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EVALUATING ORAL, NON-COMBUSTIBLE POTENTIAL REDUCED EXPOSURE PRODUCTS MARKETED TO SMOKERS

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EVALUATING ORAL, NON-COMBUSTIBLE POTENTIAL REDUCED EXPOSURE PRODUCTS MARKETED TO SMOKERS

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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List of Abbreviations

1-HOP	1-hydroxypyrene
ANOVA	analysis of variance
CO	carbon monoxide
HSD	Honestly Significant Difference
LOQ	limit of quantification
mg	milligram
min	minute
ml	milliliter
ng	nanogram (0.0000000001 grams)
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NRT	nicotine replacement therapy
PAHs	polycyclic aromatic hydrocarbons
ppm	concentration in parts per million
PREPs	potential reduced exposure products
QSU	Questionnaire of Smoking Urges
TSNAs	tobacco specific nitrosamines
US DHHS	U.S. Department of Health and Human Services
VAS	visual analog scale
µg	microgram

Abstract

EVALUATING ORAL, NON-COMBUSTIBLE POTENTIAL REDUCED EXPOSURE PRODUCTS MARKETED TO SMOKERS

By Caroline O. Cobb, B.A.

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

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Potential reduced exposure products (PREPs) are marketed to reduce smoking's harm, despite little information concerning their effects. This study adapts previously reported clinical laboratory methods used to evaluate combustible PREPs to investigate the acute effects of four non-combustible PREPs (Ariva, Camel Snus, Marlboro Snus, Commit nicotine lozenge) relative to own brand cigarettes, sham smoking, and one combustible PREP that delivers no measurable nicotine (Quest). Twenty-eight smokers participated in 7 Latin-squared ordered, 2.5-hr sessions in which each product was administered twice (60-minute inter-administration interval). Sessions differed by product and were separated by $\geq$ 48 hours. Plasma nicotine, heart rate, expired air carbon

monoxide (CO), and subjective effects were assessed. Relative to own brand, non-combustible PREPs decreased nicotine and CO exposure, did not suppress abstinence symptoms fully, and were less acceptable. These short-term clinical laboratory methods are reliable and provide valuable information concerning non-combustible PREPs for smokers.

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CHAPTER 1 Introduction

Tobacco use is one of the most dangerous threats to public health today. In the United States (U.S.), an estimated 45.4 million adults smoke tobacco cigarettes, a behavior that remains the leading cause of preventable death in this country (Centers for Disease Control, 2007). Diseases associated with tobacco use include cardiovascular disease and a variety of cancers (Mathers & Loncar, 2006; U.S. Department of Health and Human Services [US DHHS], 2004). These diseases are primarily caused by components of tobacco smoke such as carbon monoxide (CO) and carcinogenic toxicants (Lakier, 1992; Hoffman & Hoffman, 1995). The carcinogenic toxicants in tobacco smoke include polycyclic aromatic hydrocarbons (PAHs) and tobacco specific nitrosamines (TSNAs; Hecht, 2006) that damage genes that control the growth of cells, causing them to grow abnormally or to reproduce rapidly (US DHHS, 2004). Besides CO-induced cardiovascular disease and cancer, tobacco cigarette smoking also causes emphysema (Minai, Benditt, & Martinez, 2008), chronic obstructive pulmonary disease (Yoshida & Tuder, 2007), and a "fetal tobacco syndrome" that is related to infant morbidity, mortality, and other adverse developmental outcomes (Baird & Wilcox, 1985; Nieburg, Marks, McLauren, & Remington, 1985). In sum, reducing tobacco-caused death and disease is a national health care priority (US DHHS, 2000).

Researchers and clinicians agree that the most effective way to reduce tobacco-caused death and disease is to quit smoking (Critchley & Capewell, 2004; Schroeder,

2005; Reid, Quinlan, Riley, & Pipe, 2007). However, quitting smoking is challenging because smokers are dependent on another smoke toxicant, the stimulant drug nicotine (Benowitz, 2008). Nicotine is one of several alkaloids found in the tobacco plant, and it is the primary psychoactive toxicant in tobacco smoke (US DHHS, 1988; Zevin, Gourlay, & Benowitz, 1998). After nicotine enters the pulmonary venous circulation via inhalation, it moves quickly to the brain and binds directly to nicotinic acetylcholine receptors (NAChRs; Benowitz, 2008; Nisell, Nomkios, & Svensson, 1994). Nicotine then causes the release of a variety of neurotransmitters, most importantly dopamine (Benowitz, 2008). These properties underlie the influence of this drug upon tobacco users.

Nicotine's Role in Tobacco Use

Because nicotine is a stimulant drug that cigarette smokers self-administer, and because stimulant drugs often act as positive reinforcers (e.g., amphetamine, caffeine cocaine; Griffiths & Woodson, 1988; Higgins, Bickel, & Hughes, 1994; Rush, Essman, Simpson, & Baker, 2001) initial tobacco use episodes may be reinforced positively (i.e., produce direct effects that make subsequent drug self-administration more likely; Eissenberg, 2004). Indeed, like other drugs that act as positive reinforcers (e.g., cocaine), nicotine administration is associated with dopamine system activation (Benowitz, 2008; Watkins, Koob, & Markou, 2000). The ability of drugs to act as reinforcers has been addressed by many models, including one that suggests that the first several drug use episodes cause a brief decrease in reward threshold (Koob & Le Moal, 1997; Koob, 1999). This decrease in reward threshold leads to the perception of events that are

usually perceived as neutral to be perceived as pleasant, and events that are usually perceived as pleasant to be perceived as more pleasant. These drug-induced changes in the hedonic value of events increase the likelihood of subsequent drug self-administration (i.e., are positively reinforcing; Koob & Le Moal, 1997; Koob, 1999).

While the initial drug use episodes are reinforced positively, repeated drug administration changes the organism's underlying neurobiology and may make negative reinforcement more relevant in explaining chronic drug use. With respect to nicotine, neuroadaptations in the dopaminergic system occur with repeated nicotine exposure (Hildebrand, Nomikos, Hertel, Schildrom, & Svensson, 1998; Fung, Schmid, Anderson, & Lau, 1996; Court et al., 1998). For example, in rats exposed to nicotine over 7 days, a reduction in dopamine was observed in the nucleus accumbens during acute nicotine abstinence (Hildebrand et al., 1998). In a similar study in which rats were exposed to nicotine over 14 days, a significant decrease in dopamine output was observed from the striatum and nucleus accumbens within the first 24 hours of nicotine abstinence (Fung et al., 1996). A study performed on human brain tissue suggested that chronic cigarette smoking was associated with a reduction of dopaminergic neuron firing in the nigrostriatal region of the brain (Court et al., 1998). These changes in neurobiology may indicate an overall increase in the baseline reward threshold in chronic nicotine users (Koob & Le Moal, 1997; Koob, 1999).

This increased reward threshold is less apparent when the drug is being administered, but becomes more noticeable when the drug is not administered. In a drug dependent organism with an increased reward threshold and no access to drug, events that

are usually perceived as neutral are perceived as unpleasant, and events that are usually perceived as pleasant are perceived as neutral (Koob & Le Moal, 1997; Koob, 1999). In terms of cigarette smoking (i.e., nicotine self-administration), chronic drug exposure alters the dopamine system and thus may increase the smoker's reward threshold. During periods of tobacco abstinence, an aversive syndrome that includes irritability, anxiety, and difficulty concentrating (e.g., Hughes & Hatsukami, 1986; Buchhalter, Acosta, Evans, Breland, & Eissenberg, 2005) may reveal the altered reward threshold. Interestingly, according to the model, drug administration has the potential to decrease reward threshold temporarily, even in the dependent organism (Watkins et al., 2000). When the reward threshold is reduced, the perception of events is altered (i.e., events perceived as unpleasant are perceived as neutral or pleasant), and this drug-induced alteration of events previously perceived as unpleasant increases the likelihood of subsequent drug self-administration. In this context, these drug administrations in the dependent organism are maintained via negative reinforcement (e.g., Eissenberg, 2004). Again, in terms of cigarette smoking, the aversive symptoms that accompany abstinence are suppressed by cigarette-delivered nicotine, and this abstinence symptom suppression is thought to maintain the smokers' behavior.

Tobacco Abstinence Symptomology and Suppression

Tobacco abstinence effects have been studied in the clinical laboratory for several decades, and the evidence shows that aversive abstinence symptoms occur reliably and that administration of a cigarette or pharmacologically pure nicotine suppresses these symptoms. For example, in an early clinical study of fifty smokers, the signs and

symptoms of tobacco abstinence during two days of ad-libitum smoking and during the first four days of tobacco abstinence were examined (Hughes & Hatsukami, 1986). Relative to days during ad-libitum smoking, participants reported increases in ratings of anxiety, craving, difficulty concentrating, eating, hunger, impatience, irritability, and restlessness during the abstinence phase. Decreases in ratings of sleep adequacy and heart rate were also noted during the abstinence phase. To verify these self-reported symptoms, observers were recruited to rate each participant on an identical scale for the following symptoms: anxiety, drowsiness, fatigue, impatience, irritability, restlessness, and somatic complaints. All observer ratings were significantly related to the corresponding participant ratings. This study demonstrated that tobacco abstinence effects can be measured reliably by both participant-reported symptoms and observational signs (Hughes & Hatsukami, 1986). In another clinical examination of tobacco abstinence, participants were asked to press a button on a keyboard to obtain puffs of a cigarette under a progressive ratio schedule (Willner, Hardman, & Eaton, 1995). Participants were either tobacco abstinent or asked to smoke normally for the four hours prior to the session. Significantly increased breakpoints were observed in the tobacco abstinent participants (i.e. abstinent participants were willing to perform more work to obtain cigarette puffs; Willner et al., 1995). These results highlight tobacco abstinence symptomology and how tobacco abstinence can influence subsequent nicotine self-administration.

Smoking-induced abstinence symptom suppression has also been studied in the laboratory, and there is little doubt that cigarette smoking suppresses abstinence effects.

For example, in a short-term clinical examination of 20 cigarette smokers who were required to be tobacco abstinent prior to each session, own brand cigarette administration was associated with a significant decrease on a subjective measure of "craving a cigarette" (Breland, Evans, Buchhalter, & Eissenberg, 2002). Similar abstinence suppression effects have been noted in other studies of tobacco abstinent smokers and the changes that occurred during own brand cigarette administrations (Buchhalter & Eissenberg, 2000; Breland, Kleykamp, & Eissenberg, 2006).

In addition, tobacco abstinence effects in cigarette smokers can be suppressed by pharmacologically pure nicotine (Molander, Lunell, & Fagerström, 2000; Evans, Blank, Sams, Weaver, & Eissenberg, 2006; Nemeth-Coslett, 1987; Kleykamp, Jennings, Sams, Weaver, & Eissenberg, 2008). A clinical examination of the effects of transdermal nicotine in smokers abstaining from tobacco/nicotine for at least 8 hours showed that transdermal nicotine induced dose-related abstinence symptom suppression (Evans et al., 2006). In addition, a study investigating the effects of nicotine gum among smokers reported that nicotine gum used prior to smoking produced dose-related decreases in various measures of cigarette smoking including number of cigarettes smoked, number of puffs taken, expired air carbon monoxide level, and ratings of smoking satisfaction (Nemeth-Coslett, 1987).

These and related findings concerning the relationship of tobacco abstinence symptom suppression and administration of pharmacologically pure nicotine has lead to the introduction of nicotine replacement therapy (NRT) as a cessation strategy for tobacco use. By reducing the aversive symptoms associated with stopping smoking with

pharmacologically pure nicotine administration, NRT may improve the likelihood of successful abstinence (Stead, Perera, Bullen, Mant, & Lancaster, 2008). Currently, the U.S. Food and Drug Administration (FDA) has approved three NRT products for over-the-counter sale: nicotine gum, nicotine patch, and nicotine lozenge (American Lung Association [ALA], 2006). Other FDA approved products for tobacco use cessation include the nicotine inhaler and nicotine nasal spray, available by prescription only (ALA, 2006; Rigotti, 2002). A recent meta-analysis of 132 NRT related human trials concluded among those attempting to quit all commercially available NRTs increased the rate of quitting by 50-70% (Stead et al., 2008). These findings support the notion that suppression of tobacco abstinence symptomology with products that are not cigarettes may reduce the likelihood of future tobacco use.

However, pharmacologically pure nicotine does not suppress tobacco abstinence effects fully. One clinical study compared the effects of transdermal nicotine patch and smoking among overnight tobacco abstinent participants. The results showed that transdermal nicotine produced lower levels of abstinence suppression when compared to smoking (Kleykamp et al., 2008). Thus, nicotine dose may not be the only factor involved in tobacco abstinence suppression in smokers. In summary, cigarette smokers experience an aversive syndrome during periods of abstinence, and this abstinence syndrome can be suppressed by subsequent smoking and, to a lesser extent, by administration of pharmacologically pure nicotine.

Abstinence effects and their suppression by smoking are thought to play a major role in relapse to smoking by smokers attempting to quit (US DHHS, 1988). For

example, relapse rates in the U.S. are as high as 80% in the first month of abstinence (US DHHS, 2004; Hughes et al., 1992). Many of these failures to maintain abstinence occur within the first 24 hours following the beginning of a quit attempt (Hendricks, Ditre, Drobes, & Brandon, 2006; Westman, Behm, Simel & Rose, 1997), and a prospective study suggests that only about 3-5% of self-quitters are able to maintain abstinence at 6-12 months after their quit date (Hughes, Keeley, & Naud, 2004). Tobacco abstinence symptoms have been shown to appear on the days prior to cessation and to increase sharply on the quit date (Allen, Bade, Hatsukami, & Center, 2008). For many smokers with continued abstinence, these acute symptoms decrease gradually to baseline within 3 to 4 weeks (Piasecki, 2006), while relapse to smoking tends to relieve these aversive symptoms more quickly (Piasecki, Jorenby, Smith, Fiore, & Baker, 2003). Data from a retrospective study showed that smokers who reported high levels of smoking to alleviate negative effects were more likely to relapse (Piper et al., 2004). In addition, recent research suggests that patterns of abstinence symptomology may predict relapse susceptibility (Allen et al., 2006; Piasecki et al., 2003). In summary, these findings indicate that tobacco abstinence symptomology and suppression are highly related to smoking behavior and the likelihood of successful cessation.

Harm Reduction

While almost all public health advocates stress the role of cessation in preventing tobacco-caused death and disease in cigarette smokers (Schroeder, 2005; US DHHS, 1990), some, cognizant of the high relapse rates described above, also have begun to explore alternative approaches. One such alternative approach involves "harm

reduction": "minimizing harms and decreasing total morbidity and mortality without completely eliminating tobacco and nicotine use" (Stratton, Shetty, Wallace & Bondurant, 2001, pp.189-193). In the context of cigarette smokers who cannot or will not quit, this reduction in harm might be possible either through a reduction in tobacco consumption (i.e. fewer cigarettes smoked per day) or the use of potential reduced exposure products (PREPs; Warner, 2002; Pisinger & Godtfredsen, 2007).

Reducing the number of cigarettes smoked each day is one potential harm reduction strategy. The central notion is that, over the long term, a reduced cigarette intake (i.e. at least a 50% decrease of daily cigarette intake) will lead to reduced risk of cigarette-caused death and disease (Hatsukami, Kotlyar, et al., 2005). Some data suggest that a substantial reduction in smoking may improve some cardiovascular risk factors (Bollinger et al., 2002), but there does not appear to be a substantial beneficial effect for lung function (Burchfiel et al., 1995; Simmons et al., 2005). An important limitation to note in smoking reduction studies is the low percentage of participants who are successful in reducing their cigarette intake. In a study concerning long term smoking reduction outcomes, only 25 out of 310 participants were successful in maintaining at least a 50% decrease in cigarettes smoked per day (Bollinger et al., 2002). These low rates may be associated with acute tobacco abstinence effects that were not suppressed due to reduced tobacco consumption. In addition, it is possible that smoking reduction will increase toxicant exposure (and potentially increase risk) because smokers may compensate for a decrease in the number of cigarettes by changing the way they puff on each of the remaining cigarettes: for example by increasing puff number and/or volume.

Because the data regarding the health effects of smoking reduction do not allow definitive conclusions, some investigators suggest that the primary value of this approach may be as a preparatory step for smokers interested in quitting (Hughes, 2000; Stead & Lancaster, 2007).

Another potential harm reduction strategy involves the use of PREPs for smokers (e.g., Stratton et al., 2001, p.189). PREPs for smokers are products intended to reduce a smoker's toxicant exposure, and might include cigarette-like combustible tobacco products, non-combustible tobacco products, and non-combustible, non-tobacco products (e.g., NRTs or products that deliver pharmacologically pure nicotine; Warner, 2002). Importantly, positive health effects ultimately define an effective harm reduction strategy: a product is "harm reducing if it lowers total tobacco related mortality and morbidity …" (Stratton et al., 2001, p.189). However, because tobacco-caused mortality and morbidity can take decades to measure, there is a growing interest in evaluating PREPs based on their ability to reduce smokers' exposure to smoke toxicants. While reduced toxicant exposure is not a definite predictor of harm reduction, it does at least have face validity. Exposure can be assessed by a variety of tobacco-related biomarkers measured in users or those passively exposed to tobacco-related products. In contrast, reduced toxicant yield (i.e., a reduction in the measured amount of toxicants in tobacco and tobacco smoke) is a demonstrably poor predictor of harm: so-called "low yield" cigarettes have been used for decades in the U.S. with no significant reductions in tobacco-caused disease (Melikian, Djordjevic, Chen, Richie, & Stellman, 2007; National

Cancer Institute, 2001). This experience highlights the importance of careful and comprehensive PREP evaluation that includes an analysis of user toxicant exposure.

