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Biochemical, demographic, and self-reported tobacco-related predictors of the acute heart rate response to nicotine in smokers

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Abstract

Understanding the stimulatory effects of nicotine on cardiovascular function in humans is of great interest given the wide-spread use of different forms of combustible and smokeless products that deliver nicotine. An intravenous nicotine infusion procedure was used to evaluate factors associated with the acute heart rate (HR) response to nicotine (0.5 mg per 70 kg bodyweight) in a sample of 213 smokers. We tested for differential response to nicotine based on demographic characteristics (race [European American vs African America], sex, body mass index and age); a set of blood-based biomarkers (baseline nicotine, cotinine and cortisol levels and nicotine metabolite ratio); and a set of self-reported measures related to tobacco use. Nicotine infusion was first noted to increase HR approximately 10 beats per minute (95% CI: 7.8–12.3) one minute post-infusion, and 13 beats per minute (95% CI: 11.0–15.2) two minutes post-infusion. Higher cortisol, lower nicotine levels, higher nicotine metabolite ratio, being female and greater withdrawal symptoms were independently associated with a potentiated increase in HR one or two minutes after nicotine infusion. Factors associated with the acute HR effects of nicotine warrant further investigation given their potential to inform the development of nicotine delivery systems as tobacco harm reduction approaches for smokers.

Keywords

Tobacco; harm reduction; nicotine; cardiovascular; cortisol

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INTRODUCTION

Nicotine is a stimulant drug present in tobacco products, including traditional tobacco cigarettes, and most smokeless tobacco products such as electronic cigarettes (Benowitz and Jacob, 1984, Goniewicz *et al.*, 2014). Tobacco smoking is the leading cause of preventable death worldwide with smokers having elevated risk for several types of cancer, cardiovascular and respiratory diseases and many other medical disorders compared to nonsmokers (Carter *et al.*, 2015). The chemicals released by tobacco combustion other than nicotine contribute to much of the negative health effects attributed to tobacco smoking, while the specific contribution of nicotine, aside from its reinforcing effects on smoking behavior and strong addictive potential, are less clear (Benowitz and Fraiman, 2017). As a potent stimulant drug, nicotine activates the sympathetic nervous system, which causes increases in heart rate (HR), cardiac contractility, blood pressure, and increases levels of circulating catecholamines (Benowitz and Gourlay, 1997, Haass and Kubler, 1997). These nicotine-induced effects could contribute to the development of cardiovascular disease (CVD) in smokers, including smokers attempting to quit (Benowitz and Fraiman, 2017, Dollerup *et al.*, 2017, Mills *et al.*, 2014, Vidyasagaran *et al.*, 2016). As such, understanding the cardiovascular effects of nicotine in humans is of great interest, especially given the wide-spread use of different forms of smokeless nicotine delivery systems and their potential use for tobacco harm reduction approaches (Piano *et al.*, 2010): that is, we know a lot about the dangers of smoking *per se* but we know much less about the extent to which those dangers are specifically attributable to nicotine.

Nicotine's pharmacological effects in the central and peripheral nervous systems are mediated by nicotinic acetylcholine receptors (nAChR), which are ligand-gated ion channels that are formed by homomeric or heteromeric pentameric complexes of nAChR subunits. Of the 16 distinct nAChR subunits encoded by the human genome, the nAChRs involved in regulating the sympathetic and parasympathetic components of the autonomic nervous system contain either $\alpha 3\beta 4$, $\alpha 7$ or $\alpha 4\beta 2$ subunits (Gotti *et al.*, 2009, Rollema *et al.*, 2014). Sympathetic stimulation causes release of norepinephrine within the heart, which activates beta-adrenergic receptors to rapidly increase HR. HR is also controlled by norepinephrine that is released into circulation via sympathetic nerves within the adrenal glands. Complex interactions between the sympathetic and parasympathetic nervous system, with additional input from the baroreceptor reflex (baroreflex) and carotid body chemoreceptors that are sensitive to nicotine and other chemicals (e.g. oxygen, carbon dioxide), fine-tune HR to adjust the cardiac output to meet the requirements of the environment and or to adapt to stimuli (Fagerlund *et al.*, 2010, Gordan *et al.*, 2015, Lachman *et al.*, 2008).

