td>
</tr>
<tr>
<td>P duration</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>P amplitude</td>
<td>Increased ○</td>
<td>Increased ○</td>
</tr>
<tr>
<td>QTc and JTc</td>
<td>Decreased ✗</td>
<td>Decreased ✗</td>
</tr>
<tr>
<td>QRS duration</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>Q amplitude</td>
<td>Decreased ✗</td>
<td>Decreased ✗</td>
</tr>
</tbody>
</table>

Effect of β-Blocker

- None
- Abolished
- Attenuated
**Sympathetic activation**

Consistent with previous reports, our study found that smoking a single cigarette acutely increased heart rate and blood pressure, and decreased HRV and PR segment [90, 240, 241, 320] – consistent with increased sympathetic activation. The duration of increased heart rate up-to 20 minutes, dissipating after 30 minutes, recapitulates observations by Ramakrishnan et al, among 31 male smokers with atypical chest pain and 24 h Holter monitor [238]. However, we showed for the first time that acute nicotine exposure causes these changes, with dose-proportionate effects that were diminished by propranolol. Nicotine is a known sympathomimetic that acts on nicotinic receptors to increase sympathetic tone and catecholamine release. Thus, it is not surprising that we also found a dose-proportionate increase in cardiac sympathetic activity from oral nicotine administration. Our observations of nicotine-mediated sympatho-excitation add to recent literature on the acute physiological effects of nicotine alone [321-323] or within e-cigarette aerosols [321] and identify a plausible mechanism by which tobacco product use increases sympathetic activity. Indeed, sympathetic dominance strongly predicts cardiovascular morbidity and mortality [303, 324, 325], including for arrhythmia-related events [326], and can acutely provoke arrhythmia through numerous pathways [327]. Importantly, our observations of acute sympathetic dominance with either nicotine oral spray or conventional smoking identify nicotine as a plausible mediator of tobacco product-induced cardiovascular morbidity and mortality.
**Atria**

P wave morphology is dependent on mainly four factors 1) intra-atrial conduction abnormalities, 2) left atrial hypertension, 3) left atrial distention, and 4) chronicity disease [328]. Another novel finding of the study was that cigarette smoking and nicotine spray, within minutes, increased P wave amplitude, and propranolol prevented these effects. We also found non-significant shortening of P duration with cigarette and nicotine use. Both these observations are similar those of Goldberg et al., who noted that isoproterenol-induced β-adrenergic stimulation significantly shortened the P wave in healthy subjects [58]. Sympathetic stimulation increases peripheral vascular resistance, venous return and cardiac pressures [329], effects also seen with cigarette smoking [330, 331]. Accordingly, the significant increase in P amplitude induced by NicS and Cig, and inhibited by β-blockade, indicate that nicotine increases atrial pressures through β-adrenergic receptors. The acute increase in atrial pressures together with increased dromotropy, may represent two distinct pathways with possible synergistic effects of tobacco products induced increased atrial arrhythmia and thrombosis and stroke risk [283, 312].

**Ventricles**

To the best of our knowledge, this study demonstrates for the first time that smoking acutely induces ST-depression. Smoking caused a gradually worsening ST-depression that peaked at 9-12 minutes, abated by about 30 minutes post-exposure, and vanished with propranolol pretreatment. Recently, Ramakrishnan noted gross alterations in ST-T morphology with smoking (1-2 mm changes in ST segment or T wave), but only in one tenth of men with prior atypical chest pain and with onset at 8-12 minutes after smoking
in [238]. The cardio-protective effects of β-blockers among patients with stable angina is well described [332]. However, cigarette smoking induced ST-segment depression at rest, via β-adrenergic receptors activation complements the propositions of others that enhanced sympathetic tone mediates ST-segment depression in general [333-335].

Interestingly, we also found that cigarette smoking caused significant albeit minimal shortening of QTc. The relationship between QTc and the ANS is particularly complex [299]. However, several studies have shown that acute adrenergic stimulation shortens QTc in humans [64, 336], possibly by reducing transmural dispersion of repolarization [337]. Chronic smoking is associated with higher potassium levels [338], however, acutely it inhibits potassium channels [85] and/or may decrease potassium levels via direct sympathetic activation [339]. The effects of potassium levels and/or ion channels may be more pronounced in the ventricles than in atria, resulting in hypokalemia related ECG changes and ST-segment depression [339]. Unlike the dose-dependent effects of nicotine (in either NicS or Cig) on atrial and atroventricular (AV) electrophysiology, there were no significant effects of nicotine on ventricular depolarization and repolarization. We speculate this may derive from disproportionate autonomic control of the atria, sinus node, and AV node, which have a greater density of cardiac autonomic ganglia than the ventricles [340, 341]. Alternatively, because of distinct atrial-specific ion-channel and intercellular coupling properties and distribution, atrial myocardium may be more susceptible to architectural or structural changes with a substantial impact on cardiac performance, arrhythmia occurrence, and stroke risk [342].
**Strengths and Limitations**

It is well accepted that many of the effects of cigarette smoking, including addiction and acute alterations in cardiovascular physiology, stem from the rapid delivery of nicotine. However, there are very few studies that have directly compared the effects of nicotine vs cigarette, and those studies found no significant effect of nicotine replacement therapy, in contrast to cigarette exposure [343], we used a novel oral nicotine spray with pharmacokinetic properties for nicotine similar to those of cigarettes [314, 315, 344, 345], and also perhaps electronic nicotine delivery systems. We also recorded continuous standard 12 lead ECG during exposure, and systematically analyzed the interval and amplitudes of each beat with an automated software, to investigate the acute temporal relationship of smoking and nicotine exposure on ECG.

One potential source of variability in this study was the outdoor setting during mid-exposure ECG monitoring. However, to diminish the influence of outdoor conditions (e.g., temperature, humidity, air pollutants etc), most of the study visits were performed on consecutive days. Similarly, we saw no differences in ECG variables between BB and non-BB days during the first minute of outdoor recording. To facilitate detection of the early acute effects under typical smoking conditions and the delayed acute effects under conventional clinical conditions, participants were assessed for ECG changes while seated at mid-exposure, but while supine at pre- and post-exposure. We also performed the same protocol on 10 healthy non-smokers, where the exposure consisted of periodically inhaling through an empty straw for 5 minutes to simulate smoking, and demonstrated that there were minor changes related to position. We did not exclude any coronary artery disease among the study participants, however, because of our eligibility criteria, participants had
no known cardiac medical condition, nor any active symptoms and were healthy. Another limitation is that the oral nicotine spray may be pungent and taste unpleasant for some participants, which may result in significant changes in heart rate. However, these changes are usually transient and less potent than that seen during our study (i.e., increase in heart rate of 7.1–13.6% for various taste stimuli, with maximum effect ~25 s and returned to pre-exposure after 80 and 100 seconds [346]. Lastly, since this is a human in vivo experimental study, we were unable to differentiate direct vs indirect effects from nicotine and cigarette smoking (e.g., heart rate may have increased due to direct effects of nicotine and non-nicotine constituents on receptors in the sinoatrial node or central nervous system, or instead via reflexes from increased cardiac pressures secondary to increased peripheral vascular resistance and systemic pressures).

Conclusion

Smoking and nicotine alone acutely induce myriad changes in electrophysiology that are known to be pro-arrhythmic. Smoking may acutely promote arrhythmia via nicotine-mediated acceleration of heart rate and ventricular repolarization (QTc), with β-adrenoceptors mediating only the latter. Unlike acute nicotine, cigarette smoke rapidly depresses ST segment and shortens PR segment via β-adrenoceptor stimulation, indicating smoking transiently induces ischemia and accelerates dromotropy by sympathetic activation. Nicotine in cigarette smoke, via β-adrenoceptors, mostly seems responsible for transient increase in atrial pressures, which may reflect possible mechanism for tobacco induced atrial arrhythmia and stroke risk.
CHAPTER IV

IMPLICATIONS AND CONCLUSIONS

Clinical Implications

Cigarette smoking is the most significant modifiable risk factor for cardiovascular morbidity and mortality. Although smoking rates in the United States have declined over the past five decades to historic lows, efforts to protect public health are far from complete. The burden of combustible tobacco use in the United States remains high, especially in vulnerable populations. This persistence of cigarette smoking, coupled with the advent and dramatic rise in the use of new tobacco products, such as electronic cigarettes, especially among adolescents and young adults, are of significant concern [347]. These products may benefit by helping some smokers quit or transition to a less harmful product; however, the long-term health effects of these products and the net public health effect associated with their use remain unclear and widely debated [348]. Evidence is mounting that the use of these products may catalyze the transition to the use of other tobacco products or recreational drugs, particularly in young adults [349]. The use of certain newly regulated tobacco products has skyrocketed, particularly among youths. For example, from 2011 to 2018, the rate of e-cigarette use among high school students ballooned from 1.5% to 20.8% [350], and then up to 27.5% in 2019, according to preliminary results from the Centers for Disease Control and Prevention’s annual National Youth Tobacco Survey.
Nicotine is addictive and present alongside other chemicals in ENDS. Several studies have revealed the existence of toxicants in both the liquid and aerosol of e-cigarettes. Recent studies suggest that, although adverse experiences such as chest pain, palpitations, coronary heart disease, lung injury [351], and increased risk of myocardial infarction [352] are associated with e-cigarette use, further investigation is required to evaluate whether any causal relationships exist. The FDA has included the discovery of biomarkers to assess exposure and harm or toxicity of non-cigarette tobacco products, including ENDS, as well the as short- and long-term health effects of tobacco products among its research priorities [348]. The series of studies presented in this dissertation directly relate to those scientific domains—to help establish sensitive biomarkers to detect short-and long-term subclinical cardiovascular injury from tobacco and other nicotine-related products, and potentially guide regulation of nicotine levels in recreational products [347].

