

HORMONES RULE THE ROOST: FLUCTUATIONS OF OLFACTORY FUNCTIONING
THROUGHOUT THE MENSTRUAL CYCLE AND DURING PREGNANCY

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ABSTRACT

The relationship of olfactory functioning and hormones is complex, and, for the most part, heterogenous. The present study aims to clarify and or define aspects of this relationship by examining how olfactory thresholds fluctuate in relation to changing hormone levels in two populations: menstruating and pregnant women. A longitudinal study was implemented to assess women at two different times. A total of 72 non-pregnant and 7 pregnant women participated in the first assessment, with a total of 62 non-pregnant and all 7 pregnant women returning for the second assessment. During each examination participants had blood drawn and were administered a threshold test. The Wheeler-University of Tennessee at Chattanooga odor threshold test (WUTC) allowed for the measurement of odorant sensitivity of 4 distinct odorants. Hormone levels were analyzed using a method of gradient high-performance liquid chromatography (HPLC). Multiple analyses yielded a conclusion that the changes in estrogen levels influence olfactory ability to an extent that as estrogen levels increase throughout the menstrual cycle or peak during the first trimester of pregnancy, olfactory ability is significantly heightened ($p < .05$). This research has provided evidence supporting the idea that hormone levels do influence olfactory ability fluctuation during the menstrual cycle and in portions of pregnancy.

DEDICATION

To my parents for your unending support and dedication to my education, and to myself for your perpetual to do lists and sacrifices along the way.

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LIST OF ABBREVIATIONS

WUTC, Wheeler-University of Tennessee at Chattanooga

HPLC, High Performance Liquid Chromatography, Reverse-Phase

CHAPTER I

INTRODUCTION

As human beings, perception of the environment is based on the sensory information provided by external stimuli to the five senses. Sight is a product of electromagnetic radiation filtering to the retinas in the eyes. The sense of touch is stimulated by the resulting pressure from physical contact to an object. Fluctuations in air pressure result in perceiving different sounds. The chemical nature in the surroundings informs the senses of taste and smell. These two senses are chemically influenced by the most common environmental component – air. Yet, the research of these two senses, particularly that of olfaction, is comparatively limited.

Beyond the external, internally there are multiple facets that influence our perception of the environment. One of the major influences of bodily function, and therefore a dominant contributor to the perceived world around us is hormones. Hormones, both in type and amount, can and do dictate many changes within the body, possibly even, changes in the ability to smell.

Menstrual Cycle

The possible influence of hormonal state on olfactory function has long been an interest of researchers (Doty, Deems & Stellar, 1988). This interest has led to several studies investigating olfactory perception during natural states of hormonal fluctuation and the menstrual cycle.

Researchers have since studied the menstrual cycle in depth and examined it in a variety of ways. Across various studies the menstrual cycle has been divided into two (e.g., Hertz & Jensen, 1975), three (e.g., Englander-Golden, Change, Whitmore & Dienstbier, 1980), four (e.g., Graham & Sherwin, 1993), five (e.g., Hart, 1960), and six (e.g., Bancroft, Sanders, Davidson & Waller, 1983) phases. In the current study, four phases of the menstrual cycle will be differentiated: follicular phase, ovulation, luteal phase and menses.

During the menstrual cycle the female body undergoes a variety of changes including fluctuations in basal body temperature (Church, Hedricks, LeFevre & McClintock, 1994) and hormone levels (Wallen & Zehr, 2004). In healthy females, gonadal hormone alterations occur in a cyclic fashion, changing from phase to phase throughout the menstrual cycle. The basal body temperature, rising and falling as a result of hormonal fluctuation also follows a cyclic pattern (Church et al., 1994). During the first phase of menstruation, the follicular phase, estrogen exponentially rises to a peak level relative to each individual's baseline. If the basal body temperature changes during the follicular phase, a decrease within half a degree will be measured. The relatively high levels of estrogen cause a surge of two hormones: luteinizing hormone and follicle-stimulating hormone. The spike of these two hormones indicates the second phase, ovulation. Upon ovulation, completion estrogen, luteinizing and follicle-stimulating hormones drop and return to baseline. Through the development of the third phase, the luteal phase, progesterone increases, up to tenfold, and remains consistently high throughout the phase. A rise in estrogen occurs, however, levels do not peak rapidly, nor do they surpass those of progesterone. It is after ovulation, when progesterone is rising, that basal body temperatures tend to increase up to a degree and a half above an individual's baseline. At the

onset of the fourth phase of the menstrual cycle, menses, both estrogen and progesterone levels have dropped back to baseline (Reichman, 2009).