Interindividual differences affect nicotine response. For example, genetic and population differences are associated with nicotine metabolism, nicotine consumption, nicotine's subjective effects, nicotine reinforcement, and variation in tobacco smoking behavior (Benowitz *et al.*, 1982, DeVito *et al.*, 2014, Hancock *et al.*, 2015, Jensen *et al.*, 2015a, Jensen *et al.*, 2016b, Jensen *et al.*, 2017, Liu *et al.*, 2010, Loukola *et al.*, 2015, Schoedel *et al.*, 2004, Sofuoglu *et al.*, 2012, Ware *et al.*, 2016). These differences have been linked to the negative health consequences of smoking, such as lung diseases (Thorgeirsson *et al.*, 2008). Research based on these associations have also characterized key biological mechanisms linked to the

development of tobacco product misuse (Fowler *et al.*, 2011, Jensen *et al.*, 2015a). Although potentially informative for evaluating the health consequences of different tobacco products, interindividual variation in the cardiovascular effects of nicotine has not been extensively studied (Benowitz and Fraiman, 2017, Vidyasagaran *et al.*, 2016). A study by Swan and colleagues investigated genetic and environmental sources of variation in HR response to nicotine in a twin sample (n= 278) (Swan *et al.*, 2007). The study assessed HR change during a 30-minute nicotine infusion period (starting at minute 5) and for thirty minutes after the infusion ended. They reported no significant associations with HR increase at minute 5, although age, current smoking status, body mass index, and nicotine clearance were associated with HR levels over the full 60-minute assessment period (Swan *et al.*, 2007).

The goal of this study was to evaluate individual differences that might be associated with the cardiovascular response to nicotine as measured by the acute increase in HR following rapid intravenous (IV) nicotine infusion (0.5 mg nicotine at 0.24 ug per kg body weight per second). We focused on HR given prior work linking smoking intensity to changes in HR, but not blood pressure (Linneberg *et al.*, 2015). The 0.5 mg nicotine dose is approximately equivalent to nicotine intake from smoking half of a cigarette. We analyzed data from 213 current tobacco smokers that participated in an IV infusion protocol that followed overnight smoking abstinence. For many smokers, relative to other periods of the day, the time following overnight abstinence is when nicotine consumption is highest (Payne *et al.*, 1994). Thus, it is important to understand the HR effects of nicotine following overnight abstinence. The IV infusion protocol is a pure nicotine delivery system that allows us to determine the effects induced by precise doses of nicotine in the absence of sensory cues that are typically associated with tobacco product use, and in the absence of products of tobacco combustion. The sample included males, females, and European American and African American subjects. We focused on individual differences not previously characterized with respect to their association to the acute HR response to IV nicotine. These differences included demographic characteristics (Race [European American vs African American], sex, body mass index (BMI) and age); a set of blood-based biomarkers (baseline nicotine, cotinine, nicotine metabolite ratio and cortisol); and measures related to tobacco use, dependence and withdrawal symptoms, which were each analyzed for associations to HR response. The findings might help in the evaluation of cardiovascular risks of nicotine.

Materials and Methods

Subjects

Two hundred and thirteen non-treatment-seeking smokers were recruited from the New Haven, Connecticut area for the nicotine infusion study. All subjects provided written informed consent before participating in the study and subjects were paid for their participation. Institutional review boards at Yale University and the VA Connecticut Healthcare System approved the study. Subsets of this sample have been included in previous studies, including genetic studies related to smoking behavior (DeVito *et al.*, 2014, Herman *et al.*, 2013, Jensen *et al.*, 2015a, Jensen *et al.*, 2015b, Jensen *et al.*, 2017, Sofuoglu *et al.*, 2012). Inclusion criteria included expired breath carbon monoxide levels of 10 parts per million at initial screening and self-reported smoking frequency of 10–25 cigarettes per

day for the past year. The subjects were relatively healthy with no major-medical problems as determined by a self-report, a physician's health check and laboratory testing. Subjects that met criteria for a current psychiatric disorder based on the Structured Clinical Interview for DSM-IV (First *et al.*, 1996) were excluded from the study; this exclusion criterion included current dependence on alcohol or drugs (other than nicotine). A urine drug screen confirmed no recent use of commonly abused drugs other than nicotine. Individuals on psychotropic medication, or who were pregnant or breastfeeding were also excluded. Demographic variables are described in Table 1.