Besides reduced toxicant exposure, another outcome related to the effectiveness of a PREP as a harm reduction strategy involves the ability of a PREP to suppress abstinence effects. As discussed previously, abstinent smokers experience a variety of aversive effects and smoking own brand cigarettes suppresses these abstinence effects. To the extent that PREP use also suppresses these abstinence effects, the PREP may be a viable harm reduction strategy. In contrast, if the PREP does not suppress these aversive abstinence effects, smokers who try it may return to their own brand cigarettes (or supplement PREP use with own brand cigarette smoking). Thus, in addition to toxicant exposure, understanding PREP tobacco abstinence suppression is an important component of a comprehensive PREP evaluation.

Categorizing PREPS

As several PREPs for smokers have been introduced in the U.S. and elsewhere, methods to evaluate them comprehensively have become more important. In terms of combustible products, these PREPs include Accord (Philip Morris; uses electronics and is marketed as a product that heats rather than burns tobacco), Advance (Brown and Williamson; uses specially cured tobacco and novel filter technology and is marketed to reduce carcinogens and other toxicants), Eclipse (R.J. Reynolds; uses a carbon element and is marketed to primarily heat tobacco and reduce toxicant exposure and decrease disease risk), Omni (Vector Tobacco; uses novel filter technology and is marketed to

reduce carcinogen exposure), and Quest (Vector Tobacco; uses genetically altered tobacco and is marketed to lower nicotine exposure).

Non-combustible tobacco based PREPS for smokers include Ariva (Star Scientific; a tablet made of compressed tobacco and marketed to be low in TSNAs) and a variety of snuff products made from and/or modeled after Swedish "snus", which are marketed to be low in some tobacco toxicants (e.g., Liggett's Grand Prix snus; Lorillard's Triumph snus; Philip Morris' Marlboro Snus; R.J. Reynolds' Camel Snus). In addition, non-combustible, non-tobacco based products are marketed to smokers, either as cessation medications (e.g., nicotine patch, gum, inhaler, or lozenge) or products that might replace cigarettes (e.g., Crown 7 and NJOY; electronic products that are marketed as a means to deliver vaporized nicotine without combustion).

Overall, there are many PREPs for smokers now available, with little information regarding their ability to reduce user toxicant exposure and suppress abstinence symptoms. However, a growing body of literature demonstrates, at least for combustible PREPs, that the clinical laboratory provides a valuable setting for studying these outcomes.

Evaluating Combustible PREPs: Toxicant Exposure and Abstinence Suppression

Over the last decade, several studies have focused upon the user toxicant exposure and abstinence symptom suppression associated with combustible PREP use. This literature is described below.

Cigarette smokers inhale hundreds of smoke toxicants (Hoffman & Hoffman, 1997), though exposure to only a subset of these can be measured conveniently in a

clinical setting. This subset includes TSNAs and PAHs (exposure is assessed via metabolites measured in urine), CO (measured in expired air), and nicotine (measured in blood). Several variations of clinical methodology have analyzed the toxicant exposure associated with combustible PREP use. For example, one study compared the toxicant exposure associated with four weeks' use of Omni and own brand cigarettes (Hatsukami et al., 2004). Outcome measures included a PAH metabolite (1-hydroxypyrene, or 1-HOP) and a TSNA metabolite (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol or NNAL), as well as expired air CO. Relative to own brand, Omni reduced NNAL levels by 20% (p <.001), but did not reduce 1-HOP or CO significantly (Hatsukami et al., 2004). This study demonstrates the feasibility of measuring PREP toxicant exposure.

Several studies have shown that measurement of toxicant exposure and abstinence symptom suppression can be combined in a single design. For example, one study assessed the effects of Advance, a combustible PREP that uses specially cured tobacco and a "trionic filter," and is marketed to reduce TSNA exposure (Breland, Acosta, & Eissenberg, 2003). Twelve smokers completed three, 5-day conditions in which they smoked either own brand, Advance, or no cigarettes, and smokers' urine was collected on days 1, 3, and 5 of each of these conditions. Relative to own brand, both Advance and no smoking produced significant decreases in urine NNAL levels and CO on day 5. Following day 1 of each condition, tobacco abstinence symptoms were elevated in the no smoking condition relative to own brand and Advance across study days. This observation indicated that own brand and Advance may produce similar abstinence symptom suppression (Breland et al., 2003). Similar results were obtained from 35

participants in a second study using nearly identical methods, though blood sampling also revealed that the nicotine exposure associated with Advance and own brand cigarettes did not differ (Breland et al., 2006). In addition, this second study also included a condition in which participants used Eclipse, a combustible PREP marketed as a means of reducing smokers' toxicant exposure. Relative to when using own brand, participants' NNAL levels and abstinence symptom ratings did not differ, though blood nicotine was lower and expired air CO was higher when using Eclipse (Breland et al., 2006). These two studies, as well as others (e.g., Hughes, Hecht, Carmella, Murphy, & Callas, 2004; Fagerström, Hughes, Rasmussen, & Callas, 2000; Fagerström, Hughes, & Callas, 2002) demonstrate the feasibility of measuring toxicant exposure and abstinence symptom suppression associated with PREP use in a single study.

Measurement of the toxicant exposure and abstinence symptom suppression associated with PREPs can be measured in a laboratory setting after relatively brief periods of use. These studies of PREP acute effects offer some advantage in that they can be completed relatively quickly and are less labor intensive than longer-term examinations. An acute laboratory study was conducted to investigate three varieties of Quest, a combustible PREP made from tobacco containing varying nicotine levels (Strasser, Lerman, Sanborn, Pickworth, & Feldman, 2007). The researchers aimed to determine the effect of Quest nicotine yield (0.6, 0.3, and 0.05 mg) on outcomes including expired air CO. Fifty participants who had no prior Quest experience were administered masked Quest cigarettes in a random, counter-balanced order. Post hoc analyses indicated that CO boost for the 0.3 mg nicotine cigarette was significantly

greater than the 0.6 mg nicotine cigarette, while the CO boost for the 0.05 mg cigarette was not significantly greater than the boost for the 0.6 mg or 0.3 mg cigarette (Strasser et al., 2007). This study shows how an acute examination can provide information concerning PREP-associated toxicant exposure.

Acute studies can also be used to assess toxicant exposure and abstinence suppression concurrently. To identify the effects of Advance, a combustible PREP, researchers recruited 20 smokers of light or ultra-light cigarettes to participate in three, Latin-square ordered, laboratory based sessions (Breland, Evans, et al., 2002). In each 2.5-hour session, participants completed an 8-puff smoking bout from their own brand, Advance, or an unlit cigarette (sham smoking) four times. Outcome measures included participant-rated measures of tobacco abstinence symptoms, CO, cardiovascular response (heart rate), and plasma nicotine concentrations. Relative to own brand, Advance use was associated with similar levels of participant-rated tobacco abstinence suppression and cardiovascular effects. In addition, relative to own brand, Advance use was associated with lower levels of CO exposure and higher levels of plasma nicotine. The researchers determined the abstinence suppression and lower CO levels were positive outcomes, but the higher nicotine concentrations observed with Advance may produce greater levels of nicotine dependence, thus making tobacco cessation more challenging (Breland, Evans, et al., 2002). Nonetheless, this acute study demonstrates that the toxicant exposure and abstinence symptom suppression produced by a single PREP can be measured in a laboratory setting after brief periods of PREP use.

These same methods have been used to evaluate the toxicant exposure and abstinence suppression of several PREPs within the same study. For example, in a study with four, Latin-square ordered, laboratory based sessions, the effects of three combustible PREPs were examined using 20 smokers (Breland, Buchhalter, Evans, & Eissenberg, 2002). Overnight abstinence was required prior to each condition, which included participant's own brand cigarettes and three combustible PREPS (Accord, denicotinized cigarette, and Eclipse). Each product was used four times during each session. Outcome measures included participant-rated measures of tobacco abstinence symptoms, expired air CO, cardiovascular response (heart rate) and plasma nicotine concentrations. Relative to own brand, Accord was less effective at suppressing tobacco abstinence symptoms and was associated with minimal CO exposure. In contrast, Eclipse suppressed abstinence symtomology fully and increased CO by approximately 30%. All products except for denicotinized cigarettes increased plasma nicotine concentrations (Breland, Buchhalter, et al., 2002). This acute clinical study demonstrates that comparisons of multiple combustible PREPs are practical and can provide valuable information concerning toxicant exposure and tobacco abstinence suppression.

Evaluating Non-combustible PREPs: Toxicant Yield, Toxicant Exposure, and Abstinence Suppression

Clinical laboratory methods to evaluate non-combustible PREPs for smokers are still developing, but there have been preliminary toxicant yield analyses performed for many non-combustible PREPs (Ayo-Yusuf, Swart, & Pickworth, 2004; McNeill, Islan, Malkhatib, & West; Stepanov, Jensen, Hatsukami, & Hecht, 2006; Stepanov, Jensen,

Hatsukami, & Hecht, 2008). These types of analyses use chemical assays to determine TSNA, PAH, pH, and nicotine yield of a variety of tobacco and non-tobacco products. Higher pH levels in a product aid the transformation of nicotine into an unprotonated or "free" form, which moves easily across membranes such as the skin or mouth (Tomar & Henningfield, 1997). Thus, products with increased pH levels achieve faster rates of nicotine absorption into the body. Like with other drugs of abuse that have a rapid onset, this pharmacokinetic profile appears to have greater abuse liability potential (Balster & Bigelow, 2003; Farré & Camí, 1991; Tomar & Henningfield, 1997). Although the information obtained by these chemical analyses does not reveal actual toxicant exposures or probability of nicotine dependence in humans, it does provide some idea of the potential toxicant exposure of these products.

One analysis compared the TSNA concentrations for a variety of products marketed to smokers including several non-combustible PREPs (Stepanov et al., 2006). The non-combustible PREPs included NRTs (nicotine patch, gum, and lozenge), Ariva (tobacco tablet), and General (Swedish snus product). The NRT products had non-detectable or extremely low concentrations of TSNA (e.g. <.01 μg/g wet weight of product), and in comparison to full-flavor cigarettes, both Ariva and General contained lower levels of TSNA (see Table 1; Stepanov et al., 2006). The results of these analyses may help inform potential users of these products, though they do not address the level of toxicant exposure and abstinence symptom suppression that might be associated with product use.

Table 1

TSNA yield for non-combustible PREPs and conventional cigarettes

Product	Total TSNA µg/g wet weight
Non-combustible PREPs	
Ariva (tobacco tablet)	0.19
Commit (nicotine lozenge)	Not detected
General	2.0
NicoDerm CQ (nicotine patch)	0.008
Nicorette (nicotine gum)	0.002
Conventional cigarettes	
Camel full flavor	5.2
Camel light	4.6
Camel ultra-light	4.8
Marlboro full flavor	6.3
Marlboro light	4.6
Marlboro ultra-light	4.8
Newport full flavor	3.9

Note. Data as described in Stepanov et al., 2006.

Another more recent toxicant analysis of smokeless tobacco products including non-combustible PREPS assessed TSNAs, nicotine (unprotonated and total), PAHs, and pH levels (Stepanov et al., 2008). Non-combustible PREPs included General (snus product marketed in Sweden and elsewhere by Swedish Match), Camel Snus (snus product marketed in the U.S. by R.J. Reynolds), Taboka (snus product marketed in the U.S. by Philip Morris), and Marlboro Snus (snus product marketed in the U.S. by Philip Morris). Total TSNA concentrations for all varieties of Taboka, Marlboro Snus, Camel Snus, and General were lower than that of conventional smokeless tobacco products (see Table 2 for product specific results). Levels of unprotonated nicotine and pH were low in Taboka and Marlboro Snus, while General and Camel Snus unprotonated nicotine levels and pH levels were similar to that of conventional smokeless tobacco products (see Table 2). PAH levels of all non-combustible PREPs were lower than that of conventional smokeless tobacco products (Stepanov et al., 2008). These results indicated that many of the non-combustible PREPs contained lower levels of PAHs, TSNAs, and nicotine (with the exception of General and Camel Snus) than conventional smokeless tobacco products. These results also display the advantage of comparing multiple products to conventional tobacco products but, again, do not address actual user toxicant exposure or abstinence symptom suppression.

Although clinical laboratory studies of non-combustible PREPs are less common, many use similar methodology to that of combustible PREP evaluations. For example, one clinical study measured the plasma nicotine concentrations following 12 hours of regular use (1 dose administered every hour) for four commonly used brands of Swedish

Table 2

TSNA, pH, and nicotine (total and unprotonated) yield for non-combustible PREPs and conventional smokeless tobacco products

Product	Total TSNA µg/g dry weight	pH	Total nicotine mg/g dry weight	Unprotonated nicotine mg/g dry weight
Non-combustible PREPS				
General	3.10	7.95	16.7	7.69
Camel Snus				
Original	1.73	7.46	28.2	6.09
Spice	1.75	7.75	25.4	9.16
Frost	1.68	7.59	23.7	6.40
Marlboro Snus				
Rich	1.98	6.83	17.8	1.08
Mild	1.98	6.47	12.8	0.35
Spice	2.06	6.85	17.9	1.13
Mint	3.72	6.58	20.0	0.701
Taboka				
Original	1.50	6.65	21.1	0.8
Green	1.33	6.85	19.9	1.2
Conventional smokeless products				
Copenhagen Snuff	7.79	7.45	23.0	4.88
Skoal Long Cut	7.96	7.51	25.6	6.03
Kodiak Wintergreen	12.0	8.23	19.6	12.1

Note. Data as described in Stepanov et al., 2008

snus and the 2-mg nicotine chewing gum (Lunell & Lunell, 2005). Twelve overnight tobacco abstinent, non-smoking snus users participated in this within subjects design. Each session lasted 12 hours, and each product administration lasted 30 minutes. Plasma nicotine concentrations obtained during the last dosing period were used to determine the mean maximum nicotine level for each of the Swedish products and the nicotine gum: General maximum = 29.0 ng/ml, Catch Licorice maximum = 23.8 ng/ml, Catch Licorice Mini maximum = 21.0 ng/ml, Catch Dry Mini maximum = 10.9 ng/ml, and nicotine gum maximum = 12.8 ng/ml. For this measure, all Swedish products except for Catch Dry Mini delivered significantly more nicotine than the nicotine gum. General reliably produced the highest mean plasma nicotine concentration following each dosing procedure. This study demonstrates one method for examining levels of nicotine exposure from a variety of non-combustible PREPs (Lunell & Lunell, 2005), although the study sample (snus users) does not allow any conclusions regarding the effects of smokeless tobacco products on suppression of smokers' abstinence symptoms.

Nicotine exposure and tobacco abstinence suppression can be measured concurrently in a clinical laboratory study of non-combustible PREPs. One study compared the effects of 2 mg sublingual nicotine tablets to placebo tablets (no nicotine) in a double-blind randomized crossover study of two 2-day smoke-free periods in a population of 20 smokers (Molander et al., 2000). Participants were given an allotment of tablets to use during each 2-day, non-smoking period. Tobacco abstinence symptoms were assessed multiple times over each 2-day period and plasma nicotine concentrations were measured in the afternoon of each study day. In addition, a blood sample was taken

in the afternoon of a 2-day period during own brand smoking for comparison. Mean plasma nicotine levels during own brand smoking days were 25.7 ng/ml on day 1 and 24.6 ng/ml on day 2. During active nicotine tablet use, mean plasma nicotine levels were 7.5 ng/ml on day 1 and 7.7 ng/ml on day 2. During the placebo condition, mean plasma nicotine levels were 0.8 ng/ml on day 1 and 0.5 ng/ml on day 2. Compared to placebo, active nicotine tablet condition was significantly better at decreasing craving and other tobacco abstinence symptoms; mean tobacco abstinence symptom scores were reduced by approximately 50% when using the active nicotine tablet. One limitation of the study was the lack of abstinence symptom assessment during the own brand smoking condition. This omission of assessment restricts these results in distinguishing differences in abstinence symptom suppression between non-combustible PREPs and own brand smoking. Nonetheless, the data showed that the nicotine tablet was able to provide partial relief of tobacco abstinence symptoms while delivering lower levels of nicotine than own brand cigarettes. This study also demonstrates that nicotine exposure and tobacco abstinence suppression can be assessed in a single study involving smokers using non-combustible products that deliver active nicotine doses (Molander et al., 2000).

A pair of related studies used crossover methods to measure the toxicant exposure and tobacco abstinence suppression of two tobacco based non-combustible PREPS (Ariva and Exalt) as well as a non-tobacco non-combustible PREP (nicotine lozenge; Mendoza-Baumgart et al., 2007). Participants recruited were adult cigarette smokers that were motivated to quit smoking. In one of the pair of studies, participants (N=39) completed one week of baseline smoking assessment and then were asked to quit

smoking and assigned randomly to use Exalt or a nicotine lozenge for two weeks. After two weeks, participants were crossed over to use the other PREP product for an additional two weeks. The second of the pair of studies used an identical design except that participants (N = 26) were randomized to use Ariva (instead of Exalt) and the nicotine lozenge. Outcomes included TSNA exposure (urine NNAL concentration), nicotine exposure (urine cotinine concentration), and subjective, physiological, and behavioral responses. Results showed that urine NNAL levels were greater from Exalt than from the nicotine lozenge and were comparable between the nicotine lozenge and Ariva. Tobacco abstinence symptom suppression and physiological effects were similar among Exalt, Ariva, and the nicotine lozenge. At the conclusion of the study when participants were asked to pick their preferred product, Ariva was preferred over the nicotine lozenge, which was preferred over Exalt (Mendoza-Baumgart et al., 2007). This study demonstrates how long-term clinical laboratory studies can be used effectively to measure the effects of multiple PREPs concurrently. Studies of non-combustible PREPs also can provide valuable information with shorter PREP exposure periods.

