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Effects of Hookah Smoking on Vascular Regulation: Novel Insights into Endothelial Function

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Nursing

by

Mary Rezk Hanna

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ABSTRACT OF THE DISSERTATION

Effects of Hookah Smoking on
Vascular Regulation: Novel Insights into
Endothelial Function

by

Mary Rezk Hanna
Doctor of Philosophy in Nursing
University of California, Los Angeles, 2016
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Hookah (water pipe) smoking is a major new understudied epidemic of tobacco abuse particularly affecting youth. Hookah's rapidly growing popularity is due to unregulated expansion of hookah cafes near college campuses and social media marketing to young adults as a safer avant-garde alternative to cigarettes. What distinguishes hookah from all other tobacco products is that burning charcoal is used to heat the tobacco. As a result, hookah smoke delivers a large exposure not only to carbon monoxide (CO), but also to carbon-rich nanoparticles that constitute putative vasoconstrictor stimuli. To determine if hookah smoking acutely impairs endothelial and vascular function, in 23 healthy young adult hookah smokers who do not smoke cigarettes (age 25±5 years, mean±SD; 6 women, 17 men; BMI 23.6±2.3 kg·m2), we

measured conduit vessel endothelium-dependent flow-mediated dilation (FMD) by high-resolution ultrasound as well as exhaled CO and plasma nicotine before and immediately after 30 minutes of *ad lib* hookah smoking in a custom-built smoking chamber. Additionally, we measured micro-vessel endothelial function by way of reactive hyperemia peripheral arterial tonometry (EndoPAT) and central arterial stiffness by carotid-femoral pulse wave velocity (cfPWV). With hookah smoking, exhaled CO increased from 3.5±0.4 to 27±2.4 ppm (mean±SE, $p<0.001$ pre-vs. post-hookah), which approximates the CO boost of a typical *ad lib* hookah smoking session in hookah bars and indicates an exposure to fine and ultrafine particles that is 10 times greater (and to total particles that is 250 times greater) than that with cigarette smoking. The increased heart rate and blood pressure (Δ heart rate +14±2, $p<0.001$; Δ mean arterial pressure +5±1, $p<0.001$) were accompanied by increased plasma nicotine (0.6±0.1 to 6.4±1.2 ng/mL, $p<0.001$). On ultrasound and in contrast to our hypothesis, brachial artery FMD did not decrease with hookah smoking but, surprisingly, increased from 6.9±0.5 to 9.7±0.6%, $p<0.001$: a 49.6±9.3% relative increase while micro-vessel endothelial function did not change: EndoPAT index 2.08±0.14 to 1.99±0.12, $p=0.633$). Moreover, cfPWV increased significantly (7.47±0.20 to 8.04±0.22, $p<0.001$) suggesting central arterial stiffness. Further studies are indicated to elucidate the major underlying mechanisms underpinning the augmented flow-mediated dilation and central arterial stiffness.

The dissertation of Mary Rezk Hanna is approved.

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Dedication:

To David, who made my dreams come true.

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CHAPTER 1

Introduction, Specific Aims, and Significance

Introduction

Hookah (i.e. waterpipe) smoking is a historically Middle-Eastern and Indian form of tobacco use that is fueling a major contemporary epidemic of tobacco abuse and a global public health crisis, particularly among young adults (Centers for Disease Control and Prevention, 2013; World Health Organization (WHO), 2005; American Lung Association, 2007). For centuries, Hookah smoking was defined as a cultural phenomenon and a masculine habit that is endemic only to male Middle-Eastern culture. Today, Hookah smoking is a rampant social phenomenon plaguing the United States, and other western countries. It has evolved to include both genders, and is primarily marketed to young adults of all ethnic backgrounds as a safer, non-addictive alternative to cigarette smoking (Sameerur et al., 2012; Akl et al., 2010; American Lung Association, 2007). Despite numerous public health efforts to promote a tobacco free environment, Hookah smoking is the only form of tobacco smoking that is not regulated in the United States. Its exemption from clean indoor air legislation, such as the California Clean Air Act (California Indoor Clean Air Act, 1976), is contributing to its rapidly growing popularity.

Recent epidemiological studies reveal hookah use has the highest prevalence amongst youth (Gilreath et al., 2016; Montgomery et al., 2015), counteracting the success of tobacco control efforts on cigarette smoking. According to the California Tobacco Survey, Hookah use increased by more than 40% from 2005 and 2008, with 24.5% of young adults reporting ever smoking Hookah (Smith et al., 2011). In 2011,

data from the U.S. National Youth Tobacco Survey revealed that 8% of adolescents and young adults smoke Hookah. Currently, an astounding one in five U.S. high school seniors report smoking Hookah (Amrock, Gordon, Zelikoff, Weitzman, 2014; Palamar, Zhou, Sherman, Weitzman, 2014). In 2014, the Centers for Disease Control and Prevention, called for regulation of Hookah smoking, while the American Heart Association called for regulation of electronic cigarettes (e-cigarettes) but failed to mention Hookah smoking, which is far more prevalent among young adults (Centers for Disease Control and Prevention, 2014; American Heart Association, 2014; Gilreath et al., 2016; Hampson et al., 2015).

By comparison to other forms of tobacco use such as cigarette smoking, little is known about the cardiovascular effects of Hookah smoking. Cigarette smoking transiently increases heart rate and blood pressure, with the acute pressor response mediated by both vascular and neural mechanisms including inflammation, oxidative stress, sympathetic vasoconstriction, vascular stiffening and endothelial dysfunction (Narkiewicz et al., 1998; Grassi et al., 1994; Haass & Kubler, 1997; Cai & Harrison, 2000; Powell, 1998; Rhee, Na, Kim, Lee, Kim, 2007; Jatoi, Jerrard-Dunne, Feely, Mahmud, 2007; Lekakis et al., 1997; Ijzerman, Serne, van Weissenbruch, de Jongh, Stehouwer, 2003). Whether Hookah smoking shares the same —or even worse— cardiovascular toxicity remains unclear. The widespread popular belief is that the Hookah smoke becomes detoxified as it passes through the water (Heinz et al., 2013; Jamil, Elsouhag, Hiller, Arnetz, Arnetz, 2010; Amin, Amr, Zaza, Suleman, 2010; Asfar, Ward, Eissenberg, Maziak, 2005; Rezk-Hanna, O'Connell, Woo, 2014). In contrast, it is hypothesized that Hookah smoking is an even worse vascular toxin than cigarette

smoking for two main reasons: 1) not only is the acute tar and nicotine exposure of a single Hookah smoking session 100-200 times greater than that of a single cigarette (Shihadeh, Azar, Antonios, Haddad, 2004); 2) but also the burning charcoal (used to heat the Hookah tobacco) causes the Hookah smoker to inhale a large amount of carbon monoxide (CO) and carbon-rich nanoparticles such as fine particulate matter ($PM_{2.5}$; defined as less than 2.5um in hydrodynamic diameter) and ultra-fine particulate matter (UFP; defined as less than 0.1um in hydrodynamic diameter) that have been implicated in the cardiovascular toxicity of air pollution (Daher et al., 2010; Pope et al., 2009; Eissenberg & Shihadeh, 2009; Schubert et al., 2011; Brook & Rajagopalan, 2010; Brook et al., 2010).

Given the gaps in knowledge about Hookah smoking and the unknown cardiovascular toxicity effects on health, the proposed dissertation project used state-of-the-art clinical research methodology to test the following novel major hypothesis:

Major New Hypothesis: In 26 young adult regular Hookah smokers, each 30-minute smoking session evokes significant acute endothelial dysfunction. It is further hypothesized that the endothelial dysfunction involves the entire vascular tree from the most distal microvessels to the large conduit vessels, resulting in a more rapid return of the arterial pulse wave from the peripheral circulation to the central aorta thus augmenting aortic systolic pressure.

In overtly healthy young adults who are habitual Hookah smokers but not cigarette smokers, interrogation of endothelial-dependent vasodilation and central aortic pressure before and after a typical session of Hookah smoking was done, in a controlled laboratory environment, to accomplish the following specific aims:

Specific Aims

Aim 1: Determine the acute effects of Hookah smoking on large-vessel endothelial vasodilator function as measured by brachial artery flow-mediated dilation (FMD).

Hypothesis: Hookah smoking evokes acute large vessel endothelial dysfunction evidenced by impaired endothelial-dependent brachial artery vasodilation.

Aim 2: Determine the acute effects of Hookah smoking on micro-vessel endothelial function as measured by reactive hyperemia peripheral arterial tonometry (RH-PAT).

Hypothesis: Hookah smoking evokes acute microvessel endothelial dysfunction evidenced by endothelial-mediated low pulse volume amplitude scores.

Aim 3: Determine the acute effects of Hookah smoking on pulse wave velocity and central aortic stiffness measured by pulse tonometry.

Hypothesis: Hookah smoking evokes an acute increase in vascular stiffness evidenced by a faster carotid-femoral pulse-wave velocity and an increase in central aortic systolic pressure.

Significance

Research findings of the proposed project will begin to fill in large knowledge gaps regarding the potential toxic effects of Hookah smoking on the human cardiovascular system. It is hypothesized that each session of Hookah smoking poses an immediate threat to the blood vessel wall as evidenced by acute diffuse deterioration in endothelial-dependent dilation throughout the vascular tree. Over time, repeated exposure of young blood vessels to this toxic smoke could set the stage for premature vascular aging with atherosclerosis and systolic hypertension, thereby increasing the risk of myocardial infarction, peripheral artery disease, stroke, and death. Future studies

will pursue chronic structural—as well as acute functional—cardiovascular consequences of Hookah smoking. By addressing a critical public health issue, the proposed research findings could impact regional, national, and international public policy and help stave off the growing Hookah smoking epidemic.

CHAPTER 2

Review of the Literature

History

Hookahs originated in India and have been used to smoke tobacco, opium or hashish for more than four centuries. Hookah is said to have been invented by the Indian physician Hakim Abul Fath, who introduced the unsubstantiated belief that tobacco smoke is harmless when passed through water (Chattopadhyay, 2000). A Hookah consists of three main parts: head, body, and bowl (**Figure 1**). For many centuries, Hookah smoking was largely limited to masculine Middle-Eastern culture. However, with the introduction of fruit flavored tobacco, trendy water pipes and aggressive marketing, the perception of Hookah as a non-addictive, healthier alternative to cigarette tobacco has been established among its young adult users (Rezk-Hanna, O'Connell, Woo, 2014; Heinz et al., 2013; Jamil, Elsouhag, Hiller, Arnetz, Arnetz, 2010; Amin, Amr, Zaza, Suleman, 2010; Asfar, Ward, Eissenberg, Maziak, 2005).

Hookah tobacco is generally moist, sweetened and associated with numerous flavored fruits or candies. Unlike cigarette tobacco, Hookah tobacco does not remain lit independently; therefore, charcoal must be placed on top of the tobacco to maintain its continuous burn throughout the smoking session. In addition, the process of smoking Hookah differs from smoking cigarettes in several ways, mainly because of the mode and the unique manner of a Hookah (World Health Organization, 2005; 2006). The temperature of the burning hookah tobacco is much lower than that of cigarettes (450^{o}C. for hookah vs. 900^{0}C. for cigarettes), therefore tobacco and other toxicant constituents produced may differ qualitatively as well as quantitatively from cigarette

smoke. Additionally, the burning charcoal increases the concentration of specific toxins such as carbon monoxide, benzene, and other carbon-rich nanoparticles far beyond that of cigarette smoke (discussed below). Other differences that lead to greater tobacco and toxicant exposure in Hookah include longer length of a smoking session, greater depth of smoke inhalation and higher frequency of puffing. A regular Hookah smoking session typically lasts 30-120 minutes, rather than the few minutes that it takes to smoke a single cigarette (Smith-Simone, Maziak, Ward, Eissenberg, 2007; Ahmed, Jacob, Allen, Benowitz, 2011).

Prevalence

Prevalence of Hookah smoking among high school, college students and young adults is increasing dramatically throughout the U.S and around the world. According to the Centers for Disease Control and Prevention, current trends in other forms of tobacco use such as cigarette smoking among adolescents are the lowest in 22 years; from 27.5 in 1991 to 15.7 in 2013 (Centers for Disease Control and Prevention, 2013). The Centers for Disease Control and Prevention warns that this drop is being offset by increases in other forms of tobacco use such as the current surge in Hookah smoking.

In the U.S. between 2012 and 2013, Hookah use significantly increased among adolescents from 18.3% to 21.4% (Johnston, O'Malley, Miech, Bachman, Schulenberg, 2014). The California Tobacco Survey revealed that Hookah use increased by more than 40% from 2005 and 2008, with 24.5% of young adults reporting ever smoking Hookah (Smith et al., 2011). Astoundingly, recent U.S. surveys estimate that 1 in 5 high school seniors report smoking Hookah (Palamar, Zhou, Sherman, Weitzman, 2014; Amrock, Gordon, Zelikoff, Weitzman, 2014; Smith et al., 2011).

Recent U.S. demographic studies reveal the mean age of Hookah smokers to be 22 years, 43% women and the majority of smokers to be Caucasian (33%) and Latino (22%) with African Americans and Asians on the rise. Remarkably, 49% reported they smoke Hookah either every day or every week (Aljarrah, Ababneh & Al-Delaimy, 2009). It was also revealed that from 2005 to 2008, Hookah use among adults increased by more than 40% with a much higher proportion of use among young adults age 18-24 years; 24.5% among young men compared to 11.2% among men older than 24 years of age (Smith, Edland, Novotny, Hofstetter, White, Lindsay & Al-Delaimy, 2011).

Hookah smoking has expanded rapidly among those of college age. Sutfin et al. conducted a cross-sectional web-based survey of 3,770 college students from eight universities in North Carolina to estimate the prevalence of Hookah use. The study revealed 40% of college students reported ever smoking a Hookah (Sutfin et al., 2011). Another study that used an internet-based survey of first-year university students (n=744) revealed that Hookah smoking within the past 30 days was reported by 20% (Eissenberg, Ward, Smith-Simone, Maziak, 2008)

Hookah Lounges and Cafes

Currently, Hookah cafes and lounges are exempt from the Indoor Clean Air Act. This exemption allows their unchecked expansion and may contribute the growing prevalence of Hookah smoking. Research demonstrates that even though factors such as tobacco tax policies and advertising regulations are crucial for tobacco control, clean air laws seem to be the largest policy-related contributor to public health (Martinasek, McDermott, Martini, 2011; Akl et al., 2011; Aldrich et al., 2014). Primack et al (2012) conducted a study to assess how a representative sample of U.S. tobacco control

policies may apply to Hookah smoking. The authors obtained data on tobacco-related policies from the U.S. Tobacco Control Laws Database and mainly focused on clean air legislation at the municipal, county, and state levels. The study revealed that 73 of the largest 100 cities disallow cigarette smoking in bars and nearly 90% (n=69) of these cities permit Hookah smoking by exemptions (Primack et al., 2012).

According to the Tobacco Public Policy Center (2007) “Hookah bars may claim to be exempt from clean indoor air ordinances for various reasons”. One of the main reasons is related to the vague or lack of a definition of smoking in clean indoor air laws (Tobacco Public Policy Center, 2007). For example, the California Indoor Clean Air Act of 1976 does not define smoking or tobacco. Because Hookah tobacco is not directly burned in the smoking process, many existing control measures do not apply and it is unclear whether smoking through a Hookah would be considered smoking under the Act (California Indoor Clean Air Act, 1976). Also, exemptions are granted for retail tobacco shops, operators who are self-employed, and family-owned-and-operated businesses (Tobacco Public Policy Center, 2007). Hookah lounges and cafes could get exemptions because they sell tobacco and therefore are classified primarily as tobacco retail establishments or because they do not serve alcohol on the premises (Noonan, 2010).

The number of Hookah lounges has almost doubled in the last seven years in the United States. In 2006, the American Lung Association Tobacco Policy Trend Alert estimated that there were 200-300 Hookah lounges and cafes operating in the U.S. (American Lung Association, 2007). However, a recent online search for “Hookah” and “California” at the Better Business Bureau (www.bbb.org) and Hoovers full business

database (www.hoovers.com) Web sites revealed a total of 2,242 shops that sell Hookah tobacco and related products, in addition to 270 Hookah lounges and cafes in California alone, of which a disproportionate majority are located in Los Angeles near universities and college campuses. In fact, two of Los Angeles' busiest Hookah lounges, are located within one block of our University of California, Los Angeles (UCLA) campus.

Myths and Misconceptions

A review of the literature indicates that the majority of young adults who are smoking Hookah have false perceptions of Hookah as being a healthier alternative to cigarette smoking. In preparation for the proposed dissertation, a descriptive study was conducted in southern California to assess young adult Hookah smokers' attitudes, perceptions and beliefs towards their choice of smoking and to identify predictors of Hookah smoking. The study revealed that the majority of subjects (57%) believe that Hookah smoking was not harmful to one's health. Subjects (n=91) had a mean age of 24$\pm$3 years and 35% were young women. The widespread popular belief was that Hookah smoke becomes detoxified as it passes through water. This belief was found to be an independent predictor of heavy smoking ($\geq$ 3 times per week) verses light Hookah smoking (Rezk-Hanna, O'Connell, Woo, 2014).

Remarkably, the majority of Hookah users are convinced about their ability to quit smoking; most believe Hookah tobacco smoking is less addictive, less harmful and delivers less nicotine than cigarettes. For example; among a sample of 241 university student Hookah smokers at a large U.S. Midwestern university (54% women), 58.9% perceived Hookah to be less addictive and 31.5% perceived it to be less harmful than

cigarette smoking (Noonan, Patrick, 2013). In a sample of 86 university students who are Hookah smokers, 89.5% were convinced about their ability to quit Hookah smoking anytime and 89.5% believed Hookah smoking to be less addictive than cigarette smoking (Asfar, Ward, Eissenberg & Maziak, 2005). Similarly, among 201 university students Hookah smokers, 80% were confident in their ability to quit and 87% did not believe they were dependent on Hookah. In addition, 67% believed that Hookah smoking was less harmful, 79.2% less addictive, and 65.5% delivered less nicotine than cigarettes (Smith-Simone, Maziak, Ward, Eissenberg, 2007).

Multiple factors have been associated with Hookah smoking. In a study by Maziak, Ward, Soweid, Eissenberg (2004), female study subjects perceive Hookah use more positively than cigarette smoking. They noted its highly positive-related attributes of being familiar, traditional, and being social (Maziak, Ward, Soweid, Eissenberg, 2004). Positive correlates of current Hookah use included: male gender, freshman class, cigarette smoking, alcohol or marijuana use, perceiving Hookah smoking to be less harmful than regular cigarettes, and having a commercial waterpipe venue near campus (Sutfin et al., 2011). It was also estimated that 21% of Hookah smokers perceived harmfulness of secondhand smoke from Hookah exposure (Jamil, Elsouhag, Hiller, Arnetz & Arnetz, 2010). The vast majority of Hookah smokers appear to be less knowledgeable about the demonstrated harmful effects of Hookah smoking with the widespread popular belief that Hookah smoke becomes detoxified as it passes through water (Amin, Amr, Zaza & Suleman 2010; Aljarrah, Ababneh & Al-Delaimy, 2009). In contrast to these misconceptions, the dangers of Hookah smoking are supported by

current literature demonstrating enormous exposure to not only nicotine and tar but also high levels of carbon-rich nanoparticles, CO and other harmful chemicals.

State of the Science:

Nicotine, Charcoal and Carbon-Rich Nanoparticles

A Hookah is not only an efficient delivery system for nicotine but also for harmful chemicals and carbon-rich particulates from the burning charcoal and Hookah tobacco. In comparison to cigarettes, Hookah smoking exposes the smoker to far more smoke over a much longer period of time. It has been estimated that cigarette smokers typically take 8-12, 40-75 milliliter puffs and inhale 0.5 to 0.6 liter of smoke over 5-7 minutes (Schubert, Bewersdorff, Luch, Schulz, 2012). In contrast, Hookah smokers take approximately 50-200 puffs which range from about 0.15 to 1 liter each with the smoking session lasting anywhere from 30-180 minutes (Shihadeh, Azar, Antonios, Haddad, 2004). Each Hookah session exposure is equivalent to the tar and nicotine in smoking at least 100 cigarettes. Burning charcoal exposes the Hookah smoker to CO, $PM_{2.5}$ and UFP not found in cigarettes. Investigators estimate that exposure to total particulate matter during a single Hookah smoking session is approximately 250-fold higher than from a single cigarette (Schubert et al., 2011; Schubert, Bewersdorff, Luch, Schulz, 2012). It is important to note that these studies used smoking machines, rather than human smokers, to measure levels of exposure. Each smoking session consisted of 171 puffs of 530 ml each and 2.6 seconds duration every 20 seconds (Schubert, Bewersdorff, Luch & Schulz, 2012; Schubert et al., 2011). This may underestimate the true magnitude of the exposure because the typical Hookah smoking session lasts 30

minutes to three hours. Future studies utilizing human smokers to estimate Hookah smoke exposure levels are needed.

The air pollution literature provides ample precedent that fine $PM_{2.5}$, small enough to enter the lung parenchyma, is a major cause of environmental cardiovascular disease and reduced life expectancy (Gold, 2000; Pekkanen et al., 2002; Pope et al., 2004; VanHee et al., 2009; Pope, Ezzati, Dockery, 2009; Brook et al., 2010; Pope et al., 2014). A study conducted to investigate the association between ambient PM and cardiovascular function in Boston residents revealed that exposure to $PM_{2.5}$ with an average concentration of 15.5 $\mu g/m^3$ was associated with decreased vagal tone and reduced heart rate variability (Gold, 2000). The Exposure and Risk Assessment for Fine and Ultrafine Particles in Ambient Air (ULTRA) study revealed that elevations in PM predicted risk for exercise-induced ST-segment depression in subjects with coronary artery disease (Pekkanen et al., 2002).

