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# Chemosensory Function in Chronic Smokers: Findings from NHANES 2011-2014 and an E-Cigarette Intervention

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# Chemosensory Function in Chronic Smokers: Findings from NHANES 2011-2014 and an E-Cigarette Intervention

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**Chemosensory Function in Chronic Smokers: Findings from NHANES 2011-2014 and an E-Cigarette Intervention**

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# DEFINITION OF VARIABLES

The following variables were measured and evaluated for this research. The definition for each variable is provided; differences between chapter two and three on the variable are noted.

## Demographic Characteristics

The following variables were measured by self-report through survey questionnaires.

**Age and Gender:** Participants self-reported age in years and gender as male or female.

**Race/Ethnicity:** Race was classified as Mexican American, Other Hispanic, Non-Hispanic white, Non-Hispanic black, Non-Hispanic Asian, or other non-Hispanic/Multirace based on the National Health and Nutrition Examination (NHANES) categories[1] for chapter two. For chapter three, race was classified as American Indian, Asian, Hawaiian, Black, White, or Multi-Race.

**Education:** Participants were asked to mark their highest level of educational attainment based on grade levels and college education. Education level was dichotomized to below high school, or high school and above.

**Marital Status:** Participants self-reported being married, living with a partner, divorced, widowed, separated, or never married (single). Marital status was dichotomized to married/cohabitating, or not (widowed, divorced, separated, or never married/single).

**Income Status:** Participants were asked to report their annual household/family income either by $5,000 increments or $10,000 increments from zero dollars to $100,000 or above. For chapter two, this information was viewed as an income to poverty ratio based on The Department of Health and Human Services (HHS) poverty guidelines[2]. These are yearly defined guidelines used to determine eligibility for federal supplemental programs such as SNAP. A ratio of $\leq 1$ is considered below the poverty line, and >1 is above the poverty line. For chapter three it was viewed in dollar amount.

## Health History Characteristics

The following variables were measured by self-report through survey questionnaires to assess the presence of risk factors for chemosensory issues. Noted is if each variable is used in both, or one study.

**Self-rated health status:** Participants were asked to self-rate their overall general health as excellent, very good, good, fair, or poor as part of the NHANES questionnaires[3]. Health status was dichotomized to excellent/very good/good vs. fair/poor. This variable was not assessed in chapter three.

**Sinonasal problems:** Participants were asked if they experienced either frequent nasal congestion, or a cold/flu that lasted longer than a month during the past year. The variable was dichotomized to presence/absence of these sinonasal issues in both studies.

**Xerostomia:** Participants were asked if they experience persistent dry mouth or not enough saliva during the past year (yes/no). This variable was used in both studies.

**Serious head or face injury:** Participants were asked if they ever experienced a broken nose or serious face injury, or lost consciousness due to a head injury. This was dichotomized to yes/no in both studies.

**Tonsillectomy:** Participants were asked if they ever had their tonsils removed, either as an adult or as a child (yes/no). This variable was used in both chapter two and three.

**Frequent Ear infections:** Participants were asked if they have ever had three or more ear infections (yes/no). They were asked to think back to when they were a child and include these instances as well. This variable was not used in chapter three.

**Alcohol consumption:** Participants were asked about their alcohol consumption as part of the examination section of NHANES[4]. A heavy drinker was classified as reporting having 4/5 drinks on almost every day, either formerly or currently. This variable was not used in chapter three.

**Body Mass Index (BMI):** Body Mass index was calculated based on participant's self reported height and weight. The equation is:

$$\text{BMI} = \frac{Weight\,(kg)}{Height^2\,(m^2)}$$

BMI was classified with the CDC definitions for underweight, normal weight, overweight, obese grade I, obese grade II, and obese grade III[5].

## Cigarette Use

The following variables were measured by self-report and/or objective measure to assess current and former cigarette smoking use.

**Cigarette Smoker:** In chapter two, participants were classified as never, current, or former cigarette smokers based on self-reported use. A smoker was classified as ever smoking 100 cigarettes in their lifetime. In chapter three, an inclusion criterion for the study was smoking at least ten cigarettes a day. Participants in both studies were asked to self-report average cigarettes smoked per day as well as number of years they currently or previously smoked. Smokers were further classified in chapter two based on the following measurements, which are described in more detail in the methods section of chapter two:

*Pack Years:* Determined based on packs smoked/day * years smoked.

*Time to first cigarette (TTFC):* Based on self-reported time to first cigarette upon waking in the morning. This is classified as within 30 minutes of waking, or after 30 minutes of waking.

*Serum Cotinine Level:* Blood serum cotinine level was measured through a blood draw. Cotinine is a metabolite of nicotine used to measure recent cigarette use or exposure. A value of less than 10 ng/mL or ≥ 10 ng/mL was used.

**Menthol Status:** In chapter three, smokers were further classified by menthol status. Responses to brand of cigarette used classified participants as menthol or non-menthol smokers.

**Preferred Electronic Cigarette Flavor:** In chapter three, cigarette smokers trialed five electronic cigarette flavors (Tobacco, menthol, cherry, chocolate, no flavor), rating the most preferred flavor to least preferred flavor.

## Chemosensory Function

The following variables were measured through survey questionnaires to assess self-rated chemosensory function. In addition, measured variables of chemosensory function were used in chapter three. Noted is if each variable is used in both, or one chapter.

**Self-reported olfactory function:** NHANES questions are formulated to assess self-rated olfaction and taste function[6] and used in both chapters. Participants were asked about perceived smell problems within the past 12 months [yes/no], phantom odor sensations [yes/no], and changes since age 25 in smell function [no change, better now, worse now]. Participants who answered 'yes' to either of the first two questions or 'worse now' to the last question were classified as having an olfactory alteration. This classification based on the three questions has proved to have test-retest reliability[7] with fair sensitivity and specificity when compared with a single measure of olfactory function[8].

**Self-reported taste function:** This variable was examined only in chapter three. Based on the NHANES protocol, participants were asked about perceived problems in the past year, loss or

change in function since age 25, for taste (salt, sweet, bitter, sour) and for flavor (chocolate, vanilla or strawberry), as well as the presence of dysgeusia (taste things when nothing should be there) [6]. Participants who answered 'yes' to problems, or 'worse now' since age 25 were classified as having a taste alteration.

**general Labeled Magnitude Scale (gLMS):** All sensory intensity ratings described below for chapter three were made on the gLMS scale, which ranges from 0=*nothing* to 100=*strongest sensation of any kind*, with intermediate labels of 6=*barely detectable*, 17=*moderate*, 35=*strong*, and 53=*very strong*. This scale generalizes ratings to all sensations, and has shown consistency with magnitude matching[9], the gold standard for measuring perceived intensity.

**Measured Olfactory Function:** A sixteen-item odor identification and intensity rating task was used to measure olfactory function. Participants were classified as anosmic/severe hyposmic (0-7 odors identified correctly), hypsomic (8-12 identified correctly) or having a normal sense of smell (13-16 correctly identified). This was only examined in chapter three.

**Measured Taste Function:** Taste function was measured using the NHANES protocol[10]. Participants sampled concentrated quinine hydrochloride (QHCl - 1mM) as well as concentrated sodium chloride (NaCl - 1M and .32 M) drawn across the tongue tip and then sampled with the whole mouth. Participants also reported the intensity of concentrated propylthiouracil (PROP - 1 and 3.2 mM) sampled with the whole mouth. Intensity ratings on the gLMS scale were compared to a control group. This was measured only in chapter three.

**Measured Retronasal Function:** Retronasal function was measured using a jelly bean test and included four jelly bean flavors (cherry, coffee, chocolate, and Tabasco®). Participants first rated the sweetness/flavor intensity of the jelly bean with their nose plugged using the gLMS. Then participants were told to unplug their nose and again rate the intensity of sweetness, flavor

intensity, and level liking or disliking of the flavor on the gLMS. For the Tabasco® jelly beans, participants also rated the intensity of the burn or irritation feeling with the nose unplugged.

# CHAPTER ONE

## INTRODUCTION

### *1.1 Overview*

There are a total of four chapters in this thesis. Chapter one serves as an introduction to the research including specific aims and hypotheses. Chapter two and three report on studies of cigarette smokers and their chemosensory abilities, focusing on olfaction and taste. Specifically, chapter two reports on a secondary data analysis of the 2012-0214 National Health and Nutrition Examination Survey Data (NHANES), testing the classification of smoking status used to examine the association with self-reported olfactory alterations. Chapter three reports on baseline data from an NIH-funded Electronic Cigarette Study being conducted at UConn Health. The study population included male and female chronic cigarette smokers. Both chapters two and three include an introduction, methods, results, discussion, conclusion, and references sections. Chapter four serves as an overall conclusion, summarizing important results and implications for future research.

### *1.2 Background*

The chemosenses refer to the sense of smell and taste, two sensory systems relied on by humans daily. The sense of smell functions through stimulation of the olfactory receptors by volatile chemicals that pass through the nasal cavity or the nasopharynx. Once at the receptors, olfactory neurons send the information to the central nervous system[11]. Odorants passing through the nasopharynx play a key role in flavor perception via retronasal olfaction, linking taste and smell together closely. Taste perception is mediated through taste buds located on the papillae of the tongue, which contain taste receptors to identify each of the unique five tastes (salty, sweet,

sour, bitter, and unami). Multiple nerves contact taste receptors throughout the tongue and throat to transfer taste information to the central nervous system[11]. Some differences in taste perception, specifically bitter taste perception, are second to polymorphisms in up to 26 different taste receptor genes, including the most studied *TAS2R38* receptor gene [12]. Additionally, taste perception plays a key role in flavor preference[13], including influencing differences among smokers in choice of nicotine products, such as mentholated cigarettes or electronic cigarettes.

Disorders or alterations of these senses include depressed/loss of smell (hyposmia, anosmia) and loss of taste (hypogeusia, ageusia), as well as distorted senses (parosmia) and phantom sensations (phantosmia)[11]. Alteration of the olfactory and/or taste systems has been linked to increased risk of exposure to hazards such as fire, fumes, or spoiled food[14], as well as poorer dietary quality[15] and reduced quality of life[16,17]. Olfactory dysfunction is more common than taste dysfunction, due to the redundancy of taste nerves that carry sensory information to the central nervous system, yet individuals often have trouble distinguishing the two dysfunctions[18]. Prevalence estimates of olfactory hyposmia/anosmia in population-based studies range from 3.8% to 19.1%[10,19,20]. Taste hypogeusia/ageusia prevalence estimates are much less, with one study of a clinic population reporting 0.85% have true generalized taste loss[21]. Taste changes are more likely to be localized to an area of taste nerve innervation, present as oral pain, and/or flavor changes as result of an olfactory dysfunction[22,23].

Main causes of olfactory and/or taste alteration include sinonasal conditions[17], head trauma[24], neurogenerative diseases[25], upper respiratory tract infections[23], aging[26,27], as well as certain medications[28,29]. Alteration stems from changes or damage to receptors and the inability to transmit signals to the central nervous system[30,31]. Treatments for olfactory alterations vary

with the etiology, and include surgery or oral steroid use[32-34]. However, the outcome of many treatments, as well as the long-term effectiveness, is still largely unclear[32].

Because of the limitations of treatment for olfactory and taste alteration, prevention by limiting exposure to modifiable risk factors is an important avenue to explore. One modifiable risk factor that is a potential cause of olfactory and taste alteration is cigarette smoking. Cigarette smoking has remained a serious issue in the United States for decades. In 2014, an estimated 16.8% of U.S adults were current smokers[35]. Of cigarettes sold in 2012, 31% were a mentholated brand[36] and used more frequently by younger individuals and minority groups, specifically African Americans[37]. Although the detrimental effects of cigarette and tobacco use are well known, approximately 480,000 individuals die every year from smoking, and an additional 16 million are living with a smoking related disease[38] in the U.S. Additionally, 289 billion dollars are lost in the U.S. due to cigarettes each year, including 133 billion dollars from direct medical costs and over 156 billion dollars in lost productivity[38]. Healthy People 2020 has cigarette related goals and objectives, including reducing illness and death related to tobacco and second hand smoke exposure[39].

The U.S. Surgeon General report of the 50 Year Progress on Health Consequences of Smoking shows research over the decades has linked cigarette smoking to diseases in nearly all organs of the body[38]. It is a direct cause of several cancers, including lung cancer[40], and is associated with heart disease[41], chronic obstructive pulmonary disease[42], diabetes[43], rheumatoid arthritis[44], and a weakened immune system[45]. Smokers have also reported significantly lower health-related quality of life compared to non-smokers. They are more likely to be heavy drinkers, report depressive symptoms, be less physically active, and have poorer intake of fruits and vegetables[46].

Conflicting results of cigarette smoking on olfactory/taste alterations have been found in population and community-based studies. Several studies examining olfactory function in adults via standardized measured identification tests found no association between smoking and olfactory dysfunction[19,47,48]. However, other population-based studies have found smoking to be a risk factor for olfactory dysfunction, ranging from examining this relationship in current smokers and based on heaviness of smoking[49,50]. Similarly, studies have shown smokers to have altered taste function, however the type of alteration found varies between studies. Studies have found elevated taste sensations[22] or greater taste thresholds[51,52], as well as greater oral pain[53] among smokers. Conversely, a study examining risk factors for taste alterations and increased taste thresholds did not find an association with smoking[54].

There are several potential reasons for the conflicting findings of the association between cigarette smoking and olfactory/taste alterations, which points to the central aim of the current thesis. First, the way smoking is measured between studies is inconsistent. Population-based studies range in methods to characterize smoking as only current smokers to smoking heaviness by reported number of cigarettes or pack years (packs/day * years smoked). Second, measured function and single questions about olfactory/taste function do not always capture phantom sensations, and perceived changes in function with age, which both effect chemosensory abilities and must be accounted for. The NHANES 2012 results find smoking as a protective factor for olfactory function, but characterize smoking only as reported current, former, or never. Inconsistencies throughout population-based studies may account for varied findings in the literature. Cigarette smoking may require sophisticated characterization, probing further than current/former/never status. Additionally, olfactory and taste function must be measured comprehensively, both by self-report and measured function, in order to fully understand the

risks associated with altered functioning. Finally, cigarette smoking is associated with other health behaviors and health risks, which also may combine to impact olfactory and taste function. Cigarette smoking is linked to heavier alcohol use[55], as well as more frequent sinus and throat/mouth issues[56-58]. These unique and shared risks of being a cigarette smoker must be fully examined to understand the relationship with olfactory and taste alterations.

### *1.3 Purpose*

The purpose of this research is to utilize a nationally-representative sample as well as a clinical sample of well-characterized cigarette smokers to improve the understanding of the chronic smoking effects on self-reported and measured olfactory and taste function. First, we assessed what measure of smoking in large population-based studies helped to best assess the relationship between smoking status and self-reported olfactory alteration and additional risk factors that contributed to the smoking-olfaction relationship, both directly and indirectly. Measures of smoking that captured chronic use, heaviness, and nicotine dependence were tested. Second, we aimed to assess olfactory and taste function of chronic smokers through objective measures, and assessed differences compared to non-smokers to determine variation among chronic smokers, specifically in regards to menthol status.

### *1.4 Specific Aims*

1) To use the National Health and Nutrition Examination Survey (NHANES) 2011-2014 data to assess the direct and indirect relationship between cigarette smoking and self-reported olfactory alteration, testing different measures of smoking to determine the most helpful measure/combination of measures.

2) To use NHANES to assess what other olfactory-related risk factors and health behaviors (e.g., heavy alcohol use) contribute to the relationship between smoking and self-reported olfactory alteration.
3) To use a well characterized clinical sample of cigarette smokers to assess measured olfactory and taste function and compare dysfunction rates to non-smokers and national prevalence estimates.
4) To identify if the favorite flavor of electronic cigarettes of new electronic cigarette smokers was associated with their chemosensory function or use of mentholated cigarettes.