Similar to acute combustible PREP evaluations, short term studies of a single non-combustible PREP can provide important information concerning toxicant exposure and tobacco abstinence symptom suppression in cigarette smokers. In a single-session study examining the acute effects of Ariva, 10 overnight-abstinent cigarette smokers were administered Ariva tablets in increasing dosages (i.e. 1 tablet, 2 tablets, 3 tablets; Blank, Sams, Weaver, & Eissenberg, 2008). Each dose was separated by a 90 minute inter-administration interval. Results showed that Ariva delivered nicotine in a dose-

dependent manner: mean plasma nicotine level at baseline was 2.4 mg/ml, and mean peak level after one tablet was 4.1 ng/ml, after two tablets was 8.0 ng/ml, and after three tablets was 11.1 ng/ml. There were also dose-related decreases in ratings of urges to smoke and increases in ratings of nausea. This acute short-term evaluation demonstrated that Ariva exposes users to nicotine and has effects on tobacco abstinence related symptomology. The study also showed that clinical laboratory methods can be used to assess the toxicant exposure and abstinence symptom suppression associated with acute administration of a non-combustible PREP in smokers (Blank et al., 2008).

Another group conducted a short-term study, which combined the measurement of nicotine pharmacokinetics and subjective effects of several non-combustible PREP products in a population of smokeless tobacco users (Kotlyar et al., 2007). Ten subjects who were all regular users of U.S. smokeless tobacco products completed a randomized within-subject study, where products included Ariva, Copenhagen (U.S. moist snuff brand), and a nicotine lozenge. Prior to session, participants were tobacco abstinent, and after a baseline measurement, products were placed in the participant's mouth for 30 minutes. Outcome measures included plasma nicotine, tobacco abstinence symptoms, and ratings of product effects. Craving during use of Copenhagen decreased significantly compared to the other products assessed. Additionally, Copenhagen produced the highest maximal nicotine concentration (16.1 ng/ml). The nicotine lozenge had a maximal nicotine concentration of 7.2 ng/ml and Ariva's was 2.7 ng/ml. This study, along with others (i.e., Blank et al., 2008), highlights the value of clinical laboratory methods and the acute administration of non-combustible products in understanding PREP toxicant

exposure and other effects in a variety of populations (i.e., smokers and smokeless tobacco users).

Methods to Evaluate PREPs

The evaluations described in the preceding sections demonstrate many techniques and methods that are useful to assess combustible and non-combustible PREPs. While several different study designs have been used, within-subject methods are most common (e.g., Blank et al., 2008; Breland et al., 2006; Kotlyar et al., 2007; Mendoza-Baumgart et al., 2007). Outcome measures also differ across studies, though plasma nicotine, expired air CO, cardiovascular response, and subjective ratings are particularly common in assessing PREP toxicant exposure and other effects. The population being sampled is clearly a function of the study hypotheses, such that smokers are used in studies where PREPs marketed to that population are tested.

Study duration is also an important variable, as some outcomes require longer PREP exposure periods (i.e., NNAL's half-life, measured in days, requires a multi-day exposure period; Hecht et al., 1999). Shorter exposure periods are particularly efficient when the research question concerns PREP-associated exposure to nicotine and CO as well as PREP-induced tobacco abstinence suppression and physiological effects. Study duration is also relevant to the issue of compensatory behaviors (i.e. switching to a lower nicotine cigarette impacts the way cigarettes are smoked). Short term examinations have shown smokers switching to lower nicotine cigarettes may show compensatory behaviors (i.e., smoking more intensely; Hammond, Fong, Cummings, & Hyland, 2005; Strasser et al., 2007) while longer term studies have shown conflicting results concerning smoking

compensation (Benowitz et al., 2007). Blood and breath samples collected before and after PREP use address the toxicant exposure associated with acute administration (e.g. Blank et al., 2008; Breland, Evans, et al., 2002). Similarly, subjective questionnaires administered before and after PREP use provide valuable information concerning a product's potential abstinence suppression, direct effects (e.g., nausea, lightheadedness), and acceptability. Short term methods often involve repeated PREP administration within a single experimental period (i.e., 4 smoking bouts in 2.5 hours; Breland, Evans, et al., 2002). These repeated administrations offer an opportunity for examining within-session PREP effect reliability. Thus, clinical laboratory studies that use acute exposure methods can address many questions related to PREP use. These studies are particularly valuable when they employ rigorous control procedures, including the positive and negative control conditions.

Several clinical laboratory studies of PREP effects demonstrate the value of a variety of control conditions (e.g., Breland et al., 2006). A positive control condition is one in which the anticipated effect is observed and, in the context of PREP evaluation, may include having participants use conventional tobacco products (e.g. own brand cigarettes) that deliver nicotine and other toxicants and suppress abstinence symptoms effectively. The positive control condition is most valuable when included as part of the study randomization scheme (in between-subjects designs) or as one of the experimental conditions (in within-subjects designs). In these cases, the PREP effects can be compared statistically with effects produced by the positive control condition. A negative control condition is one in which the anticipated effects is not observed and, in

the context of PREP evaluation, may include no tobacco use or sham tobacco use (i.e., puffing on an unlit cigarette; Breland, Evans, et al., 2002). Again, the negative control condition is most valuable when it is incorporated as part of the overall study design. In studies of cigarette smokers, sham smoking may be particularly valuable, as it controls for motoric components of smoking, while delivering no tar, carbon monoxide, or other components of tobacco smoke, as well as few sensory stimuli (Morris & Gale, 1994; Robinson, Houtsmuller, Moolchan, & Pickworth, 2000). Another useful control condition that may provide information concerning PREP effects is a denicotinized cigarette. This condition can provide experimental control for the effects of smoke in the absence of nicotine. Previous studies have shown that denicotinized cigarette can provide acute abstinence suppression similar to that of an own brand cigarette (Buchhalter, Schrinel, & Eissenberg, 2001; Butschky, Bailey, Henningfield, & Pickworth, 1995). In sum, with appropriate control conditions, acute PREP exposure in the clinical laboratory can provide valuable information for understanding PREP effects.

Statement of the Problem

Tobacco cigarette smoking causes death and disease and is considered a major threat to public health in the U.S. Along with prevention, smoking cessation is a cornerstone of the public health response to this threat, yet many cigarette smokers are unable to quit due to tobacco/nicotine dependence. For this reason, interest in harm reduction via PREP use has grown, and combustible and non-combustible PREPs for smokers are now marketed in the U.S. Methods have been developed to evaluate the effects of combustible PREPs, and results from clinical laboratory studies highlight the

value of understanding toxicant exposure and subjective effects associated with PREP use. For example, PREPs that increase rather than decrease toxicant exposure are generally considered unsuitable for a PREP-based harm reduction strategy (e.g., Eclipse, see Breland, Buchhalter, et al, 2002), as are PREPs that do not adequately suppress tobacco abstinence symptoms (e.g., Accord, Buchhalter et al., 2001; Hughes & Keely, 2004). To date, there has been little clinical evaluation of non-combustible PREPs, particularly tobacco based non-combustible PREPs for smokers. The present study was designed to explore how clinical laboratory methods used to evaluate combustible PREPs might be adapted to evaluate non-combustible PREPs for smokers.

The Present Study

This study adapts clinical laboratory methods used to evaluate combustible PREPs for smokers to investigate the acute effects (toxicant exposure, cardiovascular response, and subjective effects) of three tobacco based non-combustible PREPs (Ariva, Camel Snus, and Marlboro Snus) and an non-tobacco based non-combustible PREP (nicotine lozenge; Commit) relative to own brand cigarettes (positive control), sham smoking (negative control) and one combustible PREP (denicotinized cigarette; Quest). Own brand cigarettes are included to compare PREP effects to normal levels of toxicant exposure, cardiovascular response, and subjective effect (particularly tobacco abstinence suppression). Sham smoking is included to control for motoric components of smoking, while delivering no tar, carbon monoxide, or other components of tobacco smoke, as well as few sensory stimuli, while Quest is included to control for the effects of smoking-related stimuli in the absence of nicotine.

Statement of Hypothesis

The primary goal of the study is to explore methods for evaluating non-combustible PREPs relative to own brand cigarettes, and the primary hypothesis reflects this goal: relative to own brand, non-combustible PREPs will expose users to lower levels of CO and nicotine, produce smaller heart rate increases, and provide lower levels of subject-rated abstinence symptom suppression and acceptability.

CHAPTER 2 Method

Selection of Subjects

Thirty-three male and female cigarette-smoking community volunteers completed this within-subject, Latin-square ordered study, though, through an administrative error, five of these participants duplicated already-completed Latin-square condition orders. Data from these five were excluded from further analyses. Thus, a total of 28 participants were included in all subsequent analyses.

This sample size is appropriate based on power analysis completed on data collected from a similarly designed study (i.e., Breland et al., 2006). In that four-condition (own brand, no tobacco, Advance, or Eclipse), within-subject study, medium to large effect sizes were observed on many outcome measures. Given the within-subjects design and the expectation of a medium effect size, 28 participants should be sufficient to have an 80% chance of detecting an effect assuming a small to moderate (i.e., 0.30 to 0.50) correlation among repeated measures (i.e., power $\geq$ 0.8, alpha $\leq$ .05; Barcikowski & Robey, 1984).

The 28 study participants (17 men, 14 non-white) were included if they were healthy, between 18 and 55 years of age (mean [*M*] = 32.2 years, standard deviation [*SD*] = 10.1), and reported a cigarette intake of at least 15 cigarettes per day (*M* = 22.4, *SD* = 7.5) for at least 1 year (*M* = 10.8 years, *SD* = 9.9). This criterion of smoking status was used to insure that the study population reflected a group of current tobacco users, which

is an important methodological issue in PREP evaluation (Hatsukami, Giovino, et al., 2005). Smoking status was confirmed objectively with an afternoon expired air CO level of at least 15 parts per million (ppm; $M = 25.7$ ppm, $SD = 10.7$; as in Breland, Evans, et al., 2002) and a semi-quantitative urine cotinine analysis result of at least 4 on a seven point (0-6) scale ($M = 6$, $SD = 0$; NicAlert, Nymox Corp., Maywood, New Jersey; see Acosta, Buchhalter, Breland, Hamilton, & Eissenberg, 1994).

Participants were excluded if they reported a history of chronic health problems or psychiatric conditions, current breastfeeding, or current pregnancy (assessed by urinalysis). Individuals who reported current attempts to quit smoking or active menopause were also excluded (menopause may cause tobacco/nicotine abstinence-like symptoms, such as depression, Parry & Newton, 2001). Current use of smokeless tobacco in combination with smoking cigarettes or previous experience with Ariva, Camel Snus, or Marlboro Snus (i.e., more than one individual container) were exclusions for potential participants as this prior experience may have influenced measures of abstinence symptomology and product acceptability.

Screening and Informed Consent Procedures

All interested participants took part in a two-part screening process. The first part consisted of a phone interview where potential participants responded to questions about health and tobacco use (see Appendix A). Individuals whose responses suggested that they may be eligible were invited to appear in the laboratory for an in-person screening. Prior to completing the in-person screening, these individuals provided their informed consent to participate in the study (see Appendix B). Then individuals provided

information concerning their health, tobacco use, and basic demographics. They also provided a urine sample for immediate semi-quantitative analysis of cotinine content and, for women, a pregnancy test. Lastly, participants provided a breath sample for analysis of expired air CO level.

Materials

Combustible products. Two combustible products were used in this study, a low nicotine combustible PREP called Quest (QUEST; Vector Tobacco Inc., Durham, NC) and own brand cigarettes. The low nicotine product uses genetically modified tobacco, and three progressively lowered nicotine varieties are marketed (Level 1: 0.6, Level 2: 0.3, and Level 3: 0.05 mg; measurements determined via the Federal Trade Commission [FTC] method). The lowest level (0.05 mg nicotine and 4 mg tar; via FTC method) was used in this study and cigarette flavor was matched to participant's own brand (i.e., menthol/non-menthol). Own brand cigarettes were used in the own brand (OWN) and sham (SHAM) sessions. Participants identified their own brand at screening, and this brand was purchased prior to own brand/sham sessions. According to the FTC method, on average, OWN yielded 1.1 mg nicotine (*SD* = 0.3), 15.5 mg tar (*SD* = 3.3), and 15.2 mg CO (*SD* = 2.8). Cigarette brand identifiers were masked, thus participants were blinded to cigarette brand/type during QUEST, OWN, and SHAM sessions.

Non-combustible products. Four non-combustible products were used in this study. Three were tobacco products (Marlboro Snus, Camel Snus, and Ariva) while the fourth was a pharmaceutical nicotine lozenge marketed as a NRT (Commit).

Marlboro Snus (MS) is marketed by Philip Morris Inc. (Richmond, VA) and is produced in the U.S. The product is comprised of a small pouch containing pasteurized tobacco and is available in four flavors ("mild", "mint", "rich", and "spice"). The pouch is meant to be placed between the lip and gum (see Kotlyar et al., 2007; Lunell & Lunell, 2005), and "mild" was chosen arbitrarily as the flavor to be used in this study. This product was released for retail sale in the Dallas/Fort Worth test market area in August of 2007, and a supply was purchased from retail outlets at that time and subsequently stored at -4^{o}C. Independent analysis reveals that from dry weight this product contains 12.8 mg/g total nicotine and 0.350 mg/g free (unprotonated) nicotine (Stepanov et al., 2008).

Camel Snus (CS) is marketed by R.J. Reynolds Tobacco Company (Winston-Salem, NC) and is produced in Sweden. The product is comprised of a small pouch containing pasteurized tobacco and is available in three flavors ("original", "frost", and "spice"). "Original" was chosen arbitrarily as the flavor to be used in this study. Camel Snus was first marketed in select U.S. cities in summer, 2006 and a supply was provided to this laboratory by R.J. Reynolds Tobacco Company at that time. This 2006 supply was stored at -4^{o}C since the day it was obtained. In spring of 2008 marketing of the product began in Richmond VA, thus making available a fresh supply. Relative to the 2006 version, the 2008 version is packaged differently and weighs more (approximately 0.1 g more). Because the two versions of the product may differ in other characteristics, this study compared them: half of the participants used the 2006 (frozen) version and half used the 2008 (fresh) version. Independent analysis of the 2006 version reveals that in

dry weight this product contains 28.2 mg/g total nicotine and 6.1 mg/g free (unprotonated) nicotine (Stepanov et al., 2008).

Ariva (ARIVA) is manufactured by Star Scientific Inc. (Chesterfield, VA) and is a tablet made of compressed powdered tobacco that is said to be low in tobacco specific nitrosamines. Available in "mint", "wintergreen", and "java", "mint" was chosen arbitrarily as the flavor to be used in this study. This product has been marketed in Virginia for several years, and a supply was obtained from retail sources. Independent analysis reveals that this product contains 0.6 mg/g nicotine per tablet (Hatsukami, Ebbert, Feuer, Stepanov, & Hecht, 2007). Research with tobacco users reveals that one tablet produces a maximal nicotine concentration of approximately 2.7 ng/ml nicotine (Kotlyar et al., 2007). Similarly, Blank et al. (2008) report that one tablet produces a plasma nicotine increase of 1.4 ng/ml by 45 minutes post administration. Relative to use of normally marketed cigarettes, Ariva use is associated with lower levels of nitrosamine exposure (Eissenberg et al., 2008).

The Commit lozenge (COMMIT) is a pharmaceutical product marketed by GlaxoSmithKline (Pittsburgh, PA) as a smoking cessation aid. The product has been marketed in the U.S. for several years, and currently is available in "original", "mint", and "cherry" flavors in 2 and 4 mg doses. The 2 mg "original" lozenge was chosen for use in this study in order to limit the potential for nicotine intoxication that might occur when administering two lozenges in a single 2.5-hour session. A supply was obtained from retail sources. Research with tobacco users reveals that one 2 mg lozenge produces a maximal plasma nicotine concentration of approximately 4.4 ng/ml (Choi, Dresler,

Norton, & Strahs, 2003). Because the product delivers pharmaceutically pure nicotine, is considered a safe and effective as a smoking cessation aid, and is intended for smokers, it is used as a comparison condition for the tobacco based non-combustible products.

Procedure

After all screening procedures were completed (including informed consent), the first of seven experimental sessions were scheduled, with each session corresponding to one of the seven product conditions (i.e., QUEST, OWN, SHAM, MS, CS, ARIVA, and COMMIT). During each condition, each product was administered twice with a 60-minute inter-administration interval. This repeated administration procedure was used in order to observe the extent to which the acute effects observed for each product were reliable.

Prior to each session,, participants reported to VCU's Clinical Behavioral Pharmacology Laboratory and their expired air CO was assessed as a measure of compliance with the overnight tobacco abstinence criteria (CO $\leq$ 10 ppm is an indicator of overnight abstinence; see Breland, Evans, et al., 2002; Buchhalter et al., 2001; Schuh, Schuh, Henningfield, & Stitzer, 1997). If the initial CO assessment did not indicate abstinence, participants could wait until their CO level reached criterion, or they could reschedule the session for another day. Participants were discontinued from participation if they appeared on more than three occasions with CO levels higher than 10 ppm.