Environmental studies have found evidence of cardiovascular harm from $PM_{2.5}$, similar to that found in Hookah smoke. A study that included 3,827 participants who underwent cardiac magnetic resonance imaging between 2000 and 2002 found that individuals living within 50 miles of a major roadway had a higher cardiac function-left ventricular mass index associated with $PM_{2.5}$ elevation, indicating chronic vascular end-organ damage from traffic-related environmental exposure (VanHee et al., 2009). Also, in an epidemiological study conducted by Pope et al (2004), $PM_{2.5}$ exposure revealed to be a risk factor for cause-specific cardiovascular disease mortality via mechanisms that include altered cardiac autonomic function, systemic inflammation, and accelerated atherosclerosis (Pope et al., 2004).

Smoke Constituents of a Single Hookah Session

Investigators have compared the quantity and types of ultrafine particle emissions, carcinogenic polyaromatic hydrocarbons (PAH), volatile aldehydes, and CO that result from cigarette and Hookah smoking. According to a research study from Lebanon that utilized smoking machines, a single one hour Hookah smoking session emits in the sidestream smoke approximately four times the carcinogenic PAH, four times the volatile aldehydes, and 30 times the CO of a single cigarette (Daher et al., 2010). The authors concluded that during a 60 minute Hookah smoking session, a smoker emits as much aldehydes and PAH into the immediate environment as do two cigarette smokers, and as much CO as ten cigarette smokers (Daher et al., 2010). Other studies revealed that in comparison to one cigarette, a single Hookah smoking session is associated with a 3.75-fold greater carboxyhemoglobin (COHb), approximately 4 times the carcinogenic PAH and 4 times the volatile aldehydes (Shihadeh, Azar, Antonios, Haddad, 2004; Eissenberg & Shihadeh, 2009; Schubert et al., 2011; Schubert, Bewersdorff, Luch, Schulz, 2012; Cobb, Shihadeh, Weaver, Eissenberg, 2011).

A similar study was conducted to examine the systemic absorption of nicotine, CO, and carcinogens from one Hookah smoking session. Jacob et al (2011) measured expired CO, COHb levels, plasma samples for nicotine concentrations, and analyzed urine samples for the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanol (NNAL) and PAH metabolite biomarker concentrations. The study recruited 16 healthy subjects with a mean age of 22.9 years old. The majority of participants (n=13) were Hookah smokers and did not smoke cigarettes, while three smoked both

Hookah and cigarettes. All subjects were invited to smoke Hookah as desired for 30-60 minutes on a clinical research ward. Expired CO and blood samples were collected at 15, 30, 45, 60, and 90 minutes, and at 2, 3, 4, 6, 8, 12,16 and 24 hours after the time of initiating smoking. Urine was collected from 0–4, 4–8, 8–12, and 12–24 hours after starting smoking. The study revealed that with an average of 39 minutes of Hookah smoking, systemic intake of nicotine was estimated to be 1.8 mg for all Hookah-only smokers and 5.4 mg for mixed tobacco users. Among Hookah only smokers there was a significant correlation between the number of puffs of Hookah taken and the maximal plasma nicotine concentration ($r = 0.59$, $p = 0.03$). The expired CO boost averaged 33.5 ppm, and the mean COHb boost was 6.2% for Hookah only smokers. After Hookah smoking, there was approximately a doubling of all PAH metabolites values on average for 2-naphthol, 2-hydroxyfluorene, and the sum of hydroxyphenanthrenes. The boost in 1-hydroxypyrene was 50% greater than the baseline. Among Hookah-only smokers there was a significant correlation between number of puffs of Hookah taken and the maximal urine 1-hydroxypyrene concentration ($r = 0.59$, $p = 0.045$). The study concluded that absorption of nicotine in amounts comparable to cigarette smoking indicates a potential for addiction, and absorption of significant amounts of carcinogens raises concerns of cancer risk in Hookah smokers (Jacob et al., 2011).

In regards to the remarkable increases in COHb and CO levels, multiple case reports of CO poisoning secondary to Hookah smoking have been reported. Misek and Patte presented a case of a 24 year-old male who worked at a Hookah lounge serving and lighting Hookah for customers. After working, he was admitted to the emergency room with severe CO poisoning. Initially unconscious, the patient's COHb level was

found to be 33.8% (a normal level is <1.5%) (Misek, Patte, 2014). Another study revealed similar results with a 16 year old female who smoked Hookah for approximately three hours before reporting to the emergency room with symptoms of headache, dizziness, nausea, and weakness, followed by syncope. Her COHb level was 24% which resolved following treatment with 100% oxygen via a non-rebreather mask (La Fauci, G., Weiser, Steiner, Shavit, 2014). Other case studies revealed similar CO poisoning cases in adolescents and young adults resulting from Hookah smoking with COHb levels between 24-32.7% (Ozkan, Ozturk, Ozmen, Durukan, 2013; Cavus, Rehber, Ozeke, Ilkay, 2010; Lim, Lim, Seow, 2009; Uyanik, Arslan, Akay, Ercelik, Tez, 2011).

Studies conducted to provide laboratory comparison of the toxicant exposure related to Hookah and cigarette tobacco smoking concluded similar results. According to Eissenberg and Shihadeh (2009), participants (N=31) completed a crossover study in which they each smoked Hookah for a maximum of 45 minutes or a single cigarette. Outcomes measures included expired air CO five minutes post smoking session, blood COHb, plasma nicotine, heart rate, and puff topography. The study revealed that in comparison to cigarettes, CO increased by 23.9 ppm for Hookah (SD=19.8) verses 2.7 ppm for cigarettes (SD=1.8). In parallel, Hookah tobacco peak COHb levels (SD=2.5) were three times higher than with cigarettes and mean total puff volume was 48.6 liters for Hookah in comparison to 1.0 liter for cigarettes (Eissenberg, Shihadeh, 2009).

In addition to the noted detrimental Hookah smoke components, it has been concluded that Hookah smokers have impairment effects on blood cells. According to Cobb et al. (2011), Hookah smoke is associated with 3.75-fold greater COHb and 56-

fold greater inhaled smoke volume than cigarette smoke. A total of 54 individuals who reported Hookah and cigarette smoking completed two, 45-minute, counter-balanced sessions in which they completed a Hookah smoking episode (mean smoking time = 43.3 min) or a cigarette (mean = 6.1 min). Outcomes measured included plasma nicotine, COHb and subjective effects including information on predicting dependence potential. The investigators concluded that mean peak COHb concentration of Hookah was 4.5% ± 0.3% compared to cigarettes which was 1.2% ± 0.1%. However, it was also revealed that relative to cigarette smoking, Hookah smoking was associated with similar peak nicotine exposure (Cobb, Shihadeh, Weaver, Eissenberg, 2011).

Carcinogen exposure is another major concern with Hookah smoking. In a cross-over study to assess daily nicotine and carcinogen exposure with Hookah and cigarette smoking, researchers found that in comparison to cigarettes, exposure to benzene was 2.5 times higher (Jacob et al., 2013). This study is supported by recent research documenting significant uptake of benzene among Hookah smokers (Kassem et al., 2014). Benzene is a well-known human hematotoxicant that is associated with increased risk of cancers, in particular acute myelogenous leukemia (Rinsky et al., 1987; Hayes et al., 1997; Hayes, Songnian, Dosemeci, Linet, 2001). It is classified as a Group 1 carcinogen with no safe level of exposure to humans (WHO, 2010; International Agency for Research on Cancer (IARC), 1987; U.S. National Toxicology Program, 2011). The literature has systematically identified benzene as a predominant aromatic compound emitted from glowing charcoal (Olsson, Petersson, 2003; Barrefors, Petersson, 1993; IARC, 2012*;* National Institute for Occupational Safety and Health, 2009). Since Hookah is the only form of tobacco use that involves the use of burning

charcoal and while the longitudinal, chronic effects of Hookah smoking are unknown, future studies are needed.

The data presented in this section demonstrates Hookah smoking to be the new explosive, yet understudied worldwide epidemic of tobacco abuse, particularly among young adults. The data presented also provide a comprehensive overview of Hookah smoke's significant overall exposure that includes not only tar and nicotine, but also large amounts of CO, fine PM and other toxic emissions. By virtue of the significant exposure to toxicants, Hookah smoking is likely to be a much more potent cardiovascular stimulant and vascular toxin, than other forms of tobacco use, and emerging evidence supports this assumption.

A Potent Cardiovascular Stimulant

Far less is known about the acute cardiovascular effects of Hookah smoking than cigarette smoking. Clinical studies conducted on cigarette smoking suggest that components of smoke other than nicotine are the most important causes of cardiovascular events (Benowitz, 1997). Cigarette smoking transiently increases blood pressure and heart rate with the acute pressor response being mediated by both neural and vascular mechanisms. Neural mechanisms include sympathetic vasoconstriction through the release of catecholamines epinephrine and norepinephrine (Narkiewicz et al., 1998; Grassi et al., 1994; Haass, Kubler, 1997). The catecholamine release has important effects on both cardiac function as well as vascular tone. Vascular mechanisms include inflammation, oxidative stress, endothelial dysfunction and vascular stiffening (Cai & Harrison, 2000; Powell, 1998; Rhee, Na, Kim, Lee, Kim, 2007;

Jatoi, Jerrard-Dunne, Feely, Mahmud, 2007; Lekakis et al., 1997; Ijzerman, Serne, van Weissenbruch, de Jongh, Stehouwer, 2003).

As nicotine is absorbed through the systemic circulation, the consequent release of catecholamines binding with alpha-1 adrenergic receptors on vascular smooth muscles results in vasoconstriction. In a healthy endothelium, this vasoconstriction response is counterbalanced by shear stress causing local release of nitric oxide (NO), which is produced by endothelial nitric oxide synthase (eNOS). NO, a lipid-soluble molecule, formerly known as the endothelial derived relaxing factor, promotes smooth muscle relaxation, which is critical to the maintenance of normal vascular tone in healthy vessels. Cigarette smoking increases eNOS acetylation and expression while decreasing and uncoupling eNOS activity (Barua, Ambrose, Srivastava, DeVoe, Eales-Reynolds, 2003; Jaimes, DeMaster, Tian, Raij, 2004; Arunachalam, Yao, Sundar, Caito, Rahman, 2010). Uncoupling eNOS results in the production of superoxide anion, which causes disruption of the endothelial production of NO, therefore resulting in a decrease in vasodilatory function in both large and small vessels. These changes occur acutely, even after smoking a single cigarette and are considered to be the earliest injurious manifestations of endothelial dysfunction (Powell, 1998; Rhee, Na, Kim, Lee, Kim, 2007; Jatoi, Jerrard-Dunne, Feely, Mahmud, 2007; Lekakis et al., 1997). In addition to endothelial dysfunction, cigarette smoking has been shown to acutely evoke arterial stiffness resulting in a faster pressure wave traveling through the vasculature and a higher pulse wave velocity (Rhee, Na, Kim, Lee, Kim, 2007; Jatoi, Jerrard-Dunne, Feely, Mahmud, 2007).

Initial studies conducted in the Middle-East support the hypothesis that Hookah is a potent cardiovascular stimulant. A research study done to assess the acute effects of Hookah smoking on the cardiovascular and respiratory systems revealed Hookah smoking to have significant stimulant effects. Participants (n=202 males) aged 17 years or older were recruited and blood pressure, heart rate and respiratory rate values of each participant, before and after a 30 minute Hookah smoking session were measured. The study revealed significant increases in systolic blood pressure by 16 ± 1 mmHg, diastolic blood pressure by 2 ± 0.7 mmHg, heart rate by 6.30 ± 0.60 beats per minute and respiratory rate by 2 ± 2 breathes per minute ($p < 0.001$) (Shaikh, Vijayaraghavan, Sulaiman, Kazi, Shafi, 2008). A second recent cross-sectional study found similar results. Hookah smokers (n=61; 49 males and 12 females) ages 18-25 years of age were recruited from six Hookah cafes. Participants smoked Hookah for a period between 45-90 minutes and pre- and post-smoking blood pressure, heart rate and CO levels were measured. The study revealed a significant increase in mean arterial blood pressure by +12 mmHg and heart rate by +14 beat/ minute ($p < 0.001$). The investigators reported that even though CO increased from an average of 3 to 35 ppm ($p < 0.001$), correlation analysis showed no relationship between CO and the other indices measured. Due to the weak correlation, it was concluded that CO levels did not contribute to the significant increases in all other variables measured (Kadhum, Jaffery, Haq, Bacon, Madden, 2014).

A third, related research study, conducted in Israel, revealed comparable results. The prospective study aimed at evaluating the acute effects of a single 30-minute Hookah smoking session on COHb levels, pulmonary function test results, vital signs,

fractional exhaled NO levels, and exhaled breath condensate cytokine levels in participants smoking in an outpatient setting on an open-air balcony. Hookah tobacco was prepared by one of the investigators and the Hookahs were of similar size. In addition, all subjects smoked 10 grams of Hookah flavored from the same brand and tobacco was lit with the same instant-light charcoal disks. Subjects were instructed to smoke at their own regular pace and pattern. After assessing 45 participants (30 men, 15 women), aged 32.35 ± 15.33 years, the study revealed that smoking a single 30 minute Hookah smoking session resulted in a statistically significant increase in COHb levels from 1.47% ± 0.57% to 9.47% ± 5.52%. Systolic and diastolic blood pressure levels increased from 119±12 to 132±18 mmHg and 75±8 to 83±12 mmHg. In addition, heart rate increased from 80 to 96±17 beats per minute and respiratory rates increased from 14±2 to 17±2 beats per minute ($P < .001$). It was also noted that forced expiratory flow decreased between 25% and 75% of Forced Expiratory Volume, peak expiratory flow rate, fractional exhaled NO levels, percentage of eosinophils in peripheral blood, and 8-isoprostane levels in exhaled breath condensate. (Hakim, Hellou, Goldbart, Katz, Bentur, Bentur, 2011).

A fourth study by Shafagoj & Mohammed (2002) reported similar results. The study involved 18 healthy habitual Hookah smokers who were invited to smoke a single 45 minutes Hookah smoking session. Participants were invited to smoke in a student's laboratory at the Department of Physiology at the University of Jordan in Amman. The study revealed that in comparison to baseline, heart rate, systolic blood pressure, diastolic blood pressure and mean arterial blood pressure increased by 16±2.4 beats per minute, 6.7±2.5 mm Hg, 4.4±1.6 mm Hg and 5.2±1.7 mm Hg respectively ($P<.05$).

Although all four studies presented reveal comparable results, it is important to note that having Hookah studies conducted at an outpatient setting on an open-air balcony may underestimate the magnitude of smoke exposure. This is because many Hookah lounges or cafes do not have an open-door policy or proper ventilation systems. In addition, the type of charcoal used during the smoking session affects the tobacco combustion product and may directly relate to the extent of toxin exposure. Future studies assessing Hookah smoke exposure using different charcoal types are needed.

Other studies that were done to evaluate the acute effects of Hookah smoking on heart rate, blood pressure and baroreflex control of heart rate, reported remarkable results. In 20 healthy, chronic Hookah smokers (20 men, 27 $\pm$ 6 years (mean $\pm$ S.D.), Hookah smoking evoked an acute increase in systolic blood pressure (from 110±13 to 123±12 mmHg; p value 0.004) and diastolic blood pressure (67±11 to 81±11 mmHg; p value 0.0002). Importantly, this study demonstrates that Hookah smoking markedly impaired baroreflex control of the heart rate causing a significant decrease in its baroreflex sensitivity (from 9.16±4 to 5.67±3 ms/mm Hg; p value 0.003) (Al-Kubati, Al-Kubati, Al Absi, Fiser, 2006). Arterial baroreflexes have a pervasive influence throughout the entire body and provide a crucial role in defending homeostasis. For example, to prevent large fluctuations in blood pressure, arterial baroreceptors as well as afferent nerves found on the aortic arch and carotid sinus send inhibitory signals to the brain that decrease central sympathetic efferent nerve outflow and increase vagal outflow (Benarroch, 2008).

The results of this study are supported by Cobb et al. (2012) who also demonstrated that Hookah smoking alters the balance of the autonomic nervous system.

Importantly, the investigators revealed that not only does Hookah smoking cause a temporary decrease in heart rate variability but also that smoking tobacco-free Hookah eliminates the increase in blood pressure and heart rate but produces even greater decrease in heart rate variability (Cobb, Sahmarani, Eissenberg, Shihadeh, 2012). Using a 32 participant cross-over study design, the study included two smoking groups; Hookah-tobacco and Hookah tobacco-free and outcome measures were done before and after a 45-minute Hookah smoking session in both groups. Outcome measures included comparison in heart rate variability measures, exhaled breath CO, plasma nicotine, and puff topography. Heart rate variability was analyzed through spectral analysis of the beat-to-beat changes in heart rate, which are thought to be indicators of autonomic function. High frequency (HF) power which is believed to be a measure of efferent cardiac parasympathetic activity, low frequency (LF) which is thought to represent sympathetic activity along with the ratio of low-frequency to high-frequency (LF/HF) power were measured. The study revealed that relative to baseline, LF and LF/HF increased significantly for both tobacco-based and tobacco-free Hookah products by the end of the 45-minute smoking session (all $p < 0.01$). HF did not change significantly by condition or time. In addition, plasma nicotine, blood pressure, and heart rate increased only with the smoking Hookah tobacco ($p < 0.01$). The study also revealed that the level of CO increased in both groups ($p < 0.01$). (Cobb, Sahmarani, Eissenberg, Shihadeh, 2012). This study adds to the limited body of knowledge that even smoking a non-tobacco Hookah product reduces heart rate variability, supporting the concept that PM and other toxic chemicals, not just the nicotine, interact adversely with the cardiovascular system specifically the autonomic nervous system

Sibai et al (2014) conducted a retrospective study to examine the association between Hookah smoking and coronary artery disease confirmed by angiography. The study included a total of 1210 individuals, ages 40 years and older who had no history of smoking-associated diseases or history of cardiovascular procedures and who were admitted for coronary angiography. The authors summarized the extent of coronary artery disease as diseased (≥50% and ≥70% occlusion in at least one main coronary artery) and non-diseased (entirely normal coronaries). In addition, a score of Hookah-years, capturing intensity and lifetime duration of exposure, was estimated for each individual based on Duke Coronary Artery Disease Prognostic Index. The authors reported that a lifetime exposure exceeding 40 Hookah-years was associated with a threefold significant increase in the odds of having severe stenosis (≥70%) compared to non-smokers (OR = 2.94, 95% CI 1.04-8.33) as well as with the CAD Index (β = 7.835, p-value = 0.027). A dose-response relationship between Hookah-years and percent stenosis was established (Sibai et al, 2014).

Tolerance, a well-known phenomenon in smoking-exposure studies, should be considered in the study of Hookah. It is defined as a state in which repeated exposure to smoking results in lowered responses, or increased doses of smoke are required to achieve similar effects as observed with the first dose. This is specifically important to the proposed dissertation because the population proposed are habitual Hookah smokers. One may argue that tolerance could reduce the acute effects of Hookah smoking in habitual smokers. In fact, a large body of the literature reveals that tolerance to the effects of nicotine develops rapidly. A study conducted by Benowitz (1982) showed that a constant intravenous infusion of nicotine increased heart rate even

though nicotine levels in the blood were relatively low. As the infusion continued, heart rate reached a plateau, despite a progressive rise in nicotine blood levels (Benowitz, Jacob, Jones, Rosenberg, 1982). This initial study was supported by other experiments demonstrating that smokers obtain similar cardiovascular effects from each subsequent nicotine exposure (Perkins et al., 1995; Perkins et al., 1994). Also, analysis of the acceleration of the heart rate indicates a half-life of development and regression in tolerance of 35 minutes (Benowitz, 1988). These observations explain why the heart rate in smokers increases most with the first few cigarettes of the day but does not vary thereafter in relation to the amount of nicotine consumed. Importantly, this literature provide key data revealing that nicotine's biological effects are characterized by a rapid onset of tolerance and this aspect of tolerance to nicotine does not dissipate over time.

Based on the scant emerging research studies presented in this section, Hookah smoking constitutes a potent cardiovascular stimulant. More studies are needed to document and confirm the magnitude of Hookah smoking on cardiovascular function. In addition, future studies are needed to dissect the relative contribution of nicotine from fine PM or other toxic substances from burning charcoal in mediating the noted cardiovascular response of Hookah smoking.

Endothelial and Vascular Dysfunction

Whereas the literature is replete on endothelial dysfunction effects of cigarette smoking, nothing is published about the acute effects of Hookah smoking on endothelial and vascular function. The endothelium plays a central part in regulating vascular tone and function. Endothelial dysfunction is considered to be an important early phenomenon in atherogenesis that precede plaque formation and an independent

predictor of vascular disease events (Schachinger, Britten, Zeiher, 2000; Celermajer et al., 1992; Egashira et al., 1993; Shinozaki et al., 2001; Balletshofer, et al., 2000; Clarkson, et al., 1996; Gaenzer, et al., 2002; Makimattila et al., 1996; Makimattila et al., 1997). Through several decades of cigarette research, investigators have concluded that cigarette smoking is an important, modifiable risk factor for the development of atherosclerosis in cerebral, coronary, and peripheral arteries (Toborek, Kaiser, 1999; Celermajer et al., 1996; Lekakis et al., 1997). Indeed, smoking a single cigarette has been shown repeatedly to cause endothelial dysfunction (Neunteufl et al., 2002; Amato at al., 2013; Papamichael at al., 2004; Lekakis et al., 1997). Identifying the presence of endothelial dysfunction provides a number of essential clinical implications related to the development of a variety of pathological conditions, such as peripheral atherosclerosis and coronary artery disease, and their known risk factors, such as hypercholesterolemia, hypertension, diabetes, and insulin resistance (Celermajer et al., 1992; Egashira et al., 1993; Shinozaki et al., 2001; Balletshofer, et al., 2000; Clarkson, et al., 1996; Gaenzer, et al., 2002; Makimattila et al., 1996; Makimattila et al., 1997).