*1.5 Hypotheses*

1) Smoking is a risk factor for self-reported olfactory alteration, but the significance of findings will be based on the measure of smoking used.
2) The most helpful measure of smoking to assess the relationship with self-reported olfactory alteration will include markers of chronic use (pack years), dependence (time to first cigarette), and biomarkers of heaviness (serum cotinine).
3) The combination of smoking and heavy drinking will increase the odds of a self-reported olfactory alteration even greater than being a smoker alone.
4) An indirect relationship will also exist between chronic smoking/smoking and drinking and self-reported olfactory alteration through known olfactory-related pathologies.
5) Measured olfactory dysfunction, along with self-reported alteration, will be greater in chronic smokers, compared to general population estimates, and vary by menthol status.

6) Measured taste function of chronic smokers will show more impairment compared to non-smokers, and vary by menthol status.
7) Electronic cigarette flavor preference will vary by menthol status or PROP taster profile.

*1.6 Significance*

The effects of cigarette smoking and taste/olfactory alteration have a significant impact on health, health care costs, and quality of life. The relationship between the two is still largely unclear due to inconsistent measures, which highlights the importance of further exploration. The significance of the research is first to provide insight into measures and characterization of smoking to understand those at highest risk for chemosensory alteration. Secondly, the findings from this thesis may provide information on additional benefits of smoking cessation as well as information on prevention of chemosensory function through avoiding chronic smoking. Finally, this research provides baseline chemosensory data of chronic smokers, allowing for examination of changes in function with smoking cessation.

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# CHAPTER TWO

## Cigarette Smoking's Association with Self-reported Olfactory Alterations: Analysis of the 2011-2014 NHANES.

### 2.1 Abstract

**Background:** Population-based studies show inconsistent effects of cigarette smoking on olfactory function. We aimed to identify direct and indirect associations between measures of smoking exposure/dependence and altered olfaction in a nationally-representative sample of adults. **Methods:** NHANES 2011-2014 (n=7,418) participants (mean age=57.8±12.2 years) self-reported olfaction and related health and demographic risks. Affirmative answers to three questions defined altered olfaction (olfactory problems in past year, worse ability since age 25, phantosmia). Smoking (never, former, current) was self-reported by chronicity (pack years, PY) and dependency (time to first cigarette upon waking), and measured by serum cotinine. Associations were tested with logistic regression, reporting odds ratios (OR) and 95% confidence intervals (CI), and by mediation models. **Results:** Estimated prevalence of altered olfaction was 22.3% and showed age-related increases. Nearly half of the sample was former/current smokers (47.4%). Controlling for olfactory-related risks, ≥10 PY smokers (current and former) had significantly greater odds of altered olfaction versus never smokers [OR 1.36, CI: 1.06-1.74]. Current smoking with ≥10 PY failed to show greater odds of altered olfaction unless they smoked within 30 minutes of waking [OR 1.41, CI: 1.01-1.99]. Light smokers (≤10 PY smokers) did not show increased odds versus never smokers. Current smokers who also were heavy drinkers (≥4 drinks/day) had greatest risk for altered olfaction (OR 1.96, CI: 1.20-3.19). Olfactory-related pathologies (sinonasal problems, serious head injury, tonsillectomy, xerostomia) partially-mediated the association between smoking and altered olfaction.

**Conclusion:** Chronic cigarette smoking was associated with increased risk of self-reported olfactory alterations, directly and indirectly via olfactory-related pathologies.

## 2.2 Introduction

According to the National Health and Nutrition Examination Survey (NHANES) 2011-2012, olfactory dysfunction is a prevalent problem, affecting nearly 13%[1] of U.S adults ≥40 years of age based on performance on odor identification and 23%[2] by self-report. Olfactory dysfunction can range from partial (hyposmia) to complete (anosmia) loss, as well as alterations perceived with age and phantom olfactory sensations[2]. Olfactory dysfunction may result from changes to the olfactory receptors, inability of odors to reach and bind these receptors, interrupted transmission of odors from the periphery to the central olfactory systems, or inability to correctly identify and label odors[3]. As shown in clinical and population-based studies[2,4-7], common causes of olfactory dysfunction include frequent sinonasal problems[8], trauma to the head or face[9], exposure to certain chemicals[10], medications[11], neurodegenerative disorders[12], and advanced age[13]. Individuals with olfactory dysfunction have greater risk of hazardous exposure related to depressed ability to detect warning signs of fire, fumes, leaking gas, or spoiled foods[14]. They can suffer from poorer dietary quality and nutritional status[15,16], as well as reduced quality of life[17]. Healthy People 2020 has added chemosensory disorder-related goals, including increasing the proportion of adults who seek diagnosis and treatment for these disorders[18]. With growing attention to olfactory function, screening, assessment and treatment options should be expanded (e.g.[19-22]).

Olfactory dysfunction could be prevented by limiting modifiable risk factors. One potential modifiable risk factor is cigarette smoking. In animal models, chronic exposure to aqueous cigarette smoke decreases functional olfactory receptor neurons[23]. However, these

findings have not been consistently generalized to large community and population based studies. Baseline results from the Epidemiology of Hearing Loss Study indicated that current smokers (relative to former and never smokers) had greater odds of olfactory dysfunction by odor identification task [4], yet the 5-year follow-up found no significant association between baseline smoking status and incidence of olfactory dysfunction[24]. A cross-sectional population-based study of 1,300 Swedish adults found no association between measured odor identification ability and cigarette smoking, whether defined as current smoking, heavy smoking, or pack years[25]. Yet a cross-sectional population based study in Spain (n=9,348) utilizing self-administered odor identification task and self-reported function, found that former or current smoking was a mild protective factor for olfactory function[5]. Conversely, a population-based study in Germany of 1,312 individuals found current smoking to be a risk factor for measured olfactory dysfunction, with a dose-response relationship between cigarettes/day smoked and frequency of impairment[26]. Similarly, a dose-response relationship was reported between chronic smoking and odor impairment in a community-based study of 638 adults [27], yet the combined effects of other olfactory-related risk factors on this association was not tested. A recent clinical study also identified greater levels of olfactory dysfunction in chronic smokers (thoroughly characterized for smoking behaviors) than that found in a nationally-representative sample of U.S. adults [28].

Long-term cigarette exposure also may have an indirect effect on olfactory function through other known risk factors for dysfunction including upper respiratory track infections[29], sinonasal problems[30,31], and dry mouth (xerostomia)[32]. Smokers may be more susceptible to developing viral respiratory colds [33]. A comprehensive review also found that smoking was associated with acute and chronic rhinitis and increased nasal inflammation[34]. Additional studies

have found smokers more likely to experience xerostomia[35, 36]. These same risk factors associated with increased risk of olfactory alteration in NHANES 2011-2012. Significant risk factors for self-reported olfactory alteration were persistent cold/flu, persistent xerostomia, frequent nasal congestion, and history of head injury as well as heavy alcohol consumption[2]. Greater alcohol consumption has been noted among smokers [37]. Excessive alcohol consumption has been linked to depressed olfactory function measured by odor identification[38] and/or odor discrimination[39,40], and dysfunction has been noted among those with Korsakoff Syndrome (a neurological complication of alcohol dependence)[41].

Using the NHANES 2011-2014 data, the goal of the present study was to examine the independent and joint effects of smoking status and other olfactory-related risk factors on self-reported olfactory alteration in a nationally-representative sample of U.S adults 40 years and older. The majority of studies to date have examined only self-reported current, never, or past smoking. Here, we examined measures of self-reported smoking exposure including chronicity (duration and amount smoked captured in pack years), as well as time to first cigarette of the day (TTFC), which serves as a proxy for nicotine dependence that links with negative health outcomes[42,43]. Additionally, we defined current smoking (at the time of the NHANES assessment) by serum cotinine, the main metabolite of nicotine, which is regarded as the best biomarker of smoking status and exposure[44]. We hypothesized that defining smoking status by chronicity, dependence and a nicotine biomarker would strengthen its association with self-reported olfactory alteration. We also hypothesized a synergistic effect of dependent smoking and heavy alcohol consumption on olfactory alteration. Finally, we hypothesized that smoking would have an indirect effect on self-reported olfactory alteration through other olfactory-related pathologies (e.g., sinonasal problems).

## 2.3 Methods:

### *2.3.1 NHANES Data Source and Participants*

The NHANES is conducted each year by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). The survey utilizes cluster, multistage sampling to randomly select households from across the United States. The sample is nationally representative of civilian, non-institutionalized residents, selected for assessment of health and nutrition via interview questionnaires, laboratory tests and physical examinations. The data collected give insight to emerging health issues, risk factors for diseases, and changes in health problems over time[45].

For this study, the continuous NHANES 2011-2012 and 2013-2014 waves were merged and adults, aged 40 years and older (n=7418) who answered questions on olfactory-related problems, cigarette smoking, and other potential risk factors, were included in the analysis. The NCHS Research Ethics Review Board approved all procedures. All participants provided written, informed consent.

### *2.3.2 Measures*

<u>Chemosensory (CSQ) Questionnaire</u>

The NHANES CSQ questions were formulated to capture perceived taste and olfactory function. The olfactory-related questions included self-reported ability, symptoms and medical treatment for dysfunction, and presence of any related risk factors for dysfunction. Affirmative responses to three questions were used to define self-reported olfactory alteration: perceived olfactory problems within the past 12 months [yes], phantom odor sensations [yes], and perceived changes in function since age 25 [worse now]. This index of self-reported olfactory

alteration has proved reliable[46] with 54.4% sensitivity and 78.1% specificity for identifying anosmia/severe hyposmia[1]. The dichotomous measure [‘yes’ or ‘no’] for self-reported olfactory alteration was the outcome variable in data analyses in the present study.

Cigarette Smoking

The smoking portion of the NHANES home interview included questions about daily cigarette use, history of use, and related details including length of time being a smoker and time to first cigarette upon waking in the morning[47]. Serum cotinine was measured as part of the laboratory procedure in the NHANES mobile examination center. The interview responses and cotinine levels were used to formulate five classes of smoking status (**Table One)**.

Smokers were classified based on an affirmative response to ever smoking 100 cigarettes in their lifetime; never-smokers answered “no.” Current smokers answered “yes” to the question “do you now smoke cigarettes” whereas former smokers answered “no”. Former smokers also reported the length of time since quitting cigarettes, which was converted into a continuous measure (years, portion of years).

Packs smoked per year (packs/day X years smoked) defined smokers as light (<10 pack years, n=1,343) or chronic (≥ 10 pack years, n=1,922) smokers. Chronic smokers were classified further as current (n=915) or former (n=1,007). Years smoked was calculated for current (interview age – age reported started smoking) and former [interview age – (age reported started smoking – reported number of years since quitting)] smokers.

Adding a proxy for nicotine dependence, smoking status was further defined by incorporating time to first cigarette of the day (TTFC). Current chronic high dependent smokers were defined as ≥10 PY and time to first cigarette (TTFC) within thirty minutes of waking (n=582). Light smokers were either <10 PY or did not smoke within thirty minutes (n=697). PY

also was combined with the available cotinine measure from the NHANES 2011-2012 subset. Typical levels among non-smokers are <1 ng/mL and those with heavier exposure (e.g., secondhand smoke) are 1-10 ng/mL[44]. In the current study we used a level of ≥10 ng/mL cotinine to define a smoker[48] and to distinguish false self-reports of non-smoking. Thus, chronic active smokers were defined as ≥10 PY smokers with cotinine levels ≥10ng/mL (n=418) and current light smokers were <10PY or had <10 ng/mL cotinine (n=422). Never/former smokers were defined as having serum cotinine <10 ng/mL. Because cotinine metabolism varies between race/ethnicity groups[49], multivariate analyses were also verified using race/ethnicity-specific cotinine exposure levels [50].

Smoking status also was defined with TTFC and available cotinine levels to compare to never/former smokers who had cotinine <10 ng/mL. High dependent active smokers reported TTFC within thirty minutes of waking and had cotinine levels ≥10ng/mL (n=297); light smokers did not smoke within thirty minutes (n=450) or had <10 ng/mL cotinine (n=450).

Alcohol Consumption

The alcohol use questionnaire was asked in the NHANES examination and probed both current and lifetime alcohol use trends. A heavy drinker was defined as reporting current or history of drinking ≥4/5 drinks on most/every day [yes/no]. This variable was examined independently as a risk factor for olfactory alteration as well as combined with high dependent smoking, defined as <30 minute TTFC. Adults were classified as either never/former smoker and heavy drinker (n=628), high dependent smoker and non-heavy drinker (n=391), or high dependent smoker-heavy drinker (n=214) to compare with neither smokers nor heavy drinkers (n=4,642).

**Table One: NHANES smoking status class definitions and comparison groups**

| Smoking status measure | Measure definition | Adults (n=) in smoker group versus comparison group |
|---|---|---|
| Chronic Smoker<br><br>(≥10 PY Smokers) | Current or former chronic smokers based on ≥10 PY (packs smoked per day * years smoked); Former/current light smokers <10 PY | Chronic smokers (915 current and 1,007 former) or light smokers (1,343 ) versus never smokers (3,942) |
| Current Chronic High Dependent Smoker<br><br>(≥10 PY, <30 minutes TTFC smokers) | Current chronic high dependent smoker based on ≥10 PY who report smoking within 30 minutes of waking (TTFC); Current light smoker based on <10 PY or >30 minute TTFC | Current chronic dependent smokers (582) or current light smokers (697) versus never/former smokers (6,058) |
| Chronic Active Smokers<br><br>(≥10 PY, ≥ 10 cotinine smokers*) | Chronic active smokers based on ≥10 PY smokers who also have cotinine levels ≥10 ng/mL; Current light smokers based on <10 PY or cotinine <10 ng/mL | Chronic active smokers (418) or current light smokers (422) versus never/former smokers (2,468 ) |
| High Dependent Active Smokers<br><br>(<30 min TTFC, ≥ 10 cotinine smokers*) | High dependent active smokers based on reporting smoking within 30 minutes of waking (TTFC) and cotinine levels ≥10 ng/mL; Current light smokers based on >30 minute TTFC or cotinine <10 ng/mL | High dependent active smokers (297) or Current light smokers (450) versus never/former smokers (2,468) |
| High Dependent Smoker-Drinker<br><br>(<30 min TTFC smoker and heavy drinker) | Dependent smokers (<30 minutes TTFC) and who report having ≥4/5 alcoholic drinks on most/every day | High dependent smokers-drinkers (214) or never/former smokers and drinkers (628) or smokers and non-drinkers (391) versus never/former smokers and non-drinkers (4,642) |

**Mean cotinine levels by smoker classification* (ng/ml)
Chronic Active Smokers: Never/former 0.13±0.73, Light 148.52±125.82, Chronic Active 265.89±138.05
High Dependent Active Smokers: Never/former 0.13±0.73, Light 193.24±133.68, Dependent Active 275.15±129.66

Olfactory-related Pathologies and Socio-demographic Risk Factors

A number of potential risk factors of olfactory alterations were assessed and examined as covariates, including socio-demographic variables and olfactory-related pathologies. Education status was dichotomized to below a high school education or high school education and above. Race was classified as Mexican American, Other Hispanic, Non-Hispanic white, Non-Hispanic black, Non-Hispanic Asian, or Other Non-Hispanic/Multi-Race. Income to poverty ratio (family income divided by federal poverty threshold) also was dichotomized as below (≤ 1) or above (>1) the poverty line. Marital status was defined as married or not (widowed, divorced,

separated, or never married). Self-rated health status was dichotomized to poor/fair health or excellent/very good/good health. A sinonasal problem was defined as report of persistent cold/flu or frequent nasal congestion in the past twelve months. Other examined risk factors included xerostomia (persistent dry mouth) during the past twelve months, history of serious head or face injury, history of tonsillectomy, and history of frequent ear infections (3 or more).