Most investigations into the pharmacokinetics of nicotine from ENDS have yielded variable results, perhaps owing to the large variability of study protocols. Moreover, several studies have lacked generalizability, as they incorporated a single standard e-cigarette and nicotine concentrations with a protocol for vaping at fixed intervals [353]. Furthermore, they were unable to investigate the differential impacts of variations in experimental parameters, such as battery output voltage or coil resistance, which are known to impact delivery of nicotine and production of potentially toxic constituents [354-356]. Further complicating the relevance of past ENDS studies, e-cigarette devices have rapidly evolved to more effectively deliver nicotine. A recent pharmacokinetic study revealed that an ad-lib session with third-generation e-cigarettes is able to achieve the dose and speed of
nicotine delivery similar to conventional cigarettes [357]. Another study revealed that newer generation e-cigarettes (“mod”-type cigarette) delivered nicotine at levels close to, or even exceeding, those of combustible cigarettes (highest average Cmax - 43.6 ng/ml) [358]. The parent company of JUUL, the relatively new pod device that has claimed 70%-80% of the U.S. e-cigarette market [359], noted in their initial patent filing that their device achieved 36% higher peak levels of plasma nicotine and higher increases in heart rate with more rapid onset than full-flavored cigarettes. This is due in major part to the use of nicotine benzoic acid salts, which further enhance nicotine delivery [359]. In our experimental trial, the plasma levels were drawn about 45 minutes after the start of exposure, and the nicotine levels increased an average of 7.83 ng/ml after cigarette use, and 6.98 ng/ml after nicotine spray, which is similar to that achieved by newer e-cigarettes [353]. The JUUL brand is one of the newer entries into the market and utilizes prefilled EC fluid “pods,” being more similar to the “cig-a-like” products than to the recently available tank/box mod styles. Nicotine concentrations (average 60.9 mg/mL) were significantly higher in JUUL than those of any EC products previously analyzed by Omaiye et al. [360]. Such high nicotine levels are not observed after use of NRTs, with doses recommended to reduce withdrawal symptoms and cigarette cravings [344]. Despite the large variability in the pharmacokinetic profiles and levels of nicotine due to varying e-cigarette device designs, e-liquid concentration, and use behavior, the results of our study provide valuable insight into the likely cardiovascular effects and risks of e-cigarettes.

We conducted three studies that may elucidate the cardiac impacts of nicotine-containing e-cigarettes. In our cross-sectional observational study, we found that chronic nicotine or tobacco exposure leads to dose-dependent shortening of PR segment and QTc,
and that an extremely high burden of exposure (measured by the nicotine metabolite, cotinine) leads to QRS shortening and JT prolongation. Although our analyses suggested a role for nicotine in these effects, the design of this study made it impossible to delineate between effects from exposure to cigarette smoke and exposure to nicotine. In our follow-up acute exposure study, we found evidence that nicotine mediates increased sympathetic activity (as measured by decreased HRV), shortened JTc, and increased P amplitude. Taking these findings into consideration—and extending to the possible nicotine-related effects from ENDS on cardiac electrophysiology—we suggest that e-cigarettes both acutely and chronically increase sympathetic activity, as noted in recent observations [321, 361]. Other studies have also found that conventional smoking chronically increases sympathetic modulation of the heart [72, 241]. Moreover, our results suggest that nicotine in e-cigarettes may both acutely and chronically shorten ventricular repolarization while also acutely increasing atrial pressures (indicated by P amplitude). Notably, a few studies also observed an increased P amplitude among smokers at baseline [212, 213, 362, 363], similar to our findings in which cotinine (proxy for tobacco use) was associated with P amplitude (particularly in deep terminal negativity of the P wave in V1 [DTNPV1]) in the chronic state [364]. However, the mechanisms for abnormal P wave indices during acute and chronic exposure may differ while also carrying different prognostic value. As acute increases in P amplitude may reflect increased cardiac filling pressure, chronic elevations in P amplitude may reflect atrial hypertrophy/distention.

Such baseline pathophysiological changes in human cardiac electrophysiology have clinically important implications. Increases in sympathetic activity [303], atrial cardiopathy [365, 366], and shortened rate of ventricular repolarization [367-369] have
been associated with increased risk for adverse outcomes, such as all-cause mortality, arrhythmia, and stroke. In this regard, a strategy formed by the FDA to limit nicotine levels in ENDS might help reduce some of the long-term harmful cardiovascular effects. Our study featuring oral nicotine spray indicated that the nicotine levels should be lower than those currently found in newer generation e-cigarettes (e.g., JUUL and other pod devices). However, more studies are required to establish whether the nicotine levels are directly related to increased risk for short- or long-term adverse cardiovascular events.

**Conclusion**

The acute and chronic proarrhythmic effects of smoking and their underlying mechanisms are currently unknown. To address this gap in the knowledge, we assessed the effects of chronic cigarette exposure on ECG, using serum cotinine (as a sensitive and specific marker of tobacco use) from the NHANES database, and urinary cotinine from cardiovascular patients from an outpatient clinic. From the NHANES database, higher serum cotinine levels were associated with considerably shortened PR intervals, PR segments, and QRS duration, and considerably prolonged JT intervals. We also found a negative linear relationship between cotinine and QTc, as well as between cotinine and PR segment. A similar effect on atrial electrocardiographic indices (shortened PR interval and PR segment) was also observed among the CVD patients. Interestingly, our statistical mediation analyses indicated that dopamine fully mediated the association between cotinine and the shortening of both PR intervals and segments. Collectively, the results from the chronic exposure studies suggest that habitual smoking accelerates atrioventricular conduction (possibly though dopamine) and ventricular depolarization, but
appears to exert a more complex effect on repolarization. Overall habitual smoking modestly accelerates ventricular repolarization, and simultaneously increases the risk for severe slowing of ventricular repolarization in a subset of patients.

An experimental trial to investigate β-adrenergic receptors’ role in the acute effects of smoking versus nicotine spray on ECG was also performed. Nicotine, both with and without a β-blocker, increases the sympathetic nervous activity to a similar extent as observed with cigarette smoking in the presence of a β-blocker. Nicotine increases the heart rate through receptors independent of the β-adrenergic receptors, suggesting its acute and direct action on cardiomyocytes is independent of increased central sympathetic activation. Cigarette smoking had a higher effect on heart rate; however, after treatment with a β-blocker, the heart rate increase was similar to that from nicotine (with or without a β-blocker). This suggests that the non-nicotine constituents of cigarette smoke that increase heart rate act through β-adrenergic receptors. Cigarette smoking and nicotine comparably increase P-wave amplitude without affecting P duration (which was also unaffected in chronic exposure studies), and β-blockade did not alter this effect. This increase in P amplitude may derive from increased peripheral vascular resistance with both exposures, which increases cardiac filling pressures and cardiac output. Cigarette smoking, but not nicotine, also shortened the PR segment and decreased the height of the ST segment, with both being rescued by β-blockade. This suggests that non-nicotine constituents increase dromotropy and ischemia through β-adrenergic receptors. Furthermore, the relationship between cotinine and the PR segment found in the chronic exposure studies likely derived from cotinine reflecting exposure to tobacco smoke, including non-nicotine constituents. Indeed, the tar and nicotine levels in cigarettes correspond with one another, just as
exposures to each would be expected to correspond. Consistent with our observations of the link between chronic smoking and QTc shortening, ventricular repolarization time was more acutely shortened with cigarette smoking compared to nicotine, and was abolished by the β-blockade. It is plausible that nicotine acutely accelerates repolarization via adrenergic-related mechanisms (e.g., β-adrenergic induced phosphorylation of Kv7.1), whereas chronic smoking, itself, impedes repolarization via myocardial injury (e.g., fibrosis). Alternatively, chronic smoking may impede repolarization by desensitizing adrenergic pathways that would otherwise modulate the heart-rate-dependent shortening of repolarization with sympathetic activation in healthy individuals.