To date, only two studies have been published that assess the effects of Hookah smoking on endothelial function. While the first study evaluated in-vitro effects of mainstream Hookah smoke condensate and the second focused on the chronic effects, both studies concluded that Hookah smoking is a potential risk factor for endothelial cell dysfunction. The first study assessed changes in cell viability, reactive oxygen species generation, inflammatory and vasodilatory markers and in vitro angiogenesis of human aortic endothelial cells in response to Hookah smoke condensate exposure. In addition, researchers assayed for impaired endothelium-dependent vasodilation and induced

inflammation by studying the effect of Hookah smoke on the content and activity of adenosine monophosphate-activated protein kinase, eNOS proteins and NF-κB p65 ser536 phosphorylation. Compared to control, Hookah smoke condensate induced cell cycle arrest, apoptosis, oxidative stress and inflammation as well as reduced the motility and inhibited angiogenic potential of human aortic endothelial cells (Rammah, Dandachi, Salman, Shihadeh, El-Sabban, 2013). Although smoking was generated using a laboratory machine, this study offers compelling in-vitro evidence of the potential toxic effects of Hookah smoke condensate on endothelial cells

The second study aimed to investigate the chronic effects of Hookah smoke on endothelial function compared to cigarettes in asymptomatic young adults using brachial artery ultrasound imaging. The authors compared flow mediated dilation measurements in a Hookah smoking group (n=30), cigarette smoking group (n=30) and a non-smoking group (n=10). Results of this study should be considered cautiously because it was observational in design and included only a one-time measure of flow-mediated dilation. Nonetheless, it revealed that flow-mediated dilation measurements were significantly impaired among the Hookah smoking group in comparison to the cigarette and non-smoking group (7.9 ± 3.8% vs. 12 ± 3.4% and 21.5 ± 2.5% respectively $p<0.001$). The investigators concluded that Hookah smoking has a more hazardous effect on brachial artery endothelial-dependent flow mediated vasodilation than cigarette smoking (Selim, Elia, El Bohey, El Meniawy, 2013).

In regards to the topic of tolerance, endothelial dysfunction and tobacco smoking, initial evidence suggests that, unlike changes in heart rate, acute endothelial changes are not attenuated with repeated exposure. Lekakis et al (1998) conducted a study to

investigate the possible development of tolerance produced by repeated nicotine exposure and the duration of the effects of acute cigarette smoking on the endothelium. The study revealed that acute endothelial dysfunction from cigarette smoking is maintained for 60 minutes after smoking. Most importantly, endothelial dysfunction induced by the second cigarette smoking was similar in magnitude and duration with that observed during the first exposure to nicotine (Lekakis et al., 1998). Since this is the only study to investigate tolerance to endothelial change and since the study involved cigarette smoking only, findings should be interpreted cautiously. Further study is needed to determine how endothelial function is influenced over time by Hookah smoking.

Summary

In summary, based on the presented literature review, the cardiac risks of Hookah smoking have not been systematically studied. Considering the widespread and increasing use, particularly among young adults, in addition to the lack of policy regulation, the presence of nicotine, tar, CO and a significant amount of PM exposure, coupled with reports that even low exposures to $PM_{2.5}$ confer significant cardiac risk, physiological studies of the acute vascular and endothelial function effects of Hookah smoking in humans are needed. Therefore, the key unanswered question that was addressed in my doctoral dissertation was whether the scant but compelling literature could be translated to create a new conceptual framework for understanding the acute effects of Hookah smoke on the young adult human cardiovascular system.

CHAPTER 3

Theoretical Underpinnings and Conceptual Framework

General Adaptation Syndrome

The hypothesized relationships between Hookah smoking and the proposed study aims were formulated from the General Adaptation Syndrome (GAS) Theory introduced by Hans Selye (Selye, 1951). The theory helped formulate holistic (macroscopic) as well as molecular (microscopic) pathways underpinning the proposed dissertation study. In regards to the macroscopic, holistic pathway, the GAS focuses on identifying physiologic processes and changes that occur related to mental or physical some type of stresses. Selye's theoretical propositions have been cited by many professionals as scientific bases for both nursing and medical theories (Okonta, 2012; Oberg, 2009; Smith, 2000; Sharp, 1996; Murphy, 1993). In addition, the GAS empirical model has helped shape practice, science and clinical outcomes and revealed an important and crucial relationship between stressors and the evolution of physiological diseases (Selye, 1951).

According to Selye (1951), the GAS is a three stage model that forms an integrated holistic syndrome of interconnected adaptive responses to stress. The three stages include the alarm reaction, the stage of resistance, and the final stage of exhaustion (Selye, 1951). The theory suggests that during the alarm phase, when a stressor is introduced to the body, the body attempts to compensate by implementing the "fight or flight" response to maintain optimal function and homeostasis (Selye, 1978, p.160). This response is triggered by the sympathetic nervous system which results in; activating the secretion of stress hormones (adrenaline and noradrenaline), triggering

the increase in heart rate, diverting blood to skeletal muscle with the end goal of preparing the body for either the “fight or flight” response.

During the second stage, the stage of resistance, the body strives to adapt to the stressor by implementing changes in order to decrease the effect of the stressor and maintain homeostasis. The majorities of the characteristic manifestations that occur during the alarm phase disappear or are reversed during the stage of resistance. Body changes often involve multiple body systems. However, Selye highlights and focuses primarily on the autonomic nervous system and the endocrine system.

In the GAS, Seyle proposes further that if the stressor persists or is repeated overtime, then the body’s energy gets depleted, which is outlined during the final stage of exhaustion. As a result, pathological adaptations and diseases, which can be life-threatening (e.g., heart attack, stroke, cancer), take place as the body gets overwhelmed with the exposure of the stressor (Selye, 1978).

Application of General Adaptation Theory to Hookah Smoking

According to current research, Hookah smoking has been revealed to be a negative stressor to the body contributing to several toxic physiological changes (Mohammad, Kakah, Mohammad, 2008; Shaikh, Vijayaraghavan, Sulaiman, Kazi, Shafi, 2008; Al-Kubati, Al-Kubati, Al Absi, Fiser, 2006; Kiter, Ucan, Ceylan, Kilinc, 2000; Aydin et al., 2004; Hakim, Hellou, Goldbart, Katz, Bentur, Bentur, 2011). Selye (1973) stated that each stressor produces in the body certain physiological responses that yield a predictable, observable biological pattern as the body tries to maintain homeostasis. He concluded by stating that every human body has a restricted supply of adaptive energy to deal with stressors and the mount often declines with chronic exposures to the same

stressor (Selye, 1951). For the purpose of the dissertation project, the GAS served as a useful paradigm and an organizing theoretical framework for identifying the physiological effects of Hookah smoking on the human cardiovascular system. Because the dissertation study focused on acute vasodilatory responses initiated during a single Hookah smoking experience, the following discussion focuses on the first stage of the GAS, the alarm phase.

Alarm Phase

The hypothesis for the study was that the alarm phase of the GAS is initiated with the introduction of the physical stressor (Hookah smoking) to the body in general and to the vascular system in particular. The body attempts to compensate by stimulating the sympathetic nervous system with the consequent release of the catecholamines epinephrine and norepinephrine. Through binding with alpha-1 adrenergic receptors on vascular smooth muscles, these catecholamines trigger cardiovascular responses, which result in muscle contraction and vasoconstriction and increased cardiac contractility, heart rate and blood pressure. Vascular effects was empirically evident through acute functional changes seen throughout the body's endothelium -- from the most distal microvessels to the large conduit vessels. Based on a review of the literature, I hypothesized that each session of Hookah smoking would pose an immediate threat to the blood vessel wall as evidenced by acute diffuse deterioration in endothelial-dependent dilation throughout the vascular tree, with an acute stiffening of the central aorta.

The molecular (microscopic) underpinning of the hypothesized Hookah smoking-induced endothelial dysfunction was assessed through the eNOS pathway (**Figure 2**).

Endothelium-dependent vasodilation is, to a great extent, maintained by NO. NO, a lipid-soluble molecule with a half-life of a few seconds is crucial for the vasculature and eNOS is responsible for most of its production. In a healthy endothelium, eNOS metabolizes the substrate l-arginine to produce l-citrulline and NO. This process requires the presence of l-arginine and tetrahydrobiopterin (BH_4), a critical cofactor for NO production by eNOS. After production, NO is released into the bloodstream to inhibit platelet aggregation and the release of vasoconstricting factors such as serotonin and thromboxane. Additionally, NO diffuses into the media and dilates blood vessels by stimulating soluble guanylyl cyclase and increasing cyclic guanosine monophosphate in smooth muscle cells (Forstermann et al., 1994; Munzel et al., 2008). Derangements in the eNOS pathway represent the most important cause for reduced NO bioavailability. In cardiovascular disease risk factors, such as hypertension, atherosclerosis, or diabetes mellitus, production of reactive oxygen species in the vascular wall is increased and NO bioavailability is decreased, which is implicated in initiation and evolution of the disease process. It is hypothesized that hookah smoking acutely reduces NO bioavailability through the process of oxidative stress and generation of reactive oxygen species (**Figure 3**).

Oxidative stress and generation of reactive oxygen species will be explored in the future to investigate the mechanism underlying Hookah smoking-induced endothelial dysfunction. Vascular oxidative stress is a state resulting from excessive vascular reactive oxygen species production and either unchanged or reduced antioxidant defenses. In oxidative stress, excessive reactive oxygen species are mediated primarily by a combination of increased activity of the oxidant enzyme,

nicotinamide adenine dinucleotide phosphate oxidase, and eNOS uncoupling (Paravicini, Touyz, 2008; Mueller at al., 2005). Reduced antioxidant defenses may be related to the down-regulation of endogenous antioxidant enzymes, such as the main vascular superoxide neutralizing enzyme, superoxide dismutase (Kacmaz, Ozturk, Çete, Kavutcu, Durak, 1997).

The excessive availability of vascular reactive oxygen species such as superoxide impairs NO-dependent endothelial dilation by reacting with NO to form peroxynitrite (ONOO), thus directly reducing the bioavailability of NO in both large and small vessels. In addition, ONOO which is a potent reactive oxygen species readily oxidizes BH_4 to its inactive form (BH_2), causing eNOS to become "uncoupled", a state in which the enzyme produces more superoxide and less NO in a vicious cycle. As a result, oxidative injury to the endothelium occurs, which is hypothesized to be manifested by the acute diffuse deterioration in endothelial-dependent dilation and vasomotor tone from Hookah smoking, proposed to be studied through this dissertation.

Resistance Phase

After the Hookah smoking session and during the second stage of the GAS (resistance), adaptation will be attempted through maintaining homeostasis and the increased variables during the alarm phase will slowly return to normal levels. It is unknown how long it may take for the hypothesized stimulant effects of Hookah smoking to return to normal levels. According to Rhee et al (2007), who tested the acute effects before and 5, 10, and 15 minutes after smoking one cigarette, heart rate and blood pressure appeared to be declining but nowhere back to baseline by 15 minutes (Rhee, Na, Kim, Lee, Kim, 2007). By virtue of the significant exposure to toxicants and

since Hookah smoking is hypothesized to be a much more potent vascular toxin, it may take longer for the effects to return to normal levels. Future studies are needed to investigate recovery measures and the magnitude of the insult caused by Hookah smoking on cardiovascular function.

Exhaustion Phase

As previously described, the literature clearly demonstrates that: (1) endothelial dysfunction is an important early phenomenon in atherogenesis (Celermajer et al., 1992; Egashira et al., 1993; Shinozaki et al., 2001; Balletshofer, et al., 2000; Clarkson, et al., 1996; Gaenzer, et al., 2002; Makimattila et al., 1996; Makimattila et al., 1997) and; (2) increased arterial stiffness is recognized as an independent predictor of all-cause and cardiovascular mortality (Laurent et al., 2001; Blacher et alk., 1999). Over time, repeated exposure of young blood vessels to this toxic smoke could set the stage for premature vascular aging with atherosclerosis and systolic hypertension, thereby increasing the risk of myocardial infarction, peripheral artery disease, stroke, and death. This will be evident through the exhaustion phase, which constitutes sustained and chronic changes in endothelial vascular function resulting from Hookah smoking. Future longitudinal studies based on the initial findings of this study will be done to identify physiological and chronic structural changes that may take place as the body fails to compensate for the negative effects of Hookah smoking. Fortunately, the literature clearly suggests that the increased mortality from cardiovascular disease declines after cigarette smoking cessation (Armstrong et al., 2014; Bakhru, Erlingerm, 2005; Clair et al., 2013) providing further evidence in support of the health education and benefits of smoking cessation.

Summary

The value of utilizing the GAS concept for the proposed research is that it will serve as a guide in identifying the physiological endothelial and vascular toxicity effects of Hookah smoking. Given the gaps in knowledge about Hookah smoking and the unknown cardiovascular toxicity effects on human health, using the GAS to guide this study will inform interpretation of findings related to Hookah smoking as a cause of acute endothelial dysfunction on the young adult human cardiovascular system. The hypothesis of the proposed study is that Hookah smoking evokes profound acute endothelial dysfunction involving the entire vascular tree from the most distal microvessels to the large conduit vessels. This endothelial dysfunction is directly related to a reduction in NO bioavailability. The proposed dissertation study will provide initial foundational data for future studies designed to explore mechanistic underpinnings of Hookah smoking-induced endothelial dysfunction, including the eNOS pathway and carbon-rich nanoparticles effects on cardiovascular toxicity that are distinct from those of CO, tar or nicotine.

CHAPTER 4

Research Methodology

Purpose

The purpose of this study is to determine the acute effects of Hookah smoking on endothelial and vascular function.

Hypotheses

Based on a review of the literature and the application of GAS theory, I hypothesize that each Hookah smoking session evokes profound acute endothelial dysfunction in young adults who are regular Hookah smokers. A further hypothesis is that the endothelial dysfunction involves the entire vascular tree from the most distal microvessels to the large conduit vessels, which results in a more rapid return of the arterial pulse wave from the peripheral circulation to the central aorta and yields increased aortic systolic pressure (**Figure 4**).

Aim 1: Determine the acute effects of Hookah smoking on large-vessel endothelial vasodilator function as measured by brachial artery flow mediated dilation.

Hypothesis: Hookah smoking evokes acute large vessel endothelial dysfunction evidenced by impaired endothelial-dependent brachial artery vasodilation.

Aim 2: Determine the acute effects of Hookah smoking on micro-vessel endothelial function as measured by reactive hyperemia peripheral arterial tonometry (RH-PAT).

Hypothesis: Hookah smoking evokes acute microvessel endothelial dysfunction evidenced by endothelial-mediated low pulse volume amplitude scores.

*Aim 3***:** Determine the acute effects of Hookah smoking on pulse wave velocity and central aortic stiffness measured by pulse tonometry.

Hypothesis: Hookah smoking evokes an acute increase in vascular stiffness evidenced by a faster carotid-femoral pulse-wave velocity and central aortic systolic pressure augmentation.

Design

The proposed study is an experimental study with a pre-test/post-test design. There are three aims with the following primary endpoints (underlined): Aim 1 - To determine the acute effects of Hookah smoking on large-vessel endothelial vasodilator function as measured by brachial artery flow mediated dilation (FMD); Aim 2 - To determine the acute effects of Hookah smoking on micro-vessel endothelial function as measured by pulse volume amplitude scores; Aim 3 - To determine the acute effects of Hookah smoking on pulse wave velocity and central aortic pressure measured by pulse tonometry.

Operational Definitions. Definitions for each variable of interest are as follows:

Hookah smoking: A 30-minute Hookah smoking session using a standard charcoal-heated waterpipe prepared with 12.5 grams of flavored Hookah tobacco. Two natural charcoal cubes (20 x 20 mm) by CocoNara were be used to heat the tobacco. Hookah flavored tobacco by Starbuzz manufacture with a nicotine level of 0.05% was used.

Flow-Mediated Dilation (FMD): Endothelium-dependent process facilitating the relaxation of the brachial artery in response to increased shear stress. FMD reflects NO release in large-vessels in response to reactive hyperemia. FMD is reported as absolute (mmΔ) and percent (%Δ) change between baseline brachial artery diameter and peak

brachial artery diameter and calculated as: FMD (%) = (Peak Diameter – Baseline Diameter) / Baseline Diameter x 100.

Blood Flow: Brachial artery blood flow was calculated as: mean blood velocity (cm/s) x πr^2 x 60; where r is radius.

Baseline Hyperemic Blood Velocity: The average of 15 resting end-diastolic cardiac cycles prior to cuff inflation.

Peak Hyperemic Blood Velocity: The average of the first 15 end-diastolic cardiac cycles immediately post cuff release.

Reactive Hyperemia: The transient increase in organ blood flow that occurs following a brief period of ischaemia (5-minute cuff occlusion) is reported as percent change in flow during hyperemia (in the first 15 seconds after cuff deflation) compared to baseline.

Baseline Brachial Artery Diameter: Brachial artery end-diastolic diameter during rest is calculated as an average of all frames calculated during end diastole within the resting recording period (45 seconds).

Peak Brachial Artery Diameter: Maximal increase in brachial artery end-diastolic diameter after reactive hyperemia and averaged over three consecutive cardiac cycles.

Time to peak diameter: The time interval (in seconds) calculated from cuff deflation to maximum peak brachial artery diameter.

Shear Stress: The frictional force of blood on the arterial wall and the trigger for endothelial release of NO and flow-mediated dilation. Shear stress is directly related to the viscosity and velocity and inversely related to the diameter and: shear stress = viscosity x velocity / diameter. Given our laboratory setting and after an overnight fast,

viscosity could be considered constant and of negligible value. Therefore, shear rate is calculated as: (8 x time averaged peak velocity)/ occlusion diameter, based on a large centered sample volume from the first 15 velocity envelopes following cuff release.

Pulse Volume Amplitude Score: Measurements reflecting NO dependent changes in vascular tone and is the ratio of the average amplitude of the digital pulse wave volume signal over a 1-min time interval starting 1 min after cuff deflation divided by the average amplitude of the digital pulse volume signal of a 3.5-min time period before cuff inflation (baseline).

Pulse Wave Velocity: An established index in assessing central arterial stiffness. It is a direct method, trans-cutaneously calculating the speed of the pressure wave travelling through the carotid and femoral arteries (Laurent S, Cockroft European Heart Journal 2006 Expert consensus on arterial stiffness). It is calculated as: distance (meter) / transit time (second). The distance is defined as the surface distance measured manually between the two sites, and the transient time is measured by the foot-to-foot method of the waveforms, where the foot of the wave is defined at the end of diastole.

Central aortic systolic pressure: Non-invasive central systolic blood pressure calculated through a validated generalized transfer function (proprietary software) to convert radial waveforms – measured by applanation tonometry – to a derived central aortic blood pressure waveform.

Sample and Setting

A sample of 26 research participants were used. Research participants are overtly healthy young adult Hookah smokers. Non-smokers were not studied to avoid the risk of tobacco addiction.

Subject Recruitment

Subjects were recruited both from our earlier descriptive study of attitudes, perceptions, and beliefs we have completed and via advertisements at UCLA college campus. Twenty participants from our previous descriptive study agreed to be contacted for future studies, and we have had at least the same number of responses to our advertisement for pilot studies. To insure a generalizable sample, an equal number of female (n=13) and male (n=13) subjects will be recruited. In regards to ethnicity and based on previous experience, we anticipate that the sample include: 39% White (Non-Hispanic or Latino) (n=10), 15% African American (n=4), 15% Asian (n=4), 15% Hispanic or Latino (n=4), 8% American Indian or Alaska Native (n=2), and 8% Multi-Racial/Multi-Ethnic (n=2).

Inclusion criteria: Research participants who meet the following inclusion criteria will be invited to participate in the study: 1) age 18 to 39 years; 2) no evidence of cardiopulmonary disease, diabetes or dyslipidemia (by self-reported history); 3) BP < 140/90 mmHg; 4) BMI >18.5 or <30 $kg \cdot m^2$; 5) resting heart rate < 100 beats/min; 6) taking no prescription medication; 7) have smoked Hookah at least 12 times in the past 12 months; 8) have not smoked any cigarettes in the past 12 months and smoked <100 cigarettes in their life; 9) end-expiratory CO < 10 ppm prior to study; 10) no history or evidence of psychiatric illness, claustrophobia (by self-reported history).

Exclusion criteria: Research participants will be excluded if they meet any of the following criteria: 1) age younger than 18 or older than 39; 2) any evidence of cardiopulmonary disease, diabetes or dyslipidemia (by self-reported history); 3) systolic BP > 140 mmHg or diastolic BP > 90 mmHg; 4) BMI <18.5 or >30 kg·m2; 5) resting heart rate > 100; 6) taking any prescription medication; 7) does not smoke Hookah at all or did not smoke Hookah at least 12 times in the past 12 months; 8) is a past or current cigarette smoker; 9) exhaled CO > 10 ppm; 10) any history or evidence of psychiatric illness, clusterphobia (by self-reported history).

Setting: The proposed study will take place at Cedars-Sinai Hypertension & Vascular Biology Clinical Research Center (CRC) where there is a Hookah smoking chamber that is custom-built specifically for Hookah tobacco-related research. The chamber is fully functional and has been approved for use in clinical research by the Cedars-Sinai Environmental Health and Safety Office showing that smoke is fully contained within the chamber without escaping to the surrounding room. The construction company completed an air balance report showing that the ceiling exhaust fan met design specifications to continuously clear the smoke from the chamber and recirculate fresh air. The Plexiglass and aluminum smoking chamber has an enclosed procedure chair. A fan within the exhaust system continuously pulls air out through the vent in the ceiling. Multiple air-tight rubber ports on the front and side-panels allow wires and tubing to be connected to recording equipment outside the closed chamber (**Figure 5**).

Research Methods

Following approval of the protocol by the Human Subjects Protection Committee at UCLA and Cedars-Sinai Medical Center and the obtaining of informed consent, subjects will be studied at Cedars-Sinai Hypertension & Vascular Biology Clinical Research Center (CRC) early morning.

Prior to initiation of the study protocol, the following experiments will be conducted to insure reproducibility of measures.

Test-Retest Reproducibility Experiments: To assess and document reproducibility for assessing large-vessel endothelial function, subjects (n=12) will be asked to undergo brachial artery FMD, but not undergo Hookah smoking. The procedure will be performed twice on each arm with at least 20 minutes between measurements. Subjects will be asked to return for a second study visit to obtain reproducibility and validity of this technique as noted by the Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery (Corretti et al., 2002).