Laboratory procedure

The laboratory session started at 0800 following an overnight abstinence from smoking that was confirmed by expired carbon monoxide levels less than 10 parts per million. The study procedure has been described in detail elsewhere (Sofuoglu *et al.*, 2012). Subjects were administered an IV dose of saline, followed by two escalating doses of nicotine (0.5 mg per 70 kg of body weight and 1 mg per 70 kg of body weight) in uniform order. Each infusion was delivered over 1 minute and separated by 30 minutes. Each dose of nicotine corresponds to the dose of nicotine delivered by an average smoker smoking 0.5–1.0 standard tobacco cigarette (Djordjevic *et al.*, 2000).

Heart rate outcomes

The focus of this investigation was on factors associated with the acute HR response to nicotine, specifically the rapid increase in HR that occurs following IV nicotine administration. HR was assessed continuously using a HR monitoring device. The HR response induced by this protocol has been reported in earlier studies that were based on a subset of the subjects in this sample (Sofuoglu and Mooney, 2009). For each subject, a nicotine-induced change in HR (in beats per minute (BPM)) was calculated based on the HR during the nicotine infusion condition and the HR during the saline infusion condition at the same minute post infusion initiation (i.e. nicotine HR minus saline HR). The BPM change was analyzed for the first two timepoints post nicotine infusion (minute 1 and 2), where there is a rapid BPM increase relative to the saline condition. The maximum HR was achieved at minute 2 post nicotine infusion for the 0.5 mg and 1.0 mg per 70 kg body weight doses (Figure 1). The number of subjects with complete data and the distribution of the change scores for each outcome is shown in supplemental Figures S1.

Biochemical assessments

Baseline (pre-saline infusion) nicotine, cotinine and 3'-hydroxycotinine levels were determined using HPLC interfaced with tandem mass spectrometry (LC/MS/MS) with deuterium-labeled internal standards as previously described (Sofuoglu *et al.*, 2012). Cortisol levels were determined with a radioimmunoassay. Baseline measures were collected after IV lines were established but before saline infusion. As noted in our prior studies, cortisol levels change modestly during the laboratory procedure (Jensen *et al.*, 2015b). To account for these changes, we analyzed cortisol levels at the pre-saline infusion and in a sensitivity analysis considered cortisol levels measured ~30 minutes later prior to nicotine infusion. Nicotine, cotinine and 3'-hydroxycotinine were only collected at baseline.

Tobacco-related outcomes and demographics

Withdrawal symptom severity and smoking urges were assessed before and after the infusion session using the Minnesota Nicotine Withdrawal Scale (MNWS) and the Brief Questionnaire on Smoking Urges (B-QSU), respectively (Cox *et al.*, 2001, West *et al.*, 2006). The MNWS is an 8-item questionnaire with values of 0–10 for each item. The B-QSU is a 10-item questionnaire with values of 0–7 for each item. The Fagerström Test for Nicotine Dependence (FTND) was administered to determine severity of dependence (Heatherton *et al.*, 1991). Cigarettes per day (CPD) smoked was self-reported average number of daily cigarettes smoked. With the exception of FTND and cigs per day, all smoking-related measures were collected after IV lines were established but before saline infusion. The laboratory subjects were classified as belonging to either European American (EurAm) or African American (AfrAm) ancestry groups based on a previously described ancestry informative genetic marker method in those subjects where genotype data were available ($n=124$) or based on self-report (Herman *et al.*, 2013). Seven subjects that were not EurAm or AfrAm were excluded from the analyses of race. Four subjects with a self-report that was discordant with the genetic marker method were re-assigned to the ancestry group defined by the genetic markers method.