Once abstinence was determined, a catheter was inserted into the participant's forearm vein and the session began (at time 0) with continuous recording of physiological measures. Thirty minutes after session onset (time +30), participants rated tobacco

abstinence symptoms by responding to four subjective questionnaires (see below) and 7 ml of blood was sampled. After the blood sampling, a session-specific product was administered. For the next 45 minutes, participants responded to subjective measures and 7 ml blood was sampled at 5, 15, 30, and 45 minutes post-administration. In addition, expired air CO was measured 15, 30, 45 minutes post-administration (CO cannot be measured reliably until at least 5 minutes after the last puff from a cigarette has been taken; Woodman, Wintoniuk, Taylor, & Clark, 1987). The identical measurements occurred after the second product administration, which was preceded (at time +60) by subjective measures and 7 ml blood sampling. During times when participants were not completing subjective measures or having blood/CO sampled, they were allowed to read or sit quietly. After the last blood sample was taken, the catheter was removed, and the participant was assessed by the study nurse for any residual session effects. Once any residual effects were resolved, the participant was paid for their time, and, if necessary, the next session was scheduled. During a single session, the total amount of blood taken was 70 ml and over the entire 7-session study was 490 ml. The total time spent in the laboratory was 17.5 hours with 10 hours of smoking abstinence required before each session. To compensate participants for their time in the laboratory and maintaining abstinence, those who completed the study were paid a total of $450.

Administration Instructions

All products were administered in a single-blind procedure and no brand information was provided to participants. During OWN and QUEST, products were smoked *ad libitum*. For SHAM, because participants might not otherwise puff from an

unlit cigarette, they were asked to complete ten "puffs" approximately 20 seconds apart (similar to Breland, Evans, et al., 2002). Ten puffs is the average number of puffs smokers take from an own brand cigarette (see Breland et al., 2006). For MS and CS, participants were asked to place the pouch between their lip and gum; the product remained in the participant's mouth for 15 minutes during each administration. For ARIVA, participants were asked (per package instructions) to place the product in mouth and allow it to dissolve, without chewing or swallowing it. For COMMIT, participants were asked (per package instructions) to place the product in their mouth and allow it to dissolve, without chewing or swallowing it.

Physiological Measures

During each session, blood was sampled, centrifuged, and plasma stored at -70 C^{o} for later analysis of nicotine and its metabolite cotinine; assays were conducted using established procedures (as in Evans et al., 2006; Kleykamp et al., 2008). The limit of quantitation (LOQ) of this assay is 2.0 ng/ml. Heart rate was measured every 20 seconds and blood pressure measured every 5 minutes and stored electronically by noninvasive computerized equipment (Noninvasive Patient Monitor Model 506, Criticare Systems, Waukesha, WI). Expired air CO was assessed at baseline and periodically throughout in each session (BreathCO monitor, Vitalograph, Lenaxa, KS).

Subjective Measures

During each session, participants responded to computerized questionnaires (each questionnaire was administered a total of 10 times per session). These questionnaires consisted of the Tiffany-Drobes Questionnaire of Smoking Urges Brief (QSU Brief;

Cox, Tiffany, & Christen, 2001), a set of tobacco/nicotine abstinence symptoms described by Hughes and Hatsukami (1986; for example, see Eissenberg, Griffiths, & Stitzer, 1996), and two scales measuring the direct effects of nicotine and tobacco related effects, as described below.

Tiffany-Drobes QSU Brief. The QSU Brief (see Appendix C) consisted of 10 smoking-related items and has been empirically validated (Cox et al., 2001). Participants rated each item on a 7-point scale ranging from 0 (Strongly disagree) to 6 (Strongly agree). The items form two factors: Factor 1 (intention to smoke) and Factor 2 (anticipation of relief from abstinence symptoms). This measure is demonstrably sensitive to the effects of tobacco abstinence in cigarette smokers (Cox et al., 2001).

Hughes-Hatsukami Withdrawal Scale. The Hughes and Hatsukami (1986) scale assesses tobacco/nicotine abstinence effects and consists of 13 items. In this study, an adapted version (see Breland, Evans, et al., 2002, Buchhalter et al., 2005) was used and was composed of 11 items: "Urges to smoke", "Irritability/Frustration/Anger", "Anxious", "Difficulty concentrating", "Restlessness", "Hunger", "Impatient", "Craving a cigarette/Nicotine", "Drowsiness", "Depression/Feeling blue", and "Desire for sweets". These items were presented as a visual analog scale (VAS) with a word or a phrase centered above a horizontal line; the line is anchored on the left with "Not at all" and on the right with "Extremely." Participants responded to each item by moving a computer mouse-controlled cursor to any point on the line and clicking a mouse button, producing a vertical mark on the horizontal line. This mark could be further adjusted as necessary. The score for each scale was the distance of the vertical mark from the left anchor,

expressed as a percentage of total line length (i.e., 0-100). This measure is sensitive to tobacco abstinence, has been used in previous evaluations of cigarette-like PREPs (e.g., Breland, Evans, et al., 2002, Breland, Buchhalter et al., 2002, Breland et al., 2003; Breland et al., 2006), and has the advantage of allowing for differentiation between individual abstinence symptoms (Eissenberg et al., 1996; Buchhalter & Eissenberg, 2000; Buchhalter et al., 2001).

Direct Effects of Nicotine Scale. This scale was developed to measure potential differences in the direct effects of nicotine-containing PREPs and to assess the incidence of any nicotine related side effects (see Evans et al., 2006; Gourlay, Forbes, Marriner, Pethica, & McNeil, 1995; Pullan et al., 1994). The 10 VAS (0-100) items were: "Nauseous", "Dizzy", "Lightheaded", "Nervous", "Sweaty", "Headache", "Excessive salivation", "Heart pounding", "Confused", and "Weak".

Direct Effects of Tobacco Scale. This scale was developed with items reported in studies of smoking's subjective effects (e.g., Foulds et al., 1992; Pickworth, Bunker, & Henningfield, 1994). The original scale consisted of 14 VAS (0-100) items and was adapted for evaluating oral PREPs for smokers, by changing the word "cigarette" in each item to "product" (see Breland et al., 2006). The 14 items were "Was the product satisfying?", "Was the product pleasant?", "Did the product taste good?", "Did the product make you dizzy?", "Did the product calm you down?", "Did the product help you concentrate?", "Did the product make you feel more awake?", "Did the product reduce your hunger for food?", "Did the product make you sick?", "Did the product taste like your own brand of cigarette?", "Did the product feel like your own brand of cigarette?",

"Did the product feel as harsh as your own brand of cigarette?", "Did the product feel as mild as your own brand of cigarette?", and "Would you like more of the product RIGHT NOW?"

Data Analysis Plan

Prior to analysis, heart rate values were averaged to produce a single value for each 5-minute period prior to each product administration and prior to each blood draw. This pattern of averaging is performed to reduce interference of increases due to blood sampling and completion of subjective questionnaires. For plasma nicotine, results below the LOQ were replaced with the LOQ (i.e., 2.0 ng/ml). In the event of missing data, an average of the value before and after the missing value was used (less than 0.07% of data were missing).

Plasma nicotine and cotinine, subjective, and physiological data were analyzed initially using a mixed repeated measures analysis of variance where Camel Snus version (2006 or 2008) was entered as a between-subjects factor and the three within-subjects factors were Condition (seven levels; QUEST, OWN, SHAM, MS, CS, ARIVA, and COMMIT) by dose episode (two levels; first, second) by time (number of levels depended upon outcome measure). Dose episode refers to the first and second product administrations that occurred during each session. Of 320 main effects and interactions involving the between subjects factor (Camel Snus version), only 6 were significant ($p < .05$). Because these few significant results may reflect chance rather than a real difference between the effects of the two Camel Snus versions, the between-subjects factor was dropped and all analyses were repeated using the within-subjects factors only.

Huynh-Feldt corrections were used to correct for violations of sphericity (Huynh & Feldt, 1976) and Tukey's Honestly Significant Difference (HSD; Keppel, 1991) test was used to explore differences between means, as in Breland et al., 2006.

CHAPTER 3 Results

Statistical analyses (main effects and interactions) for all measures are displayed in Table 3. Interactions that involve the condition factor are most relevant, as they indicate that the results observed differed across products and at least one other factor.

Physiological Measures

As Table 3 shows, significant condition by dose, and condition by time interactions were observed for plasma nicotine (Fs > 10.2; Ps < .001). As seen in Figure 1, relative to baseline (collapsed across all conditions, 2.4 ng/ml, standard error of the mean [*SEM*] = 0.2) OWN was associated with significant increases in plasma nicotine level at nearly every time point ($p < .05$, Tukey's HSD). The greatest mean increase was observed five minutes after the first ($M = 20.7$ ng/ml, $SEM = 2.8$) and second ($M = 20.6$ ng/ml, $SEM = 2.0$) product administration. In contrast, plasma nicotine levels did not increase reliably during QUEST or SHAM. For the non-combustible products, relative to baseline, mean plasma nicotine level in the CS condition was significantly greater 15 minutes after the second product administration (7.6 ng/ml, $SEM = 1.1$; $p < .05$, Tukey's HSD). At that same time point, mean plasma nicotine level during the MS condition was 2.9 ng/ml ($SEM = 0.3$), during ARIVA was 3.4 ng/ml ($SEM = 0.3$), and during COMMIT was 4.6 ng/ml ($SEM = 0.5$; all n.s. relative to baseline, Tukey's HSD). Relative to mean plasma nicotine levels in the OWN condition, levels observed in all other conditions were

Table 3
Statistical analyses results for all measures

	Condition (C)		Dose (D)		Time (T)		C X D		C X T		D X T		C X D X T	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Physiological effects														
Plasma nicotine[a]	48.5	<.001	44.6	<.001	56.6	<.001	10.3	<.001	47.6	<.001	3.5	<.05	1.7	n.s.
Heart rate[a]	7.0	<.001	35.8	<.001	62.9	<.001	2.3	<.05	24.3	<.001	18.0	<.001	2.9	<.001
Expired air CO[b]	59.7	<.001	38.8	<.001	116.4	<.001	147.8	<.001	156.4	<.001	2.1	n.s.	1.9	n.s.
Subjective effects														
QSU Brief[a]														
Factor 1	11.4	<.001	11.8	<.01	31.3	<.001	3.1	<.05	10.7	<.001	2.1	n.s.	1.0	n.s.
Factor 2	4.9	<.01	1.1	n.s.	8.7	<.01	2.2	n.s.	4.4	<.001	2.0	n.s.	1.4	n.s.
Hughes-Hatsukami[a]														
Urges to smoke	10.4	<.001	6.1	<.05	37.2	<.001	1.2	n.s.	6.7	<.001	4.3	<.05	1.0	n.s.
Irritability/Frustration/Anger	2.6	<.05	1.9	n.s.	7.2	<.001	1.9	n.s.	1.6	n.s.	3.2	<.05	1.5	n.s.
Anxious	2.5	<.05	0.1	n.s.	7.4	<.001	0.5	n.s.	0.8	n.s.	6.8	<.01	0.7	n.s.
Difficulty concentrating	1.6	n.s.	0.0	n.s.	3.7	<.05	1.5	n.s.	0.9	n.s.	1.4	n.s.	1.3	n.s.
Restlessness	1.4	n.s.	1.2	n.s.	6.6	<.01	2.2	n.s.	1.1	n.s.	3.9	<.05	0.9	n.s.
Hunger	1.3	n.s.	7.9	<.01	5.8	<.01	1.8	n.s.	1.5	n.s.	0.8	n.s.	1.2	n.s.
Impatient	3.2	<.05	0.2	n.s.	6.2	<.01	1.4	n.s.	1.5	n.s.	4.1	<.01	1.2	n.s.
Craving a cigarette/Nicotine	11.3	<.001	9.6	<.01	29.2	<.001	2.9	<.05	6.9	<.001	5.8	<.01	1.4	n.s.
Drowsiness	0.4	n.s.	0.1	n.s.	4.9	<.01	1.5	n.s.	1.0	n.s.	3.6	<.05	1.5	n.s.
Depression/Feeling blue	0.8	n.s.	0.4	n.s.	0.8	n.s.	1.1	n.s.	1.1	n.s.	0.6	n.s.	1.2	n.s.
Desire for sweets	1.8	n.s.	0.5	n.s.	2.7	n.s.	2.0	n.s.	1.3	n.s.	2.8	<.05	0.8	n.s.
Direct Effects of Nicotine[a]														
Nauseous	2.9	<.05	0.0	n.s.	4.7	<.05	1.8	n.s.	2.5	<.05	1.9	n.s.	1.4	n.s.
Dizzy	2.1	n.s.	0.1	n.s.	4.4	<.01	1.1	n.s.	1.6	n.s.	1.5	n.s.	2.3	<.05
Lightheaded	1.7	n.s.	0.1	n.s.	5.0	<.01	1.0	n.s.	2.3	<.05	0.5	n.s.	1.2	n.s.
Nervous	0.7	n.s.	0.4	n.s.	1.1	n.s.	1.9	n.s.	1.5	n.s.	1.3	n.s.	1.1	n.s.
Sweaty	0.7	n.s.	1.9	n.s.	1.7	n.s.	1.1	n.s.	2.0	n.s.	1.1	n.s.	1.0	n.s.
Headache	1.6	n.s.	4.1	n.s.	2.2	n.s.	1.3	n.s.	2.1	<.05	1.9	n.s.	1.0	n.s.
Excessive salivation	5.9	<.001	0.8	n.s.	17.0	<.001	1.5	n.s.	4.8	<.001	2.8	<.05	1.6	n.s.
Heart pounding	3.2	<.05	0.9	n.s.	1.2	n.s.	0.5	n.s.	0.9	n.s.	1.1	n.s.	1.2	n.s.
Confused	2.4	n.s.	2.0	n.s.	0.7	n.s.	1.0	n.s.	0.9	n.s.	1.1	n.s.	0.6	n.s.
Weak	1.3	n.s.	2.9	n.s.	0.9	n.s.	0.7	n.s.	1.0	n.s.	1.0	n.s.	0.8	n.s.

Table 3
Continued

	Condition (C)		Dose (D)		Time (T)		C X D		C X T		D X T		C X D X T	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Direct Effects of Tobacco [a]														
Satisfy	35.9	<.001	31.1	<.001	54.5	<.001	4.4	<.01	15.5	<.001	30.7	<.001	7.7	<.001
Pleasant	30.9	<.001	16.6	<.001	59.2	<.001	3.7	<.01	13.5	<.001	34.4	<.001	8.7	<.001
Taste good	24.9	<.001	16.4	<.001	50.6	<.001	6.6	<.001	9.5	<.001	38.4	<.001	6.6	<.001
Dizzy	6.3	<.001	0.5	n.s.	8.2	<.001	2.5	<.05	1.8	n.s.	11.0	<.001	3.6	<.01
Calm	11.3	<.001	1.3	n.s.	26.0	<.001	2.5	<.05	5.1	<.001	27.7	<.001	3.7	<.001
Help concentrate	9.5	<.001	5.6	<.05	13.8	<.001	1.3	n.s.	3.2	<.01	10.4	<.001	2.4	<.01
Feel awake	11.5	<.001	1.8	n.s.	21.3	<.001	0.6	n.s.	3.4	<.001	17.5	<.001	3.2	<.01
Reduce hunger	3.3	<.01	9.0	<.01	14.6	<.001	0.2	n.s.	1.7	n.s.	17.9	<.001	2.1	<.05
Feel sick	5.2	<.01	1.3	n.s.	11.4	<.001	0.6	n.s.	2.7	<.05	6.8	<.01	1.8	n.s.
Taste like own brand	55.8	<.001	24.1	<.001	32.1	<.001	12.8	<.001	22.9	<.001	19.5	<.001	16.3	<.001
Feel like own brand	48.4	<.001	22.3	<.001	36.9	<.001	8.3	<.001	22.2	<.001	23.3	<.001	13.7	<.001
Harsh as own brand	13.9	<.001	11.9	<.01	22.9	<.001	4.1	<.01	5.3	<.001	17.5	<.001	4.0	<.001
Mild as own brand	34.6	<.001	8.8	<.01	33.6	<.001	4.7	<.01	11.1	<.001	21.7	<.001	9.8	<.001
More of product right now	17.4	<.001	0.7	n.s.	12.6	<.001	0.8	n.s.	3.4	<.001	40.1	<.001	10.9	<.001

[a] $df_{condition}$ =(6,162); df_{dose}=(1,27); df_{time}=(4,108); $df_{C\,X\,D}$=(6,162); $df_{C\,X\,T}$=(24,648); $df_{D\,X\,T}$=(4,108); $df_{C\,X\,D\,X\,T}$=(24,648).

[b] $df_{condition}$ =(6,162); df_{dose}=(1,27); df_{time}=(2,54); $df_{C\,X\,D}$=(6,162); $df_{C\,X\,T}$=(12,324); $df_{D\,X\,T}$=(2,54); $df_{C\,X\,D\,X\,T}$=(12,324).