Additionally and after initiation of the study protocol the following experiments will be conducted to insure reliability and validity of measures.

Time Control Experiment: In order to confirm that any cardiovascular changes observed with Hookah smoking are directly attributable to the intervention itself (i.e. Hookah smoking), a subset of subjects (n=5) will be asked to undergo a “time-control experiment”. In this study visit, tests will be performed at the same time of the day as in the main study (between 07:00-09:00 am). Subjects will undergo study procedures, but

will not undergo Hookah smoking. Subjects will have the opportunity to participate right before the main study visit (on the same day).

Repeatability Experiment: In order to confirm that any baseline cardiovascular measurements are repeatable and reproducible, a subset of subjects (n=5) will be asked to undergo a “repeatability experiment”. In this separate study visit, subjects will be asked to repeat baseline study procedure measurements. Subjects will have the opportunity to participate in this study visit either before or after the main study visit day.

Study Protocol

Subjects will be instructed to fast for 12 hours and refrain from smoking hookah for at least 72 hours prior to study; compliance will be checked by exhaled Carbon monoxide breathalyzer in the laboratory. Subjects will also be instructed to avoid caffeine, high-fat foods, any vitamins or medications for at least 12 hours before the study and exercise and alcohol for 24 hours. After 15 minutes of rest, all testing will be done between 07:00-09:00 am to avoid diurnal variation in a quiet, temperature controlled environment. Subjects will be asked to smoke Hookah while seated comfortably on a comfortable outpatient procedure chair in the new custom-built Hookah smoking chamber. Subjects will be invited to smoke Hookah for 30 minutes through a standard charcoal-heated waterpipe prepared with 12.5 grams of flavored Hookah tobacco. Two natural charcoal cubes (1 inch by 5/8 inch) by CocoNara will be used to heat the tobacco. Subjects will be given the option to select one of three Hookah flavored tobacco by Starbuzz manufacture (a popular brand used throughout California). All selected Hookah flavored tobacco will have the same nicotine level (0.05%) provided by the manufacture and flavors will be selected based on popularity

among Hookah smokers. To prevent confounding factors, all female subjects will be assessed in a standardized phase of the menstrual cycle (days 1-7, when concentrations of circulating female sex estrogen hormones are lowest).

Instruments

Smoking Exposure Biomarkers: Expired carbon monoxide and plasma nicotine concentrations were measured immediately before and after 30 minutes of hookah smoking to characterize the smoking exposure.

Expired End-tidal Carbon Monoxide measurements will be done with a carbon monoxide meter (Micro Smokerlyzer, Bedfont Scientific Ltd.; Kent, UK).

Plasma nicotine levels measurements will be assayed in the Clinical Pharmacology Laboratory at San Francisco General Hospital by gas chromatography (GC) with nitrogen-phosphorus detection, using 5-methylnicotine and 1-methyl-5-(2-pyridyl)-pyrrolidin-2-one ("*ortho*-cotinine") as internal standards. This method has been modified for simultaneous extraction of nicotine with determination using capillary GC. The limits of quantitation are 1 ng/ml for nicotine (Jacob et al., 1991; Jacob et al., 1981).

Endothelium-Dependent Flow mediated dilation (FMD) measurements will be used to assess brachial artery (large-vessel) endothelial function and will be performed as described originally by Celermajer at al. (1992). The guidelines for FMD determination described by Corretti et al. (2002) will be strictly followed. While subjects are lying supine, the left arm will be adducted at heart level, placed on foam pads to avoid arm and fingers muscular activity, and the brachial artery will be located 3-7 cm above the antecubital crease. The brachial artery will be imaged using a 5-to-12-MHz

linear array transducer attached to a high-resolution ultrasound machine (Toshiba, Xario XG 2000). To ensure the location of the same arterial segment after hookah smoking and during repeated measures on subsequent study days, the arm, hand and head position, along with anatomical landmarks will be noted. Additionally, the distance between the cuff and the probe will be recorded and a thermal print of the arterial image will be taken. Once an optimal B-mode image is obtained, the ultrasound probe will be held stable and clamped to avoid involuntary movements during recordings. A rapid-inflation/deflation pneumatic cuff (Hokanson, Bellevue, WA) will be placed on the upper forearm for five minutes and inflated to suprasystolic pressures (250 mmHg). Doppler velocity will also be measured continuously with an insonation angle of 60°. Baseline diameter and velocity will be recorded for 45 seconds and resumed 30 seconds before cuff deflation and continuously for two minutes after deflation to obtain peak vasodilatory response. Recordings will be triggered and captured at the R-wave of the electrocardiogram (end-diastolic diameter) and stored for offline analysis using an edge detection software (Brachial Analyzer for Research (version 6.3.0), Vascular Imager (Version 6.0.0), Medical Imaging Applications, LLC, Iowa City, IA, USA). Repeatability and reproducibility of this technique indicated an intraclass correlation coefficient (ICC) of 0.10-0.94 (Welsch, Allen, Geaghan, 2002; Malik et al., 2004).

Peripheral Arterial Tonometry measurements will be used to assess small vessels endothelial function. Endo PAT 2000 (Itamar, Medical Ltd) is a device that consists of a finger probe to assess digital volume changes accompanying pulse-waves. A pressure of 40-70mmHg is applied by the probe to the index fingers of both hands to eliminate venous stasis and arterial pulse volume amplitude is recorded. Hyperemia will

be induced by occluding blood flow through the brachial artery for five minutes using an inflatable cuff on one arm. The pulse wave amplitude changes to reactive hyperemia will be calculated using the post and pre occlusion values, which reflects peripheral vascular function and have shown to be NO-dependent (Nohria et al., 2006). Test-retest reliability indicated an ICC coefficient of 0.74-0.78 *(*McCrea, Skulas-Ray, Chow, West, 2012; Selamet et al., 2009). In regards to sensitivity and specificity, the desirable cut-off value below 1.67 yield sensitivity and specificity for the detection of coronary endothelial dysfunction of 80% and 85%, respectively (Bonetti et al., 2004).

Pulse Tonometry measurements will be used to assess central aortic stiffness and carotid-femoral pulse wave velocity. The indices are produced through tonometry, a noninvasive, accurate and reproducible technology (SphygmoCor, ATCOR Medical, Australia) which uses brachial artery blood pressure and a generalized transfer function to convert the radial waveform, measured by applanation tonometry, to a derived central aortic blood pressure waveform. A central aortic blood pressure waveform is the sum of the pressure wave generated by the left ventricle and reflected waves from the peripheral circulation. Central systolic and diastolic blood pressure will be calculated through the validated generalized transfer function (proprietary software).

Pulse wave velocity will be determined by acquiring waveforms, using applanation tonometry, at the carotid and femoral arterial sites with electrocardiogram gating. Velocity (distance/time in m/s) will be calculated by measuring the time interval between electrocardiogram R-wave and the recorded waveforms at each site, whereas distance between sites will be measured manually.

Data Analysis

Sample size. No data from Hookah smoking exist that could inform power calculations. However, in order to obtain pilot data on each of the three specific aims, a proposed total number of 26 subjects will be recruited for the study to detect a medium effect size for the pre-to-post change. Estimated change scores proposed for each of our measurements were obtained from relevant cigarette smoking literature (Powell, 1998; Rhee, Na, Kim, Lee, Kim, 2007; Jatoi, Jerrard-Dunne, Feely, Mahmud, 2007; Lekakis et al., 1997; Hakim, Hellou, Goldbart, Katz, Bentur, Bentur, 2011; Shaikh, Vijayaraghavan, Sulaiman, Kazi, Shafi, 2008). The power analysis is summarized in **Table 1**.

Statistical Analysis of Study Aims. Data will be analyzed using SPSS release 23 for Windows (Chicago, IL). For all analysis, significance will be set at 0.05. Descriptive statistics (mean, standard deviation, median, interquartile range, minimum, maximum and frequency distribution) will be generated for demographic information, smoking and medical history to characterize the study population.

For test-retest reproducibility experiments, simple linear regressions and calculation of ICC will be performed. The agreement between brachial artery FMD indices (FMD%, baseline brachial artery diameter and peak hyperemic blood velocity) of the two scans obtained at different times will be evaluated by Bland-Altman analyses, where the mean differences for the repeated scan will be reported as bias.

Each of the three study aims will be evaluated with similar statistical methods. To review, measured outcomes for each aim are: Aim 1: Brachial artery FMD (indicating large-vessel endothelial vasodilator function); Aim 2: Pulse Volume Amplitude

(indicating micro-vessel endothelial function and obtained by RH-PAT); and Aim 3: PWV (indicating aortic stiffness) and central aortic systolic pressure obtained by pulse tonometry. For each measured outcome, summary statistics will be generated for pre- and post- time points. To evaluate pre- and post-test differences, a paired Student *t*-test will be used for each of the outcome measures (FMD, Pulse Volume Amplitude, PWV, central aortic systolic pressure). If outcome data are not normally distributed, natural log transformations will be conducted and used in the final analysis. To control for their potential effect, demographic data (ex: age, race, gender, BMI, pre-test measures such as duration and frequency of smoking) as well as smoking biomarkers exposures (expired CO and plasma nicotine concentrations) will be included as covariates in a mixed model (random intercept with an unstructured covariance structure) and a separate analysis model will be performed for each outcome measures. Bivariate correlation analysis will be used to assess the relationship between the four main study variables. Because shear rate is proposed to be the most important stimulus for FMD (Pyke, Dwyer, Tschakovsky, 2004) and may vary among subjects, we will control FMD for this covariate through an analysis of covariance (ANCOVA) as well as a linear stepwise regression analysis, as recommended by recent guidelines (Harris and Padilla, 2007). For all analyses, significance will be set at $p < 0.05$.

Limitations and Experimental Difficulties

For this dissertation study, I have considered relevant threats to all four types of validity: internal, external, construct, and statistical conclusion validity. The following measures will be taken to minimize relevant threats to each type of validity.

Internal Validity

Internal validity refers to the degree to which the study results are directly attributable to the intervention itself (i.e. Hookah smoking) and not to other experimental conditions. This is important because there could be related factors (listed below) influencing the hypothesized changes.

Testing: An important threat to internal validity is testing. It is important for study instruments to be reproducible, reliable and valid. The following measures will be taken to confirm that any cardiovascular changes observed with Hookah smoking are directly attributable to Hookah smoking and not to the experimental conditions. A subset of subjects (n=10) will be asked to undergo a “time-control experiment”. In this study visit, subjects will undergo all of the study procedures, but will not undergo Hookah smoking. Subjects will have the opportunity to participate right before the main study visit (on the same day). Additionally, repeatability experiment will be done in order to confirm that any cardiovascular changes observed with Hookah smoking are repeatable and reproducible. A subset of subjects (n=10) will be asked to repeat the main study experiment and undergo all of the study procedures.

Another “testing” effect may be related to variability in using FMD to measure large-vessel endothelial vasodilator function. The strength of the FMD technique has been questioned its lack of normalization of the primary stimulus, shear stress, which may differ significantly among individuals (Pyke, Tschakovsky, 2005; Mitchell et al., 2004; Stoner, McCully, 2011; Koller, Sun, Kaley, 1993) and influence endothelial function (Mitchell et al., 2004). To control for this potential effect, shear stress will be calculated as peak hyperemic shear rate on each subject using the following formula: (8

× time-averaged peak velocity)/occlusion diameter based on a large, wide sample volume from the first 10 velocity envelopes (first 15 s) following cuff release per recent recommendations (Harris, Nishiyama, Wray, Richardson, 2010). Further, to control for technical and interpretive limitations of FMD, the following additional measures will be taken: (1) pre and post measurements will be analyzed in a blinded fashion; and (2) edge detection software will be used for offline analysis to determine baseline and post-cuff release diameter and velocity. This software allows synchronization with the ultrasound system and ECG to allow sequential end-diastolic images to be stored, avoiding artifacts attributed to pulse-related changes in vessel diameter. Finally, to document reproducibility, within-subject test-retest experiments will be completed as described earlier.

In regards to RH-PAT, it is important to note that blood flow may be altered by room temperature changes and psychological stress. To control for these confounding variables, subjects will be tested in a quiet and climate-controlled environment after ten minutes of resting and laying supine on our laboratory bed will be done. Moreover, the proposed experimental design is such that subjects will serve as their own control – changes are assessed in both the hyperemic arm (experimental arm) and the contralateral control arm. Thus, any underlying changes in systemic vessel tone unrelated to Hookah smoking should be accounted for by this technique.

External Validity

External validity refers to the degree to which the study results can be generalized. To minimize threats to external validity, the following measures will be taken:

To insure a generalizable sample, an equal number of female (n=13) and male (n=13) subjects will be recruited. In regards to ethnicity, I anticipate that the sample will include: 39% White (Non-Hispanic or Latino) (n=10), 15% African American (n=4), 15% Asian (n=4), 15% Hispanic or Latino (n=4), 8% American Indian or Alaska Native (n=2), and 8% Multi-Racial/Multi-Ethnic (n=2). To insure the generalizability and diversity of subjects, recruitment flyers will be posted throughout college campuses where there will be a diversity of subjects representing the young adults' population that utilizes Hookah smoking.

Construct Validity

Construct validity refers to the degree to which instruments measure the characteristic proposed to be investigated. Several threats to construct validity and methods to minimize them are presented below:

Evaluation apprehension: Evaluation apprehension is based upon the tendency of participants to perform differently when anxious or under pressure. Examples of this threat may be any responses to the experimental setting, such as stress. To limit stress-induced sympathetic activity on the day of the study, subjects will be instructed to sit in the chamber for a period of 10-15 minutes to familiarize themselves with the environment before the start of the study. Before obtaining measurements, participants will remain in the position in which the study will be performed (i.e. supine) for $\geq$ 15 minutes in our quiet, climate controlled laboratory room. In addition, to maintain a neutral and consistent environment during the smoking session, all subjects will be provided to watch the same neutral National Geographic movie (e.g. the life of ducks).

Experimenter Expectancies: This threat to construct validity refers to the degree to which the researcher's cognitive bias causes them to unconsciously influence the participants of an experiment. Prior to starting the Hookah smoking session, a reminder to smoke at a similar rate that is often parallel to the subjects' regular smoking session.

Statistical Conclusion Validity

Statistical conclusion validity refers to whether or not there is a relationship that exists between two identified variables (I.e. Hookah smoking and endothelial dysfunction) based on statistical tests. The following threats to statistical conclusion validity may affect this proposed study:

Inaccurate effect size: This threat refers to an inappropriate sample size estimate for a proposed measure. No data from Hookah smoking exist that could inform power calculations. Statistics are based on estimated change scores from relevant cigarette smoking literature, which may not be reflective of Hookah smoking. In order to evaluate this threat, we will conduct post-hoc power calculations to report the actual power we obtained from this estimated sample.

Reliability of treatment implementation: This threat refers to when a specific experimental treatment or instruction is not similar for all participants. This threat may be operative if participants fail to puff sufficiently throughout the smoking session. In order to control for the frequency of puffing during the smoking session, a reminder to smoke at a similar rate that is parallel to the subjects' regular smoking session will be provided to each participant prior to smoking.

Summary

Little is known about the cardiovascular toxicity of Hookah smoking on human health. The goal of the planned dissertation project is to use clinical research methodology to test the following novel hypothesis: Hookah smoking evokes acute endothelial dysfunction that involves the entire vascular tree from the most distal microvessels to the large conduit vessels, resulting in a more rapid return of the arterial pulse wave from the peripheral circulation to the central aorta thus augmenting aortic systolic pressure. The proposed experimental study design research project is novel in several ways: (1) Focus on Hookah smoking which is the only form of smoking that exposes humans to toxic emissions from burning charcoal; (2) Focus on the targeted young and most heavily impacted national and international diverse populations; (3) Use of validated clinical research methodology to study novel specific aims on the effects of Hookah smoking on endothelial and vascular function in a controlled laboratory environment; and (4) Use of a detailed statistical analysis plan and addressing major threats to validity.

CHAPTER 4

Results

Demographic Characteristics

Forty-six potential subjects responded to advertisement in local media and college campuses and were screened for participation. Twenty-three individuals were excluded from the study for the following reasons: exhaled CO > 10 ppm on screening (n = 1); history of cigarette smoking and/or illicit drug use (n=10); medical history of chronic systemic illness including obesity, asthma or diabetes (n=8); poor image quality (n=2); and movements during data collection (n=2). The characteristics of the remaining 23 study subjects are shown in **Table 3**. This racially diverse group of young adults, including 6 women and 17 men, had a mean age of 25±5 years (mean±SD). Only two subjects were of Middle-Eastern origin. Most were college graduates (n=21 college students; n=2 high school education) who began smoking hookah between 18 to 24 years of age. They smoked hookah on average three times per week (self-reported history), with each session lasting on average 102 minutes (range, 60-120 minutes).

In total, we tested acute effects of hookah smoking on: 1) brachial artery FMD in 23 subjects; 2) reactive hyperemia peripheral arterial tonometry in 20 subjects (2 data points were excluded because of subject movements; and 1 for equipment failure); and 3) pulse wave velocity and central aortic systolic pressure in 23 subjects. Heart rate and blood pressure were measured in all subjects.

In general, outcome data for brachial artery FMD, peripheral arterial tonometry, and central aortic systolic pressure were normally distributed (Kolmogorov-Simirnov p=0.200 for all). However, in order to perform paired *t*-test, natural log transformations

were performed for pulse wave velocity because outcome data were not normally distributed (p=0.157).

Acute Hemodynamic Data

The acute effects of hookah smoking on hemodynamic data are shown in **Table 5**. In 23 subjects and immediately after smoking hookah for 30 minutes, respiratory rate was unchanged (from 17 ± 1 to 18 ± 1 breaths•min^{-1}, mean ± SE, pre- vs. post-hookah, p=0.077), while there were significant increases in heart rate (from 60 ± 2 to 74 ± 2 beats•min^{-1}, p<0.001), systolic blood pressure (from 113 ± 2 to 120 ± 2 mmHg, p<0.001) and diastolic blood pressure (from 66 ± 4 to 71 ± 2 mmHg, p=0.305).

Smoking Exposure Biomarkers

After a single 30 minutes of *ad lib* hookah smoking in our smoking chamber, the above increases in heart rate and blood pressure were accompanied by increases in plasma nicotine concentration from 0.59 ± 0.06 to 6.43 ± 1.22 ng/ml, p<0.001. Expired CO increased from 3 ± 0 to 28 ± 2 ppm, p<0.001 **(Table 5; Figure 6)**

Endothelium-dependent FMD

Brachial artery diameter and velocity responses to endothelium-dependent FMD are shown in **Table 5**. In contrast to our hypothesis, brachial artery FMD did not decrease with hookah smoking but increased, and rather substantially, from 6.9±0.5% to 9.7±0.6%, p<0.001: a 49.6±9.3% relative increase **(Table 5; Figure 8, Right Panel)**. Remarkably, our data showed a reduction in baseline brachial artery diameter after smoking hookah (from 3.30 ± 0.09 to 3.19 ± 0.09 mm; pre- vs. post-hookah, p=0.008), followed by significant increases in: (1) peak hyperemic blood velocity (from 97.51 ± 3.76 to 103.28 ± 3.33 cm/s, p=0.001); and (2) peak shear rate (from 2507.65 ± 138.22

to 2689.03 ± 136.04 s^{-1}, p = 0.001). The latter proposed to be the main stimulus for the FMD response (Pyke & Tschakovsky, 2004, Mitchell et al., 2004). These findings suggest a direct positive relationship of FMD to shear rate and peak hyperemic velocity (Pearson's *r*=0.446, p=0.033; *r*=0.426, p=0.043, respectively) (**Figure 10A**).

Given the modest correlation between FMD and shear; we attempted to correct (normalize) FMD for shear through ANCOVA as well as dividing FMD by shear, as recommended by some (Pyke & Tschakovsky, 2004; Harris & Padilla, 2007; Padilla et al. 2009), but not all guidelines. The covariate analysis confirmed that FMD corrected for hyperemic shear rate using ANCOVA did not affect the interpretation of the study findings and that FMD% remained statistically significant when adjusted for shear (p=0.001). Similarly, by dividing FMD by shear, there was still a significant increase in FMD by 37.4±8.0% (p<0.001) after hookah smoking.

Our data show that increases in blood flow during reactive hyperemia increased slightly but not significantly after the hookah smoking session (from 630.76 ± 56.36 to 718.79 ± 47.61%, p=0.139). Moreover, the time to peak after hyperemia was significantly faster post-hookah smoking, compared to pre-hookah (from 43.65±1.82 to 48.39±2.02, p<0.001).

When controlling for demographic characteristics (age, gender, ethnicity, BMI, self-reported smoking duration and frequency), there was still a significant change in FMD % over time (pre- vs. post-hookah, p=0.0007) (**Table 6**). After controlling for all other variables in the model, gender was revealed to be a significant predictor of FMD. On average, FMD was 3.24% higher in females that males, p=0.013. Smoking

biomarkers exposures (expired CO and plasma nicotine concentration) were not significantly associated with FMD changes (**Table 6**).

Internal Vasoconstrictor Control Group

The unexpected brachial artery FMD augmentation findings, lead us to amend our protocol to include a positive internal vasoconstrictor control-group to compare the paradoxical response to hookah smoking with the acute effect of cigarette smoking in cigarette smokers. Therefore, we recruited a total of 10 healthy young habitual cigarette smokers and measured brachial artery FMD (as well as exhaled CO and plasma nicotine concentration) before and immediately after smoking a single standardized regular cigarette (tar 12 mg, nicotine 1 mg). Subjects were asked to finish the cigarette in $\leq$ five minutes. Habitual cigarette smokers were defined as having smoked at least one pack year for $\geq$ 4 years.