The central illustration combines the findings of all three studies to demonstrate the role of nicotine and non-nicotine constituents of cigarette smoke, and the pathways involved in their effects on cardiac electrophysiology (Figure 4.1).
Figure 4.1 Central illustration summarizing the impacts of nicotine and non-nicotine constituents of cigarette smoke on cardiac electrophysiology, and the pathways likely involved

Key summary points

- Of the acute effects of cigarette smoking on ECG, nicotine is partly responsible for increased sympathetic activity and shortening of ventricular repolarization, but not for increased dromotropy and ischemia.
- Habitual smoking is associated with increased dromotropy (possibly via dopamine), faster ventricular depolarization, and inverse J-shaped effects on ventricular repolarization.
• Such acute and chronic effects on ECG from cigarette smoking and nicotine may promote cardiac arrhythmias or reflect a diseased state and an at-risk population.

• Future research is needed to (a) delineate the receptors used by nicotine to cause sympathetic activation, (b) identify the non-nicotine constituents in cigarette smoke that transiently increase dromotropy and myocardial ischemia, and (c) elucidate the mechanisms involved in nicotine and non-nicotine constituents of cigarette-smoke-induced complex effects on ventricular repolarization (shortening in most patients, and prolonging in a small subset of patients).
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LIST OF ABBREVIATIONS

Cardiovascular disease (CVD)
Gamma-Aminobutyric Acid (GABA)
Electronic Nicotine Delivery Systems (ENDS)
Volatile Organic Compounds (VOCs)
Electrocardiogram (ECG)
Heart Rate Variability (HRV)
T peak – T end (Tp-Te)
Food and Drug Administration (FDA)
Autonomic Nervous System (ANS)
Sympathetic Nervous System (SNS)
Parasympathetic Nervous System (PNS)
Cyclic Adenosine monophosphate (cAMP)
Protein Kinase A (PKA)
G-protein-coupled inward rectifier potassium (GIRK)
Nicotinic Acetylcholinergic Receptors (nAchRs)
Third National Health and Nutrition Examination Survey (NHANES)
QT corrected (QTc)
Chronic Obstructive Pulmonary Disease (COPD)
Transient-Receptor Potential Ankyrin-1 channel (TRPA1)
Long QT syndrome (LQTS)
Fine Particulate Matter (PM_{2.5})
Monoamine oxidase (MOA)
Root of the Mean of Squared differences of Successive NN intervals (RMSSD)
**Supplementary Table 2.1.1** The 5th and 95th percentiles of PR interval, P duration, PR segment, corrected QT interval (QTc), QRS durations and uncorrected JT interval

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<td>JT interval (ms)</td>
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### Supplementary Table 2.1.2 Patient characteristics by PR interval short (<5th percentile), reference (5-95th percentile) and long (>95th percentile) groups

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<tr>
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<td>Short</td>
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<tr>
<td>Age (years)</td>
<td>53.3±1.0</td>
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<td>177 (66.9%)</td>
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</tr>
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</tr>
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<tr>
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<td>Past</td>
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<td>Diabetes mellitus</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Dyslipidemia</td>
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<td>COPD</td>
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<td>Congestive Heart Failure</td>
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<td>β-blockers</td>
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<td>Calcium channel blockers</td>
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<tr>
<td>Antiarrhythmic drugs</td>
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</tr>
<tr>
<td>Cotinine &gt; 15 ng/ml</td>
<td>93 (36.9%)</td>
<td>1422 (27.6%)</td>
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</tbody>
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Supplementary Table 2.1.3 Patient characteristics by P duration short (<5th percentile), reference (5-95th percentile) and long (>95th percentile) groups

<table>
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<th>Reference</th>
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<td><strong>Age (years)</strong></td>
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<tr>
<td>Past</td>
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<td><strong>Diabetes mellitus</strong></td>
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<td><strong>Hypertension</strong></td>
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</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
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<td>1-4</td>
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<td>586 (14.0%)</td>
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<td>&gt;13</td>
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<td>820 (19.5%)</td>
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<td><strong>β-blockers</strong></td>
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<td><strong>Calcium channel blockers</strong></td>
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<tr>
<td><strong>Antiarrhythmic drugs</strong></td>
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<td>1436 (27.9%)</td>
<td>65 (24.2%)</td>
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**Supplementary Table 2.1.4** Patient characteristics by PR segment short (<5\(^{th}\) percentile), reference (5-95\(^{th}\) percentile) and long (>95\(^{th}\) percentile) groups