Data Analysis

Data were analyzed using the Statsmodel module (version 0.8.0) of Python (version 2.7.13). We first fit univariable linear regression models with the HR change during the 0.5 mg nicotine per 70 kg body weight infusion condition relative to saline at minutes 1 and 2 post-infusion (i.e. nicotine HR, minute 1 minus saline HR, minute 1; nicotine HR, minute 2 minus saline HR, minute 2) as the outcome variables and each of the items in Table 1 as predictor variables. Multiple regression models were then used to evaluate the independence of effects identified in the univariable models. We started with a full model including all predictors and then predictors with $p > 0.05$ were sequentially dropped from the model via backward elimination in order to obtain a more parsimonious model. To facilitate interpretation of results, standardized beta coefficients are reported for all predictors except for the categorical predictors (race, gender). Standardized coefficients are unitless and represent the change in outcome, in units of SD, associated with 1 SD change in the predictor (for univariable models, they equal the correlation between predictor and outcome). For categorical predictors, we report unstandardized rather than standardized coefficients as they provide a more natural interpretation. In all multiple regression models, there was low multicollinearity (VIF values < 10) among predictors. Because nicotine administration was in uniform order and there may be carry-over effects from the 0.5 to 1.0 mg nicotine condition, we focused our analysis on HR response at the 0.5 mg nicotine condition. The HR response at the saline condition and the HR response at the 1.0 mg nicotine condition were analyzed post hoc in a similar manner. As analyses were considered exploratory, we did not correct for multiple testing (an alpha level of 0.05 was used for all tests).

RESULTS

Acute heart rate response to rapid nicotine infusion.

During the 0.5 mg nicotine per 70 kg of body weight condition, there were increases in HR at post-infusion minute 1 and minute 2 relative to saline at post-infusion minute 1 and minute 2. The average HR increased from 65.1 BPM (saline, minute 1) to 75.0 BPM (nicotine, minute 1) ($p < 0.0001$), and from 66.5 BPM (saline, minute 2) to 79.73 BPM (nicotine, minute 2) ($p < 0.0001$). Increases of similar magnitudes were observed at post-infusion minute 1 and minute 2 of the 1.0 mg nicotine per 70 kg of body weight condition (78.01 BPM and 79.57 BPM, respectively) relative the saline condition. The distribution of the change in HR and mean of each outcome is shown in Figures 1 and S1.

Biochemical, demographic, and self-reported tobacco-related predictors of acute heart rate response to nicotine.

Table 2 shows the results for univariable association tests between each biochemical, smoking-related and demographic factor and the HR change at minute 1 and minute 2 post infusion of nicotine at 0.5 mg per 70 kg of body weight. The most robust findings were negative correlations between change in HR and baseline nicotine levels at minute 1 and minute 2 (-0.30 and -0.25 , respectively). There was a strong ($p < 0.0001$) positive correlation between change in HR at minute 1 and baseline cortisol levels (Figure 2). Also, consistent with the nicotine finding, we observed that cotinine, a nicotine metabolite, was negatively associated with change in HR at both timepoints. There was also a positive correlation between NMR and change in HR at both timepoints.

MNWS scores were positively correlated with HR change at minute 1 and minute 2, whereas another withdrawal-related measure, BQSU, was not. (Table 2). There were no significant correlations between self-reports related to smoking quantity and tobacco dependence severity and the HR change induced by nicotine.

To assess the independent contributions of these factors, we used a multiple regression model that included all biochemical, demographic and subjective factors as predictor variables. Four factors with $p < 0.05$ at minute 1 post 0.5 mg infusion (cortisol, nicotine, sex and MNWS score) and three factors for minute 2 (nicotine, sex and NMR) were selected by backward elimination to include in a separate multiple regression model. As shown in Table 3, these factors had independent associations to HR change at minute 1 or minute 2 post infusion of the 0.5 mg nicotine per 70 kg of body weight condition. At both timepoints, lower baseline nicotine levels and being a female were associated with higher changes in HR. At minute 1, after controlling for the other variables in the model, one SD increase in cortisol was associated with an estimated $\beta = 0.33$ SD (95% CI 0.19 to 0.46, $p < 0.0001$) increase in outcome. To consider the potential effect of a modest change in cortisol over the course of the laboratory procedure we also analyzed in the multiple regression model the cortisol levels just prior to nicotine infusion rather than the levels just prior to saline infusion. In this model, the association between cortisol and HR increase was of similar magnitude ($\beta = 0.27$ [95% CI 0.14 to 0.41], $p = 0.0001$). Cortisol was not selected as a predictor in the final model for minute 2 (eliminated with a p -value of 0.20).