For this sub-study, twenty-four subjects responded to our advertisements and were screened for participation. Sixteen individuals were excluded for the following reasons: history of illicit drug use (cocaine and/or ecstasy) (n=2); medical history of chronic systemic illness including obesity, asthma or diabetes (n=2); not classified as chronic cigarette smoker (n=10); and movements during data collection (n=2). The characteristics of the remaining eight study subjects are shown in **Table 4**. On average, subjects reported smoking 9 ± 6 cigarettes per day and 4 ± 4 pack years.

As expected, brachial artery FMD decreased acutely from 6.0±1.3% to 3.9±0.7%, P<0.001: a 35.7±3.7% decrease, after smoking one single cigarette **(Table 5; Figure 8, Left Panel)**, thus confirming previous reports (Neunteufl et al., 2002; Amato at al., 2013; Papamichael at al., 2004; Lekakis et al., 1997). Indeed, and in contrast to hookah

smoking, there were reductions in the vasodilatory response stimulus: (1) peak hyperemic blood velocity (from 82.31 ± 7.65 to 78.23 ± 5.28 cm/s, p=0.269); and (2) peak shear rate (from 2157.80 ± 180.28 to 2097.00 ± 159.60 s^{-1}, p = 0.533), although these changes did not reach statistical significance. These findings suggest an inverse negative relationship of FMD to shear rate and peak hyperemic velocity (*r*=-0.693, p=0.057; *r*=-0.736, p=0.037, respectively) (**Figure 10B**). Importantly, similar to hookah smoking, our data showed: (1) a reduction in baseline brachial artery diameter after smoking cigarettes (from 3.20 ± 0.22 to 3.11 ± 0.22 mm; pre- vs. post-hookah, p=0.001); and (2) the time to peak after hyperemia was significantly faster post-cigarette smoking, compared to pre-cigarette (from 38.63 ± 2.95 to 45.25 ± 2.43, p<0.019) (**Table 5**). Figure 9 demonstrate an illustrative brachial artery image in a hookah and cigarette subject before and after smoking.

Furthermore, the increases in BP and heart rate were much less with hookah than with a cigarette (ΔSystolic BP: +7 ± 1 vs. +11 ± 3 mmHg, p=0.045; ΔDiastolic BP: +5 ± 1 vs. +13 ± 2 mmHg, p=0.007; Δmean arterial pressure (MAP) +5 ± 1 vs. +12 ± 2 mmHg, p=0.004; Δheart rate: +14 ± 2 vs. +19 ± 2 beats.min^{-1}, p=0.248) and while plasma nicotine concentration were slightly higher in cigarette smokers (+6.83 ± 1.58 vs. +5.84 ± 1.21 ng/ml, p=0.472), the exhaled CO level was almost 3-fold greater after smoking hookah than after smoking a cigarette: +25 ± 3 vs. +3 ± 1 ppm, p=0.008) (**Figure 11**).

Test-Retest Reproducibility Experiments

We documented within-subject test-retest reproducibility of brachial artery FMD indices. In 12 subjects, measurements were performed twice on each arm with at least

20 minutes between measurement, which is the time needed to facilitate the return of baseline levels after cuff deflation. Out of the 12 subjects, 2 returned to the laboratory on a separate day to repeat the experiments. A total of 47 scans were completed and a total of 9 scans were excluded from analysis because of subject movements during data collection (n=3) and subjects' not willing to continue study participation (n=6).

In regards to test-retest reproducibility responses, brachial artery FMD parameters were not significantly different (FMD% scan one: 7.89 ± 0.46 vs. scan two: 7.70 ± 0.34 %, p=0.523; baseline artery diameter scan one: 3.34 ± 0.11 vs. scan two: 3.35 ± 0.12 mm, p=0.935, peak hyperemic blood velocity scan one: 91.38 ± 3.27 vs. scan two: 89.45 ± 2.89 cm/s, p=0.563, respectively). The reproducibility for baseline brachial artery diameter was superior to FMD% (ICC: 0.969 vs. 0.857), whereas intraclass correlation for peak hyperemic velocity was less superior (0.656) (**Table 2**). In regards to Bland-Altman plots, no systemic bias was detected from scan 1 and scan 2 (**Table 2 and Figure 7**). The SDs of the differences were as follows: FMD 1.37 %; baseline diameter 0.19 mm; and peak hyperemic velocity 15.50 cm/s. The mean differences for FMD, baseline diameter, and peak hyperemic velocity were -0.19 %, 0.00 mm, and -1.94 cm/s, respectively (**Table 2).**

Time Control Experiments

To verify that the unexpected changes in brachial artery FMD is associated with hookah smoking and not sitting in the chamber, time-control experiment studies were performed in a subset of subjects (n=5). Subjects were invited to sit in the chamber for a duration of 30-minutes, without smoking, and expired CO, heart rate, blood pressure

and brachial artery FMD were measured before and immediately after exiting the chamber.

As expected, the variables measured remained unchanged: expired CO (from 4.0 ± 0.3 to 3.6 ± 0.4 ppm, p = 0.177), heart rate (from 57 ± 5 to 58 ± 6, beats·min^{-1}p = 0.722), systolic blood pressure (from 116 ± 6 to 115 ± 5 mmHg, p = 0.605), diastolic blood pressure (from 70 ± 5 to 72 ± 2 mmHg, p = 0.911), and brachial artery FMD (from 6.60 ± 1.31 to 6.33 ± 1.48%, p=0.326; **Table 11; Figure 14**).

Repeatability Experiment

In order to confirm that baseline cardiovascular measurements are repeatable and reproducible, a subset of subjects (n=5) returned to our laboratory on a separate to undergo a "repeatability experiment". Baseline measurements, which include expired CO, heart rate, blood pressure and brachial artery FMD were measured.

In these 5 subjects and in comparison to day 1, there were no significant changes in expired CO (day one: 4.0 ± 0.3 vs. day two: 3.4 ± 0.4, p = 0.304), heart rate (day one: 57 ± 5 vs. day two: 54 ± 4 beats·min^{-1}, p = 0.534), systolic blood pressure (day one: 116 ± 6 vs. day two: 113 ± 3 mmHg, p = 0.380), diastolic blood pressure (day one: 71 ± 4 vs. day two: 67 ± 2 mmHg, p = 0.141), or brachial artery FMD (day one: 7.03 ± 1.56 vs. day two: 6.71 ± 1.29 %, p = 0.508) (**Table 12**).

Peripheral Arterial Tonometry

To determine the acute effects of hookah smoking on micro-vessel endothelial function, reactive hyperemia peripheral arterial tonometry was used before and after a 30-minutes *ad lib* hookah smoking session. In 20 hookah smokers, our data show that hookah smoking acutely causes no change in reactive hyperemia peripheral arterial

tonometry score **(Figure 12; 13).** Acutely and after smoking hookah for 30-minutes, pulse volume amplitude score changed from 2.09 ± 0.13 to 1.99 ± 0.12, p=0.633.

When controlling for demographic characteristics (age, gender, ethnicity, BMI, self-reported smoking duration and frequency), there was no change in pulse volume amplitude score over time (pre- vs. post-hookah, p<0.470) (**Table 7).** Additionally, smoking biomarkers exposures (expired CO and plasma nicotine concentration) were not significantly associated with pulse volume amplitude score changes (**Table 7**).

In regards to the relationship between micro-vessel endothelial function (pulse volume amplitude) and large-vessel (brachial artery FMD), our data show no significant correlation between pulse volume amplitude changes and brachial artery FMD parameters: FMD% (p=0.627; *r*=-0.116); peak hyperemic blood velocity (p=0.658; r=0.105); and peak shear rate (p=0.942; *r*=0.017).

Pulse tonometry

As noted previously, hookah smoking causes an increase in both heart rate and peripheral arterial blood pressure (Δ heart rate +14±2, P<0.001; Δ peripheral systolic BP +7±1, P<0.001; Δ peripheral diastolic BP +5±1, P=0.305; **Table 5**). To determine whether this hemodynamic response leads to central arterial stiffness, we used pulse tonometry to measure carotid-femoral PWV and central aortic systolic pressure before and after 30-minute hookah smoking. The results are summarized in **Table 10**.

Our data revealed that although central aortic systolic blood pressure increased, the degree of change was found to be statistically higher than peripheral blood pressure (Δ peripheral systolic BP +7±1 vs. Δ central systolic BP +5±1, P=0.007). Moreover, while carotid-femoral PWV, which is considered to be the gold standard to arterial

stiffness significantly increased after smoking (from 7.47 ± 0.20 to 8.04 ± 0.22, p=0.002), neither augmentation pressure nor augmentation index changed after smoking (from 3±1 to 3±1 mmHg, p=0.580; and from 8±3 to 10±2 %, p=0.672, respectively).

In a mixed model, when controlling for demographic characteristics (age, gender, ethnicity, BMI, self-reported smoking duration and frequency), there was still a significant change in carotid-femoral PWV over time (pre- vs. post-hookah, p=0.005) (**Table 8**). Importantly, after controlling for all other variables in the model, age and gender were found to be strong predictors of carotid-femoral PWV (**Table 8**). On average, our data showed that: (1) for every one year increase, there is a 0.1195 increase in carotid-femoral PWV (p=0.008); and (2) carotid-femoral PWV was 1.635 lower in females compared to males (p=0.0008). Smoking biomarkers exposures (expired CO and plasma nicotine concentration) were not significantly associated with carotid-femoral PWV changes (**Table 8**).

In regards to central aortic systolic BP, when controlling for demographic characteristics, there was still a significant change over time post hookah smoking (pre- vs. post-hookah, p=0.008) (**Table 9).** Similarly, no association between smoking biomarkers exposures and central aortic systolic BP were found (**Table 9**). No significant correlations between carotid-femoral PWV and central aortic systolic pressure were observed in our data (p=0.274; *r*=-0.244).

CHAPTER 5

Discussion

Hookah smoking has rapidly evolved and has become a global epidemic. It is heavily marketed with claims of being a healthier, non-addictive alternative to cigarette smoking. Unlike cigarettes, little is known about its potential cardiovascular toxicity. A unique feature of hookah is that burning charcoal is used to heat the tobacco product. Thus, in addition to tar and nicotine, hookah smoke also delivers large amounts of charcoal combustion products, including carbon-rich nanoparticles, CO and other toxins. Indeed, despite claims, hookah smoking is established to be a powerful cardiovascular stimulant evoking large increases in both heart rate and blood pressure acutely (Cobb, Sahmarani, Eissenberg, Shihadeh, 2012; Shaikh, Vijayaraghavan, Sulaiman, Kazi, Shafi, 2008, Kadhum, Jaffery, Haq, Bacon, Madden, 2014; Hakim, Hellou, Goldbart, Katz, Bentur, Bentur, 2011). Similar to cigarettes, we have previously shown that hookah smoking acutely increases coronary blood flow, measured by myocardial contrast echocardiography, presumably a response to increased myocardial work from nicotine (Nelson et al., 2016). Here, we hypothesized that hookah smoking evokes significant acute peripheral endothelial dysfunction, involving the entire vascular tree from the most distal microvessels to the large conduit vessels, resulting in a more rapid return of the arterial pulse wave from the peripheral circulation to the central aorta thus augmenting aortic systolic pressure and causing arterial stiffness.

Endothelium-dependent FMD

Brachial artery FMD is a powerful clinical research tool that reflects NO-mediated endothelial function, thus predicting future cardiovascular events (Vita and Keaney,

2002; Celemajer, 1997; Anderson et al., 1995; Ganz and Vita, 2003; Widlansky, Gocke, Keaney, Vita, 2003). Importantly, it has been shown to strongly correlate with coronary artery endothelial function (r=0.79, P<0.001 for brachial versus coronary FMD) (Takase et al., 1998). Whereas multiple studies using this technique have shown that smoking even a single cigarette acutely impairs endothelial function, the acute effect of hookah smoking is unknown. For hookah smoking, we hypothesized that each 30-minute smoking session would evoke significant acute endothelial dysfunction because of the large acute exposure of nicotine and carbon-rich nanoparticles, known to trigger vasoconstriction in the peripheral circulation. We validated this technique by showing: (1) a high degree of within-subject test-retest reproducibility (ICC: 0.969, 0.857 and 0.656 for baseline diameter, FMD% and peak velocity, respectively); and (2) the expected decrease in endothelium-dependent FMD after cigarette smoking–used as an internal vasoconstrictor control. We then tested our hypothesis using high-resolution ultrasound to measure endothelium-dependent FMD before and immediately after a typical hookah smoking session in young healthy adults who were chronic hookah smokers, but not cigarette smokers.

The principle new finding of our study was in contrast to our hypothesis. We found that hookah smoking—in marked contrast to cigarettes—acutely augments endothelium-dependent FMD, and rather substantially (from 6.9±0.5% to 9.7±0.6%, P<0.001: a 49.6±9.3% relative increase). These unexpected findings could potentially be explained by the different effects of nicotine versus CO on vascular endothelial function. First, the vasoconstriction seen in baseline artery diameter after hookah smoking is presumed to be due to one of the three potential biological mechanisms: (1)

nicotine-mediated catecholamine release: indeed, as nicotine is absorbed through the systemic circulation, the consequent release of catecholamines binding with alpha-1 adrenergic receptors on vascular smooth muscles result in adrenergic stimulation, muscle contraction and subsequent vasoconstriction (Rhee et al., 2007; Powell, 1998; Lekakis et al., 1997; Jatoi et al., 2007); (2) reflex increase in the sympathetic nervous system activity from stimulation of pulmonary vagal afferents (Qin, Foreman, Farber, 2007); and/ or (3) an acute release of vascular endothelin release: a potent vasoconstrictor seen after acute cigarette smoking (Haak, Jungmann, Rabb, Usadel, 1994).

Second, the substantial increase in vasodilation post-deflation after hookah smoking most likely is explained by CO. In our study, exhaled CO increased from 3±1 to 28±2 ppm, which approximates the CO boost of a typical *ad lib* hookah smoking session in hookah bars (Barnett, Curbow, Soule, Tomar, Thombs, 2011). Substantial evidence suggest that CO does not initiate atherosclerotic plaque (Smith and Steichen, 1993; Strom, Alfredsson, Malmfors, 1995), in fact, studies have demonstrated that CO serves as an endogenous gas that increases cyclic guanosine monophosphate and results in vascular relaxation (Siow, Sato, Mann, 1999; Kozma, Johnson, Nasjletti, 1997; Farrugia et al., 1998; Kozma et al., 1999; Wang, 1998; Thorup, Jones, Gross, Moore, Goligorsky, 1999). The physiological effects of endogenous CO has been shown to be enhanced and/or mimicked by exogenously applied CO, either by inhalation or use of CO-releasing compounds (Guo et al., 2004; Dubuis et al., 2002; Jaggar, Leffler, Cheranov, Tcheranova, Cheng, 2002; Gunther et al., 2002). More importantly, exposures to high levels of CO have been shown to decrease forearm peripheral vascular resistance

(Heistad and Wheeler, 1972). Regarding endothelial function, acute impairment in FMD has been demonstrated to not correlate with carboxyhemoglobin levels (Weber, Al-Dissi, Marit, German, Terletski, 2011). Taken together, we speculate that the acute hookah-induced catacholamine action of nicotine is being offset by the significant boost in CO.

Hookah smoking has been linked to many cases of acute CO poisoning (Misek, Patte, 2014; La Fauci, G., Weiser, Steiner, Shavit, 2014; Ozkan, Ozturk, Ozmen, Durukan, 2013; Cavus, Rehber, Ozeke, Ilkay, 2010; Lim, Lim, Seow, 2009; Uyanik, Arslan, Akay, Ercelik, Tez, 2011) because of hypoxia. Hypoxia results from a reduction in arterial oxygen content and tissue oxygenation from the increased affinity of carboxyhemoglobin for oxygen (Stewart, 1975). Because FMD in the brachial artery has repeatedly been shown to strongly correlate with endothelium-dependent vasodilation in the coronary circulation (r=0.79, P<0.001 for brachial versus coronary FMD) (Takase et al., 1998), it is appropriate to speculate that the coronary vasculature will respond in a similar pattern. However, it is important to keep in mind that the myocardiam is much more sensitive to CO, compared to peripheral tissues, because of its exorbitant resting oxygen demand (Ayres, Mueller, Gregory, Giannelli, Penny, 1969; Ayres, Giannelli, Mueller, 1970;). Therefore, further investigations are needed to extend our findings to the coronary circulation and to subjects with existing heart disease and/ or advanced atherosclerosis.

Our results differ from those of a previous study by Selim et al. (2013) who studied the chronic effects of hookah versus cigarette smoking on endothelial function and found that endothelium-dependent FMD is more impaired in hookah versus

cigarette smokers (7.9±3.8% vs. 12±3.4%, p<0.001) (Selim, Elia, El Bohey, El Meniawy, 2013). Although, we could not replicate this solitary report, our discrepant findings may be attributable to a number of factors. First, our study focuses on assessing the acute effects of hookah, which may result in different effects. Of more importance is the difference between our methodology and theirs in obtaining post-deflation measurement of diameter change. Through continuous assessment of arterial diameters post-deflation, our data show that time to peak diameter occurred at approximately 42 seconds. This is earlier than the traditional approach, which suggests a fixed time frame (60 seconds) for post-deflation measurement of diameter change (Celermajer et al., Lancet, 1992). Though this approach has been followed by Selim et al., as well as many investigators with publications in leading peer reviewed journals (Wisloff et al., 2007; Kathiresan et al., 2006; Heiss et al., 2005), it has been called into question by several studies demonstrating that discrete measurements at 60 seconds underestimate the maximal peak diameter and may lead to misleading conclusions (Black, Cable, Thijssen, Green, 2008; Liuni et al., 2010).

The delayed time to peak diameter we observed within groups after hookah and cigarette smoking is noteworthy. Black et al. (2008) showed a significant difference in time to peak diameter between young and older healthy individuals suggesting that delay in time to peak may be attributed to arterial stiffness and differences in vascular compliance associated with age (Black, Cable, Thijssen, Green, 2008). Indeed, previous studies have demonstrated cigarette smoking acutely lead to arterial stiffness, as measured by carotid-femoral PWV (Vlachopoulos et al., 2004; Rhee, Na, Kim, Lee, Kim, 2007; Lemogoum et al., 2006) and we have demonstrated in our current study that

hookah also does the same. It is important to mention, however, that Liuni et al., (2010) showed that intra-arterial infusion of the nitric oxide synthase inhibitor N-monomethyl-L-arginine (L-NMMA) had no effect on time to peak after hyperemia, suggesting that it is not influenced by NO. Therefore, other factors released during hyperemia are responsible for this delay. Clearly, more research is needed to further study the underlying mechanistic explanations in time to peak delay after interventions.

In healthy cigarette smokers, prior investigations have shown that smoking a single cigarette acutely impairs endothelial-dependent FMD (Powell, 1998; Rhee, Na, Kim, Lee, Kim, 2007; Jatoi, Jerrard-Dunne, Feely, Mahmud, 2007; Lekakis et al., 1997). In our study, we report a 35.7±3.7% reduction after smoking a cigarette and thus our results are in agreement with prior findings. Our study adds an interesting finding regarding the differential effects of hookah vs. cigarettes on endothelium-dependent FMD indices. Even though baseline diameter constricted (for both hookah and cigarette smoking), the stimulus for FMD was directionally opposite. Shear rate increased in hookah while it decreased in cigarette smokers. Indeed, these differential effects were subsequently followed by acute FMD augmentation in hookah and impairment in cigarettes. Because reactive hyperemia and the degree of shear response is thought to reflect the dilation of resistance vessels of the microvasculature, future studies are needed to elucidate effects on the microvasculature.

Several limitations of the current study must be acknowledged. Hookah smoking is a highly social activity with a typical smoking session lasting anywhere between 1 to 4 hours. Although the 30-minute smoking session is a small approximation to the natural environment, our plasma nicotine levels are consistent with previously reported levels

measured at hookah bars (Jacob et al., 2013). Our experimental set-up also likely contributed to lower than expected expired carbon monoxide levels (Jacob et al., 2011; Barnett et al., 2011). For example, we only studied one subject per visit and had a continuous exhaust system clearing the chamber air, limiting exposure to second-hand smoke. There are also several brands of hookah tobacco and charcoal available. While we focused our investigation on the most popular tobacco brand and charcoal used among young adults, hookah products are unregulated, opening the possibility that different brands may contain different chemicals and therefore exert different vascular effects.

Ideally it would have been desirable to measure catecholamine plasma levels in hookah versus cigarettes. We could only speculate that plasma catecholamines are responsible for some of our findings. Additionally, our study design did not: (1) include repeated measures of vascular function and recovery measures post hookah smoking; and (2) assess casual inferences and precise determination on mechanisms producing the unexpected results with brachial artery FMD. Finally, we only studied overtly healthy young adults, free of cardiovascular disease. By doing so, we may have reduced the strengths of our correlations and outcome effects.

Despite these limitations, our findings suggest that hookah pose a distinct effect on endothelium-dependent FMD compared to cigarettes smoking. Because this is the first study to investigate the acute effects of hookah smoking on endothelial-dependent vasodilation, a more complete understanding of the underlying mechanisms and the specific toxic constituents involved requires further investigation.

Peripheral Arterial Tonometry

Peripheral arterial tonometry is a new method that utilizes plethysmography in the fingertip to calculate pulsatile arterial volume amplitude changes (Celermajer, 2008; Bonetti et al., 2003; Gerhard-Herman, Hurley, Mitra, Creager, Ganz, 2002). Although this method is emerging in use, the physiology underlying its results remains unclear. In the present study, we demonstrate that hookah smoking acutely causes no change in pulse volume amplitude score. However, looking closely, our data show a slight reduction in pulse volume amplitude (2.09±0.13 to 1.99±0.12%, p=0.633), suggesting very mild impairment in peripheral microvascular function.