*2.3.3 Data Analysis*

The NHANES 2011-2014 data set is publicly available for secondary analysis. Because of the complex sampling design, sample weights were combined between waves and used to account for over-sampling, survey non-response, and post-stratification. Statistical analyses were completed using SAS version 9.4 (Cary, NC). All tests were two-tailed and p-values $<0.05$ were considered statistically significant.

Univariate associations between self-reported olfactory alterations and potential risk factors were assessed with chi square tests for categorical variables and two-tailed t-tests for the age continuous variables. Only chronic/dependent/active smokers, the population of interest, were compared to never/former smokers in univariate analysis. Post-hoc analysis was completed for chi-square tests when necessary using adjusted standardized residuals[51]. Potential risk factors for self-reported olfactory alteration, including all levels of smoking status, were examined in the unadjusted and adjusted logistic regression models. Odds ratios were considered significant if the confidence interval did not include the value one, and risk factors significant in the unadjusted model were included in the multivariable (adjusted) models. Separate multivariable models were tested for each smoking status class (**Table One).** Former smokers were grouped with never smokers for the analyses, except for the >10 PY (chronic) measurement, as the former group did

not have significantly greater risk of olfactory alteration compared to never smokers (results not shown). For the smoking status classes including cotinine, only the NHANES 2011-2012 wave was available. Due to unequal sample sizes of non-smokers versus chronic active/high dependent active smokers, logistic regression tests were verified using age and sex matched nonsmokers to smokers. Matching was completed using propensity scores via the MatchIt package in R (www.r-project.org).

Mediation models were completed in SPSS using the PROCESS macro. Two models were examined based on the smoking variables that explained the greatest odds of self-reported olfactory alteration in multivariable analysis (current chronic high dependent smokers, high dependent smoker-drinkers). Several other models and directionality of variables were tested with the displayed models best capturing the data and relationships. The mediator variable was an olfactory risk score comprised of the significant risk factors for olfactory alteration in the multivariable analysis, scored from zero to five based on "yes" responses to frequent nasal congestion, persistent cold/flu, xerostomia, tonsillectomy, or history of a serious head/face injury. The first model tested whether the olfactory risk score (m) mediated the association between current chronic high dependent smoking (x) and olfactory alteration (y). The second model tested whether the olfactory risk score (m) mediated the association between high dependent smoker-drinkers (x) and olfactory alteration (y). In the mediation models, the *a* paths represent the relationship between x and m, and the *b* paths represent the relationship m and y. The product of path *a* and *b* represents the indirect effect of chronic dependent smoking or dependent smoking-drinking on olfactory alteration; the *c'* path represents the direct effect on olfactory alteration. Path *c*, or the total effect, equals the direct plus the indirect effects. Equations between all variables were tested prior to running mediation modeling to test for

expected bivariate relationships between x and y, between x and m, and between m and y, controlling for x. Beta estimates, standard errors, and 95% confidence intervals were used through bootstrapping procedure using 5,000 resampling to estimate the mediation relationships. Relationships were considered significant if the confidence intervals did not include the value zero. The ratio of the indirect effect to the total effect was used to quantify the proportion mediated. Cases containing missing data were excluded in the analysis and covariates included in both models were age, sex, race and income-to-poverty ratio.

**2.4 Results**

Of the sample, 52.3% were never smokers, and 47.4% were former/current smokers. **Table Two** provides demographic characteristics of the total sample and by smoking status class. The mean age of the total sample was 57.8 ± 12.2 years and 47.2% were males. Most adults reported living above the poverty line and completing high school or above. Smokers, defined by all five classes, were more frequently male, non-Hispanic White, had lower education level, lived below the poverty line, and were heavy drinkers.

Of the total sample, 22.3% (n=1609) reported an olfactory alteration. Of those with an alteration, 32.4% reported loss since age 25 and 26.2% reported both a problem in the past year and loss since age 25. Among those who reported olfactory alterations, 6.5% reported all three olfactory-related problems (problems in past year, loss with aging, phantosmia) whereas 20.4% reported phantom sensations only (without reporting loss with age or smell problems). These results are comparable to prevalence estimates in the NHANES 2011-2012[2].

**Table Two: Demographic characteristics of participants in the NHANES 2011-2014 sample and stratified by smoking status class**

| | Entire NHANES sample | Current and Former Chronic Smokers | Current Chronic High Dependent Smokers | Chronic Active Smokers | High Dependent Active Smokers | High Dependent Smoker-Drinkers |
|---|---|---|---|---|---|---|
| **Number of Participants** | 7418 | 1922 | 582 | 418 | 297 | 214 |
| **Gender (%)** | | | | | | |
| *Male* | 47.2 | 56.6 | 53.7 | 58.4 | 54.5 | 70.6 |
| *Female* | 52.8 | 43.4 | 46.4 | 41.6 | 45.5 | 29.4 |
| **Age** (years) | 57.8 ± 12.2 | 59.4 ± 11.6 | 59.8 ± 9.6 | 54.5 ± 10.5 | 54.0 ± 10.2 | 53.2 ± 8.8 |
| **Race (%)** | | | | | | |
| *Mexican American* | 6.2 | 3.1 | 1.1 | 1.9 | 2.4 | 1.8 |
| *Other Hispanic* | 4.9 | 3.0 | 2.7 | 2.5 | 2.2 | 1.9 |
| *Non-Hispanic white* | 71.2 | 79.9 | 80.5 | 79.1 | 76.5 | 81.7 |
| *Non-Hispanic black* | 10.7 | 9.0 | 9.5 | 9.6 | 11.3 | 10.3 |
| *Non-Hispanic Asian* | 4.8 | 2.0 | 1.1 | 1.0 | 1.1 | <1 |
| *Other/Multi-Race* | 2.2 | 3.0 | 5.1 | 5.8 | 6.5 | 4.0 |
| **Education (%)** | | | | | | |
| *< High school* | 17.1 | 21.2 | 27.5 | 24.6 | 26.6 | 32.7 |
| *≥High school* | 82.9 | 78.8 | 72.5 | 75.4 | 73.4 | 67.3 |
| **Income to Poverty Ratio** (% ≤1) | 12.9 | 15.6 | 25.5 | 17.9 | 23.1 | 30.8 |
| **Marital Status** | | | | | | |
| *Married (%)* | 63.2 | 56.6 | 49.9 | 51.4 | 46.9 | 48.3 |
| **Heavy Drinkers (%)** | 15.5 | 30.5 | 37.4 | 33.6 | 32.7 | 100 |

*2.4.1 Risk factors associated with olfactory alteration examined by univariate analysis*

**Table Three** reports distribution of olfactory alteration by separate potential risk factors, including the five smoking status classes defined above. There was no significant difference in prevalence of olfactory alteration between males and females, however adults 80 years and older most frequently reported olfactory alteration. Post-hoc testing showed that a significantly higher

proportion of Non-Hispanic White and Other Non-Hispanic/Multi-Race reported olfactory alteration while fewer Non-Hispanic Black and Asians reported alteration. Adults who were not married, lived below the poverty line, had self rated fair/poor health, or were heavy drinkers had significantly greater reported frequency of olfactory alteration. Additionally, greater frequency of olfactory alteration was reported by those with history of serious head/face injury, tonsillectomy, ear infections, persistent cold/flu, dry mouth, and frequent nasal congestion. These significant risk factors are consistent with the NHANES 2011-2012 analysis[2]. Among the five classes of smokers, a greater frequency of the smokers reported an olfactory alteration compared to never/former smokers. Current chronic high dependent smokers reported olfactory alterations most frequently at 32.8%.

The unadjusted odds ratios and 95% CI for these risk factors also were examined prior to multivariable analysis to determine significant factors to be included in final adjusted models (see **Supplemental Materials**). Significant risk factors included age, not being married, income to poverty ratio ≤1, sinonasal problems, xerostomia, head/face injury, tonsillectomy, multiple ear infections, self-rated fair/poor health, and smoking (not light smoking) defined by all five classes in **Table One**.

**Table Three: Distribution of self-reported olfactory alteration by each potential risk factor**

| | **Total Sample** | **Reported Olfactory Alteration** | **% of Self-reported Olfactory Alteration** | | |
|---|---|---|---|---|---|
| | N | N | % Yes | % No | Test Statistic and P-value |
| **Age, years (mean)** | 57.8 ±12.2 | | 58.6 ±12.4 | 57.6 ± 12.2 | T=2.13, 0.04 |
| **Age strata** | | | | | $x^2$=16.10, 0.03 |
| *40-49 years* | 1,934 | 378 | 20.4 | 79.6 | |
| *50-59 years* | 1,852 | 402 | 22.8 | 77.2 | |
| *60-69 years* | 1,848 | 406 | 22.3 | 77.7 | |

| | | | | | |
|---|---|---|---|---|---|
| *70-79 years* | 1,069 | 216 | 22.1 | 77.9 | |
| *80+ years* | 715 | 207 | 28.3 | 71.7 | |
| **Sex** | | | | | $x^2$= 0.28, 0.87 |
| *Male* | 3,556 | 747 | 22.4 | 77.6 | |
| *Female* | 3,862 | 862 | 22.2 | 77.8 | |
| **Race/Ethnicity** | | | | | $x^2$=30.52, 0.001 |
| *Mexican-American* | 803 | 172 | 21.3 | 78.7 | |
| *Other Hispanic* | 737 | 162 | 21.9 | 78.1 | |
| *Non-Hispanic black* | 1,777 | 351 | 19.4 | 80.6 | |
| *Non-Hispanic white* | 3,047 | 761 | 23.2 | 76.8 | |
| *Non-Hispanic Asian* | 894 | 118 | 13.1 | 86.9 | |
| *Other/Multi-Race* | 160 | 45 | 30.9 | 69.1 | |
| **Marital Status** | | | | | $x^2$=10.32, 0.001 |
| *Married* | 4,301 | 844 | 21.0 | 79.0 | |
| *Not Married* | 3,108 | 762 | 24.3 | 75.7 | |
| **Education** | | | | | $x^2$=2.58, 0.23 |
| *< High school* | 1,925 | 433 | 24.0 | 76.0 | |
| *≥High school* | 5,484 | 1,176 | 22.0 | 78.0 | |
| **Income-to-poverty ratio** | | | | | $x^2$=16.71, 0.0001 |
| *IPR ≤1 (poverty)* | 1,426 | 377 | 27.9 | 72.1 | |
| *IPR > 1* | 5,322 | 1,112 | 21.7 | 78.3 | |
| **Self-rated health** | | | | | $x^2$=50.20, 0.0001 |
| *Fair or poor* | 1,793 | 514 | 30.0 | 70.0 | |
| *Excellent, v. good, good* | 4,704 | 924 | 20.8 | 79.2 | |
| ***Heavy Alcohol Use*** | | | | | $x^2$=55.91, <0.0001 |
| *Yes* | 986 | 281 | 31.8 | 68.2 | |
| *No* | 5,464 | 1,142 | 21.0 | 79.0 | |
| **Current & Former Smokers** | | | | | |
| *Chronic Smokers* | 1,922 | 544 | 29.3 | 70.7 | $x^2$=77.82, <0.001 |
| *Never-smokers* | 3,942 | 725 | 19.1 | 80.9 | |
| **Current Smokers** | | | | | |
| *Chronic High Dependent Smokers* | 582 | 181 | 32.8 | 67.2 | $x^2$=44.96, <0.001 |
| *Never/former smokers* | 6,058 | 1,243 | 21.2 | 78.8 | |
| *Chronic Active Smokers* | 418 | 134 | 30.6 | 69.4 | $x^2$=15.98, 0.048 |
| *Never/former smoker* | 2,468 | 542 | 22.0 | 78.0 | |

| | | | | | |
|---|---|---|---|---|---|
| *High Dependent Active Smokers*<br>*Never/former smoker* | 297<br>2,468 | 97<br>542 | 31.3<br>22.0 | 68.7<br>78.0 | $x^2$=13.99,<br>0.04 |
| *High Dependent Smoker-drinkers*<br>*Never/former smoker, non-drinker* | 214<br>4,642 | 70<br>936 | 37.6<br>20.5 | 62.4<br>79.5 | $x^2$=37.37,<br><0.0001 |
| **Olfactory-related risk factors**<br>**"Yes," have ever had…** | | | | | |
| *Serious head/face injury* | 1,573 | 465 | 28.2 | 71.8 | $x^2$=53.26,<br>0.0001 |
| *Ear infections, 3+ times* | 1,397 | 423 | 27.8 | 72.2 | $x^2$=44.67,<br>0.0001 |
| *Tonsils removed* | 1,853 | 476 | 26.0 | 74.0 | $x^2$=28.23,<br>0.001 |
| **"Yes," in last 12 months** | | | | | |
| *Cold/flu for >1 month* | 478 | 182 | 38.8 | 61.2 | $x^2$=76.22,<br>0.0001 |
| *Persistent dry mouth* | 1,115 | 424 | 37.5 | 62.5 | $x^2$=149.82,<br>0.0001 |
| *Frequent nasal congestion* | 2,055 | 671 | 32.2 | 67.8 | $x^2$=168.09,<br>0.0001 |

*2.4.2 Examination of smoking status as a risk factor for olfactory alteration by multivariable analysis*

The adjusted models were tested with each class of smoker. Significant independent risk factors of olfactory alteration in all models include sinonasal problems and xerostomia. The significance of other risk factors (history of a serious head/face injury, tonsillectomy, poor self-rated health, heavy alcohol use, poverty) varied between the adjusted models. In former smokers, a greater number of years since quitting smoking was not associated with lower odds of an olfactory alteration when controlling for age and sex (OR: 1.00; CI: 0.99-1.01).

In fully adjusted models, smoking status measured by PY alone and TTFC with PY remained significant risk factors for olfactory alteration (**Figure One: Model A-D).** Model A found that chronic smokers (former and current) versus never-smokers were at significantly greater odds of olfactory alteration (1.36, CI: 1.06-1.74). However, when former and current chronic smokers were examined separately (Model B) in the adjusted model, only former chronic

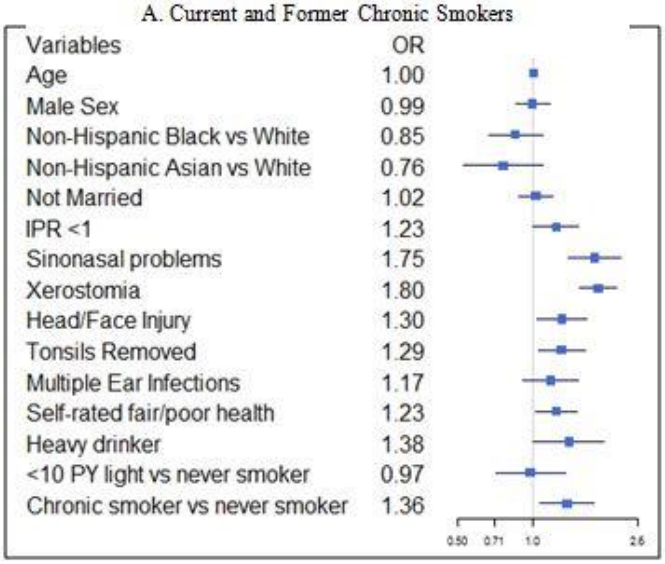
smokers remained at significantly greater odds (1.42, CI: 1.09-1.84) and current chronic smokers did not (1.29, CI: 0.93-1.80). TTFC <30 minutes was not a significant risk factor alone (1.30, CI: 0.94-1.79), but current chronic high dependent smokers were at significantly greater odds of an olfactory alteration versus never/former smokers (1.41, CI: 1.01-1.99) (Model C). No significant difference in odds of an alteration was seen between light smokers and never/former smokers in any model.

The cotinine biomarker as a measure of current smoking status did not add predictive ability. There was non-significant greater odds that chronic active smokers [1.22, CI: 0.72-2.05] or high dependent active smokers [1.22, CI: 0.77-1.93] had olfactory alteration versus never/former smokers. Chronic active smokers and high dependent active smokers compared to age and sex matched never/former smokers were also not at increased odds of an olfactory alteration. Furthermore, cotinine alone as a continuous measure of smoking, or by race specific cut-off points, was not a significant risk factor for olfactory alteration.