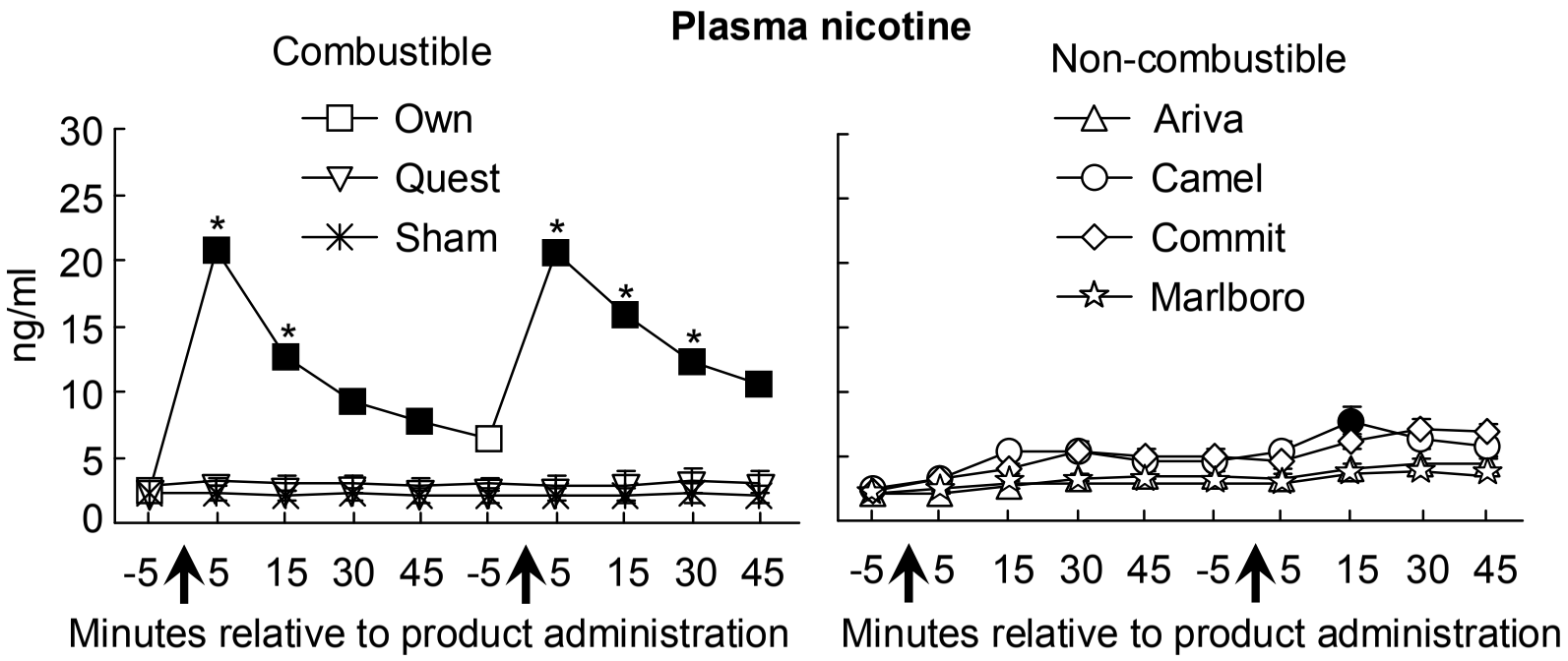

Figure 1. Mean data (±1 *SEM*) for plasma nicotine across conditions (N = 28). Arrows indicate product administration, filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN mean relative to all non-combustible product means at that time point ($p < .05$, Tukey's HSD)

significantly lower 5 and 15 minutes after the first product administration and for 5, 15, and 30 minutes after the second product administration (all Ps < .05, Tukey's HSD).

For heart rate, Table 3 shows that a significant condition by dose by time interaction was observed ($p < .001$). In the OWN condition, mean heart rate at baseline was 67.8 bpm (*SEM* = 1.9) and then increased significantly to 82.3 bpm (*SEM* = 2.3) five minutes after the first product administration; it remained significantly elevated at the 15 and 30 minute post-administration time points. Significant increases relative to baseline during OWN were also observed 5, 15, and 30 minutes after the second administration (see Figure 2; $p < .05$, Tukey's HSD). During QUEST, heart rate at baseline was 68.7 bpm (*SEM* = 2.1) and then increased significantly to 73.1 bpm (*SEM* = 2.1; $p < .05$, Tukey's HSD) five minutes after the first but not the second product administration. Mean heart rate in the SHAM condition was stable or decreased over both administration periods. For the non-combustible conditions, a significant increase in heart rate was observed during the CS condition; heart rate at baseline was 67.8 bpm (*SEM* = 2.3) and then increased significantly to 72.0 bpm (*SEM* = 2.4) 15 minutes after the first product administration ($p <.05$, Tukey's HSD). No significant increases relative to baseline were observed in ARIVA, COMMIT, and MS conditions at any time point. Relative to means observed during OWN, heart rate was significantly lower for all conditions at 5, 15, and 30 minutes after the first and second product administrations (all Ps < .05, Tukey's HSD).

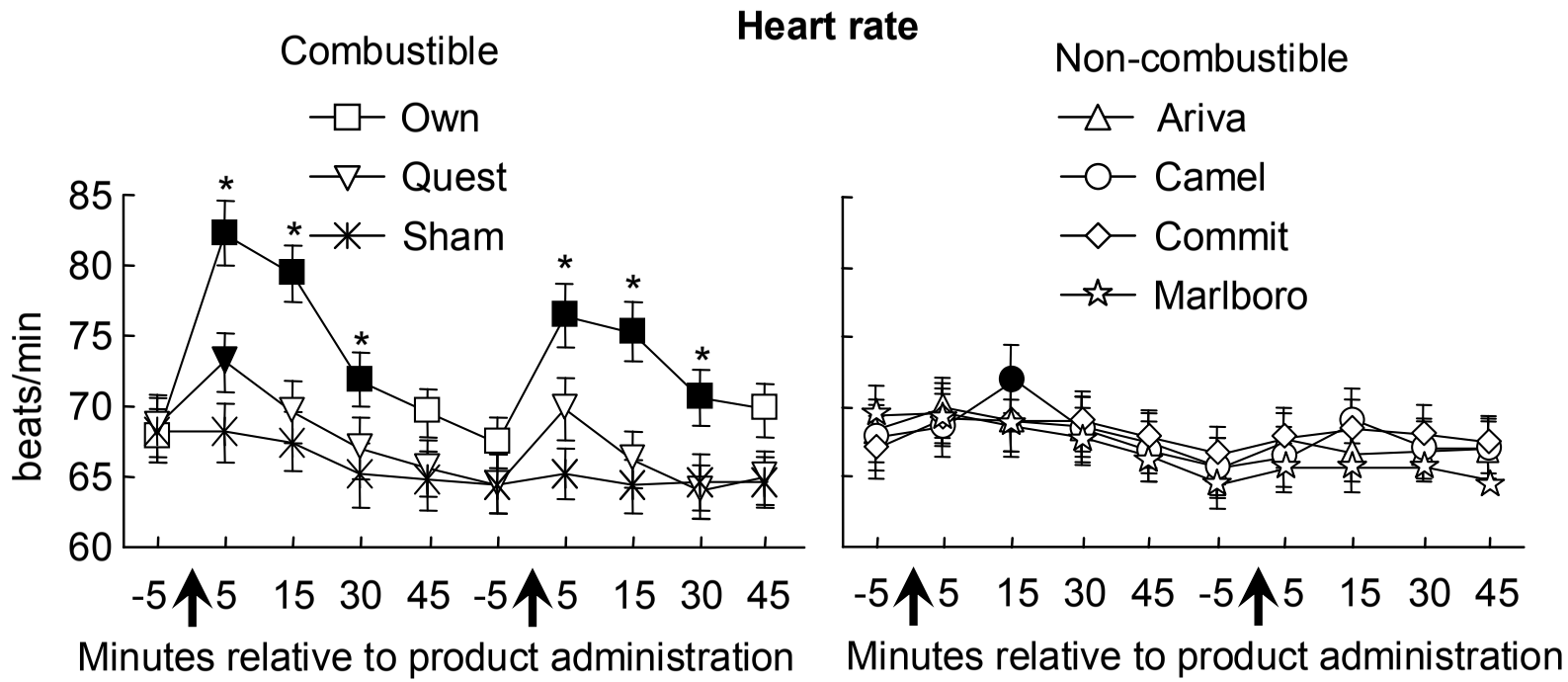

Figure 2. Mean data (±1 *SEM*) for heart rate across conditions (N = 28). Arrows indicate product administration, filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN mean relative to all non-combustible product means at that time point ($p < .05$, Tukey's HSD).

For CO, Table 3 shows a significant condition by dose interaction ($p < .001$). Relative to baseline (collapsed across all conditions, $M = 6.5$ ppm, $SEM = 0.5$), expired air CO significantly increased in the OWN and QUEST conditions after the first product administration (OWN $M = 12.8$ ppm, $SEM = 0.7$; QUEST $M = 11.0$ ppm, $SEM = 0.6$) and the second product administration (OWN $M = 17.5$ ppm, $SEM = 1.0$; QUEST $M = 14.4$ ppm, $SEM = 0.8$; Ps < .05, Tukey's HSD). There were no significant changes in any of the other conditions (see Figure 3). CO levels during all non-combustible conditions and SHAM were significantly lower relative to OWN at all post-administration time points, and during QUEST, CO levels were significantly lower after the second product administration relative to those observed during OWN (all Ps < .05, Tukey's HSD).

Subjective Measures

Tiffany-Drobes QSU Brief and Hughes-Hatsukami Withdrawal Scale. As Table 3 shows, a significant condition by time interaction was observed for both factors of the Tiffany-Drobes QSU Brief (Fs > 4.3, Ps < .001). Figure 4 displays the means for all conditions and times for Factor 1 (the factor with the higher condition by time F value; the pattern of results was similar for Factor 2). Relative to baseline, OWN was associated with significant decreases in Factor 1 scores at nearly every time point ($p <$ .05, Tukey's HSD) with the greatest mean decrease observed 5 minutes after the second product administration (mean decrease from baseline = 14.0, $SEM = 2.3$). QUEST was associated with a similar pattern of significant decreases in Factor 1 scores with the greatest mean decrease observed 15 minutes after the second product administration

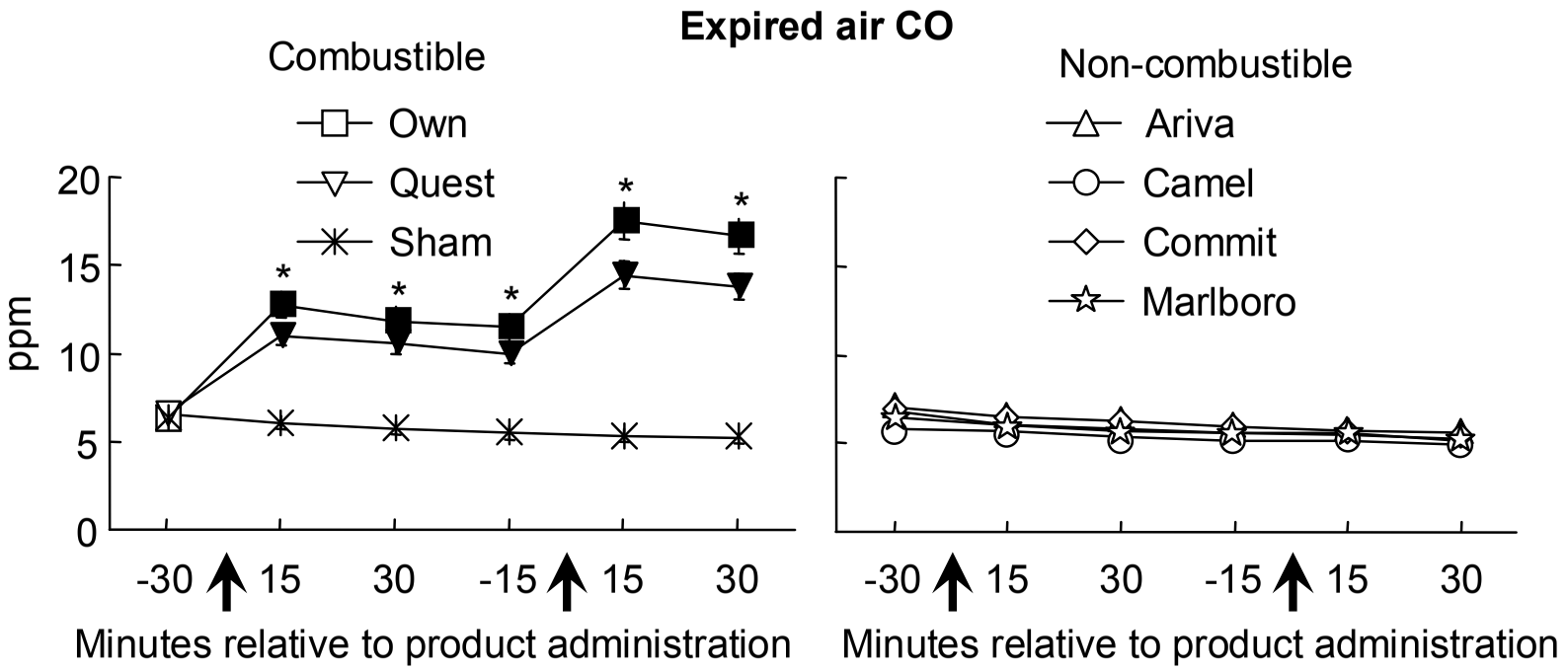

Figure 3. Mean data (±1 *SEM*) for expired air CO across conditions (N = 28). Arrows indicate product administration, filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN mean relative to all non-combustible product means at that time point ($p < .05$, Tukey's HSD).

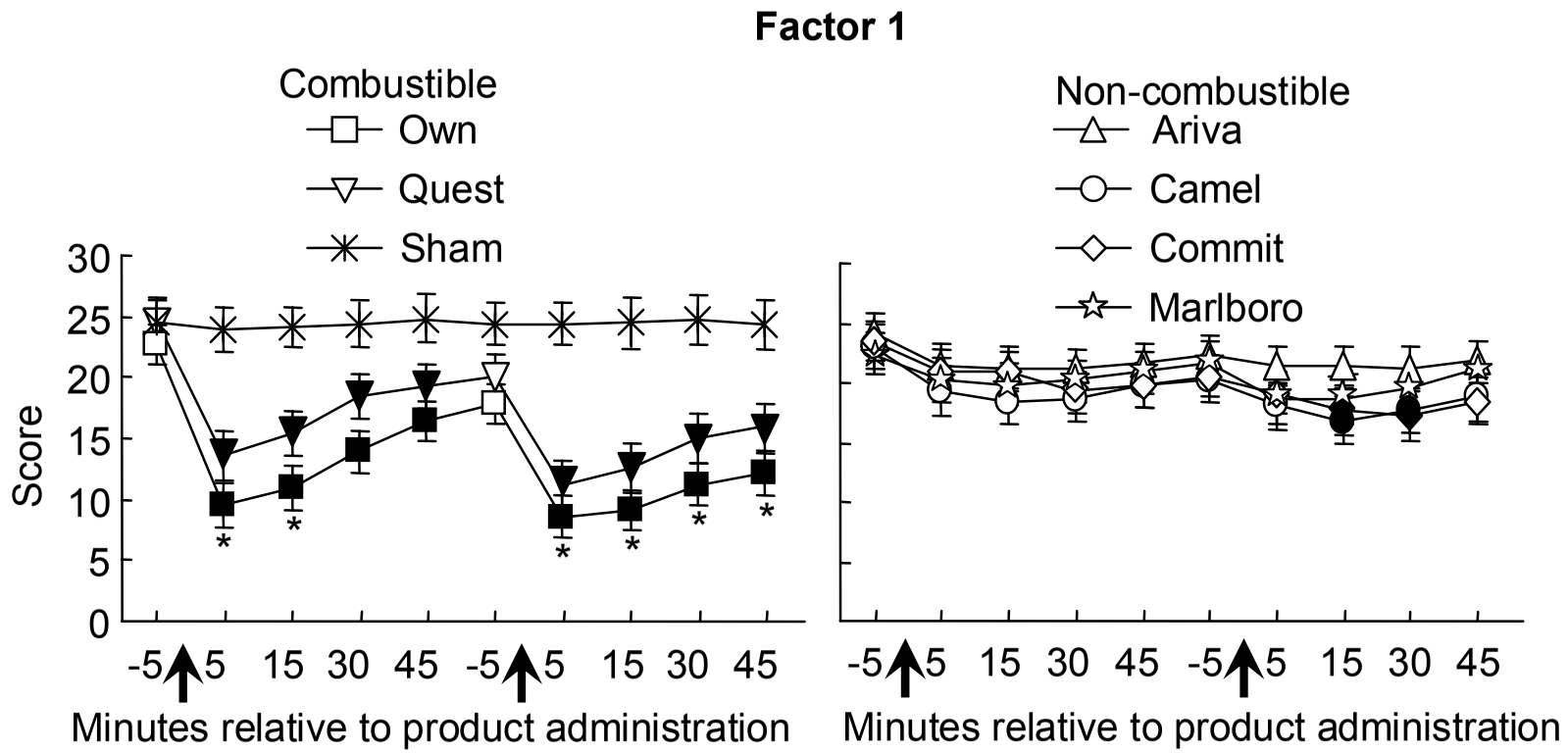

Figure 4. Mean data (±1 *SEM*) for Factor 1 of the Tiffany-Drobes QSU Brief across conditions (N = 28). Arrows indicate product administration, filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN mean relative to all non-combustible product means at that time point ($p < .05$, Tukey's HSD).