Since reactive hyperemia velocity changes in the brachial artery is highly dependent on distal resistance vessel dilation, some studies demonstrate a strong correlation between hyperemic velocity post-deflation and pulse volume amplitude changes, suggesting hyperemic velocity to be an important index of microvascular function (Martin, Gurtu, Chan, Anderson, 2013; Lee et al., AJC, 2012). In our study, we found no significant correlation between hyperemic velocity or shear rate from brachial artery FMD and peripheral arterial tonometry pulse volume amplitude score (p=0.658; r=0.105; p=0.942; *r*=0.017, respectively). Possible reasons could be attributed to differences in study methodology. For example, measurements of large-vessel brachial and micro-vessel vascular function were not performed simultaneously. We performed peripheral arterial tonometry 15 minutes after completing brachial artery FMD. Also, cuff location may contribute to this difference. We used lower arm occlusion for brachial artery FMD and upper for peripheral arterial tonometry.

Another finding of our study is that peripheral arterial tonometry, in contrast to our

working hypothesis, does not correlate with brachial artery FMD (p=0.627; *r*=-0.116). This lack of correlation is not unique to our study data, as divergence between both methods has been demonstrated before. For example, in the large, Framingham Offspring, Third Generation and Omni Cohorts study, Hamburg et al. (2011) found no correlation in subjects (n=1843) who had simultaneous peripheral arterial tonometry and FMD measurements (Hamburg et al., 2011). Similarly, other studies have demonstrated the lack of correlation in patients with and without coronary artery disease and in healthy volunteers (Nigam, Mitchell, Lambert, Tardif, 2003; Martin, Gurtu, Chan, Anderson, 2013; Lee et al., 2012). In other studies, correlation between both measures has been reported (Kuvin et al., 2003; Dhindsa, 2008).

The lack of correlation between both measures assessing endothelial function emphasizes the likelihood that physiological differences in vasodilation may differ across vessel size and testing location and that each measure may reflect distinct aspects of vascular function. Indeed, endothelium-dependent FMD is well established to reflect NO-mediated endothelial function in the conduit vessel (Singel and Stamler, 2005; Lieberman et al., 1996; Mullen et al., 2001). Peripheral arterial tonometry, which measures pulse volume amplitude changes in the fingertip of the index finger, has also been shown to be NO-dependent. One study by Nohria et al. (2006) tested this hypothesis by inhibiting nitric oxide synthesis with NG-nitro-Larginine methyl ester (L-NAME) before measuring peripheral arterial tonometry in healthy subjects (n=19) (Nohria et al., JAP, 2006). The authors found reduction in pulse volume amplitude increase by 46 ± 21% after L-NAME administration (p=0.002) (Nohria et al., JAP, 2006). Peripheral arterial tonometry has also been shown to correlate with other NO-

dependent measures of vascular function such as acetylcholine responses in coronary endothelial function - the gold standard measurement of endothelial function (Bonetti et al., 2004). These studies corroborate the notion that peripheral arterial tonometry is likely a measure that reflect micro-vessel function, which is partly mediated by NO.

It is however, important to note that the fingertips have an anatomically complex vasculature, which may respond differently to short periods of ischemia (Coffman, 1994). The fingertips consist of a dual circulation: capillaries and arteriovenous anastomoses (Coffman, 1994). Vascular tone in arteriovenous anastomoses is believed to be mainly regulated by the sympathetic nervous system and NO plays a minimal role in controlling its resting blood flow (Coffman, 1994; Noon, Haynes, Webb, Shore, 1996). Taken together, one may conclude that brachial artery FMD and peripheral arterial tonometry each evaluate and provide different physiological assessment on vascular endothelial function in large versus micro vessels. Future studies are indicated to examine the additional index each test contributes to peripheral endothelial vascular health.

Our study has several limitations. We did not administer nitroglycerin; thus we are unable to comment on the proportion of endothelium-independent vasodilation for the pulse volume amplitude mild changes observed post-hookah smoking. Also, measurements of brachial and digital vascular function were not performed simultaneously. Although it is possible that temporal factors may account for some of the lack of correlation between both methods, as mentioned above, peripheral arterial tonometry was measured within 15 minutes after measuring brachial artery FMD.

Pulse Wave Velocity

Arterial stiffness is a well-recognized powerful, independent risk factor for cardiovascular disease and plays a key role in its pathophysiology (Laurent et al., 2001; Blancher, Asmar, Diane, London, Safar, 1999). Pulse wave velocity is considered to be the gold standard measurement of central (aortic) arterial stiffness (Laurent et al., 2006; Vlachopoulos, Aznaouridis, Stefanadis, 2010). In a stiff aorta, peak systolic pressure increases from a faster return of periphery pressure waves and reduced vascular compliance (O'Rourke, Staessen, Vlachopoulos, Duprez, Plante, 2002). Indeed, acute cigarette smoking increases aortic stiffness (Vlachopoulos et al., 2004; Rhee et al., 2007; Lemogoum, Van Bortel, Leeman, Degaute, van de Borne, 2006; Kubozono et al., 2011), but the effects of hookah smoking is unknown. We hypothesized that hookah smoking would evoke an acute increase in vascular stiffness evidenced by a faster carotid-femoral pulse wave velocity.

To the best of our knowledge, the present study is the first to examine the effects of hookah smoking on arterial stiffening. Consistent with our hypothesis, our data reveal that pulse wave velocity increased significantly after smoking hookah, compared to baseline (7.47±0.20 to 8.04±0.22, $P<0.001$), suggesting acute arterial stiffening. The finding of this study most likely is explained by the direct effects of nicotine on pulse wave velocity. Indeed, nicotine administration in young healthy smokers has been previously demonstrated to acutely increase carotid-femoral pulse wave velocity (Adamopoulos et al., 2009). As mentioned above, nicotine induces catecholamines release, which results in altered arterial distensibility and compliance, leading to stiffness.

It is interesting to note that overall, our study demonstrates that hookah smoking acutely causes impairment in the central aorta without impairment in peripheral endothelial function (described above). These findings lead to an intriguing question: could hookah smoking be associated with increased stiffness of the aorta while preserving and/ or augmenting peripheral endothelial function? The answer to this question is two-fold: (1) the relative contribution of hookah mainstream smoke constituents (CO vs. nicotine) on different vascular beds could be a plausible mechanism underlying the differential central elastic and peripheral muscular vascular effects; and (2) although PWV has been shown to be NO dependent (Stewart et al., Hypertension, 2003; Fitch, Vergona, Sullivan, Wang, 2001), reactive hyperemia is a complex physiological response that involves other factors including adenosine, prostaglandin, hydrogen peroxide and others (Loscalzo and Vita, 1994; Engelke et al., 1996). All could explain the differential effects and lack of correlation between both methods in our study as well as studies by others (Koivistoinen et al., 2012; Nigam, Mitchell, Lambert, Tardif, 2003; Dhindsa et al., 2008). These results suggest that, at least in young overtly healthy adults, PWV reflects different aspects of endothelial vascular impairment than brachial artery FMD.

The finding of our study has important clinical implications. Aortic stiffness, an important determinant of left ventricular function and coronary blood flow, plays a key role in the pathogenesis of cardiovascular disease risk factors (Laurent et al., 2001; Blancher, Asmar, Diane, London, Safar, 1999). Indeed, aortic stiffening impairs tissue perfusion and ventricular performance by increasing cardiac afterload and myocardial oxygen demand (Bouthier et al., 1985; Blacher et al., Hypertension, 1999; Glasser et

al., 1998). Although the underlying mechanism is unclear and the population we focused on are overtly healthy young adults, the effects of acute hookah smoking in middle-aged Middle-Eastern populations with existing cardiovascular diseases who have been smoking hookah for decades is not known. Clearly, more studies are needed.

Summary

Substantial evidence links cigarette smoking with cardiac mortality. However, little is known about hookah smoking. To further reduce global morbidity and mortality from tobacco smoking, it is essential to pursue knowledge of the effects of novel tobacco alternative products (such as hookah smoking) on human health, particularly the cardiovascular system. Evidence is needed to fill the large gap in the literature on the potential health effects of hookah. This evidence could be used to inform policy changes and direct health promotion programs that meets the difficult challenge to make hookah smoking socially unacceptable.

The present study demonstrates important findings and indicates that, despite claims and heavy hookah tobacco marketing, hookah is not a safer alternative to cigarettes. These findings support the recent FDA proposal to regulate hookah tobacco. It is recommended that regulations not only include the hookah tobacco but the charcoal as well since it is unique to this form of tobacco use and has been shown to contribute significantly to carbon monoxide and carbon-rich nanoparticles.

Because this is the first study to investigate the acute effects of hookah smoking on the vasculature in a controlled environment, a more complete pathophysiological understanding and the mechanistic underpinnings involved require further investigation.

Indeed, the precise mechanisms responsible for the hookah-induced effects in our study on vascular and endothelial function remain unclear. In this context, the progress reported in this dissertation is viewed as the beginning rather than the end of the exploration of the cardiovascular effects of hookah smoking. Our findings set the stage for future research regarding the acute as well as chronic effects of hookah smoking on the cardiovascular system. Much work remains to be done to: (1) assess the relative contribution of nicotine-free (herbal) and charcoal-free (electronic) hookah with and without nicotine on endothelial function; (2) study the mechanistic explanations behind these findings; and (3) study the long-term effects on cardiovascular structure and function in middle-aged smokers.

Figure 1. Major Components of a Hookah**.**

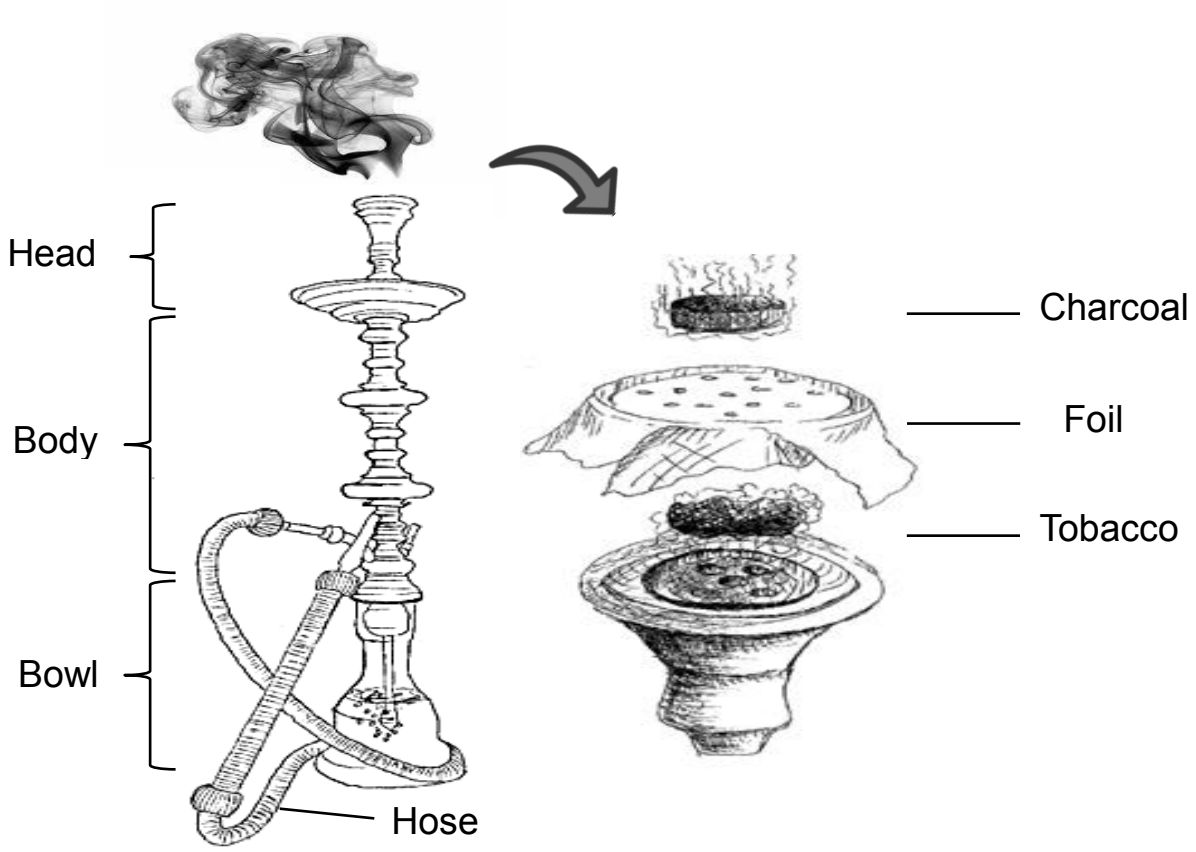

Fig. 1. *Schematic showing the major components of a Hookah.* A Hookah consists of 3 main parts; head, body and bowl. The tobacco is placed in a bowl covered with material such as aluminum foil where heated charcoal is placed on top. The bottom area, which is the bowl, is often filled with water. The aluminum foil placed between the tobacco and charcoal typically has tiny holes to allow direct exposure of the lit charcoal to mix with the Hookah. When the smoker inhales through the hose, negative pressure in the water bowl gets generated and as a result, air is pulled through the charcoal into the tobacco bowl creating smoke that is passed through the water and into the smoker's mouth, airways, circulatory and cardiovascular system (Figure adapted from Rezk-Hanna, O'Connell, Woo, 2014).

Figure 2. The Endothelium and Regulation of Vascular Tone.

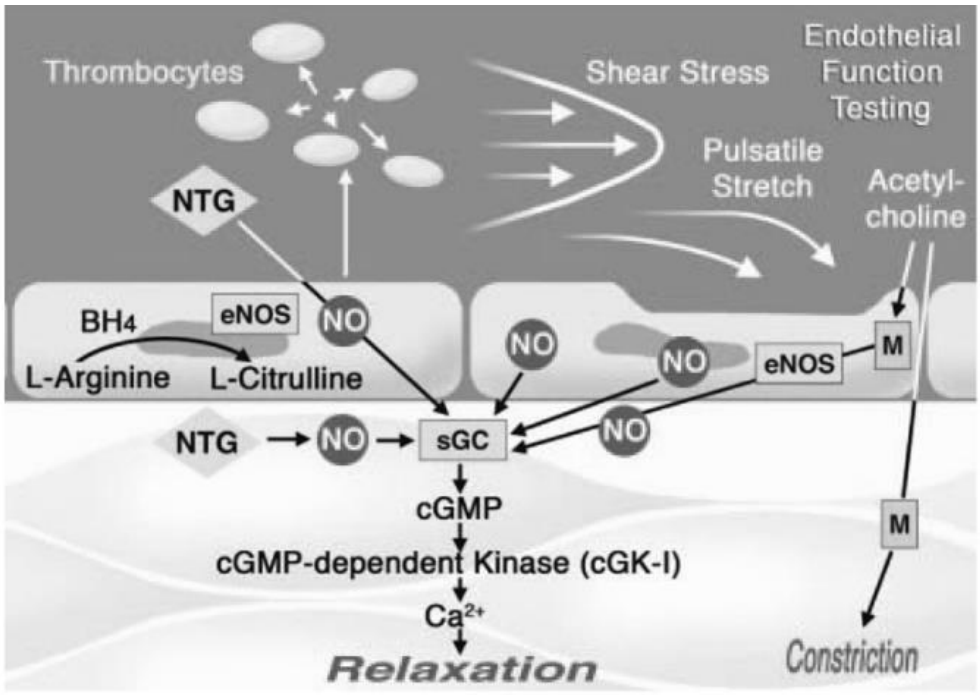

Fig. 2. *The Endothelium and Regulation of Vascular Tone.* In healthy endothelial cells, eNOS produces NO from l-arginine. NO is then released into the bloodstream to inhibit platelet aggregation and the release of vasoconstricting factors such as serotonin and thromboxane. Additionally, NO diffuses into the media and activates sGC and ultimately causing decreases in intracellular Ca and vasorelaxation. Other ways to assess endothelial function is through infusing acetylcholine which causes a dose-dependent vasodilation or constriction (in the presence of cardiovascular risk factors) and this is primarily due to stimulation of muscarinergic receptors in the media (Figure adapted from Munzel, Sinning, Post, Warnholtzm, Schultz, 2008).

Figure 3. Hypothesized Molecular Mechanism.

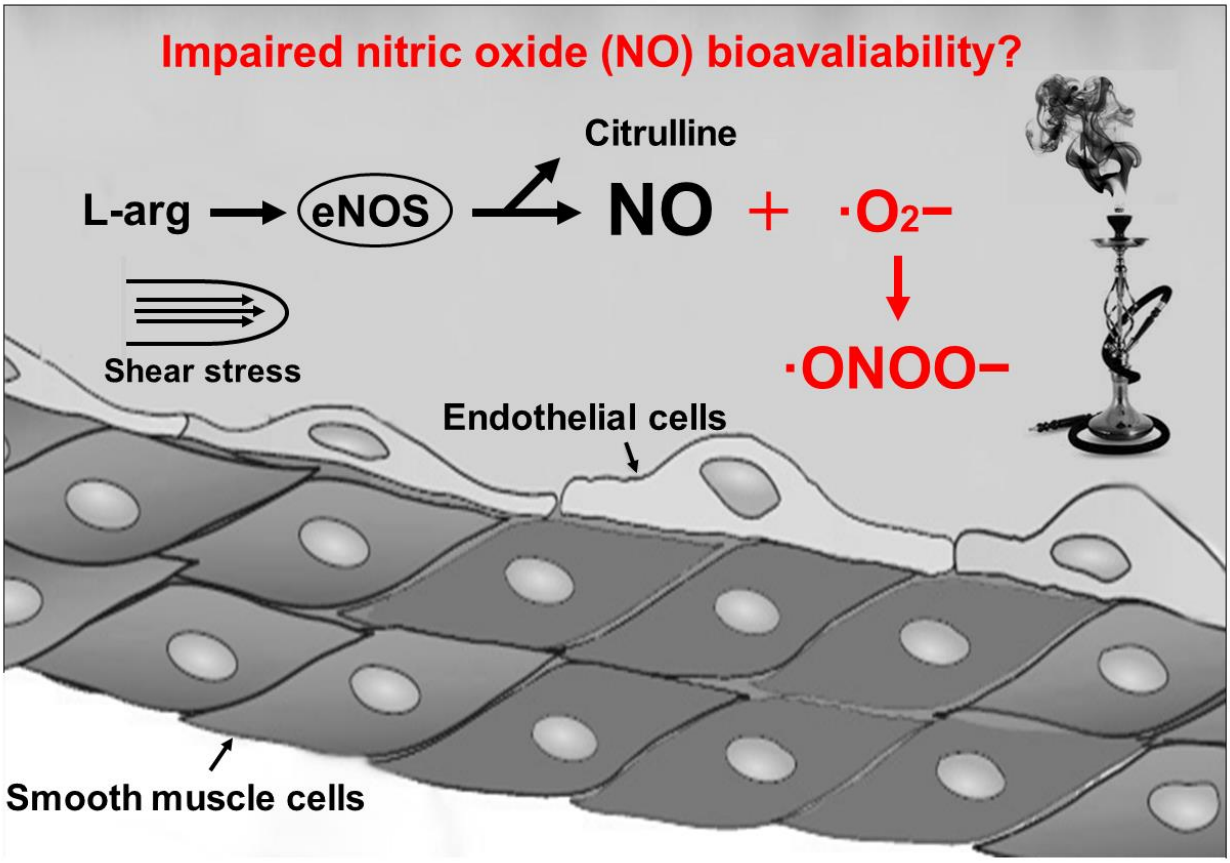

Fig. 3. Cartoon showing smooth muscle cells and the intima layer, including the endothelial cells. It is postulated that the high concentration of carbon-rich nanoparticles from burning charcoal generates reactive oxygen species such as superoxide (·O2−) that impairs NO· bioavailability leading to endothelial dysfunction, atherosclerosis, and ultimately cardiovascular disease risk factors.

Figure 4. Central Mechanistic Hypothesis.

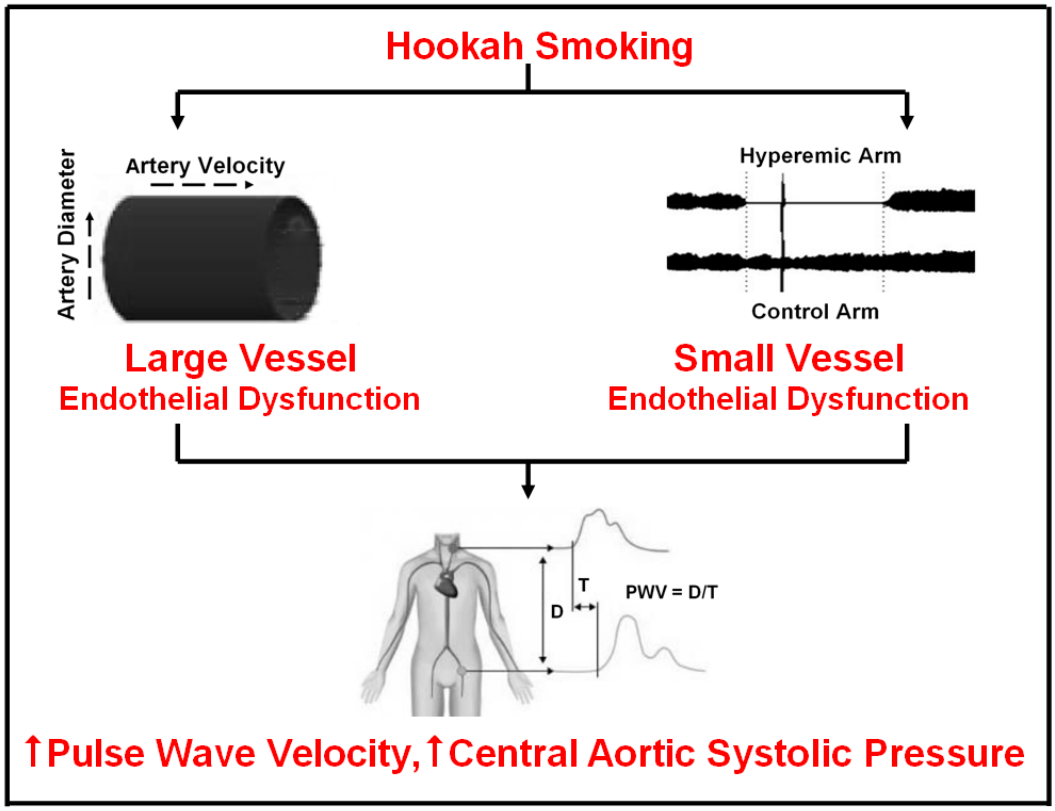

Fig. 4. *Central Mechanistic Hypothesis.* Hookah smoking is a potent stimulus to the vasculature targeted by large (Aim 1) and small (Aim 2) vessels throughout the human circulation. The acute effects of endothelial dysfunction manifested through these vessels will produce an acute increase in pulse wave velocity and an increase in central aortic systolic pressure (Aim 3; figure adapted from Complior, 2013).