Phase Classification

Studies investigating changes in olfactory perception throughout the menstrual cycle typically contribute the changes observed to the phase of menstruation and/or to hormonal fluctuations. To determine the phase of menstruation investigations have used one of five methods: self-report of menstrual cycle (e.g., Graham & Sherwin, 1993), measurement of basal body temperature (e.g., Watanabe, Umezu & Kurahashi, 2002), characteristics of cervical mucus (e.g., Church et al., 1994), urine analysis to determine levels of luteinizing hormones (e.g., Lundstrom, McClintock & Olsson, 2006), or blood analysis (e.g., O'Leary, Boyne, Flett, Beilby & James, 1991).

Olfactory Abilities

Upon menstrual phase classification researchers have attempted to understand the relationship of the phase of the menstrual cycle and possible changes in olfaction. In general, results determining a relationship are heterogeneous. It is proposed that olfactory sensitivity increases during ovulation and the luteal phase (Doty, Hall, Flickinger & Sondheimer, 1982; Doty, Snyder, Huggins & Lowry, 1981; Le Magnen, 1952; Vierling & Rock, 1967; Mair, Bouffard, Engen & Morton, 1978; Navarrete-Palacio, Hudson, Reyes-Guerrero & Guevara-Guzman, 2003), and decreases during menses (Good, Geary & Engen, 1976; Le Magnen, 1952; Mair et al., 1978; Moriyama & Kurahashi, 2000; Navarrete-Palacios et al., 2003; Schneider & Wolf, 1955). However, there are conflicting studies. Koster (1968) concluded that olfactory

sensitivity improves just before and during menses, while Henkin (1974) found that hypersensitivity occurs only during the follicular phase of the menstrual cycle. Derntl, Schopf, Kollndorfer and Lanzenberger (2012) reported increased olfactory sensitivity to occur only during the luteal phase. Furthermore, several studies have concluded that there are no menstrual cycle-dependent alterations in olfaction (Amoore, 1974; Filsinger & Monte, 1986; Herberhold, Genkin, Brandle, Leitner & Wollmer, 1982; Hummel, Gollisch, Wildt & Kobai, 1991; Hummel, Kobal, Gudziol & Mackay-Sim, 2007; Kanamura & Takashima, 1991).

The Possible Other Use of Oral Contraceptives

Few studies have attempted to directly correlate hormone level fluctuations during the menstrual cycle with olfactory changes. As previously discussed, the determination of a relationship between the phase of the menstrual cycle and changes in olfactory sensitivity is confused and inconclusive. The measurement of hormone levels has served more as a means of phase classification without the continual application and investigation of varying hormone level fluctuations and alterations of olfactory sensitivity. Rather, research has moved towards the investigation of the impact of oral contraceptives.

Oral contraceptives affect the human body in varying ways. Most commonly, oral contraceptives artificially provide heightened hormone levels to keep the body in a stall state. Typically progesterone is the primary component of oral contraceptives. This allows the body to remain in a simulated luteal phase until the hormone levels drop. Some oral contraceptives more closely mimic the body's natural menstrual cycle providing a biphasic hormone administration of estrogen and progesterone.

Caruso, Grillo, Agnello, Maiolino, Intelisann, and Serra (2001) and Doty et al. (1981) compared the changes of olfactory sensitivity of oral contraceptive users and non-users. The oral contraceptive users were taking a monophasic pill intended to mimic the luteal phase by maintaining relatively high levels of progesterone up until the day of ovulation. It was concluded that olfactory sensitivity mimicked that of oral contraceptive non-users (Caruso et al., 2001; Doty et al., 1981), suggesting that changes in olfactory function during the menstrual cycle are a result of something other than fluctuating hormone levels. Lundstrom, McClintock, and Olsson (2006) found that oral contraceptive users experienced greater olfactory sensitivity when inhaling social odors (e.g., androstadienone) and a decrease in sensitivity when inhaling environmental odors (e.g., rose).

The Pregnant Nose

As research has progressed and the relationship between hormones and olfaction has become more complex, the interests have spread to the investigation of olfactory differences during pregnancy, the results of which are, to some extent, contradictory (Laska, Koch, Heid & Hudson, 1996). Overall a majority of pregnant women self-report changes in odor perception during pregnancy (Ochsenbein- Kölbl, von Mering, Zimmermann & Hummel, 2007; Wohlgemuth, Beinder, Ochsenbein-Kölbl & Hummel, 2008). While considerable anecdotal evidence suggests that pregnant women, when compared to non-pregnant women, are more sensitive to odors, objective measurement of this sensitivity is limited and inconclusive. Some studies have presented evidence suggesting hypersensitivity during pregnancy (Broman, Olofsson, Olsson & Nordin, 2003; Lundström, McClintock & Olsson, 2006; Nordin, Broman, Bringlöv & Wulff, 2004). Yet other studies have concluded that during pregnancy there is no

olfactory hypersensitivity (Laska & Teubner, 1999; Swallow et al., 2005). Furthermore, some studies have found hyposensitivity to pervade during pregnancy, while a few have reported the occurrence of anosmia (Cameron, 2007).