(mean decrease from baseline = 12.4, *SEM* = 2.3). Scores in the SHAM condition remained relatively high and stable throughout the session. Factor 1 scores during non-combustible conditions tended to decrease after the second product administration only. Relative to baseline, scores in the CS condition were significantly decreased 15 (mean decrease = 6.3 points, *SEM* = 2.3) and 30 minutes (mean decrease = 5.4 points, *SEM* = 2.2) after the second product administration ($p < .05$, Tukey's HSD). Similarly, during COMMIT, scores were decreased significantly relative to baseline at 15 (mean decrease = 5.9 points, *SEM* = 1.9) and 30 minutes (mean decrease = 6.3 points, *SEM* = 1.9) after the second product administration ($p < .05$, Tukey's HSD). In contrast, no significant decreases were observed at any time point after an Ariva or Marlboro Snus administration. During all non-combustible conditions, Factor 1 scores were significantly higher relative to those observed during OWN at 5 and 15 minutes after the first product administration and at 5, 15, 30, and 45 minutes after the second product administration (all Ps < .05, Tukey's HSD).

Significant condition by time interactions were observed for two items of the Hughes-Hatsukami Withdrawal Scale, "Urges to smoke" and "Craving a cigarette/Nicotine" (see Table 3; Fs > 6.6, Ps < .001). Figure 5 shows the results for "Craving a cigarette/Nicotine," the item with the larger F value. For this item, OWN was associated with significant decreases in craving scores relative to baseline at nearly every time point ($p < .05$, Tukey's HSD). For example, 5 minutes after the first product

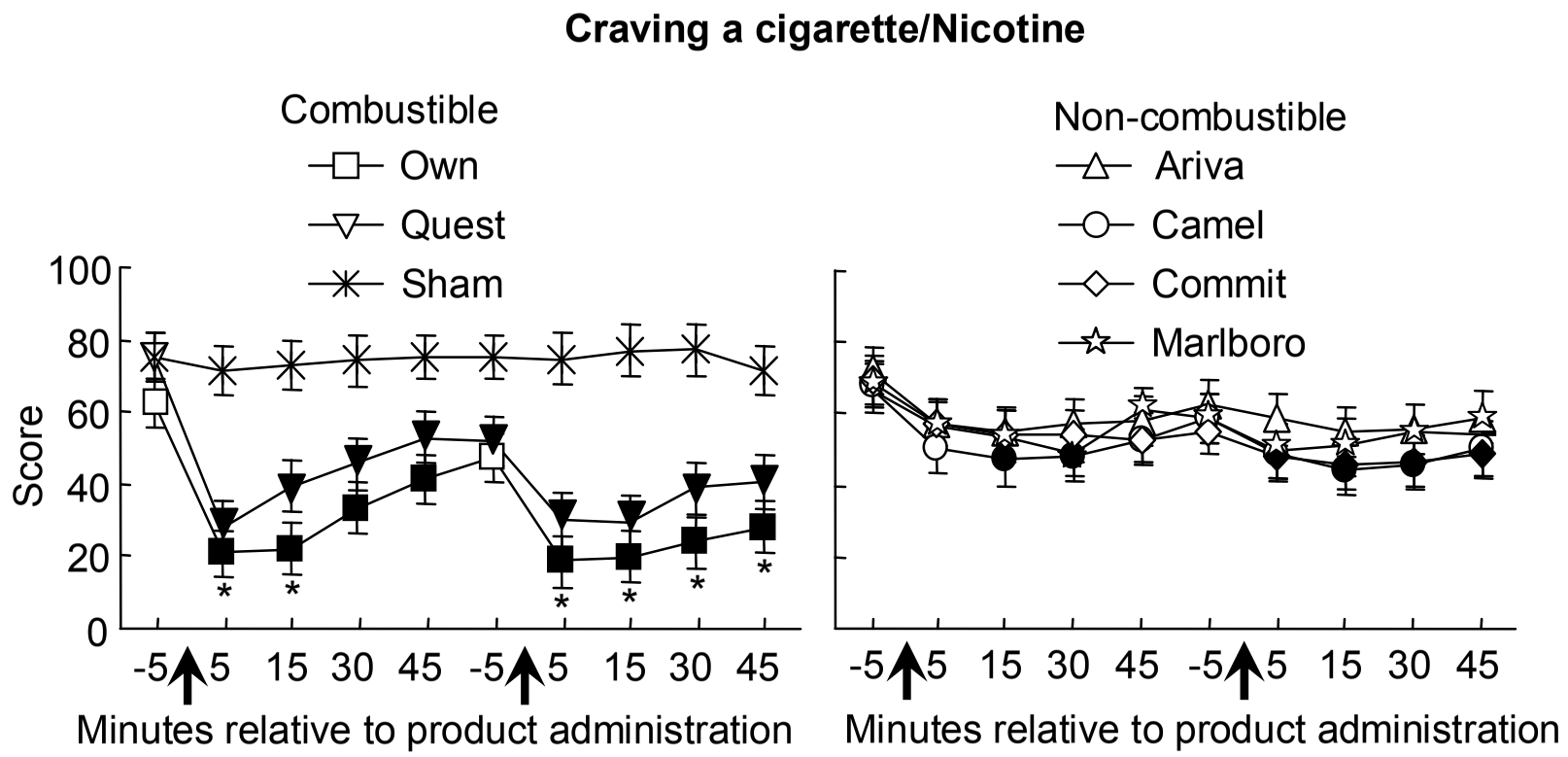

Figure 5. Mean data (±1 *SEM*) for "Craving a cigarette/Nicotine" item from the Hughes-Hatsukami Withdrawal Scale across conditions (N = 28). Arrows indicate product administration, filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN mean relative to all non-combustible product means at that time point ($p < .05$, Tukey's HSD).

administration, a mean decrease from baseline in craving score of 41.7 points (*SEM* = 8.4) was observed, with a mean decrease from baseline of a similar magnitude (44.0 points, *SEM* = 7.4) 5 minutes after the second product administration. During QUEST, a similar administration associated pattern was observed: a mean decrease from baseline of 47.5 points (*SEM* = 7.1) occurred 5 minutes after the first administration, and a mean decrease from baseline of 45.1 points (*SEM* = 8.2) was noted 5 minutes after the second product administration. In contrast, mean craving scores during SHAM were relatively high and stable over both administrations. For the non-combustible products, relative to baseline, scores in the CS condition were significantly decreased 15 (mean decrease = 20.9 points; *SEM* = 6.5) and 30 minutes (mean decrease = 20.0 points; *SEM* = 5.5) after the first product administration and 5 (mean decrease = 19.0 points; *SEM* = 6.0), 15 (mean decrease = 23.9 points; *SEM* = 6.1), and 30 minutes (mean decrease = 22.1 points; *SEM* = 6.0) after the second product administration ($p < .05$, Tukey's HSD). In the COMMIT condition, relative to baseline mean craving ratings decreased significantly at every time point after the second product administration, with the greatest mean decreases observed at the 15 minute time point (mean decrease = 23.3 points, *SEM* = 6.1). Relative to baseline, mean craving ratings in the MS condition decreased significantly only once, 30 minutes after the first product administration (mean decrease = 18.3 points, *SEM* = 5.1) while ratings in the ARIVA condition never decreased significantly. During all non-combustible conditions, craving scores were significantly higher relative to those observed during OWN at 5 and 15 minutes after the first product

administration and at 5, 15, 30, and 45 minutes after the second product administration (all Ps < .05, Tukey's HSD). A similar pattern of results was observed for the "Urges to smoke" item (see Figure 6).

Direct Effects of Nicotine Scale. As shown in Table 3, a significant condition by dose by time interaction was observed for the Direct Effects of Nicotine item assessing "Dizzy," while significant condition by time interactions was observed on items assessing salivation, nausea, heart pounding, and headache (Fs > 2.05, Ps < .05). The three-way interaction for the "Dizzy" item is explained by an increase from baseline in the OWN condition at the 5 minute time point after the first administration (mean increase = 12.3; *SEM* = 5.5; n.s., Tukey's HSD) that was not observed after the second administration and was also not observed in any other condition. For salivation, all non-combustible products produced a significant increase relative to baseline (collapsed across all conditions, 5.4 points; *SEM* = 3.1) in reports of salivation five minutes after the first product administration: ARIVA mean increase = 18.4 points, *SEM* = 5.5; COMMIT mean increase = 21.6 points, *SEM* = 6.3; CS mean increase = 18.7 points, *SEM* = 5.7; and MS mean increase = 19.4 points, *SEM* = 5.3 (all Ps < .05, Tukey's HSD). There were no significant increases on this item in the combustible conditions.

For the remainder of items with significant condition by time effects (nauseous, heart pounding, and headache), there were no ratings across conditions that increased significantly relative to baseline (Tukey's HSD). For nauseous, 5 minutes after the first administration of Ariva and Camel Snus there was a trend toward increased ratings

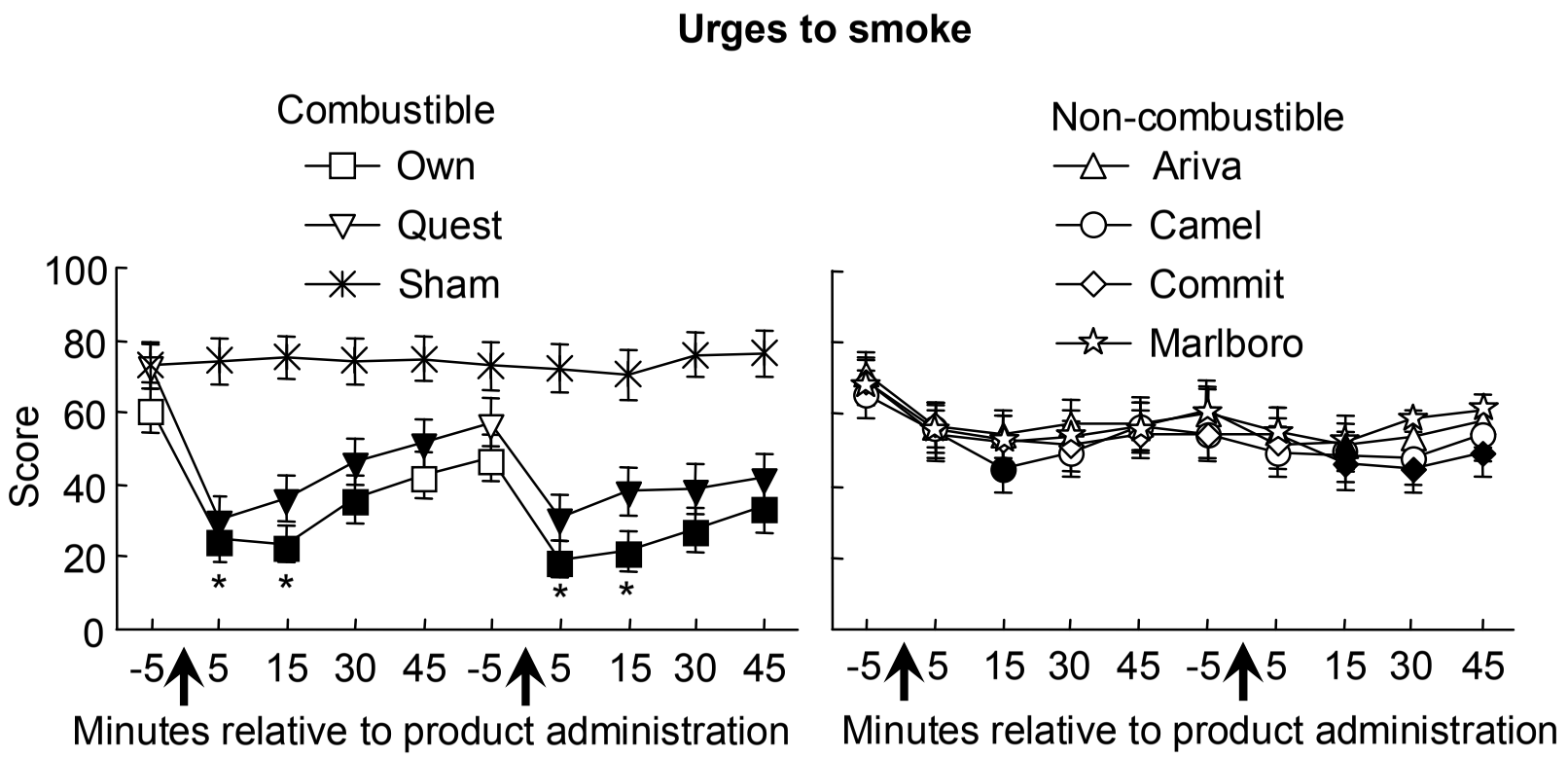

Figure 6. Mean data (±1 *SEM*) for "Urges to smoke" item from the Hughes-Hatsukami Withdrawal Scale across conditions (N = 28). Arrows indicate product administration, filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN mean relative to all non-combustible product means at that time point ($p < .05$, Tukey's HSD).

relative to baseline (ARIVA mean increase = 11.9 points, *SEM* = 5.5; CS mean increase = 14.5 points, *SEM* = 5.2; n.s., Tukey's HSD). Mean nauseous ratings observed during OWN also increased 5 minutes following the first product administration relative to baseline (mean increase = 6.7 points, *SEM* = 3.7) and stayed relatively stable over the session. Ratings during other combustible conditions had little increase or change. For heart pounding, during the ARIVA and CS conditions, a slight trend of increased ratings was observed 5 minutes after the first product administration relative to baseline (ARIVA mean increase = 4.1 points, *SEM* = 2.3; CS mean increase = 2.1 points, *SEM* = 2.1; n.s., Tukey's HSD) while other conditions' mean scores stayed relatively stable or decreased over both administrations. For headache, a trend toward increased ratings 15 minutes after the first product administration was observed during all the non-combustible conditions relative to baseline (ARIVA mean increase = 3.5 points, *SEM* = 1.6; CS mean increase = 4.1 points, *SEM* = 2.7; COMMIT mean increase = 5.9 points, *SEM* = 4.4; MS mean increase = 4.1 points, *SEM* = 2.2; n.s., Tukey's HSD). Relative to baseline, increased ratings were also observed during QUEST 45 minutes after the first product administration (mean increase = 8.4 points, *SEM* = 4.3), and during OWN and SHAM headache ratings stayed relatively stable over both administration periods.

Direct Effects of Tobacco Scale. Significant condition by dose by time interactions were observed for 13 of the 14 items of the Direct Effects of Tobacco Scale (see Table 3; Fs > 2.37, Ps < .001). Four of these items related directly to own brand cigarette smoking (e.g. "Did the cigarette taste as mild as your own brand of cigarette?"). Across these four items, a similar pattern was observed with scores during the OWN

condition consistently higher than other conditions. For example, for the item "Does the product taste like your own brand of cigarette?", in the OWN condition, the mean score at baseline was 0.3 points (*SEM* = 0.3) and then increased significantly to 67.0 points (*SEM* = 6.9) five minutes after the first product administration ($p < .05$, Tukey's HSD). During QUEST, the mean baseline score was 0.1 (*SEM* = 0.1) but then increased to 11.1 points (*SEM* = 3.4) five minutes after the first product administration (n.s., Tukey's HSD). Among non-combustible products, no significant increases were observed for this item at any time point after the first product administration. Relative to scores observed during OWN, mean scores on the "tastes like own brand" item were significantly lower for all other conditions across all post-administration time points (all Ps < .05, Tukey's HSD).