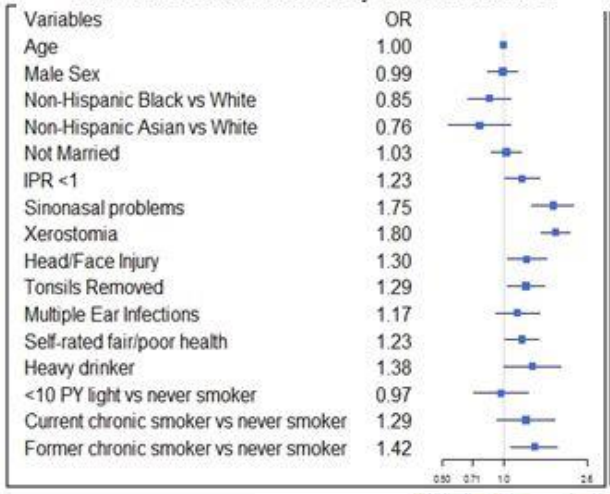
High dependent smoker-drinkers were at the greatest risk for olfactory alteration than any of the smoking measures alone (1.96, CI: 1.20-3.19) (Model D). Being a dependent smoker and non-heavy drinker or non-smoker and heavy drinker were not significant risk factors. All smoking variables were tested with heavy alcohol use; TTFC with heavy alcohol produced the highest odds ratios with olfactory alteration.

**Figure One: Forest plots of adjusted odds ratios and 95% confidence intervals of risk factors associated with self-reported olfactory alteration in U.S adults in models examining A) Current and former chronic smokers B) Chronic smokers stratified by current and former C) Current chronic high dependent smokers D) High dependent smokers-drinkers**

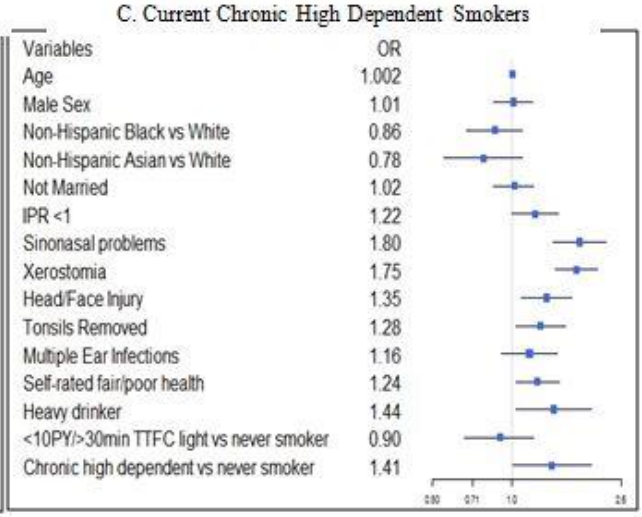
A. Current and Former Chronic Smokers



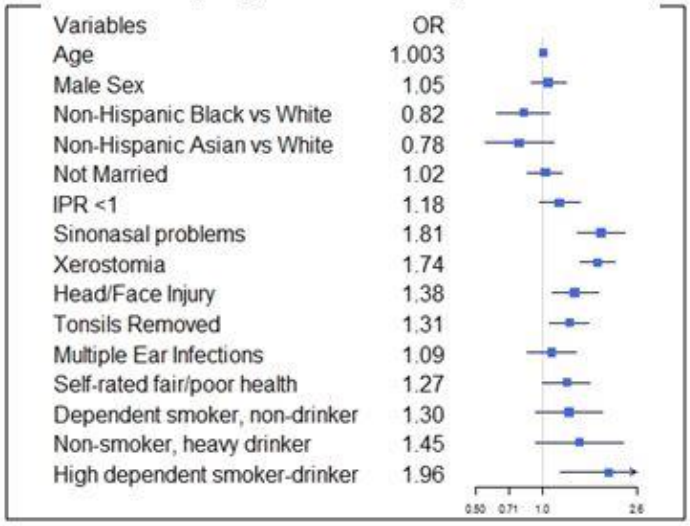

B. Chronic Smokers stratified by Current and Former




C. Current Chronic High Dependent Smokers




D. High Dependent Smoker-Heavy Drinkers



*2.4.3 Examination of the indirect relationship with olfactory alteration through mediation modeling*

**Table Four** and **Figure Two** display the two mediation models, beta estimates with 95% confidence intervals of pathways, indirect, and direct effects when controlling for age, gender, race/ethnicity, and poverty. The first model displays the indirect relationship between current chronic high dependent smokers with olfactory alteration through olfactory risk score. All of the beta estimates (path *a* and *b*) were positive, suggesting that with moving from never/former smokers to chronic high dependent smokers, there was greater number of olfactory risk factors, which resulted in greater risk of olfactory alteration. Both the direct and indirect effects were significant (0.3786, CI .1707-.5865; 0.1043, CI: .0611-.1538) indicating partial mediation. Of the relationship between smoking and olfactory alteration, 21.6% was mediated via olfactory risk factor score.

The second model showed similar, but more significant results. Moving from a never/former smoker and non-drinker to a high dependent smoker-drinker was associated with greater olfactory risk factor score, which was associated with greater risk of olfactory alteration. The direct (0.3651, CI: .0373-.6929) and indirect (0.1884, CI: .1155-.2767) effects were both significant. Of the relationship between smoking-drinking and olfactory alterations, 34.0% was mediated via olfactory risk factor score.

**Table Four: Beta estimates, standard error, and 95% confidence intervals of the mediation relationship of smoking/smoking and heavy drinking relationship with self-reported olfactory alteration**

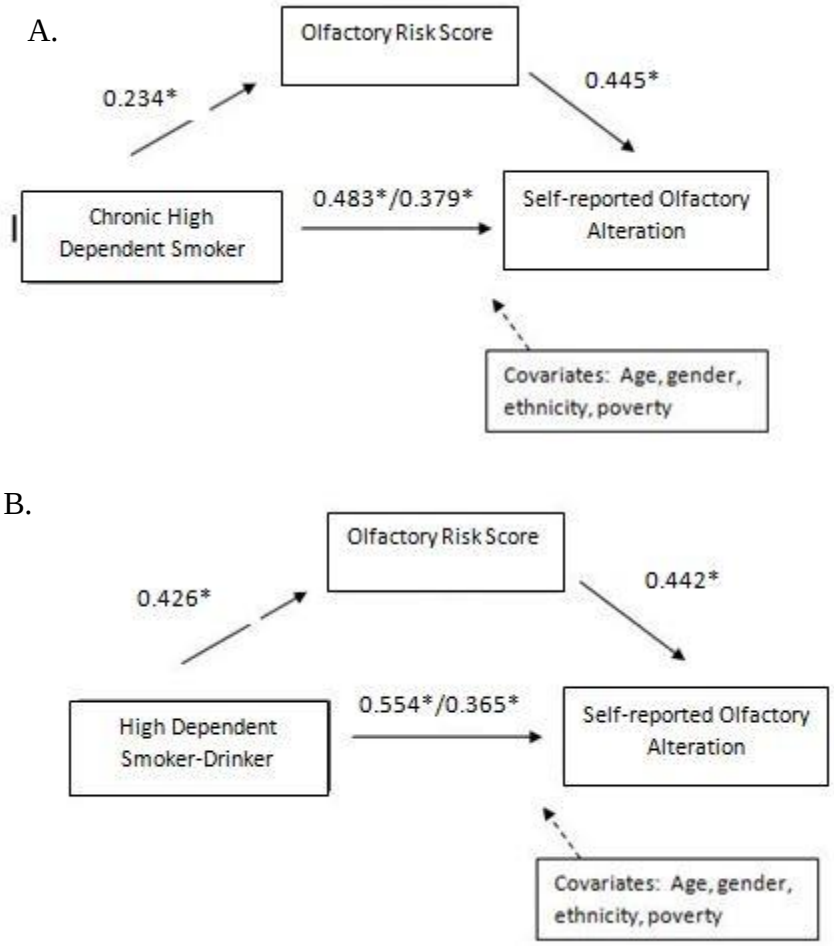
| Relationships | Estimate | Standard Error | CI |
|---|---|---|---|
| Chronic high dependent smoking→olfactory risk score (a) | 0.234 | 0.045 | 0.147-0.322 |
| Olfactory risk score→olfactory alteration (b) | 0.445 | 0.031 | 0.384-0.507 |
| Chronic high dependent smoking→ olfactory alteration (c') | 0.379 | 0.106 | 0.171-0.587 |
| Indirect Effect | 0.104 | 0.024 | 0.061-0.154 |

†Covariates controlled for include age, gender, race/ethnicity, and poverty

| Relationships | Estimate | Standard Error | CI |
|---|---|---|---|
| High dependent smoker-drinker→olfactory risk score (a) | 0.426 | 0.069 | 0.290-0.562 |
| Olfactory risk score→olfactory alteration (b) | 0.442 | 0.038 | 0.367-0.517 |
| High dependent smoker-drinker→ olfactory alteration (c') | 0.365 | 0.167 | 0.037-0.693 |
| Indirect Effect | 0.188 | 0.040 | 0.116-0.277 |

†Covariates controlled for include age, gender, race/ethnicity, and poverty

**Figure Two: Models of the association between (A) chronic high dependent smoking or (B) high dependent smoking-drinking and self-reported olfactory alterations mediated by olfactory risk score in data from the National Health and Nutrition Examination Survey 2012-2014.**




## 2.5 Discussion

With the addition of the Healthy People 2020 chemosensory goals and the data from the new chemosensory protocol in the NHANES 2011-2104, alterations in the sense of smell are

gaining more attention as a prevalent health problem. Analysis of the 2011-2012 and 2013-2014 waves revealed an estimated prevalence of altered olfaction at 22.3% across U.S adults ages ≥40 years, including problems in the past year, loss since age 25, and/or phantom olfactory sensations. This prevalence is nearly equivalent to the initial estimates from 2011-2012 NHANES (23%) [2]. Compared with never/former smokers, a significantly greater frequency of smokers reported an olfactory alteration, ranging from 29.3-32.9% depending on how smoking was characterized. High dependent smokers who also were heavy alcohol drinkers had the highest frequency of self-reported olfactory alteration at 37.6%. The association between chronic high dependent smoking or dependent smoking-drinking and olfactory alteration was partially mediated (21.6-34%) by an olfactory risk score, comprised of pathologies associated with olfactory dysfunction (frequent nasal congestion, persistent cold/flu, presence of xerostomia, tonsillectomy, or history of a serious head/face injury).

The estimated prevalence of self-reported olfactory alteration is almost double that of measured olfactory dysfunction at 12.4%[1] from the initial analysis of the 2012 NHANES. The self-report measure captures perceived changes in function with aging as well as phantosmia, neither of which are captured with a single measurement of olfactory function[52]. In contrast, two previous population-based studies reported greater measured olfactory dysfunction at 19-24.5% among adults than that with a single question about self-reported olfactory problems at 9.5-15%[4,25]. Based on previous analysis, all three questions used in the olfactory alteration index contributed to the prevalence estimated and showed a sensitivity of 54.4% (correctly identifying dysfunction) and a specificity of 78.1% (correctly identifying normal function) when compared with measured anosmia/severe hyposmia[1]. The NHANES olfactory alteration index also associated significantly with previously identified risk factors for olfactory dysfunction[2],

including sinonasal problems[8,24], xerostomia[53], history of a serious head/face injury[9], tonsillectomy[54], and poverty[55]. This self-report measure may be more beneficial than measured olfaction for the current study, as we were examining chronic cigarette use, and not just current use. The perceived changes in olfactory function over time are therefore important to capture, and measured testing does not have the ability to pick this up, or the ability to detect phantom sensation, both which contribute to olfactory issues. As previously[2], we did not observe that males self-reported higher rates of altered olfaction, in contrast to gender differences observed with measured function in NHANES 2012 (males 3 times more likely to have severe hyposmia/anosmia than females[1]), or other population-based studies [5,25].

Interestingly, in the recent analysis of the NHANES 2011-2012 olfactory data, cigarette smoking appeared as a protective factor for measured olfactory function[1] and had a non-significant effect on self-reported olfactory function[2]. However, in the current analysis, cigarette smoking was a risk factor for self-reported olfactory alteration, when characterized by chronicity (≥10 PY), dependency (time to first cigarette <30 minutes) and when combined with heavy drinking. The chronicity of smoking measured in pack years (≥10 PY) significantly increased odds of self-reported olfactory alteration in former and current smokers when examined together, but not in current chronic smokers alone. In current smokers, those who were both chronic and dependent smokers were at an increased risk for olfactory alteration versus never/former smokers. Pack years or TTFC alone as a measure of current smoking did not associate with an increased risk of olfactory alterations, and these measurements had to be looked at together to see increased odds.

To our knowledge, only four other studies have used pack years as a measure of smoking in examining the association with olfactory function. One study found no association with

olfactory dysfunction when examining pack years in former and current smokers, or heavy use (>20 cigarettes/day) among current smokers[25]. However the other three studies found a dose-related response with pack years in current smokers, who had increased olfactory thresholds[56], as well as a worse odor discrimination and odor identification[27,57]. These previous studies, however, did not control for as many other known risk factors in multivariable analysis as done in the present study (e.g., sinonasal issues, head trauma, xerostomia). None of the existing studies have used time to first cigarette as a measure of smoking to examine olfactory alterations. This measure has been used to examine numerous other outcomes, such as quitting success[58], hypertension[59] and COPD or pulmonary impairment[43,60]. TTFC corresponds well with biomarkers of smoking, such as nicotine, cotinine, and hydroxycotinine concentrations[61] and is a fast and inexpensive measure of nicotine dependence. The results from the present study suggest that it is important to characterize smoking thoroughly, capturing chronicity (PY) and dependency (TTFC), to accurately assess risk for olfactory alteration.

We did not find an association with years since quitting smoking and improvement in olfaction in former smokers, as observed by Frey et al [27]. One potential reason for the lack of findings in the present study could be the older age of our sample. Frey et al examined participants aged 17 to 69 (mean age 42.9 years), compared to the present study's mean age of 57.8 years. Advanced age is known to be associated with decreased smell function[13]. An increase in years since quitting is also accompanied by an increase in age, which may counter the positive effects of quitting on olfactory function, resulting in an insignificant finding. In addition, the Frey et al study did not control for other demographic and pathology-related risks for olfactory dysfunction.

Although TTFC measure has been shown to associate with cotinine levels, using serum cotinine as a measure of smoking status did not strengthen its association with self-reported olfactory alteration in this study. Cotinine was verified by race-specific cut off points, however there is still not a universal cut-off point verified to distinguish smokers and non-smokers, or heaviness of smoking. A systematic review examining 67 studies that measured self-reported smoking status and serum cotinine levels found that cut-off points used ranged from 8 ng/mL to 100 ng/mL[62]. Along with race, gene expression and medications competing with binding substrates[63], as well as gender[64] may all play a role in cotinine metabolism, and therefore cause variations person to person. Additionally, although cotinine has a longer half-life than nicotine, it remains in the system for only 15-20 hours[65], which still may not capture the chronic, dependent smokers who seem to be most at risk for olfactory alteration. The cotinine measure is invasive and expensive, and, according to this analysis, appears less predictive of risk for olfactory alteration than the self-reported cigarette smoking behaviors.

The combination of smoking and heavy drinking showed a synergistic effect. Being a dependent smoker and not a heavy drinker, or vice versa, was not a significant risk factor for altered olfaction in the adjusted logistic regression model, but the joint effect of the two associated with the highest risk. Although smokers tend to be heavy drinkers[37], their joint effect has not been examined previously as a risk factor for olfactory alteration. Instead, studies use smoking as a covariate when examining the association with alcohol use[40,41]. The present findings that the association between smoking and olfactory alteration depends on the smoking/heavy drinking relationship, chronicity of smoking, and/or dependency may partly explain the inconsistent reports in the literature.