Various studies reporting a change in olfactory sensitivity differ concerning the description of the time of maximum effect (Laska et al., 1996). Le Magnen (1952) concludes that pregnant women experience an increase in odor sensitivity during the first trimester, while Good, Geary and Engen (1976) stipulate hypersensitivity to occur predominantly throughout the second and third trimesters. Very few studies have emphasized or even included the third trimester of pregnancy during investigation (Ochsenbein-Kölble et al., 2007). Of these, some investigators report a decreased sensitivity later in pregnancy when compared to non-pregnant controls (e.g. Laska et al., 1996; Wohlgemuth et al., 2008), while Nordin, Broman, Bringlöv and Wulff (2007) specify that changes in olfactory sensitivity rarely occur late in pregnancy.

Odor Sensitivity

It should be noted that the aforementioned studies differ in the operational definition of odorant sensitivity. For example, Nordin et al. (2004) write of sensitivity in relation to odor identification, whereas Laska et al. (1996) discuss sensitivity in terms of ability to sense the presence of any odor. The current study refers to sensitivity as the latter. Before a certain threshold there should be no odorant detection. Above its threshold, the perception of an odor increases in magnitude relative to the increase of odor concentration (Walker, Hall, Walker, Kendal-Reed, Hood & Niu, 2003).

Evolution Could Be the Key

The potential changes in olfaction sensitivity during pregnancy could be due to strengthened hormonally modulated connections between the limbic system and the olfactory system (Cameron, 2007; Wohlgemuth et al., 2008), or this resulting effect could be in response to changes in the cognitive information processing of odorants (Kölble, Hummel, von Mering, Huch & Huch, 2001; Zou, Li & Buck, 2005). Cameron (2007) postulates that the foundation for odor detection may be more drastic during pregnancy. That is to say that a small change in odor concentration that may go unnoticed by non-pregnant women might result in a large change in odor intensity perception for pregnant women (Cameron, 2007). This enhanced odor sensitivity could provide an evolutionary advantage, discouraging pregnant women from ingesting potentially noxious substances that could be harmful to a developing fetus (Heinrichs, 2002; Profet, 1992) or enriching the perceived attractiveness of a pregnant woman's mate (Wohlgemuth et al., 2008).

Evolutionarily female fertility, in large part dictated by the endocrine system's regulation of gonadal hormones, has evolved to emphasize certain hormonal phases (Wallen & Zehr, 2004). During a normal, healthy pregnancy this regulation tends to develop in a uniform manner. Studies have shown an increase in a multitude of hormones in varying amounts throughout pregnancy. These hormones include estradiol, progesterone, testosterone, sex-binding-hormone globulin, androstenedione and 17-hydroxyprogesterone. A decrease in dehydroepiandrosterone sulfate has also been reported (O'Leary et al., 1991).

Neglected but Not Forgotten

The research conducted thus far examining the pregnant population has neglected the consideration or the empirical measure of hormonal changes experienced by pregnant females and how said changes influence olfaction. Hormonal fluctuations seemingly dictate much of the olfactory perception change during the menstrual cycle. The hormone changes that occur during pregnancy could exhibit the same type of influence over the olfactory system.

There is certain, albeit not entirely conclusive, support for the idea that pregnant women perceive some odorous substances as stronger than do non-pregnant women (Nordin et al., 2004). Also, as mentioned previously, Lundstrom et al. (2006) discussed evidence for women taking oral contraceptives sense some odorants more readily than others.

The Present Study

The literature is suggestive but contradictory and inconclusive concerning the effects of hormones on olfactory perception, particularly odorant sensitivity. The present study has been designed to examine this effect based on specific odorants and measured olfactory ability.

Odorants

In the current study, four odorants will be used – vanillin, caraway, spearmint and muscone – to assess olfactory ability. The odors were selected based on the diverse properties they possess. The odors differ by their molecular classifications as well as in their perceived qualities (e.g., sweet, pungent). The chemical formula and structure can be found in Appendix A.

Muscone is a non-polar, 15-carbon ring with a ketone functional group. Muscone is a territorial pheromone in musk deer (Jacob, Garcia, Hayreh, & McClintock, 2002), and has an

odor similar to sweet, pungent fresh earth. The chemical structure of non-polar compounds allows for the odorant to diffuse across the mucus membrane in the nose without the direct aid of olfactory binding proteins. In addition, muscone has been chosen because, even in high concentrations, it does not activate the trigeminal nerve (Jacob et al., 2002). Avoiding activation of the trigeminal nerve ensures that only odorant sensitivity is assessed (Doty, 2001).