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<th>Characteristic</th>
<th>PR segment</th>
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<tr>
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<td>Short</td>
<td>Reference</td>
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<tr>
<td>Age (years)</td>
<td>55.6±1.0</td>
<td>55.3± 0.4</td>
</tr>
<tr>
<td>Women</td>
<td>143 (60.8%)</td>
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<td>Non-Hispanic White</td>
<td>120 (82.2%)</td>
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</tr>
<tr>
<td>Calcium channel blockers</td>
<td>14 (4.2%)</td>
<td>375 (6.2%)</td>
</tr>
<tr>
<td>Antiarrhythmic drugs</td>
<td>2 (0.8%)</td>
<td>47 (0.9%)</td>
</tr>
<tr>
<td>Cotinine &gt; 15 ng/ml</td>
<td>105 (41.2%)</td>
<td>1399 (27.3%)</td>
</tr>
</tbody>
</table>
**Supplementary Table 2.1.5** Patient characteristics by corrected QT short (<5\textsuperscript{th} percentile), reference (5-95\textsuperscript{th} percentile) and long (>95\textsuperscript{th} percentile) groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Corrected QT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short</td>
<td>Reference</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.2±1.0</td>
<td>55.4± 0.4</td>
</tr>
<tr>
<td>Women</td>
<td>109 (49.9%)</td>
<td>2725 (54.8%)</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>125 (80.6%)</td>
<td>2578 (81.8%)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>104 (39.9%)</td>
<td>2273 (42.0%)</td>
</tr>
<tr>
<td>Current</td>
<td>67 (28.0%)</td>
<td>1178 (22.9%)</td>
</tr>
<tr>
<td>Past</td>
<td>91 (32.1%)</td>
<td>1658 (35.1%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>45 (11.3%)</td>
<td>549 (7.1%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>102 (31.7%)</td>
<td>1687 (30.5%)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>67 (28.9%)</td>
<td>1244 (27.7%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>50 (20.1%)</td>
<td>991 (17.3%)</td>
</tr>
<tr>
<td>COPD</td>
<td>35 (16.0%)</td>
<td>372 (7.5%)</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>89.2± 1.0</td>
<td>67.9± 0.2</td>
</tr>
<tr>
<td>Prior cardiovascular disease</td>
<td>15 (3.1%)</td>
<td>206 (3.2%)</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>15 (3.1%)</td>
<td>152 (1.6%)</td>
</tr>
<tr>
<td>Alcohol drinks per month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>164 (60.8%)</td>
<td>2842 (48.7%)</td>
</tr>
<tr>
<td>1-4</td>
<td>36 (17.0%)</td>
<td>841 (18.0%)</td>
</tr>
<tr>
<td>5-13</td>
<td>24 (8.3%)</td>
<td>586 (13.6%)</td>
</tr>
<tr>
<td>&gt;13</td>
<td>37 (13.9%)</td>
<td>828 (19.7%)</td>
</tr>
<tr>
<td>β-blockers</td>
<td>5 (2.7%)</td>
<td>341 (7.1%)</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>21 (7.5%)</td>
<td>371 (6.1%)</td>
</tr>
<tr>
<td>Antiarrhythmic drugs</td>
<td>3 (1.0%)</td>
<td>43 (0.8%)</td>
</tr>
<tr>
<td>Cotinine &gt; 15 ng/ml</td>
<td>85 (32.4%)</td>
<td>1415 (27.5%)</td>
</tr>
</tbody>
</table>
**Supplementary Table 2.1.6** Patient characteristics by QRS duration short (<5<sup>th</sup> percentile), reference (5-95<sup>th</sup> percentile) and long (>95<sup>th</sup> percentile) groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Short</th>
<th>Reference</th>
<th>Long</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56.8± 0.9</td>
<td>55.6± 0.4</td>
<td>53.6± 0.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Women</td>
<td>204 (80.3%)</td>
<td>2726 (55.1%)</td>
<td>70 (22.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>144 (78.1%)</td>
<td>2599 (81.8%)</td>
<td>117 (86.1%)</td>
<td>0.223</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td>0.197</td>
</tr>
<tr>
<td>Never</td>
<td>128 (44.5%)</td>
<td>2285 (42.1%)</td>
<td>102 (41.3%)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>65 (28.4%)</td>
<td>1163 (22.5%)</td>
<td>69 (25.3%)</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>72 (27.0%)</td>
<td>1680 (35.4%)</td>
<td>89 (33.4%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>46 (11.5%)</td>
<td>548 (7.1%)</td>
<td>28 (7.3%)</td>
<td>0.205</td>
</tr>
<tr>
<td>Hypertension</td>
<td>91 (34.1%)</td>
<td>1730 (30.9%)</td>
<td>95 (29.9%)</td>
<td>0.704</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>72 (30.7%)</td>
<td>1248 (27.5%)</td>
<td>54 (26.4%)</td>
<td>0.684</td>
</tr>
<tr>
<td>Obesity</td>
<td>44 (12.2%)</td>
<td>987 (17.6%)</td>
<td>55 (15.9%)</td>
<td>0.188</td>
</tr>
<tr>
<td>COPD</td>
<td>29 (10.6%)</td>
<td>372 (7.5%)</td>
<td>22 (13.6%)</td>
<td>0.293</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>69.7± 0.9</td>
<td>68.0± 0.2</td>
<td>65.3± 1.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Prior cardiovascular disease</td>
<td>8 (2.1%)</td>
<td>220 (3.3%)</td>
<td>11 (3.7%)</td>
<td>0.566</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>14 (1.3%)</td>
<td>161 (1.8%)</td>
<td>10 (2.1%)</td>
<td>0.618</td>
</tr>
<tr>
<td>Alcohol drinks per month</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>176 (61.2%)</td>
<td>2876 (48.9%)</td>
<td>125 (41.2%)</td>
<td></td>
</tr>
<tr>
<td>1-4</td>
<td>37 (14.6%)</td>
<td>841 (17.9%)</td>
<td>45 (18.3%)</td>
<td>0.032</td>
</tr>
<tr>
<td>5-13</td>
<td>25 (10.2%)</td>
<td>583 (14.0%)</td>
<td>32 (11.0%)</td>
<td></td>
</tr>
<tr>
<td>&gt;13</td>
<td>27 (14.0%)</td>
<td>814 (19.1%)</td>
<td>57 (29.5%)</td>
<td></td>
</tr>
<tr>
<td>β-blockers</td>
<td>12 (4.6%)</td>
<td>376 (7.6%)</td>
<td>19 (8.9%)</td>
<td>0.301</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>20 (5.6%)</td>
<td>396 (6.5%)</td>
<td>20 (7.9%)</td>
<td>0.584</td>
</tr>
<tr>
<td>Antiarrhythmic drugs</td>
<td>1 (0.1%)</td>
<td>45 (0.8%)</td>
<td>5 (2.2%)</td>
<td>0.137</td>
</tr>
<tr>
<td>Cotinine &gt; 15 ng/ml</td>
<td>79 (31.2%)</td>
<td>1416 (27.3%)</td>
<td>85 (33.0%)</td>
<td>0.213</td>
</tr>
</tbody>
</table>
**Supplementary Table 2.1.7** Patient characteristics by (uncorrected) JT interval short (<5th percentile), reference (5-95th percentile) and long (>95th percentile) groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>JT interval Short</th>
<th>JT interval Reference</th>
<th>JT interval Long</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.5± 1.0</td>
<td>55.3± 0.4</td>
<td>62.0± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>90 (34.5%)</td>
<td>2745 (55.5%)</td>
<td>165 (65.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>145 (82.0%)</td>
<td>2554 (81.7%)</td>
<td>161 (83.5%)</td>
<td>0.777</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>106 (35.4%)</td>
<td>2267 (42.3%)</td>
<td>142 (46.5%)</td>
<td>0.059</td>
</tr>
<tr>
<td>Current</td>
<td>91 (32.4%)</td>
<td>1159 (22.5%)</td>
<td>47 (19.2%)</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>107 (32.2%)</td>
<td>1653 (35.1%)</td>
<td>81 (34.3%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>52 (11.6%)</td>
<td>540 (6.9%)</td>
<td>30 (10.3%)</td>
<td>0.166</td>
</tr>
<tr>
<td>Hypertension</td>
<td>118 (34.2%)</td>
<td>1679 (30.3%)</td>
<td>119 (40.5%)</td>
<td>0.049</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>74 (27.4%)</td>
<td>1242 (27.7%)</td>
<td>58 (26.0%)</td>
<td>0.913</td>
</tr>
<tr>
<td>Obesity</td>
<td>64 (22.6%)</td>
<td>980 (17.1%)</td>
<td>42 (14.6%)</td>
<td>0.246</td>
</tr>
<tr>
<td>COPD</td>
<td>32 (12.2%)</td>
<td>379 (7.7%)</td>
<td>12 (6.2%)</td>
<td>0.118</td>
</tr>
<tr>
<td>Heart rate (beats/minute)</td>
<td>88.0± 0.9</td>
<td>67.5± 0.2</td>
<td>52.5± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prior cardiovascular disease</td>
<td>16 (2.8%)</td>
<td>206 (3.1%)</td>
<td>17 (6.6%)</td>
<td>0.107</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>17 (2.1%)</td>
<td>151 (1.6%)</td>
<td>17 (3.4%)</td>
<td>0.230</td>
</tr>
<tr>
<td>Alcohol drinks per month</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>174 (57.1%)</td>
<td>2841 (48.6%)</td>
<td>162 (51.8%)</td>
<td></td>
</tr>
<tr>
<td>1-4</td>
<td>43 (16.3%)</td>
<td>832 (17.8%)</td>
<td>48 (18.8%)</td>
<td>0.440</td>
</tr>
<tr>
<td>5-13</td>
<td>37 (9.9%)</td>
<td>582 (14.1%)</td>
<td>21 (10.3%)</td>
<td></td>
</tr>
<tr>
<td>&gt;13</td>
<td>49 (16.7%)</td>
<td>812 (19.5%)</td>
<td>37 (19.1%)</td>
<td></td>
</tr>
<tr>
<td>B blockers</td>
<td>7 (2.6%)</td>
<td>344 (7.1%)</td>
<td>56 (21.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>20 (5.4%)</td>
<td>373 (6.2%)</td>
<td>43 (14.1%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Antiarrhythmic drugs</td>
<td>4 (1.0%)</td>
<td>44 (0.9%)</td>
<td>3 (0.6%)</td>
<td>0.883</td>
</tr>
<tr>
<td>Cotinine &gt; 15 ng/ml</td>
<td>110 (37.3%)</td>
<td>1391 (27.1%)</td>
<td>79 (31.0%)</td>
<td>0.039</td>
</tr>
</tbody>
</table>
**Supplementary Table 2.1.8** Association between serum cotinine levels (dichotomous) and abnormal PR segment, QRS duration and JT interval vs the reference group among subgroups by age and sex

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>PR segment &lt;5&lt;sup&gt;th&lt;/sup&gt; percentile vs reference</th>
<th>QRS duration &lt;5&lt;sup&gt;th&lt;/sup&gt; percentile vs reference</th>
<th>JT interval &gt;95&lt;sup&gt;th&lt;/sup&gt; percentile vs reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>Interaction p-value</td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 59 years</td>
<td>1.92 (1.08, 3.42)</td>
<td>0.5661</td>
<td>1.82 (1.05, 3.15)</td>
</tr>
<tr>
<td>≥ 59 years</td>
<td>2.17 (1.14, 4.13)</td>
<td></td>
<td>1.22 (0.76, 1.95)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.26 (1.13, 4.50)</td>
<td>0.7550</td>
<td>0.76 (0.29, 1.96)</td>
</tr>
<tr>
<td>Female</td>
<td>1.82 (1.03, 3.20)</td>
<td></td>
<td>2.03 (1.37, 3.00)</td>
</tr>
</tbody>
</table>
Supplementary Figure 2.2.1 Flowchart of selection of participants for study analysis
Appendix A

ECG Measurement protocol for LHHS database

Exclude ECGs with Atrial fibrillation, Atrial flutter, paced rhythm.

The caliper measurements will be performed using electronic calipers and recorded up to 2 decimal points. Only waveforms in sinus rhythm will be included and measurements will be performed only on segments those are completely captured in that lead (ie complete QRS can be visualized in V6 and not QRS starting from V3 and transitioning into V6). Each segment will be measured and recorded in three consecutive waveforms. Incompletely recorded beats, premature atrial and ventricular beats will be excluded and the next complete sinus waveform in sequential order will be measured.

**P wave duration**

P wave duration will be measured in lead II. Measurement will be conducted from the onset of the P wave, defined as the initial deflection from the isoelectric baseline of the TP segment, to the offset of the P wave, defined as the return of the P wave to the isoelectric baseline of the PR interval.
P wave amplitude

P wave amplitude will be measured in lead II. Measurement from the onset of the P wave to its highest amplitude. Use previous T-P segment as baseline (end of T-wave to beginning of P-wave).
**PR wave duration**

PR duration will be measured in lead II. The PR interval was measured from the onset of the P wave to the onset of the QRS complex, defined as the initial deflection from the baseline of the PR interval.
QT interval

The QT interval was measured in lead V5, and determined as the onset of the QRS complex to the end of the T wave; defined as the return of the T wave to the isoelectric baseline of the TP segment.
QRS duration

QRS duration was measured in lead V6, and determined as the onset of the QRS complex to the return of the complex to the isoelectric baseline of the ST segment. If in doubt regarding the isoelectric segment, use previous T-P segment as baseline (end of T-wave to beginning of P-wave).
In cases when it is hard to identify the end of QRS because ST segment is sloped and/or QRS is wide and complex then tangent line method as below can be used: when the tracing becomes more horizontal than vertical.