Figure 5. Custom-built Hookah Smoking Chamber.

Figure 5A **Figure 5B**

Fig.5A. Custom-built Hookah smoking chamber. Figure showing the Plexiglass and aluminum smoking chamber with a procedure chair enclosed. A fan within the exhaust system continuously pulls air out through the vent in the ceiling. Multiple air-tight rubber ports on the front and side-panels allow wires and tubing to be connected to recording equipment outside the closed chamber; *Fig. 5B.* A close-up showing a mock subject inside the chamber smoking Hookah.

Figure 6. Smoking Biomarkers Exposure.

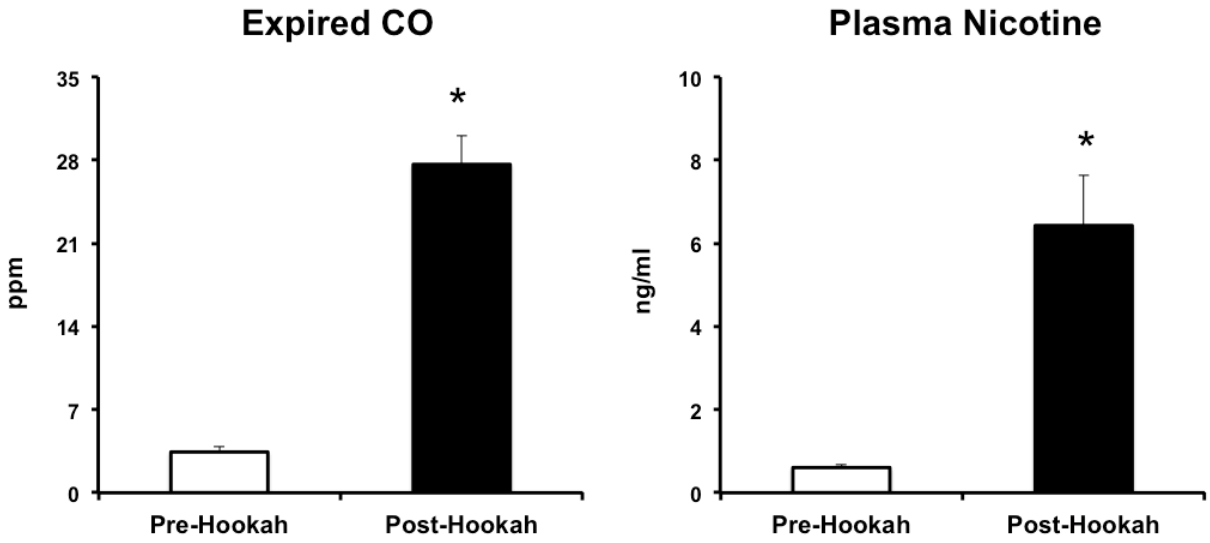

Fig. 6. *Smoking Biomarkers Exposure.* Summary data on expired CO and plasma nicotine before and after the hookah smoking session. Data are reported as mean ± SE. * Indicates $P < 0.05$.

Figure 7. Bland-Altman Plot for Assessment of Brachial Artery FMD.

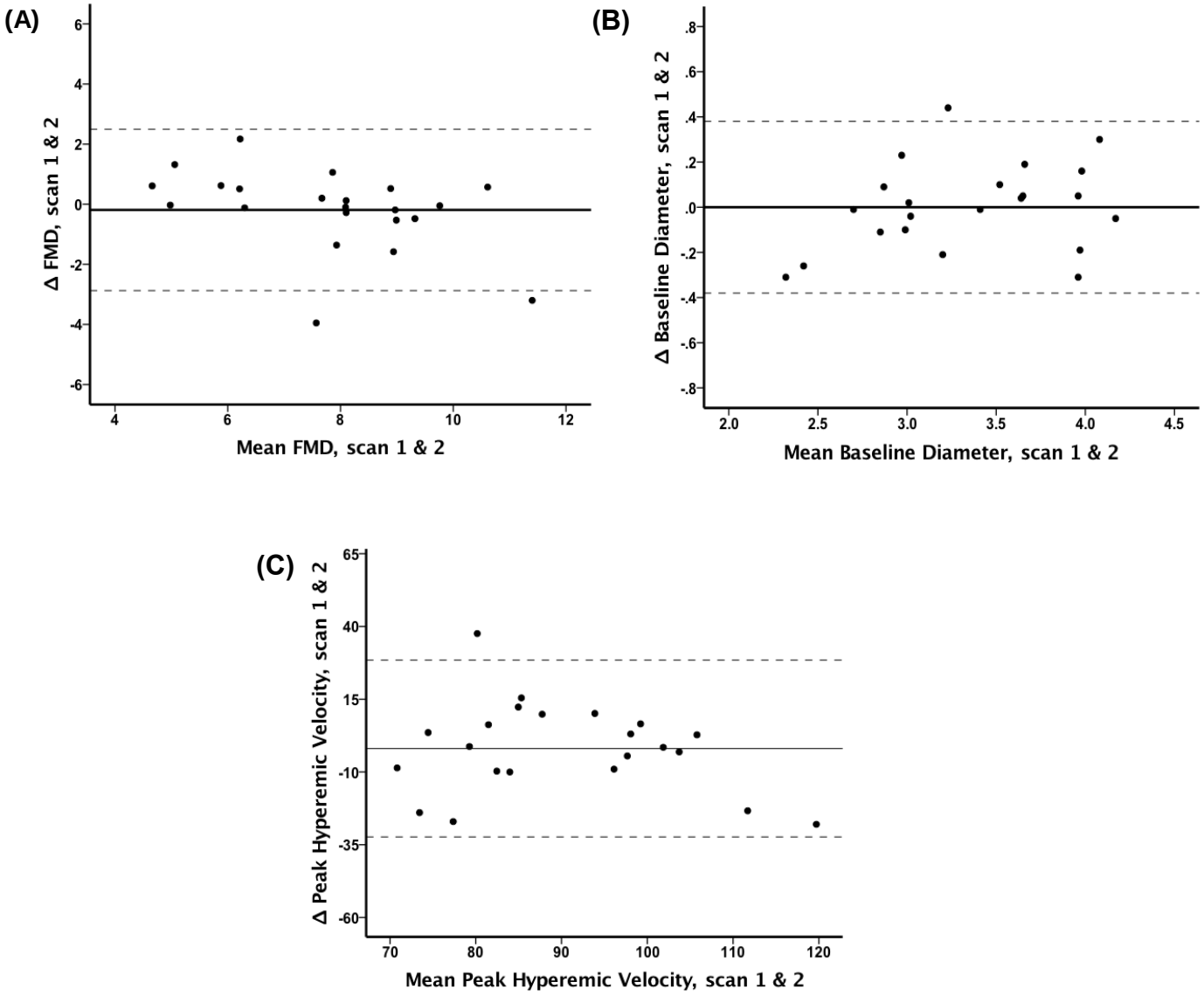

Fig. 7. Bland-Altman plots of intraobserver/ measurement variability of brachial artery FMD scan 1 and scan 2 showing no systemic bias: **(A)** FMD%, **(B)** Brachial artery baseline diameter, and **(C)** peak hyperemic blood velocity. The X-axis shows the mean and the Y-axis the differences between scans. The dotted lines represent 2 SD of the differences.

Figure 8. Differential Effects of Hookah and Cigarette Smoking on Brachial Artery FMD

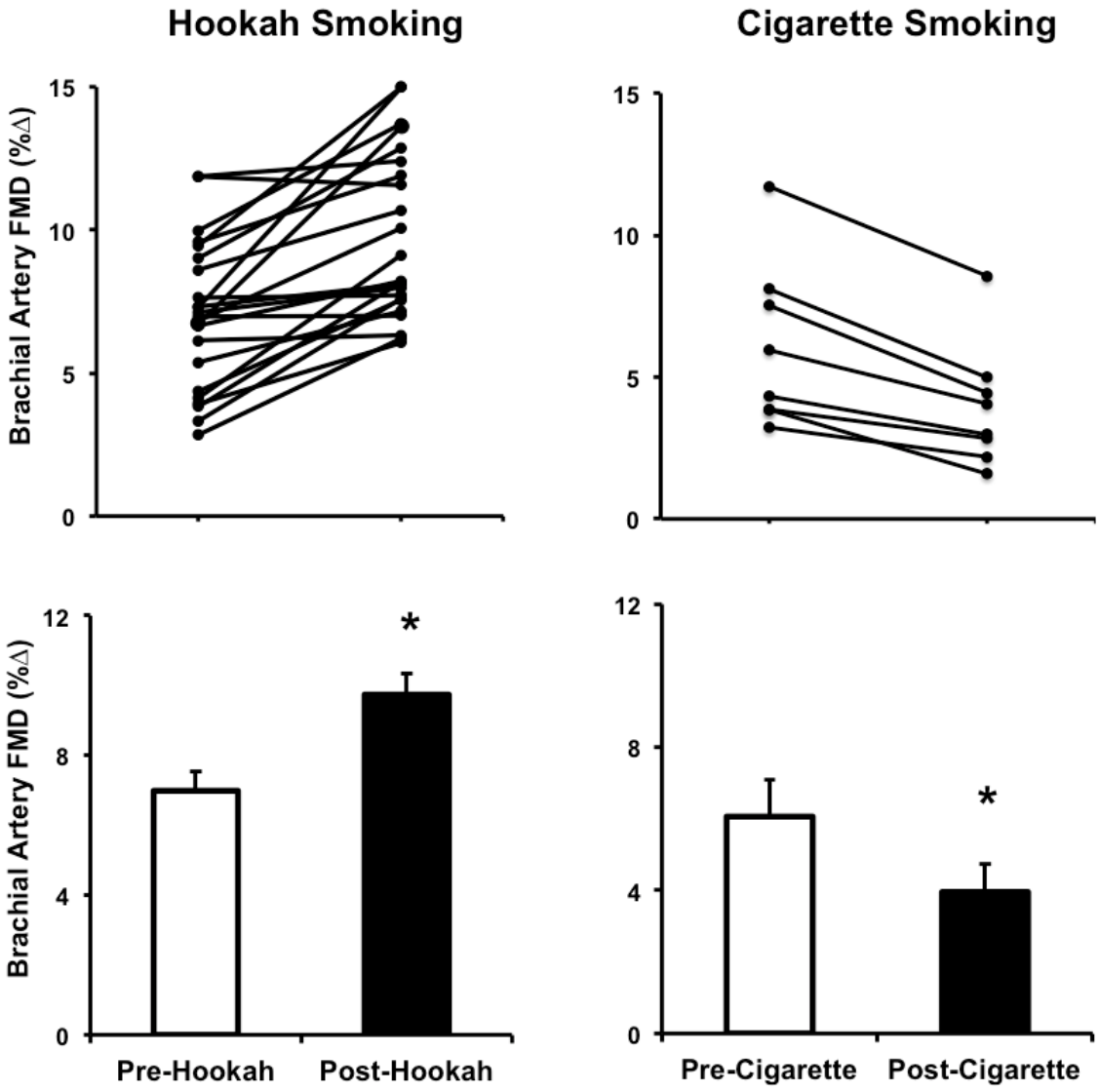

Fig. 8. Differential effects of hookah vs. cigarette smoking on brachial artery FMD. **Top panel,** Individual hookah (right) and cigarette (left); **Bottom panel**, mean percentage changes in hookah (6.9±0.5% to 9.7±0.6%%) vs. cigarettes (6.0±1.3% to 3.9±0.7%) brachial artery FMD before and immediately after smoking.

Data are reported as mean ± SE. * Indicates $P < 0.05$.

Figure 9. Illustrative Brachial Artery Images in a Hookah and Cigarette Subject Before and After Smoking

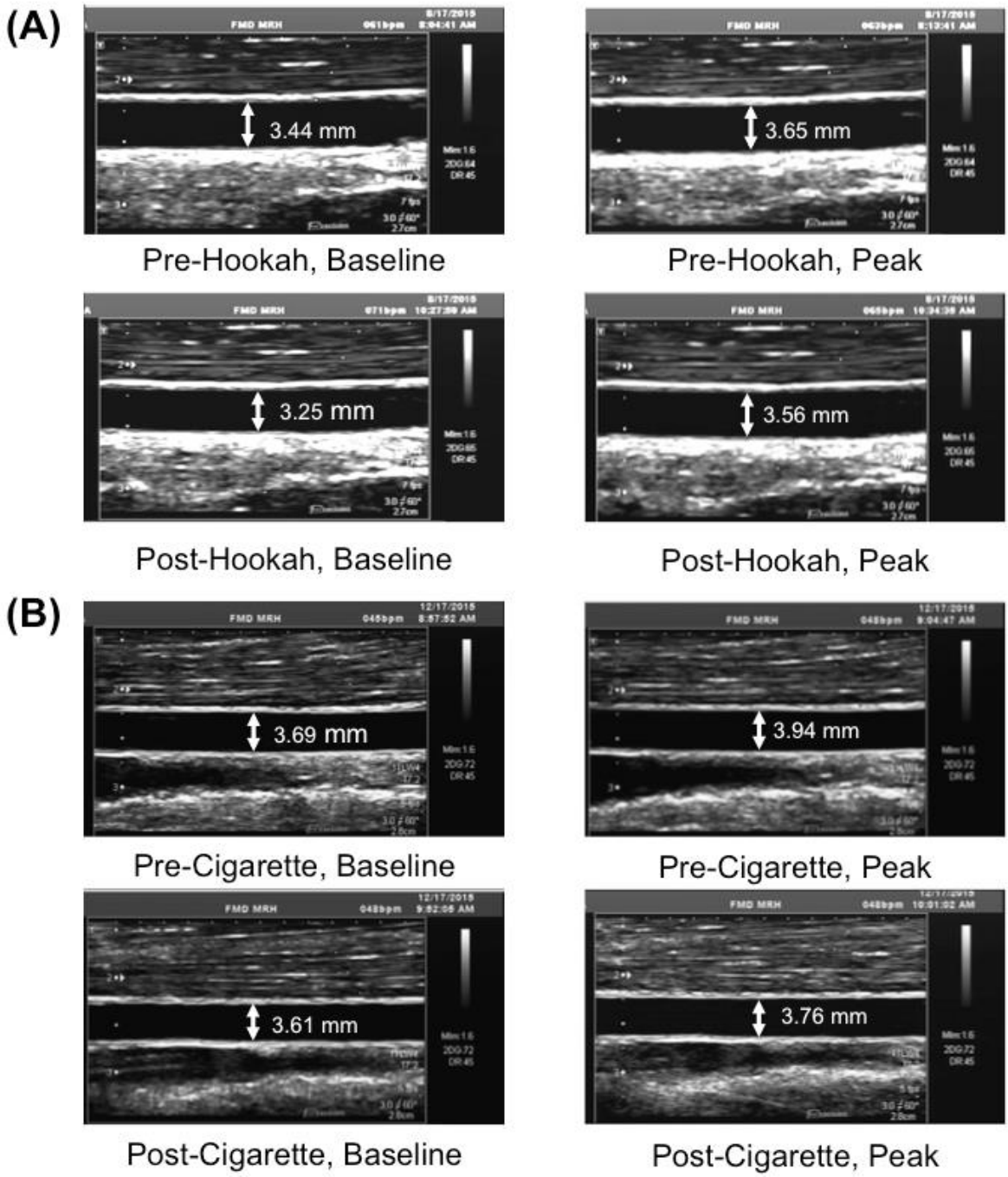

Fig. 9. Two representative brachial artery images reflecting baseline and peak diameter changes for Hookah (A) and Cigarette (B) smoking induced by hyperemia following deflation of a cuff inflated to supra-systolic pressure.

Figure 10. Relationship of FMD to Shear Rate and Peak Hyperemic Velocity in Hookah and Cigarette Smokers.

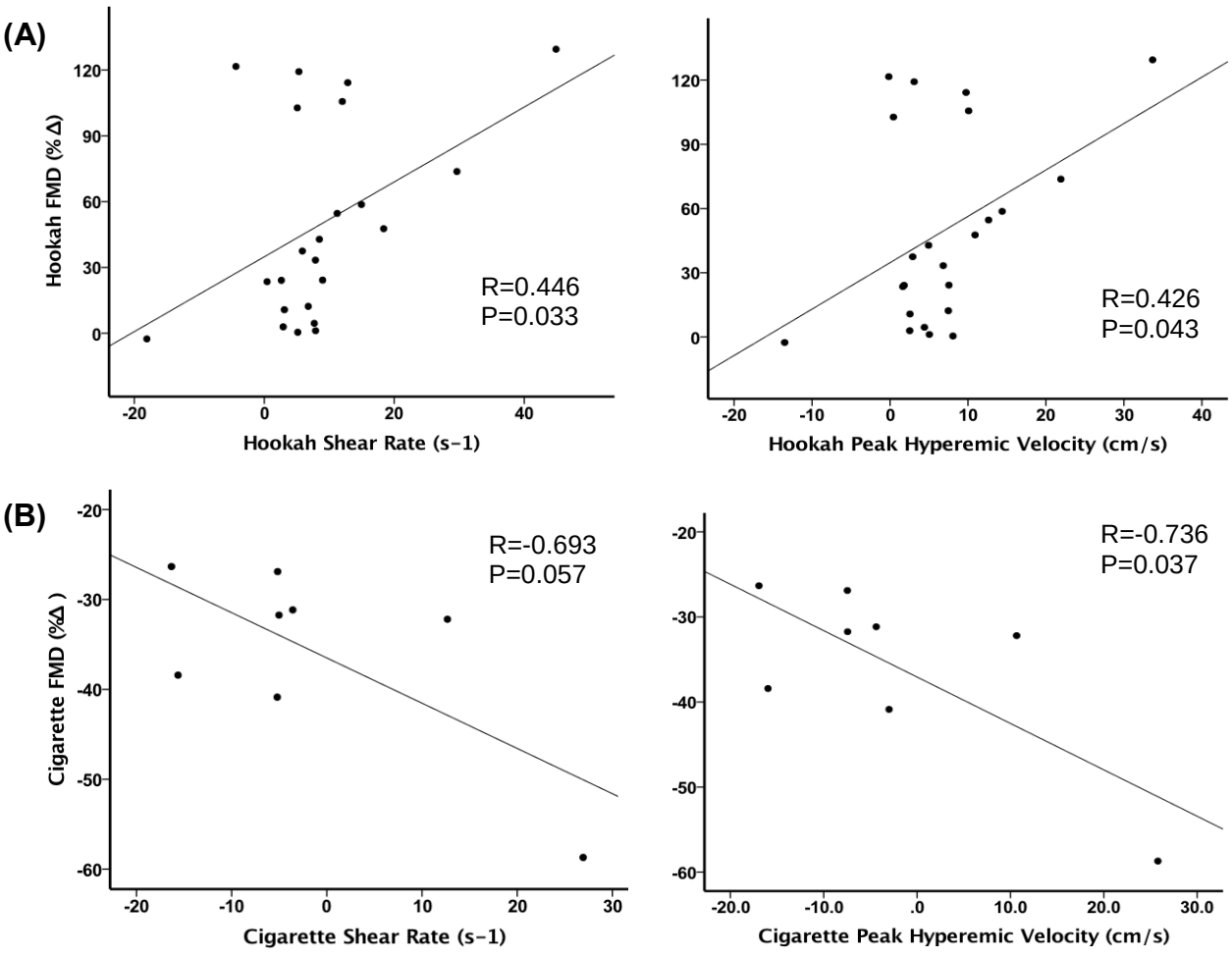

Fig. 10. **(A)** Hookah FMD *versus* peak shear rate and hyperemic velocity demonstrating a direct relationship. **(B)** Cigarette FMD *versus* peak shear rate and hyperemic velocity demonstrating an inverse relationship. Solid circles = subject values; line = fitted values by linear regression model.

Figure 11. Hemodynamic and Smoking Exposure Summary Data Before and After Hookah and Cigarettes Smoking.