Vanillin, unlike muscone, is a polar molecule used in commercial products throughout the United States. Vanillin exudes a characteristic pleasant, sweet aroma. It is also one of the first odors to be recognized and preferred by infants (Edraki, Pourpulad, Kargar, Pishva, Zare & Montaseri, 2013). Polar molecules require olfactory binding proteins to transport them across the mucus membrane. If hormone levels affect the availability or binding process of the olfactory binding proteins or olfactory binding receptors, examining the effects of both a polar and non-polar molecules has the potential to lend insight.

Caraway and spearmint each contain an optical isomer, or enantiomer, carvone. The enantiomers of carvone share an isopropenyl group at the chiral carbon, allowing for this enantiomer to differ in odor quality (Bentley, 2006; Laska, 2004). Receptors in the body are stereoselective and, as a result, typically react with only one of the compounds of an enantiomer pair. Each enantiomer, due to three dimensional molecule arrangement differences, binds to the odor receptors in a different and specific way, hence the different perception of spearmint and caraway odors. R-(-)-carvone is perceived to have the fresh, minty odor quality synonymous with spearmint, while the S-(+)-carvone odor is caraway and dill-like with a peppery undertone. Carvone enantiomeric odor pairs have also been shown to not be mediated by the activation of the trigeminal nerve (Laska & Teubner, 1999).

The chirality of these enantiomers is an aspect of olfaction unexamined in the pregnant population or within the context of the menstrual cycle. The perception of different odorant properties for these two enantiomers lends support toward olfactory receptors containing chiral groups that allow for a stronger response to one enantiomer compared to the other.

Hypotheses

The present study has been designed to examine the effect of hormones on the olfactory abilities of women on four levels by using the four aforementioned odorants. The first level examines olfactory perception across all trimesters of pregnancy, and the second is a comparison of olfactory ability between two groups: pregnant and non-pregnant women. The third and fourth levels of investigation are a comparison of the differences of odorant perception during the menstrual cycle and during pregnancy. The hypotheses are as follows:

Hypothesis 1: Women will experience higher levels of olfactory sensitivity and have lower threshold levels for all odorants as a result of increased estrogen levels.

Hypothesis 2: Overall, oral contraceptive users will have greater olfactory sensitivity and lower thresholds while taking the active hormone pill when compared to oral contraceptive users in menses.

Hypothesis 3: Olfactory abilities will decrease throughout pregnancy.

3a. Odorant thresholds will increase throughout pregnancy; therefore those at the beginning of pregnancy will have lower odorant thresholds than those toward the end of pregnancy.

3b. Olfactory sensitivity will decrease throughout pregnancy; therefore olfactory sensitivity will be higher at the beginning of pregnancy than toward the end of pregnancy.

Hypothesis 4: Pregnant women will have a greater olfactory sensitivity, thus a lower threshold, when compared to non-pregnant controls.

CHAPTER II

METHOD

The current study was conducted at two different time measurements. Assessment one data was collected in late November, and assessment two data was collected in the middle of December.

Participants

During part one of the assessment, 72 non-pregnant, female participants ages 18 to 43 years old ($M=20.11$, $SD=3.388$) and seven pregnant participants ages 29 to 41 ($M=34.86$, $SD=4.488$) were recruited from the University of Tennessee at Chattanooga campus and a medium size obstetrics and gynecology clinic in Chattanooga, Tennessee. Part two of the experiment received a total of 62 of the original 72 non-pregnant participants ages 18-41 ($M=20.21$, $SD=3.599$) and all seven of the pregnant participants returning for assessment. An overview of where each pregnant participant self-reported to be within pregnancy can be found in Table 2.1. Also, a summary of self-reported ethnicities of non-pregnant participants can be found in Table 2.2. It should be noted that each pregnant participant self-reported as Caucasian.