The findings of the present study suggest that chronic dependent smoking alone, or in combination with heavy drinking, may increase risk of olfactory alteration through pathologies associated with olfactory dysfunction. That is, 22 and 34% of the association between smoking alone or with heavy drinking was explained by an olfactory risk score comprised of five pathologies (frequent nasal congestion, persistent cold/flu, presence of xerostomia, tonsillectomy, or history of a serious head/face injury). The direct effect of chronic dependent smoking as well as smoking and heavy drinking on olfactory alterations was also significant, indicating these behaviors also likely have a direct effect on olfactory alteration. Although the relationship between smoking, olfactory alterations, and these other olfactory pathologies have been examined independently, no study to our knowledge has examined this complex relationship simultaneously. Mediation modeling allows for testing of all variables and their relationships together, displaying the complex associations between all. This provides additional information that logistic regressions cannot, as regressions look at relationships separately, not as a whole[66].

Although this study utilized a nationally-representative sample of U.S adults, there are still limitations to acknowledge, including the cross-sectional design. The present study examined a variety of risk factors for olfactory alteration, however a few other risk factors were not considered such as physical activity levels and BMI. Additionally, measures of smoking that included cotinine levels were limited in sample size as they were only available in the NHANES 2011-2012 wave and only self-reported olfactory alteration was examined. However, smoking status was measured using multiple indicators of heavy use and dependence through self-reported and measured data. Future research should utilize the shown appropriate measures of smoking to accurately capture who is at risk for olfactory alteration, including the combination

of pack years and time to first cigarette in current smokers and pack years in former smokers. In addition, the relationship between smoking and olfactory function can be examined utilizing the new measured protocol in NHANES 2013-2014[1]. Finally, other complex mediation relationships and combined lifestyle factors (e.g., heavy drinking) should be examined to fully understand the relationship of cigarette smoking on olfactory function.

## 2.6 Conclusion

In conclusion, this nationally-representative study found significant evidence that chronic dependent cigarette smoking alone or with heavy alcohol consumption was associated with increased odds of olfactory alteration, a composite index capturing self-reported problems during the past year, losses noticed with age, and phantom olfactory sensation. Some of the risk of olfactory alteration in smokers or smokers/heavy drinking was direct and some was explained by an increased frequency of pathologies associated with olfactory dysfunction (frequent nasal congestion, persistent cold/flu, presence of xerostomia, tonsillectomy, or history of a serious head/face injury). The smoking effects on risk of altered olfaction were uncovered by characterizing smoking by chronicity as well as level of dependence.

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# CHAPTER THREE

## Heightened olfactory dysfunction and oral irritation among chronic smokers and heightened propylthiouracil (PROP) bitterness among menthol smokers

### 3.1 Abstract

Chronic cigarette smoking can influence chemosensory function, which in turn affects the palatability of tobacco products and cessation efforts. We examined chemosensory function of chronic smokers in comparison to a national sample for olfaction (National Health and Nutrition Examination Survey, NHANES) and to a sample of non-smokers for taste. Chemosensation also was evaluated as a function of menthol versus non-menthol cigarette use. We expected that chronic smokers would display altered chemosensory function that would vary by menthol use. **Methods:** Chronic smokers (N=78; 49 menthol smokers) self-reported their chemosensory function (per NHANES protocol) and participated in measures of smell (16-item olfactometer identification task) and taste (quinine and NaCl intensities, NHANES protocol) function, including a taste genetic probe (bitterness of propylthiouracil, PROP). Self-reported and measured olfactory function was compared with 2011-2012 NHANES data, and taste function was compared with age- and sex-matched non-smokers (n=311). **Results:** Olfactory alterations were reported by 25% of smokers, similar to NHANES prevalence. However, measured dysfunction (22% mild, 35% moderate, 3.8% severe microsmia and 1% anosmia) exceeded that reported in NHANES, but did not differ by menthol cigarette use. Taste alteration, including altered flavor, was reported by 15% of smokers. In comparison with non-smokers, smokers reported elevated intensities from NaCl at oral-irritant concentrations. Consistent with previous *TAS2R38* taste receptor gene findings, menthol smokers reported greater bitterness from PROP

than non-menthol smokers. These findings have implications for regulation of flavorings in tobacco. As with menthol cigarette preference among smokers with genetic propensity for elevated bitter perception, other flavors may also help drive smoking in chronic smokers with altered chemosensory function.

### 3.2 Introduction

Although smoking prevalence has been declining over the past years, in 2014 over 40 million adults (16.8%) in the United States were current smokers[1]. Of all cigarettes sold in 2012, 31% were a mentholated brand[2] and used more frequently by younger individuals and minority groups, specifically African Americans[3]. Makers of tobacco-based products commonly use flavor additives to mask bitterness, irritation and other unpleasant sensations from tobacco and to produce a smooth, mellow tobacco flavor. In particular, menthol has been used to produce a smoother, cooler smoke. Genetic variation in sensitivity to flavors and taste may influence smoking choices and behaviors. Thus, we sought in the present paper to investigate olfactory and taste function among adults who are current chronic smokers, and compare chemosensory function between menthol vs. non-menthol smokers.

In population-based samples, the rates of olfactory dysfunction range from 3.8%[4,5] in the Beaver Dam Offspring Study, to 12.4% for the National Health and Nutrition Examination Survey (NHANES)[6], and l9.1% for the Skövde population-based study[7]. These rates vary with demographic factors, with increased rates in older adults[6,8,9], males[9-14], certain ethnic/racial minorities[6,9] and those with lower income/educational attainment[5,6]. Common causes of olfactory alterations include frequent nasal infections, allergies[15], injury to the head or face[16] [16], and viral infections/tonsillectomy[6]. Studies show that individuals are able to self-report

having a normal sense of smell (i.e., specificity) but are poor at self-detecting olfactory dysfunction (i.e., sensitivity)[17,18]. However, through asking a series of questions about recent smell function, loss of sense of smell with aging, and phantom sensations (i.e., phantosmia), we have been able to achieve reasonable sensitivity in tests of self-identified olfactory dysfunction[19].

Research on the association between smoking and risk of olfactory dysfunction has been inconsistent. Several studies report no association between olfactory function and smoking when examining both measured and self-reported function in current compared to former or never-smokers[7,19-21]. Other studies show an elevated risk of olfactory dysfunction among current smokers[8] and/or heavy smokers[8,22]. Recent analysis of the NHANES 2012 data, on the other hand, suggested decreased risk of olfactory dysfunction among current and past smokers, yet found an elevated risk of olfactory dysfunction among heavy drinkers[6].

Compared with olfactory dysfunction, taste dysfunction is less common[23], due to the redundancy of the three taste-related nerves that carry the sensory information from the periphery to the central nervous system. More common is regional taste dysfunction from an area of taste nerve innervations, which in turn alters whole mouth taste perception, touch/tactile and pain sensations[24]. More severe taste-related exposure could depress whole mouth taste function. Major causes of taste dysfunction include xerostomia (persistent dry mouth), facial injuries, upper respiratory and middle ear infections, surgeries to the ear, nose or throat, and aging[24]. Since individuals are usually unable to perceptually distinguish taste and retronasal olfactory sensations during ingestion, NHANES protocol queried on the ability to taste salt, sweet, sour and bitter, along with questions on changes in food flavor, and persistent tastes in the mouth (i.e., dysgeusia), which was combined into a taste alteration index[19]. The prevalence of

reported taste alteration (recent problems, loss in taste or flavor with age, dysgeusia) in adults 40 years and older was 19%[19].

There are natural variations in the ability to taste, specifically, the ability to taste PTC/PROP bitterness relates to polymorphisms in the *TAS2R38* receptor gene[25,26]. Given these taste variations, the question of interest was to determine if heightened ability to taste might be a sensory hindrance to smoking (or to smoking unflavored cigarettes), and if chronic smoking alters taste and oral sensations. Although nicotine stimulates complex taste, olfactory and somatosensory sensations[27], variation in bitter taste has been linked to differences in cigarette smoking behaviors. Nicotine stimulates bitter taste through TRPM5-dependent and independent mechanisms[28]. Fischer[29] in the 1960's reported that smokers are less sensitive to bitter taste. In recent studies of bitter taste in humans, specifically bitterness of phenylthiocarbamide (PTC) and propylthiouracil (PROP), nontasters are more likely to be smokers[30], show greater cigarette dependence[31], and less nicotine aversion[32]. Additionally, PTC/PROP nontasters by *TAS2R38* genotype show more motivation to smoke based on sensory cues than do tasters[33]. It should be noted, however, that the *TAS2R38* genotype-smoking effects are not always seen[34], and the effects may be race/ethnicity specific[35]. Menthol in cigarettes may help smokers block the negative oral sensations from cigarettes[36,37]. Females who are PTC/PROP tasters by *TAS238* receptor genotype are more likely to smoke menthol cigarettes[38].

Smoking itself may impair bitter taste perception or alter oral sensations, and smoking cessation may lead to improvements in bitter taste ability[32]. Additionally, taste perception plays a key role in flavor preference[39] and differences among smokers may influence flavor choice among nicotine products, such as mentholated cigarettes or electronic cigarettes. Long-term exposure to nicotine in rats decreased fungiform papillae size and changed their anatomical

characteristics[40], which could explain lower density of tongue-tip taste papillae (fungiform papillae) among chronic smokers[41]. Most studies report altered oral sensations among smokers, however the type of alteration varies. Studies have found elevated taste[42] and touch[43] sensations, elevated taste[44] or taste/touch thresholds[45], and greater risk of oral pain[46] among smokers. One study found no smoker effects on taste threshold[47].

Due to the complexity of smoking's potential effects on chemosensory functioning, the aim of this study was to compare olfactory and taste function in chronic smokers with the olfactory functioning of a national sample from the National Health and Nutrition Examination Survey and the taste functioning of age- and sex-matched non-smokers. Additionally, we were particularly interested in the olfactory and taste function of menthol and non-menthol smokers. Finally, because this was a baseline examination of an electronic cigarette study, we aimed to identify if the flavor electronic cigarette smokers identified as their most preferred associated with their chemosensory functioning, or menthol status. It was hypothesized that chronic smokers would display altered chemosensory function, which would vary by menthol use and that electronic cigarette flavor preference would vary by menthol status or PROP taster profile.

## 3.3 Methods

### *3.3.1 Study Design and Participants*

The analysis sample of 79 adults (42 males) was obtained from the baseline data of a study on the effects of nicotine and flavorings on use of electronic cigarettes in regular smokers. Recruited into this study were adults in the greater Hartford, CT area, ages 18 to 55 years, who responded to newspaper and radio advertisements between May 2015 and March 2016. A telephone screening protocol determined if the potential participants met the exclusion and

inclusion criteria for initial eligibility. The criteria for exclusion were: 1) unstable medical or psychiatric disorders, including uncontrolled hypertension (BP>160/100); 2) pregnancy; 3) known hypersensitivity to nicotine or to propylene glycol; 4) previous M.I. or stroke; 5) insulin dependent diabetes; 7) and known COPD or asthma. The criteria for inclusion included 1) current use of at least 10 cigarettes daily; 2) willing to abstain from cigarette smoking, and to substitute e-cigarettes, for approximately 6 weeks; 3) not currently planning to stop smoking (score <-2 on an Intentions to Quit scale[48]); and 4) able to read and sign a consent form in English. The study was approved by the institutional review board at the University of Connecticut Health Center. Participants provided informed and written consent and were paid $20 for participation in the baseline assessments.

*3.3.2 Procedure and Measures*

Adults who met the inclusion/exclusion criteria were invited to participate in the baseline measurements. During the initial screening process, participants completed the Smoking History Questionnaire [SHQ][49], a self-report questionnaire used to assess smoking history and pattern. The SHQ includes items pertaining to smoking products, brands used and smoking rate. Responses to brand used, specifically if a menthol brand is used, classified participants as non-menthol or menthol smokers. Pack years were calculated based on smokers reported average cigarettes smoked/day since being a regular smoker, converted to packs a day, and multiplied by reported years smoking. Smokers were also classified by time to first cigarette (TTFC) upon waking (within 30 minutes or >30 minutes). TTFC is a marker for nicotine dependence[50]. During baseline procedures, participants also sampled five electronic cigarette flavors, by puffing on

them for one minute each, and rated their most preferred flavor (no flavor, tobacco, menthol, cherry, and chocolate).

The chemosensory protocol took approximately 20 to 25 minutes during a 2-hour visit to the UCHC Clinical Research Center. Participants first self-reported their olfactory and taste functioning using the NHANES protocol[19,51], including problems within the past year, losses since age 25 years, and phantom sensations (dysgeusia, phantosmia/parosmia). Self-reported smell or taste alteration was an affirmative/negative response to having a problem within the past year, worse abilities since age 25, or a phantom sensation.

All sensory intensity ratings were made on the general Labeled Magnitude Scale (gLMS), which generalizes the LMS[52] to all sensations[53]. The gLMS scale ranges from 0=*nothing* to 100=*strongest sensation of any kind*, and intermediate labels of 6=*barely detectable*, 17=*moderate*, 35=*strong*, and 53=*very strong*. Participants were trained on using the generalized labeled magnitude scale (gLMS) following the procedures outlined in the NHANES but practicing the ratings with remembered sensations[54] instead of LED-generated brightness sensations used for intensity ratings[6]. Previous research has shown that the gLMS generates intensity ratings consistent with magnitude matching[53].

Olfactory function was measured using a 16-item odor identification task and intensity ratings for each odor on the gLMS[54]. The participants were instructed to identify the correct odor generated by an olfactometer (Osmic Enterprises, Inc., Cincinnati, OH) from four choices. Stimuli included food (cherry, strawberry, lemon, onion, coffee, cinnamon, chocolate, grape, vanilla), warning (gasoline, smoke, menthol) and household (soap, leather, baby powder, rose) odors. Participants who identified fewer than thirteen odors correctly were classified as having

an olfactory dysfunction (0-7 anosmia/severe hyposmia, 8-12 hyposmia). Those correctly identifying thirteen to sixteen odors were classified as normosmia.

Taste functioning was measured with the NHANES protocol[6,54]. Participants used the gLMS to report the intensity of concentrated 1mM quinine hydrochloride (QHCl) as well as 1M and .32 M sodium chloride (NaCl) drawn across the tongue tip and then sampled with the whole mouth[54]. As a probe of genetic variation in taste[26,55], participants also reported the intensity of 1 and 3.2 mM PROP sampled with the whole mouth. For analysis, participants were classified as nontasters if they reported the average intensity of PROP less than moderate on the gLMS, medium tasters between moderate and very strong, and supertasters greater than very strong.

Retronasal function was measured using a jelly bean plug/unplug taste test. Four jelly bean flavors, cherry, coffee, chocolate, and Tabasco were used. Participants first rated the sweetness/flavor intensity of the jelly bean with their nose plugged using the gLMS. Then participants unplugged their nose and again rate the sweetness, flavor intensity, and their liking or disliking of the flavor. For the Tabasco jelly beans, participants also rated the intensity of the burn or irritation feeling while unplugged. The difference in flavor intensity rating from plugged to unplugged were calculated to examine retronasal function.

### *3.3.3 Data Analysis*

Statistical analyses were conducted using SPSS version 22 (Chicago, IL) with significance criterion of $p \leq .05$. Descriptive analysis was used to compare self-rated and measured olfactory/taste functioning between smokers and the NHANES 2012[6,19] overall and by age decade.

Smokers were matched to a non-smoker sample (n=311) from our laboratory database using propensity score matching with the MatchIt[56] R package (www.r-project.org). The propensity score was estimated by logistic regressions matching for age and sex initially and then for age, sex and PROP tasting to test for differences in quinine and NaCl intensity (which vary with PROP bitterness[53,57]) using the method of nearest to match non-smokers to smokers with the closest propensity score. A 3:1 control to case ratio was used, which is preferred over 1:1 matching for increased statistical power and decreased standard errors[58,59]. Differences in taste function between smokers and non-smokers or within menthol and non-menthol were first assessed with group and sex analysis of covariance, controlling for age effects, and then assessed for differences in distribution with either chi-square or *Kolmogorov-Smirnov* test.