As shown in Figure 2C, the analysis of the 1.0 mg nicotine per 70 kg of body weight condition indicated that the cortisol effect at minute 1 ($\beta = 0.25$, $p = 0.001$, in the multiple regression model) was similar to the cortisol effect at minute 1 at the 0.5 mg nicotine per 70 kg of body weight condition ($\beta = 0.33$, $p < 0.0001$). The other predictors in the model (sex, nicotine and MNWS scores) had similar effects at the 1.0 mg nicotine per 70 kg of body weight condition as well.

Biochemical, demographic and subjective predictors of heart rate following placebo infusion.

In univariable models, HR at the placebo (saline) condition (post infusion minute 1 and 2) was positively correlated with BMI and with being female ($p < 0.05$). In a multivariable model including all predictors, only BMI remained significant, indicating that the effect of sex (female > male) on HR was mediated, at least partially, by sex-differences in BMI (female > male). Two factors with $p < 0.05$ at minute 1 and 2 post saline infusion (BMI and FTND score) were selected by backward elimination to include in a separate multiple regression model. In this model, the effect of BMI on HR was similar at each time point post saline infusion (minute 1, $\beta = 0.27$, $p = 0.0003$; minute 2, $\beta = 0.28$, $p = 0.0002$) as was the effect of FTND score (minute 1, $\beta = -0.19$, $p = 0.01$; and minute 2, $\beta = -0.22$, $p = 0.003$).

DISCUSSION

Here we systematically evaluated the association of several demographic, biochemical and self-reported outcomes related to tobacco smoking and nicotine exposure to the acute HR response to IV nicotine. Biomarkers of nicotine exposure and rate of nicotine metabolism, as well as plasma cortisol, sex and withdrawal symptoms, were independently associated with the acute HR increases following nicotine infusion. These individual differences warrant further investigation to determine their link to the negative health effects of tobacco product use.

Perhaps most interesting is the observed positive association between cortisol and nicotine-induced HR increase. Nicotine is known to stimulate the hypothalamic–pituitary–adrenal (HPA) axis to release cortisol, while smoking cessation is associated with a decrease in cortisol levels (al'Absi *et al.*, 2004, Tweed *et al.*, 2012). Hormones released by the HPA-axis regulate the behavioral response to nicotine and potentiate the activity of nicotinic receptors to nicotine, which could affect how nicotine stimulates the sympathetic and parasympathetic nervous system (Bruijnzeel *et al.*, 2007, Ke and Lukas, 1996.) Stress hormones, in particular cortisol, have been linked to blunted baroreflex, a feedback mechanism that, in addition to sympathetic and parasympathetic nervous systems, regulates HR and overall cardiac output (Broadley *et al.*, 2005, Gianaros *et al.*, 2012). Broadley *et al.* showed that blocking cortisol synthesis prevented the normal stress-induced suppression of baroreflex, resulting in reduced HR and blood pressure responses (Broadley *et al.*, 2005). This mechanism warrants further investigation given links between cortisol as well as blunted baroreflex and cardiovascular disease mortality (La Rovere *et al.*, 1998). In sum, these findings suggest the mechanism linking higher cortisol levels to greater HR response to nicotine may involve blunted baroreflex by higher cortisol levels. An individual's cortisol levels may vary over time

because of naturally occurring diurnal fluctuations, tobacco smoking, alcohol consumption, medical conditions, and perceived stress (Badrick *et al.*, 2008, Manenschijn *et al.*, 2013, McClintick *et al.*, 2013, Mendelson *et al.*, 2008, Steptoe and Ussher, 2006, Whitworth *et al.*, 2005). It will be important to consider these sources of variation when evaluating other cardiovascular effects of nicotine.