When in doubt or when two J points are visualized then the earlier J point should be used to measure QRS duration.
**T wave amplitude**

T wave amplitude will be measured in lead V5. Measurement from the onset of the T wave to its highest amplitude. In case of predominant negative T wave where the highest deflection is below the baseline then add a comment on the side and measure the amplitude from baseline to most lowest amplitude.
T peak – T end

T wave amplitude will be measured in lead V5. Measurement will be conducted peak of T-wave to the offset of the T wave, defined as the return of the T wave to the isoelectric baseline (TP segment as described above).
In cases when it is hard to identify the end of T then tangent line method as below can be used.

**R-R intervals**

Use the lead which has the longest continuous ECG recording, which is usually at the bottom of the ECG sheet. Use preferably Lead II if available otherwise use V1. Measure the duration between all R-R intervals. If R is not present then use any identical points on consecutive EKG waveforms such as the Q-Q or P-P duration. Do not measure R-R between sinus and a premature atrial or ventricular beat (defined as beat occurring 20% earlier to previous interval) and measure the subsequent waveforms.
References for ECG Measurement protocol


Jacob Melgaard et al. Automatic J-point Location in Subjects with Electrocardiographic Early Repolarization Computing in Cardiology 2014; 41:585-588

Considerations about the polemic J point location

http://bscw.rediris.es/pub/bscw.cgi/d5046031/CONSIDERACIONES%20ACERCA%20DE%20LA%20LOCALIZACI%C3%93N%20POL%C3%89MICA%20DEL%20PUNTO%20J.pdf


Tarek MohamedAbdelrahman The Egyptian Journal of Critical Care Medicine Volume 2, Issue 1, April 2014, Pages 19-27. Prognostic value of T peak-to-end interval for risk stratification after acute myocardial infarction

Supplementary Table 2.2.1 Baseline characteristics by high and low PR interval, P wave duration and PR segment

<table>
<thead>
<tr>
<th></th>
<th>PR interval</th>
<th>P wave duration</th>
<th>PR segment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 163.32 ms</td>
<td>&gt; 163.33+ ms</td>
<td>≤ 104.73 ms</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>49, 11</td>
<td>55, 8</td>
<td>51, 11</td>
</tr>
<tr>
<td>BMI (kg/m2)*</td>
<td>31, 7</td>
<td>34, 9</td>
<td>31, 7</td>
</tr>
<tr>
<td>SBP (mm Hg)*</td>
<td>133, 21</td>
<td>134, 25</td>
<td>129, 21</td>
</tr>
<tr>
<td>DBP (mm Hg)*</td>
<td>80, 10</td>
<td>81, 15</td>
<td>78, 12</td>
</tr>
<tr>
<td>Heart rate (beats/min)*</td>
<td>75, 17</td>
<td>71, 13</td>
<td>73, 16</td>
</tr>
<tr>
<td>Male gender</td>
<td>27, 42%</td>
<td>45, 63%</td>
<td>31, 44%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>37, 58%</td>
<td>40, 56%</td>
<td>44, 62%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>54, 84%</td>
<td>64, 90%</td>
<td>57, 80%</td>
</tr>
<tr>
<td>Prior MI</td>
<td>26, 41%</td>
<td>36, 51%</td>
<td>31, 44%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>18, 28%</td>
<td>25, 35%</td>
<td>20, 28%</td>
</tr>
<tr>
<td>Stroke</td>
<td>13, 20%</td>
<td>10, 14%</td>
<td>15, 21%</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>22, 34%</td>
<td>23, 32%</td>
<td>25, 35%</td>
</tr>
<tr>
<td>B blocker</td>
<td>43, 67%</td>
<td>55, 78%</td>
<td>51, 72%</td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>13, 20%</td>
<td>19, 27%</td>
<td>10, 14%</td>
</tr>
<tr>
<td>Statin</td>
<td>38, 59%</td>
<td>51, 72%</td>
<td>39, 55%</td>
</tr>
<tr>
<td>Aspirin</td>
<td>41, 64%</td>
<td>43, 61%</td>
<td>45, 63%</td>
</tr>
<tr>
<td>Diuretics</td>
<td>35, 55%</td>
<td>46, 65%</td>
<td>43, 61%</td>
</tr>
</tbody>
</table>


For each variable counts and column percentage has been reported except for where indicated * where the mean and standard deviation (SD) are reported.
**Supplementary Table 2.2.2** Distribution of urinary cotinine levels by self-reported active smoking status among all participants

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Cotinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\leq 50\text{ ng/ml} )</td>
</tr>
<tr>
<td></td>
<td>((\text{N}=73, 54%))</td>
</tr>
<tr>
<td>Current smoker (53, 39%)</td>
<td>2, 3%</td>
</tr>
<tr>
<td>Former smoker (47, 35%)</td>
<td>38, 52%</td>
</tr>
<tr>
<td>Never smoker (36, 26%)</td>
<td>33, 45%</td>
</tr>
</tbody>
</table>
**Supplementary Table 2.2.3** Comparison among participants stratified by self-reported smoking status or by cotinine level of (A) urinary cotinine and catecholamines (median [interquartile range], creatinine-normalized), and (B) high and low PR interval, P wave duration and PR segment

(A)

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Cotinine (ng/g)</th>
<th>p value</th>
<th>Dopamine (μg/g)</th>
<th>p value</th>
<th>Epinephrine (μg/g)</th>
<th>p value</th>
<th>Norepinephrine (μg/g)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active smoker</td>
<td>827.1 [527.3 - 1445.3]</td>
<td>&lt;0.001</td>
<td>176.0 [146.5 - 225.8]</td>
<td>0.24</td>
<td>5.7 [3.3 - 10.8]</td>
<td>0.31</td>
<td>42.5 [30.7 - 60.3]</td>
<td>0.07</td>
</tr>
<tr>
<td>Former-smoker*</td>
<td>3.7 [1.3 - 18.9]</td>
<td>-</td>
<td>167.1 [135.6 - 204.9]</td>
<td>-</td>
<td>4.2 [2.6 - 8.5]</td>
<td>-</td>
<td>36.2 [26.4 - 51.4]</td>
<td>-</td>
</tr>
<tr>
<td>Never smoker</td>
<td>1.6 [0.9 - 4.0]</td>
<td>&lt;0.001</td>
<td>139.7 [114.3 - 169.7]</td>
<td>0.001</td>
<td>3.7 [1.5 - 6.5]</td>
<td>0.03</td>
<td>32.4 [19.0 - 49.2]</td>
<td>0.02</td>
</tr>
<tr>
<td>Cotinine ≤ 50 ng/ml</td>
<td>1.8 [1.0 - 4.2]</td>
<td>&lt;0.001</td>
<td>160.0 [112.4 - 189.1]</td>
<td>0.003</td>
<td>4.1 [2.1 - 6.9]</td>
<td>0.08</td>
<td>33.4 [22.7 - 50.6]</td>
<td>0.02</td>
</tr>
<tr>
<td>Cotinine &gt; 50 ng/ml</td>
<td>827.1 [522.8 - 1596.0]</td>
<td>&lt;0.001</td>
<td>175.8 [142.0 - 227.9]</td>
<td>0.003</td>
<td>5.9 [3.1 - 9.2]</td>
<td>0.08</td>
<td>42.5 [30.4 - 56.4]</td>
<td>0.02</td>
</tr>
</tbody>
</table>
### Table

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>PR interval ( \leq 163.32 \text{ ms} )</th>
<th>PR interval &gt; 163.33 ms</th>
<th>P wave duration ( \leq 104.73 \text{ ms} )</th>
<th>P wave duration 104.74 ms</th>
<th>PR segment ( \leq 55.20 \text{ ms} )</th>
<th>PR segment 55.21 ms</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current smoker</strong></td>
<td>25, 39%</td>
<td>28, 39%</td>
<td>34, 48%</td>
<td>19, 30%</td>
<td>26, 40%</td>
<td>27, 38%</td>
<td></td>
</tr>
<tr>
<td><strong>Former smoker</strong></td>
<td>24, 38%</td>
<td>23, 32%</td>
<td>20, 28%</td>
<td>27, 42%</td>
<td>24, 38%</td>
<td>23, 32%</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Never smoker</strong></td>
<td>15, 23%</td>
<td>20, 28%</td>
<td>17, 24%</td>
<td>18, 28%</td>
<td>14, 22%</td>
<td>21, 30%</td>
<td></td>
</tr>
<tr>
<td><strong>Cotinine ≤ 50 ng/ml</strong></td>
<td>34, 53%</td>
<td>38, 54%</td>
<td>34, 48%</td>
<td>38, 59%</td>
<td>32, 50%</td>
<td>40, 56%</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Cotinine &gt; 50 ng/ml</strong></td>
<td>30, 47%</td>
<td>33, 46%</td>
<td>37, 52%</td>
<td>26, 41%</td>
<td>32, 50%</td>
<td>31, 44%</td>
<td></td>
</tr>
</tbody>
</table>
**Supplementary Table 2.2.4** Association between creatinine-normalized log transformed cotinine and parent catecholamines, their intermediate and final metabolites, and their metabolism, denoted by the ratios of intermediate: parent and final: intermediate metabolites