Retronasal jelly bean tests and preferred electronic cigarette ratings were examined for correlations and associations with olfactory and/or taste dysfunction as well as differences by menthol status or PROP taster classification.

**3.4 Results**

**Table One** describes the socio-demographic, lifestyle behaviors, and health conditions of the study participants by menthol smoker status. Average age of the entire sample was 35 ± 10 years. The average years smoked of the sample was 18.2 ±11 years, with an average of 11.5 ± 9.3 pack years. Eighty-one percent of the sample reported smoking within 30 minutes of waking in the morning.

Significantly more menthol smokers were single/divorced than non-menthol smokers ($x^{2=}$ 7.36, p<.01), having less than $40,000 household income ($x^2$= 10.9, p<.01) than non-menthol users, and significantly more were Black ($x^2$=4.24, p=0.04) compared to non-menthol users. In

comparison with a general population sample of adults in Connecticut from the Behavioral Risk Factor Surveillance System for Connecticut in 2013[60], fewer smokers in the current study were of normal weight (23.1 vs 35.6%, respectively), and more were obese (39.7 vs. 25%, respectively).

**Table One: Demographic and health characteristics of chronic smokers by menthol smoking status**

| Characteristics | Non-Menthol (32) | Menthol (47) |
|---|---|---|
| Gender | | |
| *Male* | 56.3% | 51.1% |
| *Female* | 43.8% | 48.9% |
| Age (years) (mean ± SD) | 35.5 ± 9.6 | 34.8 ± 10.5 |
| Marital Status | | |
| S*ingle* | 46.9% | 76.6% |
| *Married* | 53.1% | 23.4% |
| Race | | |
| *Black* | 6.3% | 23.9% |
| *White* | 93.8% | 71.7% |
| *MultiRace* | 0% | 4.3% |
| Household Income >$40,000 | 71.9% | 34.0% |
| Education Level (%) | | |
| < *High school* | 6.3% | 6.5% |
| ≥ High school | 93.8% | 93.5% |
| Average Years Smoked (mean± SD) | 19 ± 9.6 | 17 ± 12 |
| Average Cigarettes smoked/day (mean ± SD) | 12.9 ± 6.6 | 12 ± 6.5 |
| Average BMI | 28.5 | 29.9 |
| *Underweight (<18.5)* | 0% | 2.1% |
| *Normal (18.5-24.9)* | 31.3% | 23.4% |
| *Overweight (25-29.9)* | 37.5% | 34.0% |
| *Obese Grade I (30-34.9)* | 15.6% | 21.3% |
| *Obese Grade II (35-39.9)* | 6.3% | 10.6% |
| *Obese Grade III (≥40)* | 9.4% | 8.5% |
| Tonsils Removed | 18.8% | 14.9% |
| Persistent Cold/flu | 3.1% | 8.5% |
| Persistent Dry Mouth | 12.5% | 10.6% |
| Frequent nasal congestion/allergies | 25.0% | 29.8% |
| Suffered from a serious head injury | 31.3% | 25.5% |

*3.4.1 Self-reported olfactory and taste alteration*

As shown in **Table Two**, the percent of chronic smokers reporting alteration in olfaction, taste or both was very similar to that found in the NHANES 2011-2012[19]. However, there was less overlapping of the responses in forming the alteration indexes, as fewer chronic smokers reported phantom smell or taste sensations.

**Table Two: Rates of self-reported olfactory and taste alteration compared with the NHANES 2011-12[19] and olfactory dysfunction compared with the measured prevalence in the NHANES 2012[6]**

| **Self-reported Alteration**† | **% of Smoker Sample (n=79)** | **Prevalence in NHANES 2011-2012 (n=>3000)** |
|---|---|---|
| **Olfactory Alteration** | 25.3 | 23.0 |
| *Smell problem in last year* | *12.7* | *10.6* |
| *Loss since age 25* | *14.1* | *16.7* |
| *Phantom odors* | *1.3* | *6.0* |
| **Taste Alteration** | 15.2 | 18.7 |
| *Taste problem in last year* | 7.6 | 5.3 |
| *Loss since age 25 (salt, sweet, sour, or bitter)* | 1.5-5.1 | 3.6-4.7 |
| *Loss of food flavor since age 25* | 6.4 | 10.0 |
| *Dysgeusia* | 0.0 | 5.0 |
| **Combined Olfactory and Taste Alteration** | 11.4 | 10.0 |
| **Measured Olfactory Dysfunction** | **% of Smoker Sample (n=78)** | **Prevalence in NHANES 2012 (n=1281)** |
| ***Total*** | 40.5 | 12.4 |
| *Anosmia/Severe Hyposmia* | 1.4 | 3.2 |
| *Hyposmia* | 39.2 | 9.2 |
| *Normosmia* | 58.2 | 87.6 |

† Sub-scores will not sum to total alteration index because of overlapping responses.

*3.4.2 Measured olfactory function*

The rate of olfactory dysfunction among this sample of chronic smokers was 40.5%, which was higher than the 12.4% overall NHANES prevalence (8-item scratch and sniff odor identification task)[6], which included adults from the 4$^{th}$ to 8$^{th}$ decades of age (**Table Two**). The

rate of hyposmia in chronic smokers (39.2%) also exceeded the rates in NHANES among those in the 4th (2.5%) and 5th (8.1%) decades of age[6]. The percentage of chronic smokers with olfactory dysfunction increased with age; 33% in those less than 40 years of age, 40% in those under 50 and 100% in those 50 to 55. The number of odors correctly identified did not vary significantly among the female and male smokers. Rates of olfactory dysfunction among smokers did not vary by menthol status (35.5% non-menthol vs. 44.7% menthol) yet the menthol smokers tended to have greater variance in the distribution (F-test on variances=1.85, p=0.07). Rates of olfactory dysfunction among ≥10 pack year smokers (average 19.2 pack years) were 48.6% compared to 35% in <10 pack year smokers (average 4.5 pack years). This was not a statistically significant difference (p=0.25) and did not vary by menthol status.

Olfactometry indicated that prevalence of olfactory dysfunction among the chronic smokers was much higher than that suggested by self-report. Comparing the sensitivity and specificity of self-report against measured olfactory alteration, individuals had much better specificity (correct identification of normal), at >76.3%, than sensitivity (correct identification of dysfunction) at <50%. The best sensitivity and specificity was found in individuals who identified ≤10 smells correctly, indicating that individuals with more severe dysfunction are better at identifying when they have a problem than individuals with mild dysfunction.

Of the specific odors, onion (100% identified it correctly) and coffee (97.4% identified correctly) were the odors most frequently identified correctly. Chocolate (51.3% identified incorrectly) and gasoline (51.3% identified incorrectly) were the odors most frequently identified incorrectly. Of the overall sample, 24.4% did not correctly identify menthol, with more menthol smokers not identifying the odor correctly compared to non-menthol smokers (27.7% vs. 19.4% misidentified respectively); this difference did not reach statistical significance. The intensity of

the odors ranged from ≤moderate (chocolate, menthol), moderate to strong (strawberry, gasoline, smoke, lemon, cinnamon, rose, vanilla, cherry, leather, baby powder), to ≥strong (soap, grape, coffee, onion). In odors repeated in our previous study, compared with age- and sex matched non-smokers[54], these chronic smokers reported significantly lower intensities for smoke, coffee, menthol and onion but not for chocolate.

*3.4.3 Measured taste function*

Relative to non-smokers, the smokers tended to have higher PROP ratings when controlling for age and sex effects. In chi-square analysis, the chronic smokers had a higher frequency of supertasters than did non-smokers (nontasters 18.62 vs. 22.1%; medium tasters 56.3 vs. 36.4%; super tasters 25.1 vs. 41.6%, respectively; $x^2=10.2$, $p<0.01$). Because of these PROP effects, the non-smokers were matched on age, sex and PROP bitterness to test for quinine and NaCl intensity differences between smokers and non-smokers.

The smokers reported quinine intensity on the tongue tip as between moderate and strong and significantly less than NaCl intensity on the tongue tip, which averaged between strong and very strong (28.19±2.41 vs. 41.03±2.58, respectively; $t=6.24$, $p<.001$). The distribution of these tastants on the tongue tip were significantly skewed to higher intensity compared with age-, sex- and PROP- matched controls (**Figure One**). For the whole mouth, the smokers reported quinine and 1 M NaCl just above very strong and the .32 M NaCl between strong and very strong. In comparison with the age-, sex- and PROP-matched nonsmokers, the smokers were not varied in the distribution of intensity from whole mouth quinine and .32 M NaCl, but were significantly skewed to higher intensity from 1 M NaCl (**Figure One**).

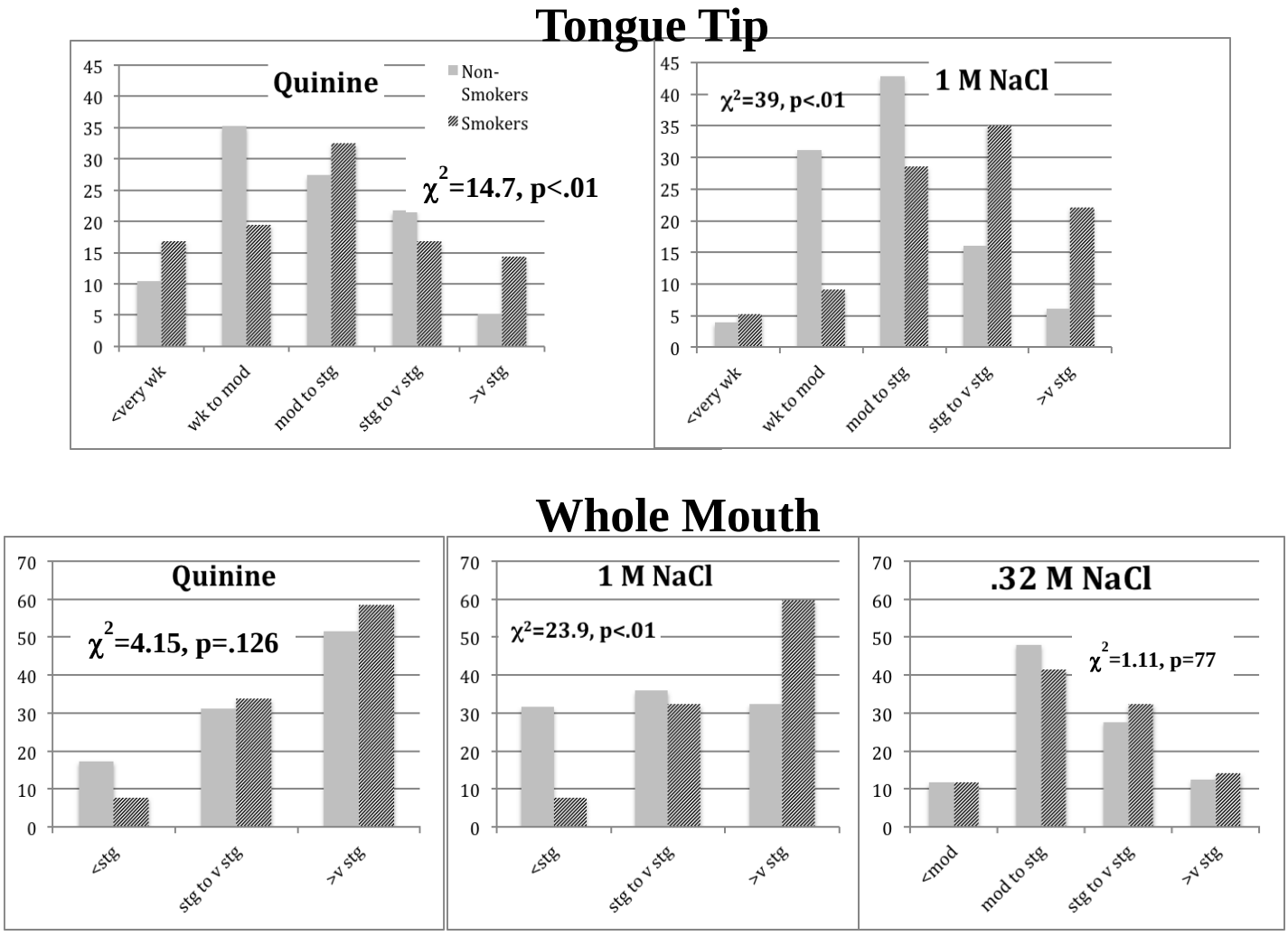


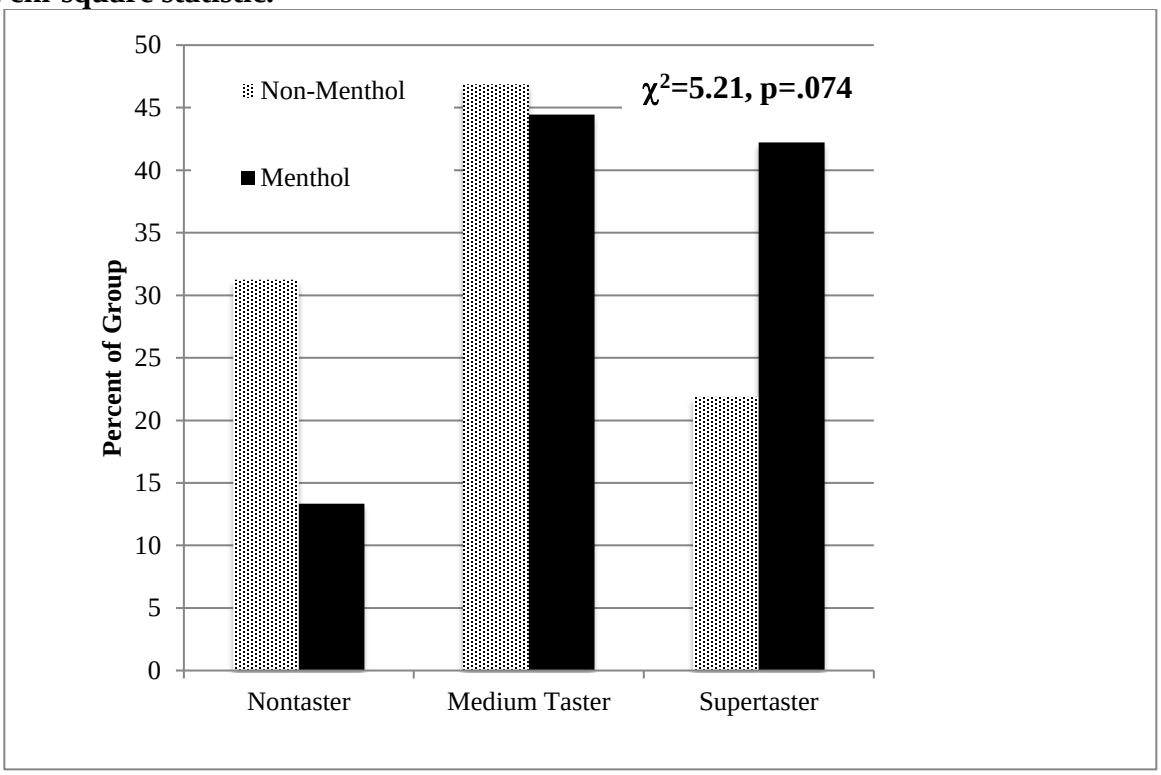

**Figure One: The distribution of taste intensity among chronic smokers (hatched bars) and non-smokers (solid grey bars) following the NHANES taste testing protocol [53] with the gLMS categories [52] on the x-axis and percent of smoker/non-smoker group on the y-axis tested by the chi-square statistics**

*3.4.4 Taste function between menthol and non-menthol smokers*

In analysis of covariance, the menthol and non-menthol smokers did not vary significantly in average or distribution in intensity of tongue tip quinine or NaCl and whole mouth NaCl (1M, .32 M) or quinine. However, the distribution of the average bitterness of 1mM and 3.2 mM PROP was significantly different in menthol smokers than among non-menthol smokers ($D$=0.31, p<0.05). **Figure Two** shows the distribution of nontasters (PROP<moderate), medium

tasters (PROP between moderate and less than very strong), and supertasters (PROP≥ very strong). There tended to be significantly fewer PROP nontasters but more supertasters among the menthol smokers than non-menthol smokers ($x^2$=5.21, p=0.074).