Table 2.1 Pregnancy Trimester During Assessment

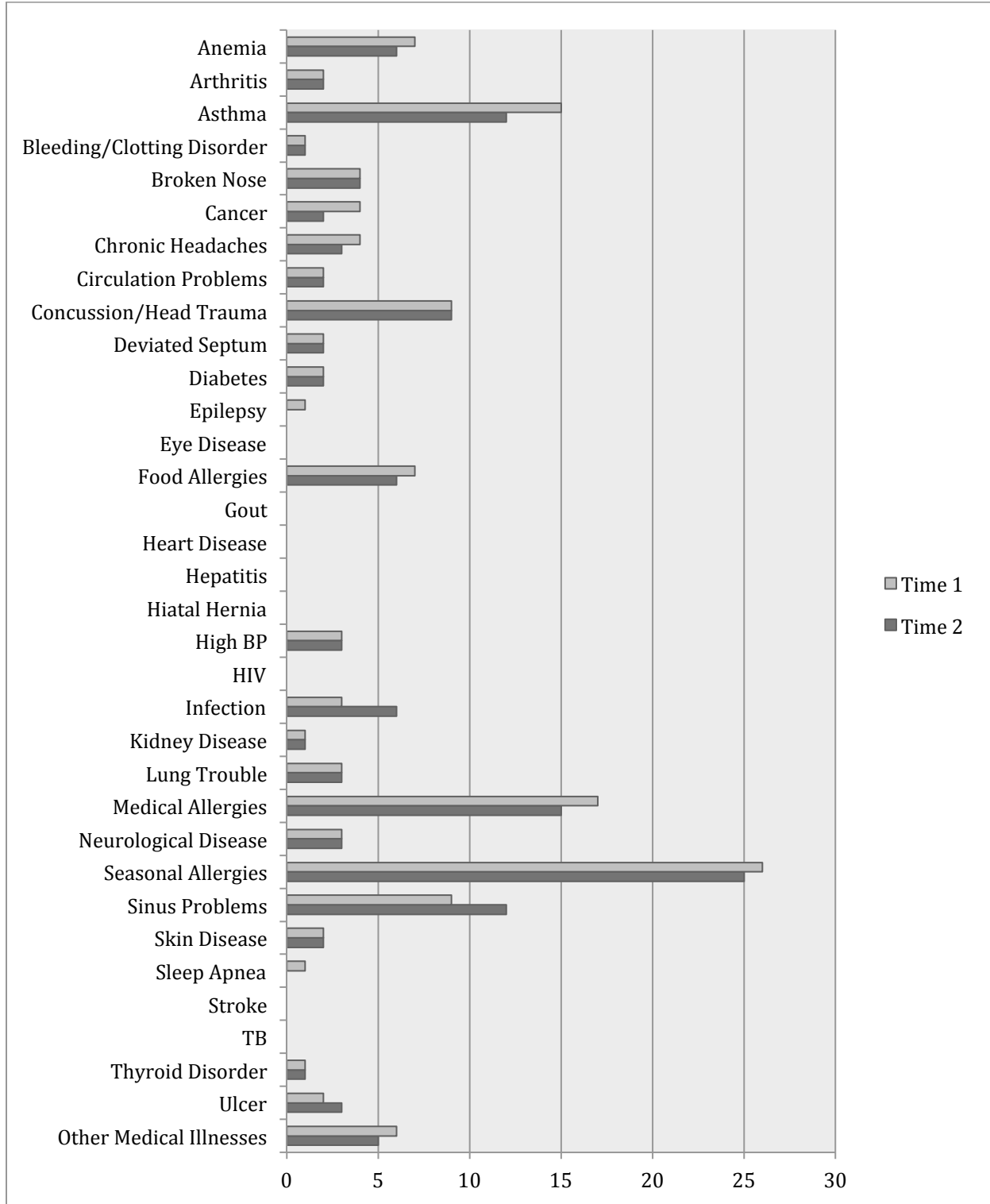
| Participant | Trimester at Assessment 1 | Trimester at Assessment 2 |
|--------------------|----------------------------------|----------------------------------|
| 1 | 1 | 1 |
| 2 | 1 | 2 |
| 3 | 1 | 2 |
| 4 | 2 | 3 |
| 5 | 2 | 3 |
| 6 | 2 | 3 |
| 7 | 3 | 3 |

Table 2.2 Ethnicity of Non-Pregnant Participants at Assessment One and Two

| Ethnicity | Assessment 1 | | Assessment 2 | |
|-------------------------|---------------------|---------|---------------------|---------|
| | Frequency | Percent | Frequency | Percent |
| Caucasian | 51 | 70.83 | 44 | 70.97 |
| African American | 10 | 13.89 | 9 | 14.52 |
| Bi-Racial | 5 | 6.94 | 4 | 6.45 |
| Latina | 3 | 4.17 | 2 | 3.22 |
| Middle Eastern | 2 | 2.78 | 2 | 3.22 |
| Asian | 1 | 1.38 | 1 | 1.61 |

Participants were asked to complete a demographic questionnaire (Appendix B) to ascertain details about past and current health at both assessment one and two. Questions concerned such things as smoking habits, past and current diseases and disorders, medications, menstruation and pregnancy history. A depiction of this data is shown in Table 2.2 for both part one and part two of the experiment. Upon completion of the second assessment, participants were given a non-transferrable, fifteen-dollar gift card from a local business.