The other items on this scale asked participants to rate their perceptions of the product on a variety of dimensions (e.g., taste, satisfy, pleasant). Figure 7 presents the responses to one such item, "Was the product pleasant?". Relative to baseline, scores on this item increased significantly for all time points after the first product administration in the OWN, QUEST, ARIVA, and MS conditions (see Figure 7; $p < .05$, Tukey's HSD), with significant increases in the CS condition at 5 and 15 minutes after the first product administration and no significant increases in the SHAM or COMMIT conditions. Relative to the scores observed during the OWN condition, mean scores on this item were significantly lower for all other conditions across all post-administration time point

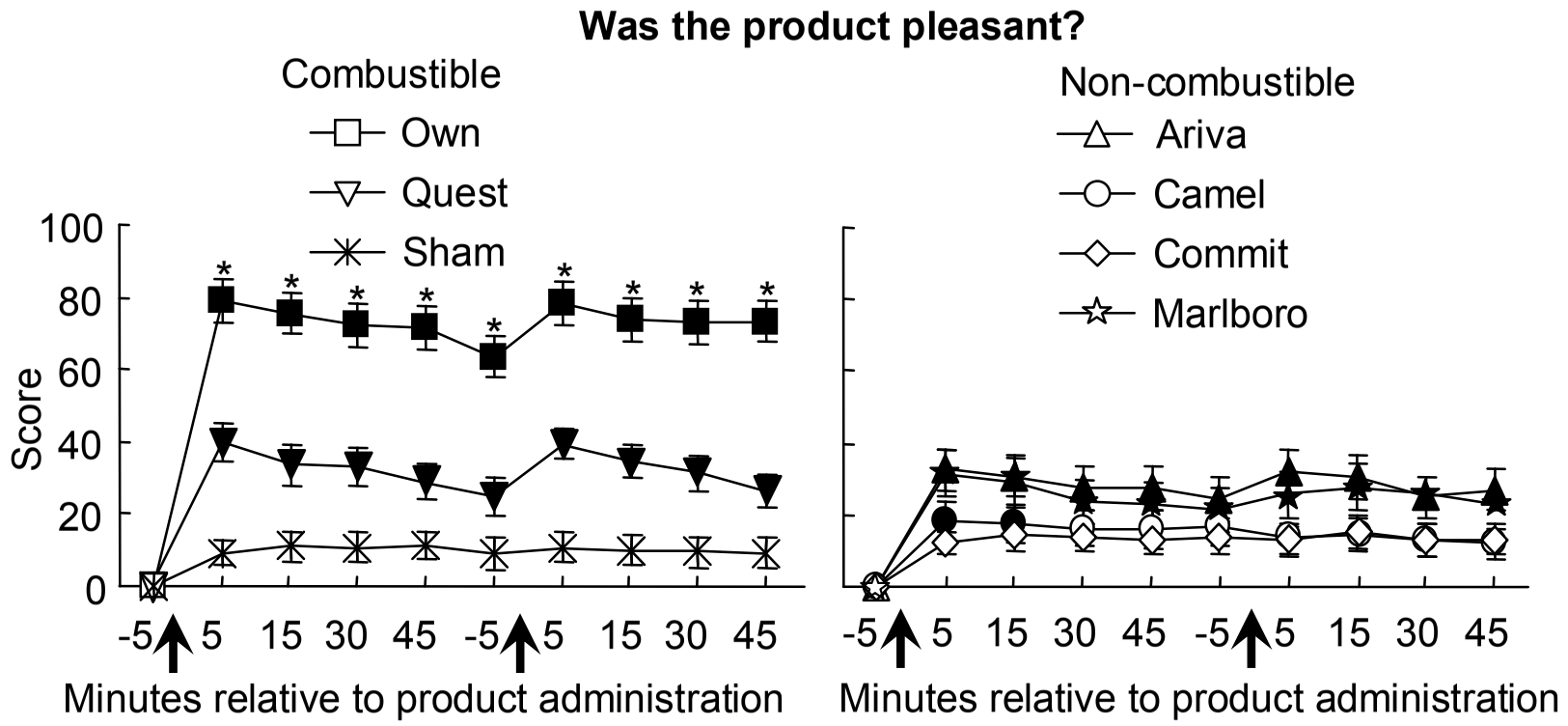

Figure 7. Mean data (±1 *SEM*) for "Was the product pleasant?" item from the Direct Effects of Tobacco Scale across conditions (N = 28). Arrows indicate product administration, filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference of OWN mean relative to all non-combustible product means at that time point ($p < .05$, Tukey's HSD).

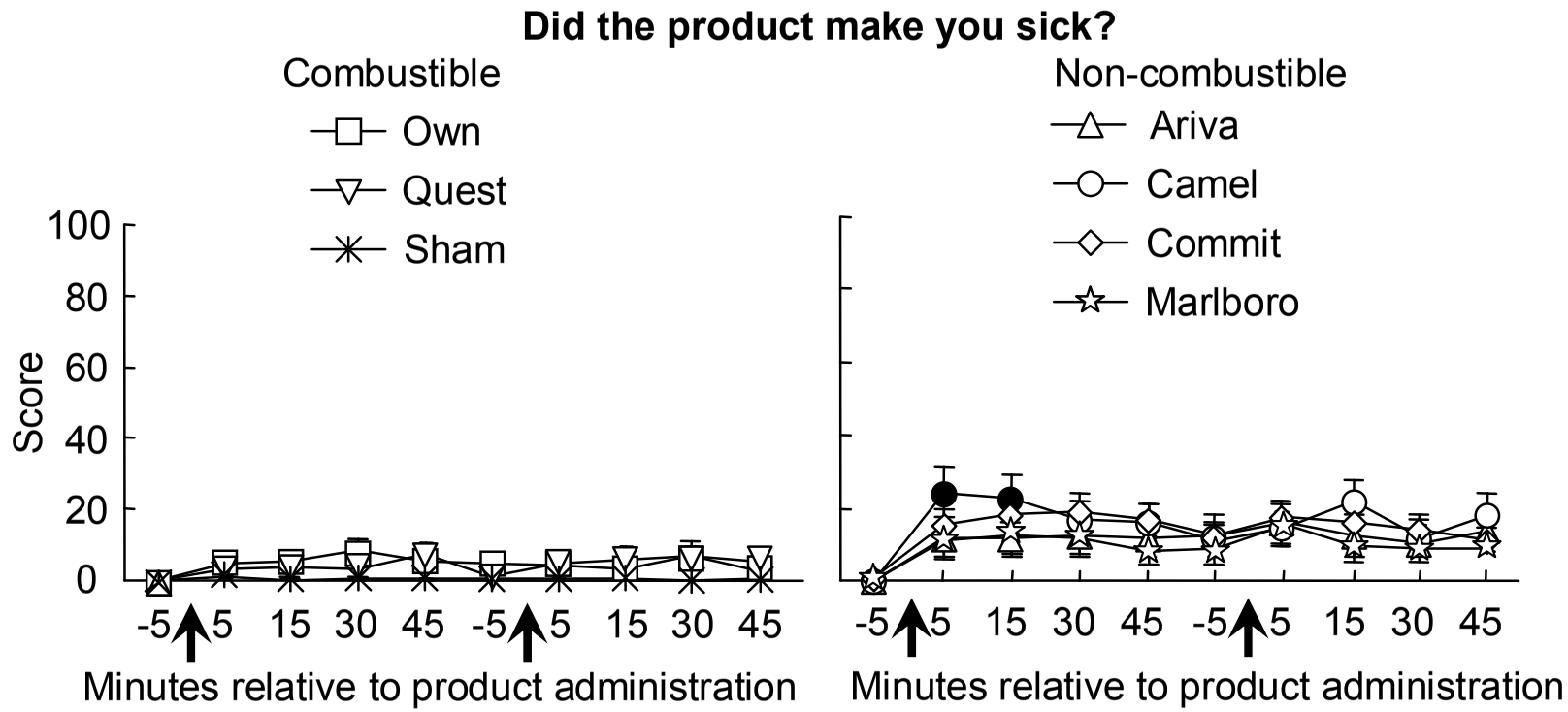

Figure 8. Mean data (±1 *SEM*) for "Did the product make you sick?" item from the Direct Effects of Tobacco Scale across conditions (N = 28). Arrows indicate product administration, and filled symbols indicate a significant difference relative to baseline ($p < .05$, Tukey's HSD). There were no significant differences of OWN mean relative to all non-combustible product means any time point.

(all Ps < .05, Tukey's HSD). This general pattern of results was also observed on items assessing "Was the product satisfying?" and "Did the product taste good?".

For items assessing product effects on ratings of "calm you down", "help you concentrate", "reduce hunger", "more awake", and "more product right now" significant increases from baseline were observed during OWN, with fewer or no significant increases relative to baseline observed in any non-combustible condition. For the item, "Did the product make you sick?", during the CS condition, relative to baseline a significant mean increase at 5 minutes (mean increase = 24.3 points, *SEM* = 6.9) and 15 minutes (mean increase = 22.8 points, *SEM* = 6.0) was seen after the first product administration (see Figure 8). Other non-combustible conditions experienced a trend towards increased "sick" scores 15 minutes after the first product administration (ARIVA mean increase = 11.1 points, *SEM* = 4.7; COMMIT mean increase = 17.9 points, *SEM* = 4.9; MS mean increase = 12.3 points, *SEM* = 4.9; n.s., Tukey's HSD). Little change was observed during the combustible conditions for this item. Lastly, ratings on the Direct Effects of Tobacco Scale item "Did the product make you dizzy?" were increased relative to baseline during the OWN condition at the 5 and 15 minute time points after the first administration that was not observed after the second administration and also was not observed in any other condition.

CHAPTER 4 Discussion

Overview

As novel PREPs for smokers continue to appear within the marketplace, efficient and comprehensive evaluation of these products is essential. Reliable methods to evaluate combustible PREPs have been reported (Breland, Evans, et al., 2002; Breland, Buchhalter, et al., 2002; Strasser et al., 2007), but there has been little clinical evaluation of non-combustible PREPs, particularly tobacco based non-combustible PREPs for smokers. This study adapted clinical laboratory methods used to evaluate the toxicant exposure and subjective effects associated with acute exposure to combustible PREPs to evaluate non-combustible PREPs for smokers, including tobacco based (Ariva, Camel Snus, and Marlboro Snus) and non-tobacco based (nicotine lozenge; Commit) PREPs. These PREPs were compared to the participant's own brand of cigarettes (positive control), sham smoking (negative control), and to a combustible PREP marketed to deliver virtually no nicotine (Quest). The study provided valuable information concerning the acute effects of non-combustible PREPs, and also highlighted the value of important control conditions in laboratory-based PREP evaluations.

Acute Effects of the Non-combustible PREPs

In comparison to the effects observed during OWN, the non-combustible PREPs reduced exposure to nicotine, did not reliably increase heart rate, and did not deliver CO. In addition, relative to the ratings observed during OWN, non-combustible PREPs were less effective in reducing tobacco abstinence symptoms and were less acceptable. Lastly,

there appeared to be no difference between the two versions of Camel Snus (2006 and 2008).

Physiological measures. The physiological measures assessed in this acute study give some indication of the toxicant exposures as well as the cardiovascular effects associated with these non-combustible PREPs. For plasma nicotine on average, five minutes after beginning to smoke a single own brand cigarette, participants' mean plasma nicotine level increased by approximately 18 ng/ml. No non-combustible PREP increased plasma nicotine level significantly at any time point after the first administration, and only one significant increase was observed after the second administration: in the CS condition, plasma nicotine levels increased relative to baseline approximately 5 ng/ml 15 minutes after the second product administration (see Figure 1). For heart rate during OWN, administration associated increases were observed (approximately 14 beats/min 5 minutes after the first product administration) while minimal changes occurred during non-combustible conditions. During CS, relative to baseline one significant increase of approximately 4 beats/min occurred 15 minutes after the first product administration (see Figure 2). The minimal heart rate increases observed during non-combustible conditions may be an effect of the low levels of plasma nicotine detected during these sessions. Also, as might be expected, administration of an own brand cigarette increased expired air CO reliably (by about 7 ppm 15 minutes after the first product administration), and no non-combustible PREPs were associated with any reliable CO increases at any post-administration time point. Thus, in terms of toxicant exposure and cardiovascular effects assessed in this short-term laboratory study, the non-

combustible PREPs decreased exposure to some smoking related toxicants (nicotine and CO) and did not reliably increase heart rate in comparison to the effects observed during own brand smoking.

Abstinence symptom suppression. Measuring effective abstinence symptom suppression in clinical laboratory evaluations of non-combustible PREPs is important. All other things being equal, PREPs that fail to suppress abstinence completely (i.e., similarly to an own brand cigarette) are less likely to achieve the goal of substituting fully for normally marketed cigarettes, and thus may have limited harm reduction potential (e.g., Breland et al., 2006; Hughes & Keely, 2004). Several non-combustible PREPs produced minimal but reliable abstinence symptom suppression at some time points, though an own brand cigarette always produced suppression of greater magnitude (see Figures 4, 5, and 6). For example, mean ratings of "Urges to smoke" decreased by approximately 20 points (on a 100-point VAS) 15 minutes after receiving the second Ariva administration, but decreased approximately 40 points at the same time point during the OWN condition (see Figure 6). CS and COMMIT were the only conditions in which significant decreases in more than one measure of tobacco abstinence symptomology were observed, but again these reductions were minimal in comparison to those seen during OWN. For example, for Factor 1 of the QSU Brief, CS was associated with a significant decrease relative to baseline of approximately 6 points 15 minutes after the second product administration; OWN was associated with a decrease of approximately 14 points at the same time point. Interestingly, in the COMMIT condition, (a comparison condition to the tobacco-based combustible PREPs), suppression of

multiple abstinence symptoms was observed only after the second product administration, whereas OWN was able to produce suppression immediately following the first product administration. Similar to the other non-combustible PREPs, the magnitude of suppression observed during COMMIT was generally significantly smaller compared to OWN. In addition, the abstinence suppression observed during COMMIT was consistent with previous work examining the effects of pharmalogically pure nicotine among smokers (Molander et al., 2000; Mendoza-Baumgart et al., 2007). Quest, a product that delivered smoking-related stimuli (i.e., taste and smell of smoke as well as CO exposure, see Figure 3) with no measurable nicotine (see Figure 1), produced more robust abstinence symptom suppression than any of the non-combustible PREPs (this placebo-induced tobacco abstinence suppression has been reported previously, see Buchhalter et al., 2005; Brauer et al., 2001). For example, Factor 1 of the QSU Brief, compared against all the non-combustible PREPs, ratings during QUEST five minutes after the first and second product administrations were significantly lower than those observed during the non-combustible conditions ($p < .05$, Tukey's HSD). Taken together, these findings among the non-combustible PREPs imply that, under the conditions reported here, these products do not suppress tobacco abstinence symptoms as completely as an own brand cigarette.

Acceptability. Another factor that may limit the likelihood that a PREP will substitute completely for normally marketed cigarettes involves acceptability – the extent to which the product provides sensory characteristics that are pleasant and/or match those provided by a smokers' usual brand of cigarettes. In this study, in virtually every

measure of acceptability, in sessions where non-combustible PREPs were administered, mean scores observed were more similar to those obtained when participants puffed on an unlit cigarette (i.e., SHAM) than when they were actually smoking their own brand of cigarette (i.e., OWN). For example, during the ARIVA and MS conditions all post administration ratings of "Was the product pleasant?" were significantly higher relative to baseline (mean increase approximately 27 points). These increases were significantly lower than mean ratings observed during the OWN condition (mean increase approximately 73 points), and a majority of these mean increases did not differ significantly from increases seen during the SHAM condition (approximately 10 points) The lowest mean ratings of non-combustible PREP "pleasantness" (approximately 14 points) were observed during the CS and COMMIT conditions, and all mean ratings measured during these two conditions were not significantly different than those seen during the SHAM condition. Other measures of product acceptability including ratings "Did the product make you sick?" were elevated for several non-combustible PREPs, while during combustible product conditions (OWN, QUEST, and SHAM) little change was seen. Based upon the results from this short-term laboratory study, the current formulation of these non-combustible PREPs is associated with low levels of acceptability in comparison to own brand cigarettes. The inability to satisfy sensory demands as effectively as a smokers' usual brand of cigarettes may limit these PREPs acceptability to U.S. smokers and may influence negatively their potential as instruments of harm reduction for tobacco users.

Camel Snus version effects. When Camel Snus version (2006 vs. 2008) was included as a between-subjects factor six significant effects involving this factor were observed. In general, many of these effects appeared to reflect a difference in baseline mean score between Camel Snus versions, rather than an effect produced by the administration of either version of Camel Snus. For example, for Factor 2 of the QSU Brief, for which a significant main effect for Camel Snus version was observed, baseline means across conditions between versions were dissimilar (2006 $M = 7.8$, $SEM = 1.3$; 2008 $M = 3.7$, $SEM = 1.3$). This difference in baseline score may have influenced the significant main effect of Camel Snus version. In addition, because there were so few significant effects involving this factor, and because the effects followed no consistent pattern, they may reflect Type 1 error rather than an actual difference between the two Camel Snus versions. That is, the alpha level of .05 used in this study reflected a 5% chance of Type 1 error on any given measure (i.e. for 100 independent ANOVAs, data consistent with rejecting the null hypothesis might be expected 5 times due to chance alone, rather than due to any effect of Camel Snus version). When the Camel Snus version factor was entered into the overall analysis, 320 independent ANOVAs were performed, thus at least 16 "significant" effects might be expected due to chance alone (and only 6 were observed). However, Type II error (i.e. a failure to reject the null when it is false) is another potential issue, because the between-subjects manipulation involved group sizes of only 14. Larger samples are required to detect small or medium effects (e.g., mean of 47 participants across the two groups; Cohen, 1977). Generally, more

powerful within-subjects designs may be the more effective method of assessing toxicant exposure and effects across and within PREP brands.

Summary of non-combustible PREP effects. Overall, the acute effects assessed during this brief examination of non-combustible PREPS indicated that compared to smoking an own brand cigarette, these products delivered reduced amounts of nicotine, were associated with little cardiovascular effect, and did not expose users to CO. Relative to own brand smoking, the non-combustible PREPs were also less effective in suppressing abstinence symptoms and were less acceptable among this population of smokers. In addition, the between-subjects factor, Camel Snus version (2006 and 2008), was removed from the analysis due to the lack of reliable effects. Although these PREPs offer significant reductions in CO and reduced exposures to nicotine, the inadequate abstinence suppression and low levels of acceptability suggest that these products might have limited harm reduction efficacy.

Clinical Laboratory Methods for Evaluating Non-combustible PREPS

The results from this study demonstrated that clinical laboratory methods can be used to evaluate the acute effects of tobacco-based and non-tobacco based non-combustible PREPS for smokers. These methods will be valuable in the future as more non-combustible PREPs are developed and marketed for smokers.