The association of cortisol with HR increase is consistent with our prior work that was based on a subset of the sample examined in the current study. The prior study focused on the gene encoding FK506 binding protein 5 (*FKBP5*) and not on cortisol, *per se*. FK506 binding protein 5 is induced by glucocorticoids, and its functions include negatively regulating glucocorticoid receptor activity via a feedback loop (Zannas *et al.*, 2016). We previously observed a positive correlation between blood cell *FKBP5* mRNA expression and nicotine-induced HR increase, and *FKBP5* mRNA levels were positively correlated with cortisol levels ($r^2 = 0.20$, $P = 0.001$). (Jensen *et al.*, 2015b) The correlation between *FKBP5* mRNA and nicotine-induced HR increase was more robust at minute 1 post infusion compared to minute 2 post infusion. Notably, the aforementioned correlation with *FKBP5* mRNA was independent of cortisol levels.

Baseline nicotine (in univariable and multivariable models) and cotinine levels (in univariable models) were negatively correlated with HR response to IV infused nicotine. This negative correlation might reflect differences in acute or chronic tolerance that are based on exposure to different levels of nicotine. The association of NMR to HR response might also reflect differences in the development of tolerance. It could also reflect a physiological response related to percent change in circulating nicotine, as opposed to final nicotine level *per se*. Subjects with low rates of nicotine clearance had an attenuated HR rate response to nicotine compared to subjects with high rates of nicotine clearance, indicating that the exposure to more nicotine via slower metabolism could lead to differences in tolerance. Our results suggest that the effects related to NMR are, to some extent, independent of baseline nicotine levels. Prior studies have reported on individual differences associated with the HR response to intravenous (IV) nicotine that are consistent with these findings. A study by Sofuoglu *et al.*, which focused on nicotine clearance, reported that nicotine clearance was positively associated with HR response to nicotine in a sample of 100 smokers (Sofuoglu *et al.*, 2012). The Sofuoglu *et al.* (2012) study used the same IV infusion procedure as described here with a subset of the current sample. A study by Perkins *et al.*, utilizing nicotine nasal spray, reported that heavy smokers (mean cigs per day (SD)=24.1(3.8), $n=10$) had an attenuated HR increase in response to a high dose of nicotine nasal spray, but not low dose of nicotine nasal spray, compared to light smokers (mean cigs per day (SD)=15.8 (1.4), $n=8$) (Perkins *et al.*, 1989). The Perkins study was conducted with overnight abstinent smokers, but the effect of differences in baseline nicotine levels between the heavy smoking and light smoking groups was not evaluated. The different laboratory (e.g. nicotine infusion versus nasal spray) and analytical methodology may be why this effect was not observed in our study. A nicotine infusion study of 14 males by Benowitz *et al.* reported no significant relationships between change in plasma nicotine concentrations during infusion and cardiovascular responses, which is perhaps due to lack of statistical power to detect effects among the 14 subjects (Benowitz *et al.*, 1982).

Females had a greater HR response to nicotine in the multiple regression model. This finding is consistent with an earlier study by DeVito et al. (2012) that used the same IV infusion procedure as described here with a subset (160 smokers (115 male and 45 female)) of the sample described in this report. The DeVito et al. study, which focused on sex-differences in the acute subjective, physiological, and cognitive response to nicotine, and did not specifically investigate the rapid changes in HR that occurred at minute 1 and 2 post-nicotine, reported that females had a greater HR response over an extended interval (DeVito *et al.*, 2014). Prior studies have suggested that females have greater sensitivity to the effects of nicotine on several outcomes, and differences in sample size and study methodology might account for some inconsistencies in the findings between these studies (Evans *et al.*, 2006, Leventhal *et al.*, 2007, Merritt *et al.*, 2012, Sofuoglu and Mooney, 2009).

Positive correlations between BMI and resting HR have been reported previously (Laatikainen, 2010). We observed a correlation between BMI and HR during the placebo condition. It is unclear based on our study whether the magnitude of this effect differs for smokers in nicotine withdrawal compared to ad-lib smokers (i.e., not in withdrawal), or non-smokers. For example, the magnitude of the correlation of BMI to HR might be exacerbated or attenuated by nicotine withdrawal among smokers.