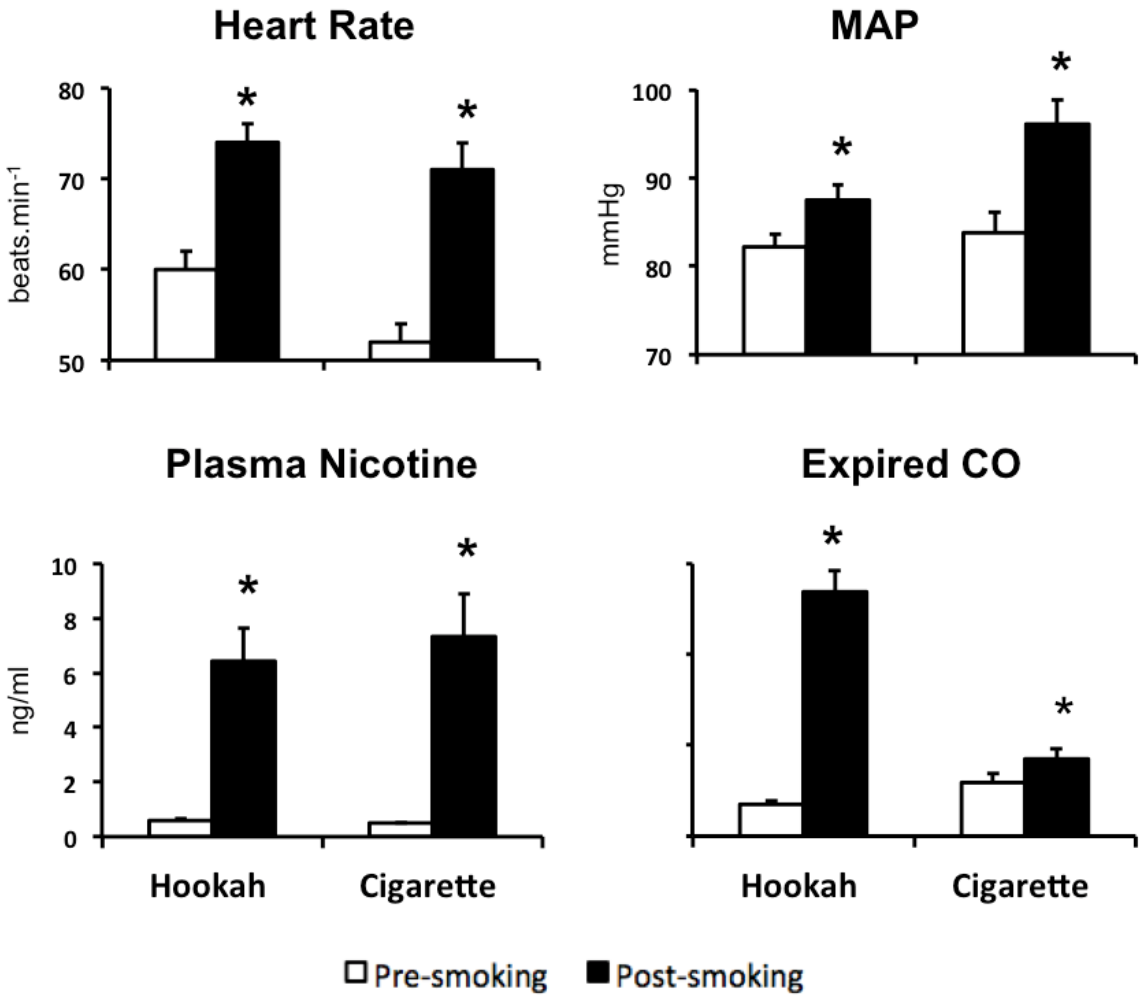

Fig. 11. Compared to cigarettes, the increases in heart rate and mean arterial pressure (MAP) were much less with hookah (ΔHR: +14±2 vs. 18±2 beats.min^{-1}; ΔMAP: +5±1 vs. +12±2 mmHg) but the expired CO level was almost 3-fold greater: +25±2 vs. +3±1 ppm). Data are reported as mean ± SE. * Indicates $P < 0.05$.

Figure 12. Acute Effects of Hookah Smoking on Micro-Vessel Endothelial Function by Peripheral Arterial Tonometry.

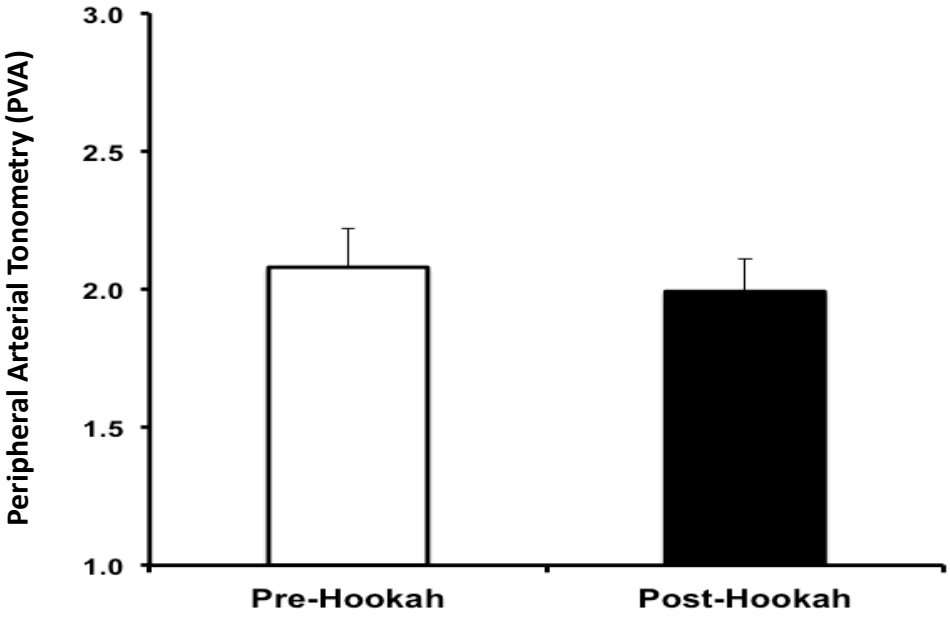

Fig. 12. Acute Effects of Hookah Smoking on Micro-Vessel Endothelial Function.

Summary data on the acute effects of hookah smoking on micro-vessel endothelial function assessed by the peripheral arterial tonometry pulse volume amplitude changes (PVA). Acutely and after smoking hookah for 30-minutes, pulse volume amplitude score changed from 2.09 ± 0.13 to 1.99 ± 0.12, p=0.633.

Figure 13. Illustrative Image Showing Pulse Volume Amplitude Changes in a Hookah Subject Before and After Hookah Smoking.

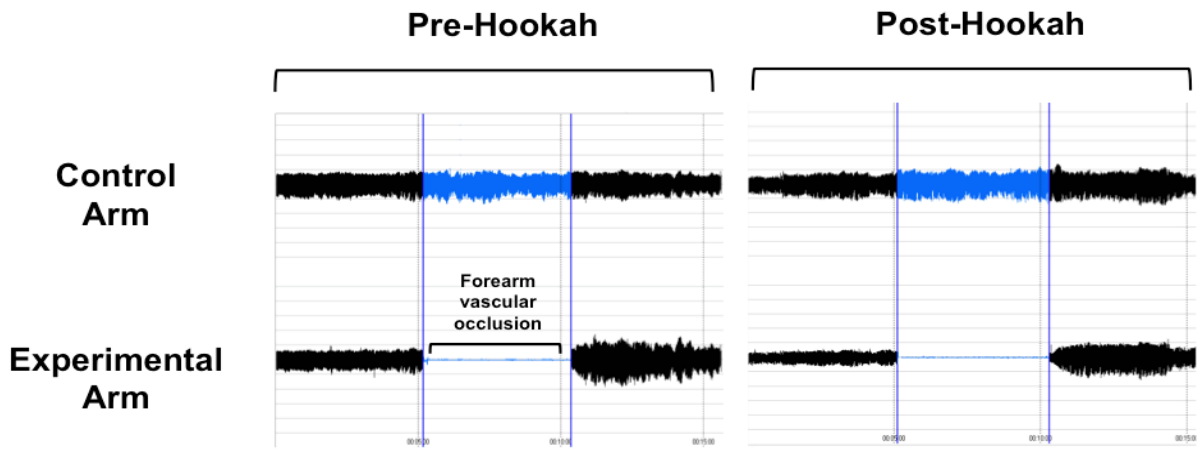

Fig. 13. Micro-vessel endothelial function measured before and after hookah smoking. In this representative 25-year-old subject, the left panel (pre-hookah) shows a normal endothelial vasodilator function evidenced by a large increase in pulse wave amplitude after forearm vascular occlusion for 5 minutes with a hyperemia index score of 1.80. The y-axis displays pulse wave amplitude. After smoking hookah for 30-minutes, the right panel reveals a reduced endothelial vasodilator function score of 1.75 evidenced by a considerable reduction of blood flow with a less than normal signal amplitude after cuff release. Measurement of the contralateral arm is used as an internal control to control for any pulse volume changes due to potential confounding/ systemic stimuli during the study.

Figure 14. Summary Data for Time-Control Experiments: Brachial Artery FMD.

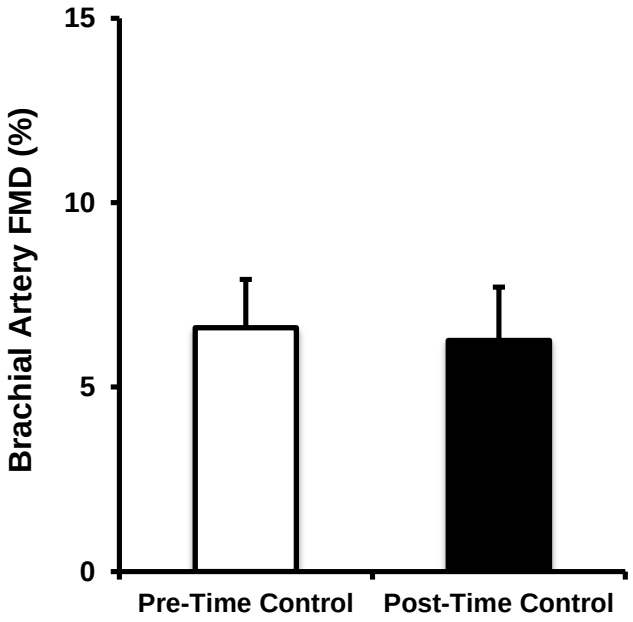

Fig. 14. After sitting in the smoking chamber, without smoking, for 30-minutes, brachial artery FMD did not change (from 6.60 ± 1.31 to 6.33 ± 1.48%, p=0.326) in time control subjects. Data are reported as mean ± SE.

Table 1. Sample Size Estimation.

Estimated power with a one-sided alpha at the 5% level			
Measurement	Estimated change	Sample size	Power
Aim 1: Brachial artery FMD	-5 ± 4%	26	>90%
Aim 2: Peripheral arterial tonometry – pulse volume amplitude	-1.27 ± 0.05	26	>90%
Aim 3: Pulse Tonometry – PWV	+2 ± 1 m/s	26	>90%
Pulse Tonometry – Central aortic Systolic Pressure	+10 ± 11mmHg	26	>90%

Table 2. Test-Retest Reproducibility of Brachial Artery FMD Indexes

Measurement	Linear Regression		Intraclass Correlation Coefficient	Bland-Altman		
	R	*P* Value		Bias ± SD	95% Limits of Agreement	
FMD, %	0.784	<0.001	0.857	-0.19±1.37	-2.87	2.49
Baseline diameter, mm	0.944	<0.001	0.969	0.00±0.19	-0.38	0.38
Peak hyperemic blood velocity, cm/s	0.496	0.009	0.656	-1.94±15.50	-32.32	28.44

Table 3. Hookah Smokers Subject Characteristics (n=23)

Variable	N or mean ± SD
Female/ Male	6/17
Age, years	25 ± 5
Body Mass Index, $kg \cdot m^2$	23.8 ± 2.3
Race/Ethnicity	
Non-Hispanic White	8
Non-Hispanic Black	2
Hispanic	2
Asian	6
Native Hawaiian/ Pacific Islander	2
American Indian/ Alaskan Native	1
Middle-Eastern Origin	2
Level of Education Attained	
High school	2
College	21
Smoking History	
Number of Hookah Sessions, week	3 ± 2
Session Duration, minutes	102 ± 42
Age of Hookah Smoking Onset, years	
$\leq$ 17	3
18-24	19
25-29	1

Data reported as number (N) or mean ± SD.

Table 4. Cigarette Smokers Subject Characteristics (n=8)

Variable	N or mean ± SD
Female/ Male	2/6
Age, years	26 ± 4
Body Mass Index, $kg \cdot m^2$	23.2 ± 2.9
Race/Ethnicity	
Non-Hispanic White	3
Non-Hispanic Black	4
Hispanic	0
Asian	1
Native Hawaiian/ Pacific Islander	0
American Indian/ Alaskan Native	0
Middle-Eastern Origin	0
Level of Education Attained	
High school	2
College	6
Smoking History	
Cigarettes, per day	9 ± 6
Cigarettes, pack years	4 ± 4
Age of Cigarette Smoking Onset, years	
$\leq$ 17	2
18-24	6
25-29	0

Data reported as number (N) or mean ± SD.

Table 5. Acute Effects of Hookah and Cigarette Smoking on Large-Vessel Endothelial Function

	Hookah Smoking				Cigarette Smoking			
	Pre-Hookah	**Post-Hookah**	**Δ (Post-Pre)**	**P Value**	**Pre-Cigarette**	**Post-Cigarette**	**Δ (Post-Pre)**	**P Value**
Biomarker Exposure								
Exhaled carbon monoxide, ppm	3 ± 0	28 ± 2	+25 ± 2	< 0.001	6 ± 1.00	9 ± 1.00	+3 ± 0.20	0.038
Plasma nicotine, ng/ml	0.59 ± 0.06	6.43 ± 1.22	+5.84 ± 1.21	< 0.001	0.50 ± 0.00	7.33 ± 1.58	+6.83 ± 1.58	0.001
Hemodynamics								
Heart rate, beats·min^{-1}	60 ± 2	74 ± 2	+14 ± 2	< 0.001	52 ± 2	71 ± 3	+18 ± 2	< 0.001
Systolic blood pressure, mmHg	113 ± 2	120 ± 2	+6 ± 1	< 0.001	117 ± 4	128 ± 4	+11 ± 3	0.003
Diastolic blood pressure, mmHg	67 ± 2	71 ± 2	+5 ± 1	0.305	67 ± 2	80 ± 3	+13 ± 2	< 0.001
Mean arterial pressure, mmHg	82 ± 2	88 ± 2	+5 ± 1	< 0.001	84 ± 2	96 ± 3	+12 ± 2	< 0.001
Endothelial Function Parameters								
Flow-mediated dilation, %Δ	6.99 ± 0.54	9.74 ± 0.61	+2.75 ± 0.46	< 0.001	6.06 ± 1.03	3.95 ± 0.77	-2.11 ± 0.33	< 0.001
Flow-mediated dilation, mmΔ	0.23 ± 0.02	0.30 ± 0.02	+0.08 ± 0.01	< 0.001	0.20 ± 0.04	0.12 ± 0.03	-0.07 ± 0.01	0.006
Baseline diameter, mm	3.30 ± 0.09	3.19 ± 0.09	-0.10 ± 0.04	0.008	3.20 ± 0.22	3.11 ± 0.22	-0.09 ± 0.02	0.001
Time to peak diameter, s	43.65 ± 1.82	48.39 ± 2.02	+4.74 ± 1.13	< 0.001	38.63 ± 2.95	45.25 ± 2.43	+6.63 ± 2.20	0.019
Peak hyperemic blood velocity, cm/s	97.51 ± 3.76	103.28 ± 3.33	+5.78 ± 1.52	0.001	82.31± 7.65	78.23 ± 5.28	-4.08 ± 3.40	0.269
Baseline flow, ml/min	92.72±11.32	81.82±8.52	-10.91±9.17	0.247	93.84±28.24	67.96±15.23	-25.88±18.30	0.200
Peak hyperemic blood flow, ml/min	568.52±30.48	595.60±29.99	+27.08±12.66	0.043	484.96±86.27	410.82±65.26	-74.14±26.08	0.024
Hyperemic flow, %	630.76 ± 56.36	718.79 ± 47.61	+88.03±57.42	0.139	591.00±103.95	601.30 ±77.65	+10.30± 61.71	0.872
Peak shear rate, s^{-1}	2507.65±138.22	2689.03±136.04	+181.38±51.5	0.001	2157.80±180.28	2097.00±159.6	-60.80 ± 92.85	0.533

Data reported as mean ± SE for 23 hookah and 8 cigarette subjects.

Table 6. Mixed Model FMD Summary Results

Variable	Estimate	Error	P Value
Age	0.05516	0.1153	0.6387
BMI	-0.2036	0.2536	0.4337
Ethnicity			
African-American/ Black	0.5711	1.8259	0.7585
Asian	-0.8448	1.7395	0.6338
Hispanic	-0.3370	1.6888	0.8443
Native Hawaiian/ Pacific Islander	3.9409	2.1763	0.0890
Caucasian/ White	Ref.	.	.
Gender			
Female	3.2448	1.1567	0.0127
Male	Ref.	.	.
Hookah Smoking Frequency	0.2366	0.4766	0.6263
Hookah Smoking Session Duration	-0.00873	0.01364	0.5310
Plasma Nicotine	0.1713	0.1692	0.3263
Expired CO	-0.01284	0.06721	0.8509
FMD, %	2.2351	0.5297	0.0007

Table 7. Mixed Model Peripheral Arterial Tonometry Summary Results

Variable	Estimate	Error	P Value
Age	0.02963	0.02842	0.3127
BMI	0.1195	0.06251	0.0740
Ethnicity			
African-American/ Black	0.1146	0.4501	0.8023
Asian	0.02312	0.4288	0.9577
Hispanic	0.1931	0.4163	0.6490
Native Hawaiian/ Pacific Islander	1.4853	0.5365	0.0137
Caucasian/ White	Ref.	.	.
Gender			
Female	-0.1168	0.2852	0.6876
Male	Ref.	.	.
Hookah Smoking Frequency	-0.02495	0.1175	0.8345
Hookah Smoking Session Duration	-0.00361	0.003362	0.2987
Plasma Nicotine	0.01366	0.04171	0.7476
Expired CO	0.01546	0.01657	0.3645
Peripheral Arterial Tonometry (PVA)	-0.1406	0.1899	0.4699

Table 8. Mixed Model Pulse Wave Velocity Summary Results

Variable	Estimate	Error	P Value
Age	0.1195	0.03912	0.0080
BMI	-0.08176	0.08602	0.3569
Ethnicity			
African-American/ Black	-0.6737	0.6249	0.2980
Asian	0.3717	0.5924	0.5398
Hispanic	-0.02357	0.6189	0.9701
Native Hawaiian/ Pacific Islander	1.4397	0.7463	0.0728
Caucasian/ White	Ref.	.	.
Gender			
Female	-1.6350	0.3924	0.0008
Male	Ref.	.	.
Hookah Smoking Frequency	0.1393	0.1627	0.4055
Hookah Smoking Session Duration	0.006819	0.004683	0.1659
Plasma Nicotine	-0.08744	0.05910	0.1597
Expired CO	-0.02053	0.02284	0.3829
Pulse Wave Velocity	0.5560	0.1694	0.0050

Table 9. Mixed Model Central Systolic Blood Pressure Summary Results

Variable	Estimate	Error	P Value
Age	0.2839	0.7301	0.7025
BMI	0.5923	1.6060	0.7171
Ethnicity			
African-American/ Black	-5.7454	11.5649	0.6261
Asian	5.5251	11.0175	0.6229
Hispanic	-0.4979	10.6966	0.9634
Native Hawaiian/ Pacific Islander	3.7653	13.7844	0.7882
Caucasian/ White	Ref.	.	.
Gender			
Female	-0.3264	7.3263	0.9650
Male	Ref.	.	.
Hookah Smoking Frequency	1.6770	3.0184	0.5862
Hookah Smoking Session Duration	0.07233	0.08637	0.4146
Plasma Nicotine	0.3311	1.0716	0.0081
Expired CO	0.05399	0.4257	0.9007
Central Systolic BP	4.5294	1.5003	0.7613

Table 10. Hemodynamic and Central Arterial Stiffness Indices in Response to Acute Hookah Smoking.

	Pre-Hookah	Post-Hookah	P Value
Respiratory rate, breath·min^{-1}	17 ± 1	18 ± 1	0.077
Heart rate, beats·min^{-1}	60 ± 2	74 ± 2	< 0.001
Peripheral Blood Pressure			
Systolic, mmHg	113 ± 2	120 ± 2	< 0.001
Diastolic, mmHg	66 ± 4	71 ± 2	0.305
Pulse pressure, mmHg	47 ± 2	48 ± 2	0.105
Mean, mmHg	82 ± 2	88 ± 2	< 0.001
Central Blood Pressure			
Systolic, mmHg	99 ± 2	104 ± 2	0.001
Diastolic, mmHg	67 ± 2	72 ± 2	0.001
Pulse pressure, mmHg	32 ± 1	32 ± 1	0.530
Mean, mmHg	78 ± 2	83 ± 2	0.001
Indices of Vascular Stiffness			
Augmentation pressure, mmHg	2.5 ± 0.8	3.1 ± 0.8	0.580
Augmentation index, %	8.4 ± 2.8	9.7 ± 2.3	0.672
Carotid-femoral PWV	7.47 ± 0.20	8.04 ± 0.22	0.002

Data are reported as mean ± SE for 23 hookah subjects.

Table 11. Summary Data for Time-Control Experiments: Expired CO, Hemodynamic Indices and Endothelial Function Parameters

	Pre-Time Control	Post-Time Control	P Value
Biomarker Exposure			
Expired carbon monoxide, ppm	4.0 ± 0.3	3.6 ± 0.4	0.117
Heart Rate and Blood Pressure			
Heart rate, beats·min^{-1}	57 ± 5	58 ± 6	0.722
Systolic blood pressure, mmHg	116 ± 6	115 ± 5	0.605
Diastolic blood pressure, mmHg	70 ± 5	72 ± 2	0.911
Mean arterial pressure, mmHg	86 ± 4	86 ± 3	0.896
Endothelial Function Parameters			
Flow-mediated dilation, %Δ	6.60 ± 1.31	6.33 ± 1.48	0.326
Flow-mediated dilation, mmΔ	0.21 ± 0.04	0.20 ± 0.04	0.208
Baseline diameter, mm	3.26 ± 0.22	3.23 ± 0.23	0.139

Data are reported as mean ± SE for 5 subjects.

Table 12. Summary Data for Repeatability Experiments: Expired CO, Hemodynamic Indices and Endothelial Function Parameters

	Day 1	Day 2	P Value
Biomarker Exposure			
Expired carbon monoxide, ppm	4.0 ± 0.3	3.6 ± 0.4	0.177
Heart Rate and Blood Pressure			
Heart rate, beats·min^{-1}	57 ± 5	54 ± 4	0.534
Systolic blood pressure, mmHg	116 ± 6	113 ± 3	0.380
Diastolic blood pressure, mmHg	70 ± 4	67 ± 3	0.237
Mean arterial pressure, mmHg	86 ± 4	82 ± 2	0.157
Endothelial Function Parameters			
Flow-mediated dilation, %Δ	6.78 ± 1.40	6.71 ± 1.29	0.822
Flow-mediated dilation, mmΔ	0.22 ± 0.04	0.21 ± 0.04	0.487
Baseline diameter, mm	3.28 ± 0.21	3.20 ± 0.20	0.093

Data are reported as mean ± SE for 5 subjects.

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