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DA 0.25 &lt;0.01 0.36 &lt;0.01 0.19 0.03 -0.01 0.95 -0.37 &lt;0.01</td>
<td>NE 0.22 0.01 0.03 0.01 -0.06 0.47 -0.14 0.11 -0.28 &lt;0.01</td>
<td>EP 0.11 0.37 &lt;0.01 0.01 -0.14 0.27 -0.37 &lt;0.01</td>
</tr>
</tbody>
</table>

Parent Intermediate Final

<table>
<thead>
<tr>
<th>Parent</th>
<th>Intermediate</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA: Dopamine</td>
<td>3-methoxytyramine</td>
<td>Homovanillic acid</td>
</tr>
<tr>
<td>NE: Norepinephrine</td>
<td>Normetanephrine</td>
<td>Vanillylmandelic acid</td>
</tr>
<tr>
<td>EP: Epinephrine</td>
<td>Metanephrine</td>
<td>Vanillylmandelic acid</td>
</tr>
</tbody>
</table>
**Supplementary Table 2.2.5** Associations of QRS duration, QT interval and corrected QT with urinary cotinine in unadjusted and adjusted linear regressions

Urinary cotinine was normalized by urine creatinine and log-transformed

<table>
<thead>
<tr>
<th></th>
<th>QRS</th>
<th></th>
<th>QT</th>
<th></th>
<th>Corrected QT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p value</td>
<td>β</td>
<td>p value</td>
<td>β</td>
<td>p value</td>
</tr>
<tr>
<td>Unadjusted Cotinine</td>
<td>0.32</td>
<td>0.80</td>
<td>4.71</td>
<td>0.11</td>
<td>3.48</td>
<td>0.14</td>
</tr>
<tr>
<td>*Adjusted Cotinine</td>
<td>-0.34</td>
<td>0.78</td>
<td>3.33</td>
<td>0.78</td>
<td>0.11</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* models adjusted for diastolic blood pressure and prior myocardial infarction (QRS); age, heart rate, systolic blood pressure, and calcium channel blocker (QT); age (QTc)
**Supplementary Table 2.2.6** Association between catecholamine and atrial or atrioventricular conduction determined by unadjusted and adjusted linear regressions

Catecholamines were creatinine-normalized and log-transformed

<table>
<thead>
<tr>
<th></th>
<th>PR interval</th>
<th>P wave</th>
<th>PR segment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p value</td>
<td>β</td>
</tr>
<tr>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>-17.13</td>
<td>&lt;0.01</td>
<td>-8.45</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>-5.47</td>
<td>0.02</td>
<td>-2.8</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>-7.31</td>
<td>0.11</td>
<td>-4.79</td>
</tr>
<tr>
<td>Dopamine</td>
<td>-12.3</td>
<td>0.02</td>
<td>-3.56</td>
</tr>
</tbody>
</table>

*Adjusted

<table>
<thead>
<tr>
<th></th>
<th>PR interval</th>
<th>P wave</th>
<th>PR segment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p value</td>
<td>β</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>-6.62</td>
<td>&lt;0.01</td>
<td>-2.73</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>-10.16</td>
<td>0.02</td>
<td>-3.39</td>
</tr>
</tbody>
</table>

* models adjust for age and gender (PR interval); body mass index, gender, hypertension, calcium channel blocker, ACE-inhibitor or angiotensin II receptor blocker (P duration); and diuretics (PR segment)
### Supplementary Table 3.1 Baseline characteristics

<table>
<thead>
<tr>
<th>Smoker ID</th>
<th>Age</th>
<th>Gender</th>
<th>Race</th>
<th>Body Mass Index</th>
<th>β-Blocker protocol</th>
<th>Sham-protocol</th>
<th>Pack-Years</th>
<th>Cigarette brand</th>
<th>Nicotine content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>Male</td>
<td>White</td>
<td>31</td>
<td>Yes</td>
<td>Yes</td>
<td>9</td>
<td>L&amp;M</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>Male</td>
<td>Black</td>
<td>29</td>
<td>Yes</td>
<td>-</td>
<td>36</td>
<td>L&amp;M</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>Female</td>
<td>Black</td>
<td>29</td>
<td>Yes</td>
<td>Yes</td>
<td>5</td>
<td>L&amp;M</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>Male</td>
<td>Black</td>
<td>23</td>
<td>-</td>
<td>Yes</td>
<td>29</td>
<td>Newport</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>Male</td>
<td>White</td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>Marlboro</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>Female</td>
<td>White</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>22</td>
<td>Pall Mall</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td>Male</td>
<td>White</td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
<td>L&amp;M</td>
<td>0.9</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>Male</td>
<td>Black</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>L&amp;M</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>Male</td>
<td>White</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>Camel</td>
<td>0.8</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>Female</td>
<td>White</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>USA Gold Reds</td>
<td>0.8</td>
</tr>
<tr>
<td>11</td>
<td>52</td>
<td>Female</td>
<td>White</td>
<td>25</td>
<td>Yes</td>
<td>-</td>
<td>22</td>
<td>High Card</td>
<td>0.8</td>
</tr>
<tr>
<td>12</td>
<td>42</td>
<td>Female</td>
<td>White</td>
<td>29</td>
<td>Yes</td>
<td>Yes</td>
<td>60</td>
<td>High Card</td>
<td>0.8</td>
</tr>
<tr>
<td>13</td>
<td>53</td>
<td>Female</td>
<td>White</td>
<td>34</td>
<td>Yes</td>
<td>-</td>
<td>42</td>
<td>Pall Mall</td>
<td>1.1</td>
</tr>
<tr>
<td>14</td>
<td>43</td>
<td>Male</td>
<td>White</td>
<td>30</td>
<td>Yes</td>
<td>-</td>
<td>18</td>
<td>American Spirit</td>
<td>0.6</td>
</tr>
<tr>
<td>15</td>
<td>49</td>
<td>Female</td>
<td>Other</td>
<td>21</td>
<td>Yes</td>
<td>-</td>
<td>35</td>
<td>High Card</td>
<td>0.8</td>
</tr>
<tr>
<td>16</td>
<td>62</td>
<td>Male</td>
<td>Black</td>
<td>28</td>
<td>Yes</td>
<td>Yes</td>
<td>20</td>
<td>Newport</td>
<td>0.8</td>
</tr>
<tr>
<td>17</td>
<td>61</td>
<td>Male</td>
<td>White</td>
<td>21</td>
<td>-</td>
<td>Yes</td>
<td>48</td>
<td>Kool</td>
<td>0.6</td>
</tr>
<tr>
<td>18</td>
<td>39</td>
<td>Male</td>
<td>White</td>
<td>26</td>
<td>-</td>
<td>Yes</td>
<td>25</td>
<td>Marlboro</td>
<td>1.0</td>
</tr>
<tr>
<td>19</td>
<td>43</td>
<td>Female</td>
<td>White</td>
<td>32</td>
<td>-</td>
<td>Yes</td>
<td>29</td>
<td>Camel</td>
<td>1.0</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>Male</td>
<td>Other</td>
<td>34</td>
<td>-</td>
<td>Yes</td>
<td>14</td>
<td>Pall Mall</td>
<td>0.9</td>
</tr>
</tbody>
</table>
**Supplementary Table 3.2** Correlation between Area under the Curve of root mean squared of successive differences (RMSSD) and rest of ECG waveform parameters

<table>
<thead>
<tr>
<th></th>
<th>Cigarette-Days and Non-smokers N=29</th>
<th>Cigarette-Days, Nicotine Days and Non-smokers N=49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td>-0.459**</td>
<td>-0.436**</td>
</tr>
<tr>
<td>ST height</td>
<td>0.430*</td>
<td>0.225</td>
</tr>
<tr>
<td>PR interval</td>
<td>-0.004</td>
<td>0.184</td>
</tr>
<tr>
<td>PR segment</td>
<td>0.164</td>
<td>0.026</td>
</tr>
<tr>
<td>P duration</td>
<td>-0.109</td>
<td>0.190</td>
</tr>
<tr>
<td>P amplitude</td>
<td>0.076</td>
<td>0.174</td>
</tr>
<tr>
<td>QTc interval</td>
<td>0.192</td>
<td>-0.002</td>
</tr>
<tr>
<td>QRS duration</td>
<td>-0.384</td>
<td>-0.217</td>
</tr>
<tr>
<td>Q amplitude</td>
<td>0.462</td>
<td>0.213</td>
</tr>
</tbody>
</table>
A) Potential participant screened (Healthy, 18-65 years, active smokers, Nicotine 0.6 – 1.2 ng/ml). Participant found eligible and consented.