Table 2.3 Health History Data for Non-Pregnant Participants at Assessment One and Two



Review Board

The current study has been approved by the University of Tennessee at Chattanooga Institutional Review Board (Appendix C). In compliance with this approval all data, including blood analysis results, threshold data and demographic information were kept confidential and stored in an encrypted data file.

Protection of Health Information

Prior to beginning data collection, all researches involved were required to complete HIPPA training and become HIPPA certified. Also, researchers were required to complete NIH and CITI certifications before having access to participant information collected during assessment.

Materials and Procedures

Modification of the WUTC

The original Wheeler-University of Tennessee at Chattanooga (WUTC) threshold test (Tewalt, 2013) contained five odorants: ethanol, L- α -pinene, vanillin, isoamyl acetate, and p-cresol. For the current study, an odorant modified WUTC threshold test was used. Four distinct odorants were selected in the modification of the WUTC threshold test: vanillin, R-(-)-carvone, S-(+)-carvone, and muscone. Each of these odorants was selected for a distinct reason as described previously.

Odorant Dilutions

In making the modified WUTC threshold test, each odorant molecule was dissolved, based on each molecule's individual solubility, in a purified water solvent to create a maximum concentration standard dilution that appeared to be visually translucent and clear. A 1:3 geometric serial dilution of the standard solution was used to create a total of nine concentrations for each odorant. The dilutions were made to allow for the middle odorant tube to contain a dilution equivalent to that of reported threshold detection norms. Ten milliliters of each dilution were contained in a sterilized glass vial. Appearance of the liquid, regardless of odorant or concentration, remained translucent and clear in nature. In total, the WUTC threshold test contained nine varying concentrations of each of the four odorants for a total of thirty-six odorant vials. In addition, nine non-odorous, "blank" vials were included. Blanks were made using ten milliliters of purified water. The final, modified version of the WUTC threshold test contained forty-five separate vials of varying concentrations of four odorants and blanks.

Threshold Test Administration

During parts one and two, participants completed the WUTC threshold test. At each presentation of the threshold test, two researchers were present. Because carry over effects from one assessment to the next can be an issue when assessing participants in a longitudinal fashion, participants were informed that the tubes would be presented in a random order at each test administration. Participants were also told that some tubes could have detectable odorants, some could have odorants that might not be potent enough to perceive, and some could have no odorant present. Once participants had read through and signed an informed consent specific to the threshold test (Appendix D), they were asked to sit, facing away from the researchers.

Participants were randomly presented with 10mL of each dilution for a total of 5 seconds at a distance of about 2 cm below the participant's nose by a researcher using a test tube holder. Upon retraction of the odorant dilution the participant was allowed 10 seconds to indicate, by stating "yes" or "no", if an odor had been detected. Out of the line of sight of the participant, a second researcher recorded the indications appropriately. This second researcher was also responsible for preparing the next tube in the randomized order for the administering researcher. In this way the administration of the test tubes was double blind. The test was considered complete when the participant had given a response for all odorant dilutions and blanks.

Blood Collection

Prior to completing the modified WUTC threshold test, blood was drawn from each participant during both parts of the experiment for the purpose of ascertaining estrogen hormone levels at the time of participation. Blood Assurance of Chattanooga agreed to draw 10 milliliters of blood from each participant during parts one and two of the experiment. In cooperation with Blood Assurance of Chattanooga, all non-pregnant participants were asked to attempt to donate blood prior to the draw of the first required 10 milliliter sample, and each participant was required to complete two informed consent forms, one specific to the 10 milliliters vial blood draw as a part of the study (Appendix E) and one provided by the staff at Blood Assurance of Chattanooga (Appendix F).

Blood withdrawals followed the standardized procedures set by Blood Assurance. After the 10 milliliters of blood was drawn into a sealed, sterilized vial, each vial was labeled with a six digit serial number. This vial number was then transferred to a master document and to the participants' specified threshold test response sheet for the purposes of linking any results

obtained from the blood analysis to individual threshold data. Once the blood was obtained from each participant for part one of the experiment it was stored at a constant temperature of -80 degrees Celsius for optimal preservation.

Three weeks after the completion of the first part of the experiment participants completed the second portion of the research. This portion of the study mimicked the material and procedures of part one, with the exception that no one was asked to donate blood prior to their blood draw. After 10 milliliters of blood was obtained from each participant, each vial was labeled with the same six digit serial number assigned to the participants during their participation in part one as recorded on the master list. To identify which blood sample was drawn first and which second for any given participant an 11 or 12 was recorded on the first and second blood draw vials respectively. Once serial numbers were confirmed to match the master list linking participants' names to the blood vial, the master list of serial numbers was destroyed to protect participant confidentiality.