**Figure Two: The distribution of propythiouracil taster status among chronic smokers who smoke menthol (black bars) and non-menthol (shaded bars) cigarettes with the gLMS categories[53] on the x-axis and percent of smoker/non-smoker group on the y-axis tested by the chi-square statistic.**




*3.4.5 Retronasal function*

Overall, the flavor intensities heightened as participants rate plugged and then unplugged jelly bean flavor intensities, with the biggest increase in sweetness intensity seen with the coffee jelly bean flavor (mean +19.3) (**Table Three)**. The flavor intensity ratings (unplugged) ranged from between moderate and strong (chocolate mean 24.5) to close to strong/very strong (Coffee mean 40.8). Cherry was the most liked jelly bean (mean liking of +35), and Tabasco was the only mean disliked jelly bean (mean disliking of -26.63). The Tabasco jelly bean's irritation

intensity was rated as a mean of 31.64. There was no significant difference in any ratings by menthol status.

Measured olfactory dysfunction (<13 correctly identified odors) was not associated with depressed change in flavor intensity rating from plugged to unplugged, and was not associated with depressed jelly bean flavor intensity ratings. There was no correlation between number of smells identified correctly by olfactometry and either difference in flavor intensity rating plugged to unplugged or flavor intensity rating alone (unplugged). However, those individuals with worse dysfunction (<10 correctly identified odors) had significantly lower Tabasco flavor intensity ratings than those without severe dysfunction (27.5 vs. 51.6 p=0.04) but no other jelly bean flavors varied. This suggests that retronasal olfaction may not be affected by smoking as orthonasal olfaction may be.

When examining jelly bean ratings by taster group (nontasters= <17, medium tasters 17-53, supertasters >54 PROP intensity ratings), there was a trend in increasing flavor intensity ratings for all jelly bean flavors between nontasters and supertasters, but this did not reach significance.

**Table Three: Change in jelly bean flavor intensity rating from unplugged rating to plugged rating**

| Jelly Bean | N | Average Plugged rating | Average Unplugged Rating | Mean Change | Std Dev | Significance |
|---|---|---|---|---|---|---|
| cherry | 79 | 15.6 | 31.1 | +15.5 | 17.8 | <0.01 |
| coffee | 79 | 13.7 | 33.0 | +19.3 | 16.3 | <0.01 |
| chocolate | 79 | 10.6 | 23.0 | +12.4 | 15.0 | <0.01 |
| Tabasco | 78 | 14.0 | 32.5 | +18.5 | 13.8 | <0.01 |

*3.4.6 Preferred electronic cigarette flavor*

The most preferred electronic cigarette flavor was cherry (31.4%), followed by menthol (30.4%) and tobacco (26.6%). Preferred electronic cigarette flavor did not significantly differ by

sex, however did differ significantly by BMI class using fishers exact test. Those who were obese most frequently rated cherry flavor as their most preferred flavor compared to normal weight individuals (p= <0.005).

There was no association between measured olfactory dysfunction and preferred electronic cigarette flavor. However, smokers who were supertasters based on PROP intensity classification most frequently reported menthol electronic cigarette as their most preferred flavor, compared to nontasters (40.6% vs. 23.5% respectively), although this did not reach statistical significance. Favorite electronic cigarette flavor also differed significantly by menthol status. Menthol smokers rated menthol the favorite flavor more frequently than non-menthol smokers (46.8% vs. 6.3%, p=<0.001) and non-menthol smokers rated tobacco the favorite flavor more frequently (59.4% vs. 4.3%, p=<0.001).

### 3.5 Discussion

This study involved chemosensory phenotyping of a sample of chronic smokers. In standardized chemosensory testing these chronic smokers showed 3 to 4-fold higher frequency of measured olfactory dysfunction than that observed in the nationally-representative sample from the NHANES[6]. Despite this high prevalence, the smokers were unaware of the dysfunction unless it was at the level of severe hyposmia/anosmia as we have seen previously[6,61]. Retronasal function did not seem to be altered by cigarette smoking as orthonasal olfaction was found to be. The chronic smokers also reported heightened taste intensities from NaCl in comparison to non-smoker controls, occurring not at concentrations where salt is primarily a taste (0.32 M), but at levels of salt as an oral irritant (1 M)[62] on the tongue tip as well as with whole mouth perception. We did not find that smokers were more likely to be nontasters of PROP (a phenotypic marker of

genetic variation in taste) in comparison to controls. However, among the chronic smokers, those who reported smoking menthol cigarettes were more likely to report elevated bitterness from PROP. Electronic cigarette flavor preference did vary by menthol-status, with more menthol smokers preferring menthol flavor and more non-menthol smokers preferring tobacco flavor, and by BMI class, however there were no statistically significant differences between preferred electronic cigarette flavor and olfactory function or taster groups.

Chronic exposure to smoking may cause direct damage to the peripheral nerve system to explain our findings of elevated olfactory dysfunction among smokers. In animal models, chronic exposure to smoking as well as ethanol is associated with olfactory epithelial neuron death, undermining the regeneration capacity to maintain functional tissues[63]. Our rates of olfactory dysfunction exceeded that in the NHANES study, which also utilized an odor identification task but with a scratch and sniff procedure[6,54]. Odor identification requires the ability to detect and correctly label an odor, which requires olfactory and cognitive processes[10]. Loss of olfaction occurs with mild cognitive impairment[10] and cognitive impairment has been noted among smokers[64]. The present study utilized olfactometer generated-odors; the odor intensities reported by the smokers were generally less than those reported by age and sex-matched non-smokers for most odors, including menthol[54]. Smoking also could have an indirect relationship with olfactory dysfunction through other risk factors (e.g., head trauma, upper respiratory tract infection, sinonasal disease)[65,66]. It is interesting that we did not observe the usual sex difference in olfactory functioning with women exceeding men in functioning. Females may suffer greater negative effects of smoking on olfactory dysfunction than males[5].

Our data did not indicate that smokers have depressed taste perception, either in ability to taste regionally on the tongue tip or with whole mouth experiences. Instead, relative to non-

smokers, the smokers here had a greater response to 1 M NaCl, an oral chemical irritant[62], which is consistent with the elevated oral irritation and pain seen in previous studies[46,67]. Other components of somatosensation, however, do tend to be depressed in smokers including thermal sensitivity[43]. The elevated intensity of NaCl irritation could not be explained by loss of taste as seen with history of middle ear infections or taste nerve damage[24]. The regional differences in taste and oral irritation could also reflect genetic variation[68].

We did not note any alteration in retronasal function, and it did not associate with orthonasal olfactory dysfunction via olfactometry. Studies have shown there are differences in the perception and processing of orthonasal stimuli versus retronasal stimuli[69], which may account for these findings. One pathway may present as normal while the other can present with dysfunction. Retronasal olfaction also interacts with the gustatory system[69], which we did not find to be depressed in smokers. In fact, because the taste functioning may be heightened in this smoker sample, this may have countered any alterations to retronasal functioning.

Genetic variation in taste as indicated by the ability to taste the bitterness of PROP was not different in our chronic smokers versus controls, but did vary by menthol cigarette preference. We did not observe more PROP nontasters among our sample of chronic smokers, which is inconsistent with previous findings of higher prevalence of nontasters among smokers[29,30]. In fact, we observed higher PROP tasting ability among these smokers by the intensity of PROP bitterness alone. Our testing employed aqueous solutions of concentrated PROP, which is more intense than commercially available PTC strips. Frequently, PROP tasting categories of nontaster, medium and supertaster are defined as the PROP to NaCl intensity ratio, with nontasters having the lowest ratios (<0.4) and supertasters the highest (>1.2)[55,70]. However,

our findings with NaCl suggest that this ratio could falsely shift the PROP/NaCl ratio toward more nontasters and fewer supertasters.

Consistent with findings that female tasters by *TAS2R38* receptor genotype were more likely to smoke menthol cigarettes[38], the present study found that, relative to non-menthol smokers, menthol smokers were skewed toward reporting that PROP had greater bitterness. This finding did not generalize to the bitterness of quinine, just for PROP, which may indicate a stronger taste genetic component to this relationship. Menthol may improve the ability to tolerate the unpleasant sensations of nicotine by providing a minty odor[71] and oral and nasal cooling[72], especially for younger smokers and minorities[73].

In regards to preferred electronic cigarette by the smokers in the study, significantly more menthol smokers rated the menthol flavor as their most preferred than non menthol smokers. Similarly, more supertasters rated the menthol electronic cigarette flavor as their most preferred. This did not reach statistical significance, but may be due to the limited sample size. As previous research notes, supertasters are more likely to smoke menthol cigarettes[38], and these current findings support this with other menthol flavored nicotine products as well. In addition, obese individuals most frequently reported the cherry flavor electronic cigarette as their most preferred. Research suggests obese individuals have a stronger attraction and preference to sweet than normal weight individuals[74].

This study had a number of limitations including a convenience sample of chronic smokers and reliance on self-reported history. The taste protocol did not include all taste qualities. Nonetheless, the sample was well characterized for smoking status and phenotyped with measures that could be compared with the new NHANES self-reported and measured olfactory functioning[6,54]. The taste testing also followed the NHANES protocol for future

comparison to national norms, including aqueous tastants compared with matched controls.

### 3.6 Conclusion

In summary, this sample of chronic smokers showed elevated levels of olfactory dysfunction but no evidence of taste dysfunction. Smokers had better sensitivity than specificity of this olfaction issue, and it did not differ by menthol status. The smokers reported heightened irritation from a concentrated NaCl solution compared to non-smokers. Menthol smokers were more likely to be bitter tasters of PROP, the probe for genetic variation in taste. Similarly menthol smokers were more likely to choose menthol flavored electronic cigarettes than non-menthol smokers, and favorite flavor also varied by BMI. These findings allow for both future insight into tobacco and nicotine product regulation and baseline data for interventions studies, particularly those that might employ flavored nicotine replacement modalities, such as electronic cigarettes.

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# CHAPTER FOUR

## CONCLUSION

### *4.1 Important Findings*

This research was conducted to further examine the chemosensory abilities of cigarette smokers. Utilizing a nationally-representative as well as a community-based sample, we were able to use sophisticated characterization of smoking, and both self-report and objective measures of chemosensory function. The research supported that chronic and dependent cigarette smokers display olfactory dysfunction/alteration more frequently than do non-smokers, but do not appear to display retronasal dysfunction or taste dysfunction. Instead, smokers reported greater oral irritation by concentrated sodium chloride solutions, and menthol smokers specifically reported heightened propylthiouracil (PROP) bitterness. In addition, electronic cigarette flavor preference varied by menthol status, PROP taster classification, and weight. These results add to the body of literature on chemosensory abilities of cigarette smokers, and help clarify potential reasons for the conflicting findings. In addition, these findings indicated another potential benefit for smoking cessation – improving chemosensory function – and suggested that smoking is a modifiable risk factor that can help prevent and decrease the prevalence of olfactory dysfunction. Finally, these results provided baseline characterization of chronic smokers, allowing for comparison of function with cessation or switching to electronic cigarettes, and can provide insight that can be used for nicotine production regulation.

Broader characterization of smoking status was crucial when examining who was most at risk for a chemosensory alteration. Both chapters two and three involved characterization of chronic and dependent smokers based on pack years and time to first cigarette of the day. In the NHANES sample, classification of smoking status by current/former/never did not result in

increased odds of an olfactory alteration in either current or former smokers compared to never smokers. Smoking status required further classification, identifying chronic and dependent smokers, defined as ≥10 pack year, <30 minute TTFC smokers, to be at increased risk of an olfactory alteration compared to never/former smokers. Former chronic smokers (≥10 pack year) also were at increased odds of self-reported olfactory alteration, compared to never smokers. However, in the e-Cigarette sample of current chronic smokers, self-reported olfactory function in smokers was not significantly different than the general population, despite having a greater frequency of olfactory dysfunction than the general population. The scientific literature has indicated that self-reported function has good sensitivity, but relatively poorer specificity, particularly in recognizing less severe olfactory dysfunction. The chronic smoker sample examined in chapter three most frequently showed measured hyposmia, which is less severe than anosmia. Therefore, this thesis points to the need to use more than one measure of chemosensory function, utilizing both self-report and objective measures when possible, to capture more individuals who may suffer from olfactory dysfunction or alteration.

In addition to the finding that cigarette smokers may be at risk for olfactory alteration, other health behaviors and issues associated with cigarette smoking influence the cigarette smoking-olfactory alteration relationship. The NHANES analysis displayed that smokers who were also heavy drinkers had the greatest odds of an olfactory alteration, even greater than being a smoker alone. In addition, a portion of the relationship between smoking alone and in addition to heavy drinking was explained through other olfactory-related pathologies, including sinonasal problems, xerostomia, head/face injury, and history of a tonsillectomy. These results highlight the importance of examining the complete relationship of risk factors and health behaviors to fully capture those at increased risk for olfactory alterations. Those individuals with multiple

addictions may be most at risk for chemosensory issues. Studies and clinical facilities must take the time to probe complete behavioral and medical histories.

Furthermore, the e-Cigarette study analysis neither revealed taste dysfunction among chronic smokers, nor showed that chronic smokers as more likely to be PROP nontasters as compared to non-smokers. Chronic smokers reported heightened irritation from a concentrated NaCl solution compared to non-smokers, not at the concentration of a tastant, but at the concentration of an oral irritant. This heightened irritation may potentially affect diet quality and quality of life, and these implications should be explored further. In addition, menthol-smokers reported elevated PROP bitterness intensity compared to non-menthol smokers, indicating genetic variations among this smoking sub-sample. These menthol/PROP supertasters, also preferred menthol electronic cigarette flavor more frequently than non-menthol/PROP non-taster smokers. This indicates that taste profiling may indicate those more likely to smoke menthol cigarettes or flavored electronic cigarettes and associated health implications.

These studies suggest that chronic smokers (10+ PY smokers), who are also nicotine dependent (<30 min TTFC), have differences in chemosensory function compared to non-smokers, including increased frequency of olfactory dysfunction and heightened oral sensations. Smoking must be characterized thoroughly in order to see these differences, pointing to a potential reason why previous literature has found such conflicting results.

### *4.2 Implications for Future Research*

Findings from this research help fill a gap in the literature, as smoking's association with chemosensory function has not been thoroughly examined utilizing sophisticated characterization of smoking, specifically focusing on chronic and dependent smokers. We found

that smokers do present with olfactory dysfunction and heighted oral irritation, which indicates another benefit to smoking cessation that can be made aware to the public. Additionally, results from these studies provide baseline data on the chemosensory abilities of cigarette smokers, which can be used in the future to examine changes in these functions with cessation or switching to electronic cigarettes. Future studies should also examine the impact these heightened taste functions have on smoker's diet quality, quality of life, and tendencies to smoke other flavored nicotine products. Furthermore, because current treatment options for chemosensory dysfunction are limited, prevention against these dysfunctions can be addressed through modifiable risk factors. Both cessation or not beginning cigarette smoking and heavy drinking can reduce the prevalence of chemosensory alterations.