Our study has limitations that warrant consideration. First, the sample includes current smokers that smoked an average of about 18 cigarettes per day and not lighter smokers (or non-daily smokers). The acute HR response to nicotine may differ for individuals that have had different levels of nicotine exposure. Second, the amounts of nicotine we used to induce a HR response were 0.5 mg and 1.0 nicotine per 70 kg body weight, which is approximately the level of nicotine in one-half or one typical tobacco cigarette, respectively. It will be important to investigate factors associated with the response to different levels of nicotine, and to nicotine delivered at different rates. Follow-up studies are necessary to see if the effects we observe with the IV nicotine infusion procedure are observed with nicotine from commercially available tobacco products, including tobacco cigarettes and smokeless nicotine delivery systems. The IV procedure used in this study mimics some (i.e. rapid nicotine delivery), but not all, aspects of nicotine delivery from tobacco products (Jensen *et al.*, 2016a). Nicotine delivery (rate and yield) varies among different types of tobacco products, with some smokeless products being capable of delivering nicotine at levels that exceed that of a combustible cigarette (Farsalinos *et al.*, 2014, Ramoa *et al.*, 2016). Many prior studies on the cardiovascular health effects associated with nicotine were based on nicotine delivered by NRT patch or gum, or smokeless tobacco (e.g. snus, a smokeless tobacco product used orally), which do not enable precise control of delivery rate, but generally deliver nicotine at slower rates than the IV nicotine procedure used here (Piano *et al.*, 2010). It will be important to carefully evaluate the effect of nicotine on HR response (and other cardiovascular responses) across a range of precisely-controlled nicotine delivery rates because the risk profile for different tobacco products might differ based on their nicotine deliver rate (Shihadeh and Eissenberg, 2015). It will also be important to consider how the observed nicotine-induced HR effects relate to other cardiovascular responses and to markers of cardiovascular disease risk, including HR response over greater time intervals, HR variability, and changes in catecholamine levels. Although our sample was well-balanced with respect to numbers of European Americans and African Americans, there

were insufficient numbers from other racial/ethnic groups (e.g., Asians) to determine if they differed in their acute HR response to nicotine. It will be particularly important to evaluate the acute HR response to nicotine among Asians given the evidence for an association between smokeless tobacco use and ischemic heart disease within this population (Vidyasagaran *et al.*, 2016). It is also important to note that our sample included subjects that were relatively healthy, and did not include smokers with comorbid cardiovascular disease or other major medical disorders. Therefore, our inclusion criteria may have excluded smokers with the greatest vulnerability to nicotine-related cardiovascular effects. Also, there may have been insufficient statistical power due to sample size to detect some associations that might be small or modest effects, and negative findings should be interpreted with caution. Finally, the variance in nicotine-induced HR increase explained by the predictors was generally modest ($< \sim 10\%$), which suggests that the predictors have limited predictive value clinically; the correlations only highlight potential mechanisms that warrant further investigation.

To summarize, we identified factors associated with the acute HR response to IV nicotine. The most robust associations involved cortisol, biochemical markers related to exposure to nicotine, nicotine metabolism, sex and nicotine withdrawal, which were independently associated with nicotine's acute effects on HR. These findings warrant further investigation given their potential to inform the development of nicotine delivery systems for harm-reduction or as smoking cessation aids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The heart rate response to saline and nicotine doses of 0.5 mg per 70 kg and 1.0 mg per 70 kg. The mean at each minute is marked by the line. The error bars reflect standard error of the mean (max SEM= 0.92).

Figure 2.

Baseline cortisol levels are associated with a potentiated increase in heart rate following nicotine infusion. The correlation between baseline cortisol and heart rate increase at minute 1 (A) and minute 2 (B) for the 0.5 mg nicotine per 70 kg body weight dose and the increase at minute 1 (C) and minute 2 (D) for the 1.0 mg nicotine per 70 kg body weight dose.