Healthy active smokers
N = 20

Visit Day 1
Cigarette smoke

Visit Day 2
Nicotine spray

Visit Day 5
Simulate Smoking

Healthy non smokers
N = 10

Visit Day 1
Simulate Smoking

10 participants will complete additional two day study

Visit Day 3
Cigarette smoke

Visit Day 4
Nicotine spray

Visit days 1 and 2: Baseline 5 minutes, 20 minutes during exposure and 5 mins post exposure
Visit days 3 and 4: Oral 80 mg propranolol, 2 hour wait, Baseline 5 minutes, 20 minutes during exposure and 5 mins post exposure
Supplementary Figure 3.1 A) Flow chart to show the study design and enrollment B) Schematic Figure to illustrate the study visit procedures on different days
Supplementary Figure 3.2 ECG morphological variables measured from Lead II
178
Supplementary Figure 3.3 The Area Under the Curve for the baseline ECG parameters for each visit day among smokers

★ : <0.05 vs Sham
#: <0.05 vs Nicotine
β: <0.05 vs non-Beta-blocker
Supplementary Figure 3.4 The raw values of several ECG parameters in pre-post and during exposure days among smokers.
Supplementary Figure 3.5 The raw ($\Delta$) and relative ($\Delta\%$) changes with standard errors of nicotine, Cotinine and 3-hyroycotinine (3-OH) levels from baseline per study visit.
Supplementary Figure 3.6 The relationship between Area Under the Curves (AUC) of ECG parameters (Heart Rate, PR segment, ST height and root mean squared of successive differences (RMSSD)), with changes in Nicotine and Baseline Cotinine levels.

X axis - Δ Nicotine (ng/ml)
Y axis – AUC of Δ ECG parameter
Z axis – Relative size of bubble indicates baseline cotinine level

- Cigarette
- NicSpray
- Non-smokers
Supplementary Figure 3.7 The changes in Blood Pressure post cigarette and Nicotine Spray use (A) and their correlation with change in nicotine levels (B).

There is no significant difference in pre-exposure and post exposure Blood pressure between Cig and NicS days (p>0.05).

(B)

<table>
<thead>
<tr>
<th></th>
<th>20 smokers (Cig-day and NicS-day) and 9 non-smokers</th>
<th>β-Blocker Cigarette and β-Blocker Nicotine days N=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure</td>
<td>0.438 P = 0.001</td>
<td>0.180 P = 0.447</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>0.374 P = 0.008</td>
<td>-0.160 P = 0.500</td>
</tr>
</tbody>
</table>
Appendix B

Visit: Day 1 □ or Day 2 □

Subject ID: _______________ DOB: ___________ Outside Temp: _______ Humidity: _______
Date: __ __/ __ __/ __ __ Time of arrival: _____:_____
Consent / abstained from food / beverages (Except water) for 8 hours and from nicotine/Cig for 8
hours? Y/N
Date and time for last Cigarette/E-cig or other nicotine product use: Date: __ __/ __ __/ __ __
Time: ______:_______ Height _____________ Weight____________

Record START time for each event

<table>
<thead>
<tr>
<th>Event</th>
<th>Time:<em><strong>:</strong></em></th>
<th>SBP____</th>
<th>DBP_____</th>
<th>HR______</th>
<th>Time:<em><strong>:</strong></em></th>
<th>SBP____</th>
<th>DBP_____</th>
<th>HR______</th>
<th>Time:<em><strong>:</strong></em></th>
<th>SBP____</th>
<th>DBP_____</th>
<th>HR______</th>
<th>Time:<em><strong>:</strong></em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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<td></td>
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<tr>
<td>2.</td>
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<td></td>
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<td></td>
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<tr>
<td>3.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>6.</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rest 5 mins (reclining) ECG 12 sec and 5 mins
Time of departure

Recorded by: _______________________________ Date: __ __/ __ __/ __ __
PI Signature: _______________________________ Date: __ __/ __ __/ __ __
Visit: Day 3 □ or Day 4 □

Subject ID: ___________ DOB: ___________ Outside Temp: _______ Humidity: _______

Date: ___/___/____ Time of arrival: ______:_____

Consent / abstained from food / beverages (Except water) for 8 hours and from nicotine/Cig for 8 hours? Y/N

Date and time for last Cigarette/E-cig or other nicotine product use: Date: ___/___/___ Time: _____:_____ Height _____________ Weight ___________

100 mg propranolol ______:_______

**BP and HR every 15 mins for 2 hours**

<table>
<thead>
<tr>
<th>Rest 5 mins (consent)</th>
<th>Rest 5 mins (reclining)</th>
<th>IV blood draw</th>
<th>Walk outside and rest 5 mins (sitting)</th>
<th>Start ECG after rest</th>
<th>Smoke/ Nicotine/ Straw outside (sitting)</th>
<th>Record 20 mins ECG outside including the exposure time (sitting)</th>
<th>Walk inside</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP and HR (sitting)</td>
<td>ECG 12 sec and 5 mins</td>
<td></td>
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<tr>
<td>SBP ______</td>
<td>DBP ______</td>
<td>Plasma</td>
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<td>Stop Time:<em><strong>:</strong></em></td>
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<tr>
<td>HR ______</td>
<td></td>
<td>C: <em><strong>:</strong></em></td>
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<td>S: <em><strong>:</strong></em></td>
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<td></td>
<td></td>
<td>Serum</td>
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</tbody>
</table>

Rest 5 mins (reclining)  | Rest 5 mins (questionnaire) | IV blood draw | Time of departure |
<table>
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</tr>
</thead>
<tbody>
<tr>
<td>ECG 12 sec and 5 mins</td>
<td>BP and HR (sitting)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Time:<em><strong>:</strong></em></td>
<td>2. Time:<em><strong>:</strong></em></td>
<td>3. Time:<em><strong>:</strong></em></td>
<td></td>
</tr>
<tr>
<td>SBP ______</td>
<td>DBP ______</td>
<td>Plasma</td>
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<tr>
<td>HR ______</td>
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<td>C: <em><strong>:</strong></em></td>
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<td>S: <em><strong>:</strong></em></td>
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</tbody>
</table>

7. Time:___:___

Recorded by: ___________________________________________ Date: ___/___/___

PI Signature: ___________________________________________ Date: ___/___/___
Appendix C

Protocol on how to use LabChart for ECG measurements

1. Open file
2. Uncheck the “Times in first Column”
3. Set the default sampling rate to 500 Hz
4. Click OK
5. Autoscale all channels
6. Analyze the entire ECG and search and record any arrhythmia, blocks, ectopic beats (PACs, PVCs) Quantify ectopic beats and time relative to exposure. Confirm QRS complex is within 80 ms.
7. Click ECG settings
8. Click ECG Beat Classifier View
Exclude the outlier beats by mapping the case-specific distribution of activity over the isoelectric noise (in millivolts) and the distribution of the form factor over the RR interval (in seconds).

Click on the marked point and view the beat to decide whether to exclude or not. If excluded then go to the next outlier closer to the data and decide about its inclusion. Keep repeating it until you find a beat which is normal and should be included. Perform this at each four quadrants and using both; Form Factor and Activity.

9. Scroll the entire ECG recording and ensure that all selected beats should be included in the analysis

10. Click ECG Table View
Appendix D

Nicotine and metabolites determination in human plasma

2.1. Chemicals and reagents

(−)-Nicotine (NIC), (−)-Cotinine (COT), and LC-MS Ultra grade ammonium formate were purchased from Sigma-Aldrich (St. Louis, MO). Trans-3'-Hydroxy Cotinine (3HC), and trans-3'-Hydroxy Cotinine-d₃ (3HC-d₃) were purchased from Toronto Research Chemicals, (Toronto, Canada). (±)-Nicotine-d₃ (NIC-d₃) and (±)-Cotinine-d₃ (COT-d₃), were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). UHPLC-MS grade water, UHPLC-MS grade acetonitrile, and LC-MS grade formic acid were purchased from Thermo Fisher Scientific Inc. (Waltham MA).

2.2. UPLC-MS/MS analysis

NIC, COT, and 3HC in human plasma samples were measured by UPLC-MS/MS using a Xevo TQ-S micro quadrupole mass spectrometer with an ESI ionization source, interfaced with Waters Acquity Class-H UPLC equipped with a quaternary pump system (Waters, MA).