Supplemental Material

**Unadjusted and adjusted odds ratios and 95% confidence intervals of risk factors associated with self-reported smell alteration in U.S adults by smoking class**

Table One: Current and former chronic smokers

| | Self-reported smell alteration | | | |
|---|---|---|---|---|
| | Unadjusted | | Adjusted | |
| **Variables** | **OR** | **95% CI** | **OR** | **95% CI** |
| Age | 1.007 | 1.001–1.014 | 1.00 | 0.99-1.008 |
| Sex (reference "female")<br>*Male* | 1.009 | 0.90–1.13 | 0.99 | 0.85-1.17 |
| Race/Ethnicity (reference "white")<br>*Mexican American* | 0.90 | 0.73–1.1 | | |
| *Other Hispanic* | 0.93 | 0.75–1.15 | | |
| *Non-Hispanic Black* | 0.80 | 0.65–0.98 | 0.85 | 0.67-1.09 |
| *Non-Hispanic Asian* | 0.50 | 0.37-0.68 | 0.76 | 0.53-1.09 |
| *Other race* | 1.48 | 0.85–2.57 | | |
| Marital Status (reference "Married")<br>*Not Married* | 1.2 | 1.07–1.35 | 1.02 | 0.87-1.20 |
| Education (reference " ≥high school")<br>*Less than high school* | 1.12 | 0.93–1.37 | | |
| IPR (reference ">1")<br>*IPR ≤ 1* | 1.40 | 1.18–1.64 | 1.23 | 1.00-1.50 |
| Sinonasal problems (reference "no")<br>*Yes* | 2.21 | 1.86–2.63 | 1.75 | 1.38-2.22 |
| Xerostomia (reference "no")<br>*Yes* | 2.40 | 2.04–2.83 | 1.80 | 1.52-2.13 |
| Head/Face Injury (reference "no")<br>*Yes* | 1.56 | 1.28–1.88 | 1.30 | 1.04-1.63 |
| Tonsils Removed (reference "no")<br>*Yes* | 1.36 | 1.12–1.66 | 1.29 | 1.05-1.60 |
| Multiple Ear Infections (reference "no")<br>*Yes* | 1.52 | 1.22–1.90 | 1.17 | 0.91-1.50 |
| Self-rated Health (reference "excellent, very good, good")<br>*Fair or poor* | 1.63 | 1.36–1.95 | 1.23 | 1.02-1.48 |
| Heavy Drinking (reference "no")<br>*Yes* | 1.76 | 1.35–2.31 | 1.38 | 1.00-1.91 |
| Smoking Status (reference "never smoker") | | | | |
| *<10 PY Light Smokers* | 1.16 | 0.86-1.56 | 0.97 | 0.71-1.34 |
| *Chronic Smokers* | 1.75 | 1.45-2.12 | 1.36 | 1.06-1.74 |

Table Two: Chronic smokers stratified by current and former smokers

| | Self-reported smell alteration | | | |
|---|---|---|---|---|
| | Unadjusted | | Adjusted | |
| **Variables** | **OR** | **95% CI** | **OR** | **95% CI** |
| Age | 1.007 | 1.001–1.014 | 1.00 | 0.99-1.007 |
| Sex (reference "female")<br>*Male* | 1.009 | 0.90–1.13 | 0.99 | 0.84-1.17 |
| Race/Ethnicity (reference "white")<br>*Mexican American* | 0.90 | 0.73–1.1 | | |
| *Other Hispanic* | 0.93 | 0.75–1.15 | | |
| *Non-Hispanic Black* | 0.80 | 0.65–0.98 | 0.85 | 0.67-1.09 |
| *Non-Hispanic Asian* | 0.50 | 0.37-0.68 | 0.76 | 0.53-1.09 |
| *Other race* | 1.48 | 0.85–2.57 | | |
| Marital Status (reference "Married")<br>*Not Married* | 1.2 | 1.07–1.35 | 1.03 | 0.87-1.22 |
| Education (reference " ≥high school")<br>*Less than high school* | 1.12 | 0.93–1.37 | | |
| IPR (reference ">1")<br>*IPR ≤ 1* | 1.40 | 1.18–1.64 | 1.23 | 1.02-1.50 |
| Sinonasal problems (reference "no")<br>*Yes* | 2.21 | 1.86–2.63 | 1.75 | 1.38-2.22 |
| Xerostomia (reference "no")<br>*Yes* | 2.40 | 2.04–2.83 | 1.80 | 1.52-2.13 |
| Head/Face Injury (reference "no")<br>*Yes* | 1.56 | 1.28–1.88 | 1.30 | 1.04-1.63 |
| Tonsils Removed (reference "no")<br>*Yes* | 1.36 | 1.12–1.66 | 1.29 | 1.05-1.60 |
| Multiple Ear Infections (reference "no")<br>*Yes* | 1.52 | 1.22–1.90 | 1.17 | 0.91-1.50 |
| Self-rated Health (reference "excellent, very good, good")<br>*Fair or poor* | 1.63 | 1.36–1.95 | 1.23 | 1.02-1.48 |
| Heavy Drinking (reference "no")<br>*Yes* | 1.76 | 1.35–2.31 | 1.38 | 1.00-1.91 |
| Smoking Status (reference "never smoker") | | | | |
| *<10 PY Light Smokers* | 1.16 | 0.86-1.56 | 0.97 | 0.71-1.33 |
| *Current Chronic Smokers* | 1.75 | 1.34-2.37 | 1.29 | 0.93-1.80 |
| *Former Chronic Smokers* | 1.72 | 1.39-2.12 | 1.42 | 1.09-1.84 |

Table Three: Current chronic high dependent smokers

| | Self-reported smell alteration | | | |
|---|---|---|---|---|
| | Unadjusted | | Adjusted | |
| **Variables** | **OR** | **95% CI** | **OR** | **95% CI** |
| Age | 1.007 | 1.001–1.014 | 1.002 | 0.99-1.01 |
| Sex (reference “female”)<br>*Male* | 1.009 | 0.90–1.13 | 1.01 | 0.85-1.19 |
| Race/Ethnicity (reference “white”)<br>*Mexican American* | 0.90 | 0.73–1.1 | | |
| *Other Hispanic* | 0.93 | 0.75–1.15 | | |
| *Non-Hispanic Black* | 0.80 | 0.65–0.98 | 0.86 | 0.67-1.09 |
| *Non-Hispanic Asian* | 0.50 | 0.37-0.68 | 0.78 | 0.55-1.09 |
| *Other race* | 1.48 | 0.85–2.57 | | |
| Marital Status (reference ”Married”)<br>*Not Married* | 1.2 | 1.07–1.35 | 1.02 | 0.85-1.21 |
| Education (reference ” ≥high school”)<br>*Less than high school* | 1.12 | 0.93–1.37 | | |
| IPR (reference “>1”)<br>*IPR ≤ 1* | 1.40 | 1.18–1.64 | 1.22 | 1.00-1.50 |
| Sinonasal problems (reference ”no”)<br>*Yes* | 2.21 | 1.86–2.63 | 1.80 | 1.43-2.26 |
| Xerostomia (reference ”no”)<br>*Yes* | 2.40 | 2.04–2.83 | 1.75 | 1.45-2.11 |
| Head/Face Injury (reference ”no”)<br>*Yes* | 1.56 | 1.28–1.88 | 1.35 | 1.08-1.68 |
| Tonsils Removed (reference “no”)<br>*Yes* | 1.36 | 1.12–1.66 | 1.28 | 1.03-1.59 |
| Multiple Ear Infections (reference “no”)<br>*Yes* | 1.52 | 1.22–1.90 | 1.16 | 0.91-1.48 |
| Self-rated Health (reference “excellent, very good, good”)<br>*Fair or poor* | 1.63 | 1.36–1.95 | 1.24 | 1.03-1.51 |
| Heavy Drinking (reference “no”)<br>*Yes* | 1.76 | 1.35–2.31 | 1.44 | 1.03-2.00 |
| Smoking Status (reference “never/former smoker”) | | | | |
| *Light Smokers (<10 PY/>30 min TTFC)* | 1.08 | 0.84-1.38 | 0.90 | 0.66-1.20 |
| *Current Chronic High Dependent Smoker* | 1.82 | 1.38-2.41 | 1.41 | 1.01-1.99 |

Table Four: Chronic Active Smokers

| | Self-reported smell alteration | | | |
|---|---|---|---|---|
| | Unadjusted | | Adjusted | |
| **Variables** | **OR** | **95% CI** | **OR** | **95% CI** |
| Age | 1.007 | 1.001–1.014 | 1.005 | 0.99-1.02 |
| Sex (reference “female”)<br>*Male* | 1.009 | 0.90–1.13 | 0.97 | 0.74-1.27 |
| Race/Ethnicity (reference “white”)<br>*Mexican American* | 0.90 | 0.73–1.1 | | |
| *Other Hispanic* | 0.93 | 0.75–1.15 | | |
| *Non-Hispanic Black* | 0.80 | 0.65–0.98 | 0.90 | 0.63-1.27 |
| *Non-Hispanic Asian* | 0.50 | 0.37-0.68 | 0.84 | 0.57-1.24 |
| *Other race* | 1.48 | 0.85–2.57 | | |
| Marital Status (reference ”Married”)<br>*Not Married* | 1.2 | 1.07–1.35 | 1.08 | 0.88-1.31 |
| Education (reference ” ≥high school ”)<br>*Less than high school* | 1.12 | 0.93–1.37 | | |
| IPR (reference “>1”)<br>*IPR ≤ 1* | 1.40 | 1.18–1.64 | 1.48 | 1.13-1.96 |
| Sinonasal problems (reference ”no”)<br>*Yes* | 2.21 | 1.86–2.63 | 1.82 | 1.33-2.49 |
| Xerostomia (reference ”no”)<br>*Yes* | 2.40 | 2.04–2.83 | 1.53 | 1.12-2.09 |
| Head/Face Injury (reference ”no”)<br>*Yes* | 1.56 | 1.28–1.88 | 1.40 | 0.92-2.14 |
| Tonsils Removed (reference “no”)<br>*Yes* | 1.36 | 1.12–1.66 | 1.30 | 0.93-1.82 |
| Multiple Ear Infections (reference “no”)<br>*Yes* | 1.52 | 1.22–1.90 | 1.13 | 0.78-1.64 |
| Self-rated Health (reference “excellent, very good, good”)<br>*Fair or poor* | 1.63 | 1.36–1.95 | 1.22 | 0.90-1.67 |
| Heavy Drinking (reference “no”)<br>*Yes* | 1.76 | 1.35–2.31 | 1.57 | 1.03-2.38 |
| Smoking Status (reference “never/former smoker”) | | | | |
| *Light smokers(<10 cotinine/<10 PY)* | 0.97 | 0.68-1.37 | 0.73 | 0.46-1.17 |
| *Chronic Active Smokers* | 1.56 | 1.00-2.49 | 1.22 | 0.72-2.05 |

Table Five: High Dependent Active Smokers

| | Self-reported smell alteration | | | |
|---|---|---|---|---|
| | Unadjusted | | Adjusted | |
| **Variables** | **OR** | **95% CI** | **OR** | **95% CI** |
| Age | 1.007 | 1.001–1.014 | 1.004 | 0.99-1.02 |
| Sex (reference "female")<br>*Male* | 1.009 | 0.90–1.13 | 0.98 | 0.71-1.35 |
| Race/Ethnicity (reference "white")<br>*Mexican American* | 0.90 | 0.73–1.1 | | |
| *Other Hispanic* | 0.93 | 0.75–1.15 | | |
| *Non-Hispanic Black* | 0.80 | 0.65–0.98 | 0.98 | 0.69-1.38 |
| *Non-Hispanic Asian* | 0.50 | 0.37-0.68 | 0.85 | 0.58-1.25 |
| *Other race* | 1.48 | 0.85–2.57 | | |
| Marital Status (reference "Married")<br>*Not Married* | 1.2 | 1.07–1.35 | 1.02 | 0.83-1.25 |
| Education (reference " ≥high school")<br>*Less than high school* | 1.12 | 0.93–1.37 | | |
| IPR (reference ">1")<br>*IPR ≤ 1* | 1.40 | 1.18–1.64 | 1.47 | 1.10-1.98 |
| Sinonasal problems (reference "no")<br>*Yes* | 2.21 | 1.86–2.63 | 1.84 | 1.34-2.52 |
| Xerostomia (reference "no")<br>*Yes* | 2.40 | 2.04–2.83 | 1.44 | 1.06-1.96 |
| Head/Face Injury (reference "no")<br>*Yes* | 1.56 | 1.28–1.88 | 1.45 | 0.95-2.22 |
| Tonsils Removed (reference "no")<br>*Yes* | 1.36 | 1.12–1.66 | 1.36 | 1.00-1.85 |
| Multiple Ear Infections (reference "no")<br>*Yes* | 1.52 | 1.22–1.90 | 1.09 | 0.76-1.57 |
| Self-rated Health (reference "excellent, very good, good")<br>*Fair or poor* | 1.63 | 1.36–1.95 | 1.27 | 0.92-1.75 |
| Heavy Drinking (reference "no")<br>*Yes* | 1.76 | 1.35–2.31 | 1.44 | 0.94-2.19 |
| Smoking Status (reference "never/former smoker") | | | | |
| *Light smokers(<10 cotinine/>30 min TTFC)* | 1.00 | 0.68-1.48 | 0.76 | 0.51-1.15 |
| *High Dependent Active Smokers* | 1.62 | 1.00-2.62 | 1.22 | 0.77-1.93 |

Table Six: High Dependent Smoker-Heavy Drinker

| | Self-reported smell alteration | | | |
|---|---|---|---|---|
| | Unadjusted | | Adjusted | |
| **Variables** | **OR** | **95% CI** | **OR** | **95% CI** |
| Age | 1.007 | 1.001–1.014 | 1.003 | 0.99–1.01 |
| Sex (reference "female")<br>*Male* | 1.009 | 0.90–1.13 | 1.05 | 0.88–1.26 |
| Race/Ethnicity (reference "white")<br>*Mexican American* | 0.90 | 0.73–1.1 | | |
| *Other Hispanic* | 0.93 | 0.75–1.15 | | |
| *Non-Hispanic Black* | 0.80 | 0.65–0.98 | 0.82 | 0.62-1.07 |
| *Non-Hispanic Asian* | 0.50 | 0.37-0.68 | 0.78 | 0.55-1.11 |
| *Other race* | 1.48 | 0.85–2.57 | | |
| Marital Status (reference "Married")<br>*Not Married* | 1.2 | 1.07–1.35 | 1.02 | 0.85-1.22 |
| Education (reference " ≥high school")<br>*Less than high school* | 1.12 | 0.93–1.37 | | |
| IPR (reference ">1")<br>*IPR ≤ 1* | 1.40 | 1.18–1.64 | 1.18 | 0.96-1.45 |
| Sinonasal problems (reference "no")<br>*Yes* | 2.21 | 1.86–2.63 | 1.81 | 1.42–2.30 |
| Xerostomia (reference "no")<br>*Yes* | 2.40 | 2.04–2.83 | 1.74 | 1.46–2.09 |
| Head/Face Injury (reference "no")<br>*Yes* | 1.56 | 1.28–1.88 | 1.38 | 1.09–1.76 |
| Tonsils Removed (reference "no")<br>*Yes* | 1.36 | 1.12–1.66 | 1.31 | 1.07-1.60 |
| Multiple Ear Infections (reference "no")<br>*Yes* | 1.52 | 1.22–1.90 | 1.09 | 0.85-1.40 |
| Self-rated Health (reference "excellent, very good, good")<br>*Fair or poor* | 1.63 | 1.36–1.95 | 1.27 | 1.00-1.62 |
| Smokers and Drinkers (reference "never/former smoker, non-heavy drinker") | | | | |
| *Dependent smoker, non-drinker* | 1.45 | 1.06-1.99 | 1.30 | 0.92-1.83 |
| *Non-smoker, heavy drinker* | 1.75 | 1.21-2.53 | 1.45 | 0.93-2.26 |
| *Dependent Smoker-heavy drinker* | 2.34 | 1.55-3.54 | 1.96 | 1.20-3.19 |