A total of 148 blood samples (10 milliliters) were stored in a freezer at the University of Tennessee at Chattanooga in the biochemistry lab.

Blood Analysis

The blood samples were analyzed through multiple procedures in randomized batches. Four days prior to the start of blood analysis vials were selected from the freezer and placed in a refrigerator. This allowed for the blood samples to slowly thaw to a temperature of 4 degrees Celsius. Once thawed one milliliter aliquots of each sample were drawn from each vial using a single, sterilized syringe and needle. The samples were subjected, six at a time, to ultracentrifugation in a Centrifree Micropartition device with a 30,000 Dalton cut-off membrane

at 2000 xg for 30 minutes. The filtrate samples were then diluted with purified methanol (CH₃OH) to make a one milliliter mixture. Each diluted filtrate sample was then labeled with a matched, six digit serial number and stored at 25 degrees Celsius prior to further analysis.

Standard solutions were made to contain single molecules of estradiol in varying concentrations with methanol in order to verify the detection of estradiol molecules based on determined retention time and corresponding peak height and area. After confirming detection criteria the refrigerated filtrate mixtures were analyzed using a Reversed-Phase High Performance Liquid Chromatography (HPLC) to separate the molecular components in the mixture. The instrument was equipped with a stationary phase 250-millimeter x 4.6-millimeter Porshell II (void volume of 1.5 milliliters); the mobile phase buffer used in separation was HPLC-grade methanol. A five minute column pre-clean, using methanol, was performed prior to each set of analyses. Also, the syringe was washed with methanol and HPLC-grade water prior to each analysis to avoid cross-contamination.

The 15 microliter injection of each filtrate mixture was loaded onto the column and subjected to elution for 5 minutes at a flow rate of 1 milliliter/minute by the methanol buffer. Component molecules of the filtrate mixture were detected by ultraviolet absorbance using a deuterium lamp at wavelengths of 280 nanometers – the wavelength at which aromatic proteins (e.g., estradiol) typically absorb. Because proteins also absorb at 214 nanometers, and organic compounds, like estradiol, absorb at 254 nanometers, each of these wavelengths was also observed. No significant differences were observed in the elution chromatograms at 214 or 254 nanometers compared to the detection profile at 280 nanometers.

CHAPTER III

RESULTS

Analysis

Blood Analysis

To determine levels of estrogen in the blood of participants at the time of completing the threshold test, samples were analyzed with an HPLC. Retention times and peaks at 280 nanometers for each sample were compared to that of the standard solutions also analyzed during the same time period. The retention time as well as the peak areas recorded varied analysis to analysis, requiring the standard solutions to be measured during each sample batch analysis. This variation can be due to factors such as ambient pressure and temperature and fluctuating pressure within the HPLC.

The analysis of these five standard solutions composed of varying levels of estradiol resulted in five peak areas from similar retention times in the HPLC output (Figure 3.1).