After the sample was thawed on ice, 20 μl of plasma was mixed with 480 μl of 75% acetonitrile with isotopic labeled internal standard (COT-d₃, NIC-d₃, and 3HC-d₃) in a 1.7 ml microcentrifuge tube. After incubation on ice for 30 min, the mixture was centrifuged at 4°C for 30 min, and 2 μl of the supernatant was applied on the UPLC-MS/MS instrument. The separation was performed on an Acquity UPLC BEH HILIC (50 mm × 2.1 mm, 1.7 μm) column (Waters, MA) with a binary gradient comprised of 95% water 5% ACN 10mM ammonium formate pH 3 (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) at a flow rate 0.4 ml/min. The gradient started at 96.5% for 1.2 min, ramped down to 30%
solvent B over a 3 min period. Then the gradient ramped down to 0% B in 0.1 min, held at 0% B for 0.3 min, return to 96.5% B in 0.1 min, and held for 5.3 min until next injection. Chromatography was performed at 40 °C. The total chromatographic run time was 10 min. The mass spectrometry detector worked in positive ion mode. Optimized cone voltage and collision energy were used for each of the individual analytes. For each analyte, three multiple reaction monitoring (MRM) transitions were set up: one for quantification, one for confirmation, and one for labeled internal standard. Transitions for NIC are 163 > 130 for quantification, 163 > 117 for confirmation, and 166 > 130 for internal standard (NIC-d3). Transitions for COT are 177 > 80 for quantification, 177 > 98 for confirmation, and 180 > 80 for internal standard (COT-d3). Transitions for 3HC are 193 > 80 for quantification, 193 > 134 for confirmation, and 196 > 80 for internal standard (3HC-d3). These MRMs were scheduled around the retention time of the analytes. No less than 15 data points were collected for each peak.

TargetLynx quantification application manager software (Waters, MA) was used for peak integration, calibration, and quantification. Analytes in plasma samples were quantified using peak area ratio based on a 7 point-standard curves which were run before and after the plasma samples.
CURRICULUM VITAE

Affan B. Irfan

WORK EXPERIENCE

7/2017- Non-invasive Cardiologist
Director, Echocardiography Laboratory, Cabell Huntington Hospital
Site Director for Cardiology Fellowship, Cabell Huntington Hospital
Assistant Professor
Department Cardiology and Clinical Translational Services
Marshall University, Huntington, WV, USA

2010 – 2011 Clinical Research Fellow
University Hospital Basel, Switzerland
Research Supervisor: Professor Dr. Christian Mueller, MD, FESC Associate
Director, Internal Medicine, University Hospital Basel.

2009 – 2010 Research Officer
Indus Hospital Research Centre, Pakistan
Research Supervisor: Dr Aamir Javed Khan, M.D., PhD. International Health Johns
Hopkins Bloomberg School of Public Health and Director, Center for Community
Development, Karachi, Pakistan

2008 Research Officer
Iberoamerican Cochrane Center, Hospital de Saint Pau, Spain Research Supervisor: Dr
Pablo Alonso-Coello, M.D.PhD,
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EDUCATION

2014-2017 General Cardiology Fellow
Level III Trained Echocardiography
University of Louisville. KY, USA

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2003-2008
Bachelor of Medicine and Bachelor of Surgery (M.B.,B.S) Aga Khan University Medical College, Karachi, Pakistan

2001-2003
General Certificate Examination Advanced Levels
St. Mary’s Catholic High School, Dubai, U.A.E.

2001
General Certificate Examination Ordinary Levels
English Medium School Dubai, U.A.E.

CERTIFICATION

2019    Board Certified American Board of Cardiovascular Diseases
2018    Board Certified American Board of Echocardiography
2018    Board Certified American Society of Nuclear Cardiology
2014    Board Certified American Internal Medicine
2011    US ECFMG Certificate
Sympathetic Activation and Nicotine Mediate the Acute Proarrhythmic and Ischemic Effects of Smoking in Humans.

Affan Irfan, George Koromia, Waiel Abusnina, Madison Crank, Lonnie Lucas, Meagen Carter, Nalini Santanam, Andrew DeFilippis, Mark Studeny, Zhengzhi, Pawel Lorkiewicz, Aruni Bhatnagar, Alex P. Carll
Upcoming

Association between serum cotinine levels and PR and QT interval and their components

Affan Irfan, Daniel Riggs, George A. Koromia, Andrew Paul DeFilippis, Elsayed Z. Soliman, Aruni Bhatnagar, Alex P. Carll
Upcoming

Effects of chronic smoking on atrioventricular conduction and catecholamine production

Affan Irfan, Claudia Arab, Andrew P. DeFilippis, Pawel Lorkiewicz, Zhengzhi Xie, Aruni Bhatnagar, Alex P. Carll
Submitted

Clinical Determinants of Myocardial Injury, Detectable and Serial Troponin Levels among Patients with Hypertensive Crisis

Giancarlo Acosta, Ahmed Amro, Rodrigo Aguilar, Waiel Abusnina, Niharika Bhardwaj, George Koromia, Mark Studeny, Affan Irfan
Submitted


Alok R. Amraotkar; Patrick J. Trainor; Affan Irfan, Charles W. Hargis; Shesh N. Rai; Aruni Bhatnagar; Andrew P. DeFilippis. Presence of multiple coronary angiographic characteristics for the diagnosis of acute coronary thrombus. Cardiol J. 2017;24(1):25-34


ABSTRACTS / POSTERS (Total 28 with 17 as first or last authors)


Affan Irfan, Majd Kanbour, Giancarlo Acosta, Mark Studney. The additional cost of inpatient two-day myocardial perfusion scan in obese patients. ICNC (International Conference on Nuclear Cardiology and Cardiac CT). Lisbon 2019


Affan Irfan, George Koromia, Waiel Abusnina, Mark Studney, Aruni Bhatnagar, Alex Carll. Comparison of the Acute Autonomic Effects of Mainstream Cigarette Smoke to Oral Nicotine Spray. AHA EPI/Lifestyle 2018


Affan Irfan, Patrick Trainor, Schultz, Andrew DeFilippis. Acrolein, smoking exposure and acute myocardial infarction. University of Louisville 2016

Affan Irfan; Raphael Twerenbold; Karin Wildi; Rubini-Gimenez Maria; Cedric Jaeger; Michael Freese; Tobias Reichlin; Christian Mueller. Association of albumin and high sensitive cardiac troponin t and i levels among chest pain patients presenting to emergency department. J Am Coll Cardiol. 2015;65(10_S)

Affan Irfan, Krishan Kataria, Bisma Irfan , Sarwar Orakzai. A case of cardiac amyloidosis with a negative biopsy from a noncardiac site. Clinical & Experimental Cardiology. 2014

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Affan Irfan; Tobias Reichlin; Raphael Twerenbold; Karin Wildi; Christian Mueller. The prognostic value of absolute and relative changes in cardiac troponin concentrations among non-acute myocardial infarction patients. J Am Coll Cardiol. 2013;61(10_S):. doi:10.1016/S0735-1097(13)60236-3

Affan Irfan; Tobias Reichlin; Raphael Twerenbold; Karin Wildi; Christian Mueller. Combination of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. J Am Coll Cardiol. 2013;61(10_S):. doi:10.1016/S0735-1097(13)60233-8


Agha SA, Georgiopoulou VV, Kalogeropoulos AP, Giamouzis G, Waheed S, **Irfan AB**, Rashad MA, Bhatt DV, Laskar S, Smith AL, Butler J. Digoxin and Outcomes in Patients with Advanced Heart Failure on Contemporary Optimal Treatment Circulation 2008;118(S_1027-b).
BOOK CHAPTER


ONLINE PUBLICATIONS


HONORS / AWARDS

<table>
<thead>
<tr>
<th>Year</th>
<th>Award Description</th>
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<tr>
<td>2019</td>
<td>International Conference on Nuclear and Cardiac CT Travel Award</td>
</tr>
<tr>
<td>2016-2019</td>
<td>University of Louisville Departmental Tuition Award</td>
</tr>
<tr>
<td>2015</td>
<td>First prize at DOC Student Research Proposal Competition</td>
</tr>
<tr>
<td>2015</td>
<td>American College of Cardiology Research Travel Award</td>
</tr>
<tr>
<td>2014</td>
<td>Best Research Scholar Award at University of Illinois</td>
</tr>
<tr>
<td>2005</td>
<td>Summer studentship at University of Toronto</td>
</tr>
<tr>
<td>2004</td>
<td>Summer studentship at University of Hamilton</td>
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GRANT

Marshall University Clinical Translational Services
Role: Principal Investigator
Project: Acute effects of Smoking and Nicotine on electrocardiogram
Award: $50,000
State: Funded for 2017-2019
West Virginia Clinical & Translational Science Institute
Role: Co-Principal Investigator
Project: Development of a statewide integrated model for analysis of clinical & economic patterns of injection drug use-associated infective endocarditis
Award: $49,938
State: Funded for 2019-2020