Table 1:

Baseline measures and demographic information

Category	Variable	Mean (SEM) or %	Total sample size [*]
Demographic:	Age	35.6 (0.62)	213
	Body Mass Index	29.00 (0.40)	207
	EuroAm (vs AfrAm)	50%	206
	Male	70%	213
Smoking-Related:	MNWS (0 to 80 scale)	24.89 (0.89)	212
	BQSU (0 to 70 scale)	44.13 (1.03)	210
	FTND (0 to 10 scale)	5.42 (0.15)	212
	Cigarettes per day	18.15 (0.65)	208
Biochemical:	Nicotine (ng/mL)	2.76 (0.21)	206
	Cortisol (µg/dL)	8.36 (0.22)	211
	Cotinine (ng/mL)	193.78 (8.83)	208
	Nicotine Metabolite Ratio	0.37 (0.02)	198

* Accounts for missing data. Abbr, EuroAm, European American; AfrAm, African American; FTND, Fagerström Test for Nicotine Dependence; MNWS, Minnesota Nicotine Withdrawal Scale; BQSU, Brief Questionnaire on Smoking Urges.

Table 2:

Factors associated with the acute heart rate response to infusion of nicotine at 0.5 mg per 70 kg body weight.

Baseline Variable		Outcome: Heart rate change (beats per minute)			
		Minute 1 post infusion		Minute 2 post infusion	
		Coefficient [95% CI]	P value	Coefficient [95% CI]	P value
Biochemical:	Cotinine	−0.25 [−0.39, −0.11]	< 0.001	−0.25 [−0.39, −0.11]	< 0.0001
	Nicotine	−0.30 [−0.43, −0.16]	< 0.0001	−0.37 [−0.50, −0.23]	< 0.0001
	NMR	0.21 [0.07, 0.35]	<0.01	0.23 [0.09, 0.37]	<0.01
	Cortisol	0.28 [0.14, 0.41]	< 0.0001	0.11 [−0.04, 0.25]	0.14
Demographic:	Age	0.01 [−0.13, 0.15]	0.85	−0.09 [−0.23, 0.05]	0.21
	BMI	0.03 [−0.11, 0.17]	0.68	−0.01 [−0.15, 0.13]	0.84
	EuroAm	0.16 [0.02, 0.29]	0.03	0.10 [−0.04, 0.25]	0.15
	Female	0.25 [−0.05, 0.55]	0.10	0.43 [0.13, 0.73]	<0.01
Smoking-Related:	BQSU	0.06 [−0.08, 0.2]	0.38	0.05 [−0.1, 0.19]	0.52
	MNWS	0.16 [0.02, 0.3]	0.02	0.08 [−0.06, 0.22]	0.24
	Cigs per day	−0.04 [−0.18, 0.1]	0.56	0.02 [−0.12, 0.16]	0.77
	FTND	−0.02 [−0.16, 0.12]	0.76	−0.02 [−0.16, 0.12]	0.82

Note: Coefficients are standardized (except for the coefficients of EuroAM and Female) and were obtained from univariable models regressing change in heart rate (nicotine minus saline) on each one of the factors. Abbr: Cigs, Cigarettes; FTND, Fagerström Test for Nicotine Dependence; MNWS, Minnesota Nicotine Withdrawal Scale; BQSU, Brief Questionnaire on Smoking Urges; NMR, nicotine metabolite ratio.

Table 3:

Multiple regression model results for the 0.5 mg nicotine per 70 kg body weight infusion condition.

Baseline Variable	Outcome: Heart rate change (beats per minute)			
	Minute 1 post infusion (N=194)		Minute 2 post infusion (N=188)	
	Coefficient [95% CI]	P value	Coefficient [95% CI]	P value
Cortisol	0.33 [0.19, 0.46]	< 0.0001	-	-
Nicotine	-0.26 [-0.39, -0.13]	< 0.001	-0.34 [-0.47 -0.21]	< 0.0001
Female	0.44 [0.14, 0.74]	< 0.01	0.41 [0.12, 0.70]	< 0.01
MNWS	0.14 [0.01, 0.27]	< 0.05	-	-
NMR	-	-	0.17 [0.03, 0.31]	< 0.05

Note: Coefficients are standardized (except for the coefficient of Female). Heart rate change was computed as nicotine heart rate minus saline heart rate. Abbr: NMR, nicotine metabolite ratio; MNWS, Minnesota Nicotine Withdrawal Scale.