There are multiple arguments for the continued use of the short term clinical laboratory methods described in this study. For one, the outcome measures used provide critical information concerning two key indicators of PREP effectiveness: toxicant exposure and abstinence symptom suppression. While the relationship between toxicant

exposure and disease risk has not been elucidated fully, there is face validity in the notion that the only PREPs that are likely to decrease tobacco-caused disease and death are those that limit users' exposure to lethal toxicants. Similarly, the only PREPs that are likely to substitute fully for a smoker's preferred brand of cigarettes are those that are able to suppress abstinence symptoms as effectively while maximizing relevant sensory characteristics. Secondly, these short term methods include valuable control conditions that, among other things, demonstrate the reliability of clinical laboratory evaluation. For example, results associated with the control conditions, OWN (positive control) and SHAM (negative control), as well as with the QUEST condition, were consistent with previous evaluations that utilized these control conditions (Buchhalter et al., 2001; Breland, Evans et al., 2002; Brauer et al., 2001). Also, these control conditions provided necessary comparison values on study outcomes that put into context the effect of non-combustible PREPs. For instance, inclusion of both control conditions makes clear that non-combustible PREPs are more like SHAM than OWN in terms of toxicant exposure, abstinence symptom suppression, and acceptability. Thirdly, in addition to offering variety of relevant outcome measures and experimental control, these clinical laboratory methods for PREP evaluation are highly adaptable. The adaptability of these methods is evidenced by the fact that they are demonstrably useful for evaluating combustible PREPs for smokers (Buchhalter et al., 2001; Breland, Buchhalter, et al., 2002; Breland, Evans, et al., 2002; Breland et al., 2006), and non-combustible PREPs for smokeless tobacco users (Gray, Breland, Weaver, & Eissenberg, 2008) and smokers (the present study). The importance of adaptability in methods used to evaluate PREPs for tobacco

users is highlighted by the design of the most recent PREPs to enter the marketplace: electronic products designed to look like cigarettes that are marketed to tobacco users as a means to deliver pure nicotine in an aerosolized form (i.e., Crown 7, NJOY). As new PREP designs are developed their effects must be evaluated reliably, and the efficient and reliable methods reported here may be adaptable enough to meet this challenge. Finally, the ability to test two versions of Camel Snus in this study demonstrates how the methods can be used to respond quickly to changes in PREP design (although a within subject manipulation would likely have greater sensitivity).

Limitations

While the laboratory-based method of PREP evaluation described here has several strengths, the short-term testing period limits the ability to measure how longer-term experience using the PREP influences study outcomes as well as any PREP-induced changes in carcinogen exposure. Particularly in the case of a non-combustible PREP, greater experience with a new product may produce a different pattern of results on items assessing abstinence symptoms and/or sensory characteristics. However, evaluating the short-term effects of PREP exposure may be especially relevant considering that, after trying a particular PREP a few times, smokers will then need choose to purchase that PREP or their usual brand of cigarettes (i.e., when their supply of PREP and cigarettes is exhausted). That choice may be guided by the knowledge that, in the short-term, the PREP failed to suppress abstinence effects and satisfy sensory factors. With regard to PREP-induced carcinogen exposure, a growing literature demonstrates longer-term methods for evaluating combustible PREPs for smokers (e.g., Breland et al., 2003; 2006)

and non-combustible PREPs for smokeless tobacco users (e.g., Hatsukami et al., 2004); these methods have been adapted for measuring the carcinogen exposure associated with non-combustible PREPs for smokers (Blank et al., under review). Longer term studies under more naturalistic conditions may also be valuable for examining PREPs-associated toxicant exposure and subjective effect. In addition, acute methods in which participants use PREPs *ad libitum* may clarify the role of dose (i.e., product number) on measures relevant to predicting harm reduction. Lastly, the population recruited may have influenced the results obtained by this study. Potential participants were excluded if they reported trying to quit smoking at the time of screening. Study outcomes, particularly subjective effect measures, might differ if smokers who are trying to quit are included because this population may be more receptive to PREPs.

Conclusions

This study demonstrates how clinical laboratory methods can be used to evaluate the acute effects of non-combustible PREPs for smokers. Results suggest that while these non-combustible products do not expose smokers to CO, they also deliver less nicotine than own brand cigarettes and fail to suppress tobacco abstinence symptoms effectively. Indeed, the subjective effects observed in this study do not support the notion that, as presently formulated, non-combustible PREPs for smokers will be a viable harm reduction strategy for the population from which this sample was drawn (i.e., U.S. tobacco cigarette smokers). Comprehensive pre-market PREP evaluation using established methods and representative samples in the context of a regulated and iterative process designed to minimize toxicant exposure and maximize abstinence symptom

suppression may be the most productive method for realizing the public health potential of PREPs for tobacco users.

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APPENDIX A

Telephone Screening Form

Interviewer: "I would like to ask you some questions about yourself and your health status as well as your use of nicotine, alcohol, and other drugs. The purpose of these questions is to determine whether or not you are eligible to participate in either the study/studies I just described or in any of the other studies being conducted in the lab. All of your responses are confidential. You are not required to answer any question and you may stop this interview at any time. May I begin the questions?"

Document caller's response by circling either: *Yes* *or* *No*

If Yes: begin form. If No: thank caller for calling.

How did you hear about us/our studies? _____

Personal Information:

1. "What is your first name?" _____

2. "What is a phone number at which you can be contacted?" _____

4. "If we call and you are not available, may we leave a message?"
Circle *Yes* *or* *No*

5. "What is your date of birth?" _____

6. "What is your height?" _____ (feet and inches)

7. "What is your weight?" _____ (pounds)

8. "Did you graduate high school?" *Circle* *Yes* *or* *No*

If Yes: Skip the next question.

9. "Did you obtain your GED?": *Circle* *Yes* *or* *No*

General health status:

10. "Do you have any chronic health concerns or problems?" *Circle Yes or No*

If Yes: "Please describe the concern or problem":

11. "Are you under a doctor's care for a medical condition?" *Circle Yes or No*

If Yes: "Please describe the condition":

12. "Are you taking any prescription or over-the-counter medications?"
Circle Yes or No

If Yes: "Please identify the medication":

13. Do you have any psychiatric conditions like depression or anxiety?
Circle Yes or No

If Yes: "Please describe the condition":

14. "Have you ever been diagnosed with high or low blood pressure?"
Circle Yes or No

If Yes: "Please indicate whether it is high or low":

Cigarette use:

15. "Do you currently smoke tobacco cigarettes?" *Circle Yes or No*

If No: Skip the remainder of this section

16. "What brand of cigarettes do you smoke?" _____

Circle:

i) Hard pack / Soft Pack
ii) Regular / Light / Ultra Lt
iii) Non-menthol / Menthol
iv) Regular / 100s / Other

17. "How many cigarettes/day do you smoke?" _____ *(num of cigs)*

[Note to interviewer: Please note exact number of cigarettes per day]

18. "For how long have you smoked this number?" _____ *(mnths or yrs)*

19. "How soon after you wake up do you smoke your first cigarette?"
Circle: Within 30 min.
After 30 min.

20. "Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., at the library, at the movies)?" *Circle Yes or No*

21. "Which cigarette would you hate to give up the most?" *Circle: 1st in the morning*
Any other

22. "Do you smoke more frequently during the first hours after awakening than during the rest of the day?" *Circle Yes or No*

23. "Do you smoke if you are so ill that you are in bed most of the day?" *Circle Yes or No*

Waterpipe use:

24. "Have you ever, in your lifetime, smoked tobacco using a waterpipe, narghile, or sheesha?" *Circle Yes or No*

If No: Skip the next question.

25. "How many days out of the last 30 have you smoked tobacco using a waterpipe?" (number of days)

Smokeless Tobacco Use:

26. "Do you use smokeless tobacco (i.e., snuff, dip, or chew)?" *Circle Yes or No*

If No: Skip the remainder of this section.

27. "What brand of smokeless tobacco do you use?" _____

28. "How many times/day do you use smokeless tobacco?" _____

29. "For how long have you used smokeless tobacco?" _____ *(mnths or yrs)*

30. "How many cigarettes have you smoked in the past 6 months?" _____

Interviewer: "I'd like to ask you some additional questions about your use of alcohol and other drugs."

Alcohol use:

31. "Have you ever been treated for alcohol abuse/dependence? *Circle Yes or No*

If Yes: "When was your treatment completed?": _____ (mnth/year)

32. "Do you use (drink) alcoholic beverages?" *Circle Yes or No*

If No: Skip the remainder of this section.

33. "How many alcoholic drinks (by alcohol I mean beer, wine, or liquor) do you have on a typical day? _____ *(num of drinks)*

34. "How many days out of the last 30 have you used alcohol?" _____ *(num of days)*

Marijuana use:

35. "Have you ever, in your lifetime, smoked marijuana or hashish?"

Circle Yes or No

If No: Skip the next question.

36. "How many days out of the last 30 have you smoked marijuana?"

_____ *(number of days)*

Other drug use:

37. "Have you used any other illegal drugs within the past month?"

Circle Yes or No

If Yes: "Please identify which drug or drugs."

For women only:

38. "Are you currently pregnant?" *Circle Yes or No*

39. "Are you currently breast-feeding a child?" *Circle Yes or No*

40. "What was the first day of your last period?" _____

Interviewer: "Thank you for responding to these questions. I need to pass on your responses to the principal investigator who will then determine whether or not you are eligible to participate in a study; someone will contact you within approximately two working days if you are eligible. If you are not eligible for any of our current studies, then you will *not* be contacted."

APPENDIX B

Informed Consent Form

Title: Evaluating the acute effects of oral tobacco products marketed to smokers.

VCU IRB Number: HM10891

Sponsor: National Cancer Institute

This consent form may contain words that you do not understand. Please ask the study staff to explain any words that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

Purpose: The purpose of this research study is to examine how different types of tobacco and nicotine products influence you.

Description of the study and your involvement: Before you join the study, we will ask you to fill out some forms about your medical history, and we will use breath and urine tests to make sure that the study is right for you. If you agree to join the study, you will participate in seven, approximately 2.5-hour sessions at the Clinical Behavioral Pharmacology Laboratory located on VCU's medical campus. Each session will begin at approximately the same time each day, and will be separated by at least 48 hours. Before each session, we will ask you to abstain from all caffeine-containing beverages, and from all foods, for 1 hour. We will also ask you to abstain from **all** tobacco products for at least 10 hours before each session. In addition, the use of any nicotine-containing products (like the gum or patch) is prohibited. We will ask you to take a simple breath test to make sure that you have complied with these restrictions. Our tests are not perfect, but they will be the only measures that we can accept to make certain that you have complied with the no tobacco/no nicotine restrictions.

Each session day you will be asked to use a tobacco or nicotine product that we provide for you. Sometimes the product will be a cigarette, sometimes it will be an oral tobacco product meant for smokers, and sometimes it will be a nicotine product used to help people stop smoking. You will use each product a total of two times during each session. In some cases you will use the product until it is all gone; in other cases we will ask you

to keep it in your mouth for 15 minutes. On one session day we will ask you to take puffs from an unlit cigarette. You may not know which cigarette brand you are using during each session, and the cigarettes that we provide may or may not be your usual brand. All products used in this study are available to adults in the U.S. without a prescription.

At the beginning of each session, and after you provide the breath sample used to assess compliance with the no tobacco restrictions, a nurse will insert a thin needle into your arm that will stay there for the entire session. This needle will be used to draw blood periodically (approximately one tablespoon per sample, 10 samples per session). We use this method because participants tell us that it is more comfortable than repeated "sticks" with a needle. Over the 7 days that you participate in this study, we will take only slightly more blood than the amount you would give in a single donation at a blood drive. In addition to taking blood samples, we will also ask you to participate in other procedures that include monitoring your heart rate, blood pressure, and breath, and responding to several questionnaires to measure how you feel before and several times after each use. You will have an opportunity to experience all of the questionnaires and physiological equipment before your first session.

Risks and Discomforts: You may experience some discomfort during sessions when you are not using your usual brand of cigarettes or during abstinence from cigarettes before each session. Side effects from products that contain tobacco/nicotine can include sweating, lightheadedness, dizziness, nausea, and nervousness. These effects are unlikely in individuals who use tobacco products regularly. Side effects from tobacco abstinence can include irritability, anxiety, restlessness, excessive hunger, difficulty concentrating, and sleep disturbance. Though uncomfortable, these feelings are not medically dangerous. You may also feel some discomfort when the nurse inserts or withdraws the needle, or when blood samples are taken. We try very hard to minimize your discomfort at these times, and the use of a trained nurse and sterile, disposable equipment enhances comfort while reducing the risk of bruising and infection. If you find any effects or data collection procedures unacceptable, you may stop your participation at any time.

Benefits. You will derive no personal benefit from this study other than the money that we pay you for the time that you spend in the laboratory. However, your participation will help us in the future as we try to understand the effects of different types of tobacco products.

Costs of Participation. There is no cost to you for participation except for your time. Participating in this study will take about 18 hours in the laboratory.

Payment for Participation. You will be paid for the time that you are not using tobacco prior to session and for your time in the laboratory: you will receive $20 after the first session, $30 after the second session, $50 after the third session, $70 after the fourth session, $80 after the fifth session, $100 after the sixth session, and $100 after the seventh session. In all, you can earn $450 for successful completion of this study.

Alternatives. This is not a therapeutic study. You have the alternative not to participate.

Confidentiality of Records. We will not tell anyone the answers that you give us; however, information from the study and the consent form signed by you may be looked at or copied for research or legal purposes by the sponsor of the research, or by Virginia Commonwealth University.

Confidentiality of your records will be maintained by keeping all data in a locked file and in a coded database. Release of this information will be withheld, consistent with the law, unless you give permission to release information. The information obtained in this study may be published, but your identity will not be revealed.

If an Injury Happens. Virginia Commonwealth University and the VCU Health System (formerly known as the Medical College of Virginia Hospitals) have no plan for providing long-term care or compensation in the event that you suffer injury as a result of your participation in this research study. If you are injured or if you become ill as a result of your participation in this study, contact your study doctor immediately. Your study doctor will arrange for short term emergency care or referral if it is needed. Fees for such treatment may be billed to you or to appropriate third party insurance. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study.

Pregnancy. Every effort will be made to have women enter this study on an equal basis with men. Tobacco use may be harmful to a fetus, and pregnant women may not participate in this study. If you suspect that you are pregnant, or if you are currently breast-feeding a baby, please inform the investigator now and do not participate. We will conduct a urine pregnancy test during the screening evaluation visit to ensure that pregnant women do not participate.

Voluntary Participation and Withdrawal. You do not have to participate in this study. If you choose to participate you may stop at any time without any penalty. You may also choose not to answer particular questions that are asked in this study. The investigators will answer any questions that you may have. If you choose not to participate or to discontinue your participation, this choice will in no way affect any medical care you receive now or in the future at this institution. If during the course of the study you experience adverse effects, or if you do not comply with the study restrictions, your participation may be stopped by Dr. Eissenberg without your consent. Any significant new findings that develop during the course of the research study that may affect your willingness to continue to participate will be provided to you.

Questions. You can call Dr. Eissenberg at 827-3562 for information about the research or about research-related injury.

Participants' Rights Information. If you have questions about your rights as a research participant, you may contact:

Office for Research Subjects Protection
Virginia Commonwealth University
Virginia Biotechnology Research Park, BioTech One
800 East Leigh Street, Suite 115
P.O. Box 980219
Richmond, VA 23298-0219
Telephone: 804-828-0868

If you agree to join this study, please print and sign your name below. You will receive a copy of this consent form.

Consent. I have read this consent form. I understand the information about this study. All my questions about the study and my participation in it have been answered. I freely consent to participate in this research study.

By signing this consent form I have not waived any of the legal rights which I otherwise would have as a participant in a research study.

_____
Participant's Printed Name

_____ _____
Signature of Participant Date

_____ _____
Signature of Person Performing Consent Date

_____
Witness's Printed Name

_____ _____
Signature of Witness Date

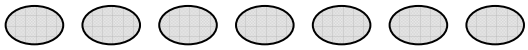
_____ _____
Signature of Investigator Date

APPENDIX C

Questionnaire of Smoking Urges – Brief

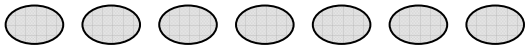
For each item, please indicate how you feel RIGHT NOW.

1. I have a desire for a cigarette right now.



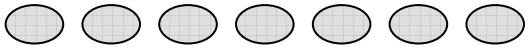
Strongly disagree — Strongly agree

2. Nothing would be better than smoking a cigarette right now.



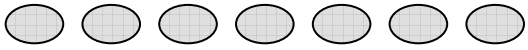
Strongly disagree — Strongly agree

3. If it were possible, I probably would smoke now.



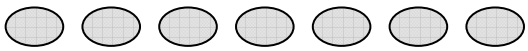
Strongly disagree — Strongly agree

4. I could control things better right now if I could smoke.



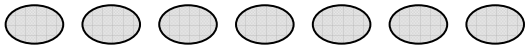
Strongly disagree — Strongly agree

5. All I want right now is a cigarette.



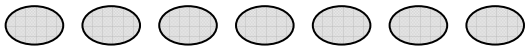
Strongly disagree — Strongly agree

6. I have an urge for a cigarette.



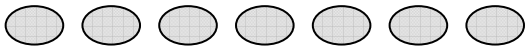
Strongly disagree — Strongly agree

7. A cigarette would taste good now.



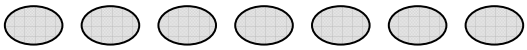
Strongly disagree — Strongly agree

8. I would do almost anything for a cigarette now.



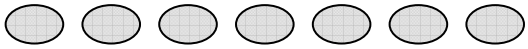
Strongly disagree — Strongly agree

9. Smoking would make me less depressed.



Strongly disagree — Strongly agree

10. I am going to smoke as soon as possible.



Strongly disagree — Strongly agree

VITA

Caroline O. Cobb was born in Henrico, VA on June 19th, 1983 and is an American citizen. She is a graduate of Trinity Episcopal School in Richmond, VA and has a B.A. in psychology from American University in Washington, DC, which she received in 2005. She began the Biopsychology Program at Virginia Commonwealth University in August, 2007.