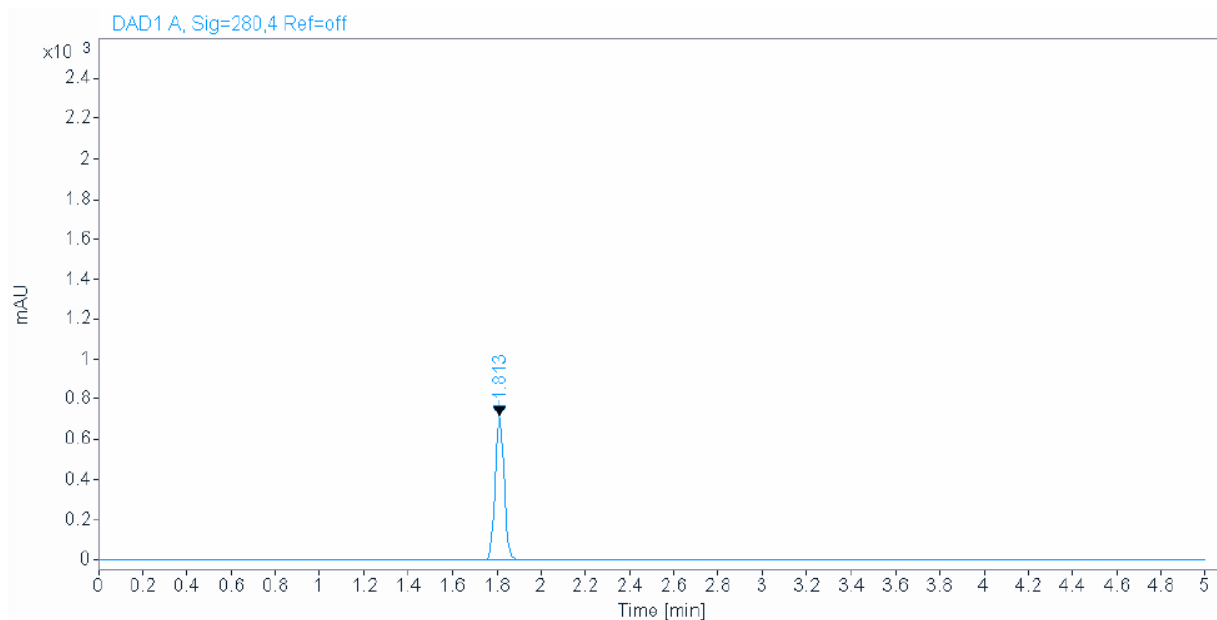


Figure 3.1 Example of HPLC Output Data

The correlation of concentration to peak area resulted in Pearson r values indicating a strong, positive relationship each time of blood analysis (Table 3.1). A scatterplot of concentration versus peak area was created for each measurement of the standard solutions, from which a prediction equation was determined.

Table 3.1 Relationship of Standard Solution Concentration to Peak Area

| Blood Analysis | Pearson r | Significance Level (p) |
|----------------|-----------|------------------------|
| Measurement 1 | .987 | .006 |
| Measurement 2 | .994 | .001 |
| Measurement 3 | .999 | <.001 |
| Measurement 4 | .997 | <.001 |
| Measurement 5 | .996 | <.001 |

Retention times within one tenth of a minute to that of the estradiol standard solutions were considered to be indicative of estrogen within the sample. These retention times and the corresponding peak areas were recorded. Each sample resulted in one peak area number that was then plugged into the equation determined from the standard solutions. This raw concentration value was then multiplied by the dilution factor by which each sample was specifically made. This final value is the estimated estrogen concentration for the analyzed blood sample.

Olfactory Ability Analysis

The modified WUTC threshold test was evaluated using multiple statistical tests. In total the threshold test is able to describe each participant's and group's olfactory ability in three distinct ways for each of the four odorants. These measures include threshold, sensitivity and response bias.

An estimated odorant threshold of each of the odors, vanillin, caraway, spearmint and muscone was obtained. To determine threshold values the "yes" and "no" responses were analyzed using logistic regression. The predicted values generated from the logistic regression were then graphically represented and the estimated threshold value was determined to be the odorant concentration value that corresponded to a p-value of 0.5 on the sigmoid curve.

To measure olfactory sensitivity the d' statistic was calculated. The d' statistic allowed for a representation of the differences in sensitivity to different odorants. This value is calculated by determining the difference between the z-scores of the hit and false alarm rates. To be considered a "hit" an individual must indicate a "yes" during the threshold test when a vial with an odorant concentration was presented. Inversely, a "false alarm" results when an individual

responds “yes” when a vial with no odorant is presented. The d' value was calculated by the following equation (Macmillan, 1993):

$$d' = z(H) - z(F)$$

The values for the d' statistic range from zero to 4.65 (Tewalt, 2013). The lower the value, the lower the sensitivity to an odorant, and the higher the value, the more sensitive to an odorant a participant is thought to be. Sensitivity was calculated for each participant for each of the four odorants of the threshold test. Their overall sensitivity to all four odorants was also calculated. Therefore, for one administration each participant produced a total of five sensitivity values.

As a way to measure the tendency of a person to respond to the presentation of a tube with either a yes or a no, regardless of whether an odorant was presented or not, response bias was calculated. The B'' value is defined by the equation (Stanislaw & Todorov, 1999):

$$B'' = \text{sign}(H - F) \frac{H(1 - H) - F(1 - F)}{H(1 - H) + F(1 - F)}$$

B'' was calculated as a measure of response bias rather than the standard response bias measure β due to B'' independence to the changes in d' (Stanislaw & Todorov, 1999). A measurement of response bias was calculated for each participant for both assessments.

To determine respondent reliability, each participant’s response data was split into first and second administrations for each odor. The reliability statistic Cohen’s kappa was then computed to determine the reliability of each participant’s dichotomous, “yes”/”no” responses. Additionally, the reliability for each odorant was calculated.

Demographics collected from each participant were analyzed for the existence of relationships with all calculated measures.

Statistical Results

Estrogen Concentration and Olfactory Ability

In order to assess the relationship of estrogen levels (Table 3.2) to olfactory sensitivity and estimated threshold values linear regressions were employed for each odorant. No significant differences were found for sensitivity levels for individual odorants. However, there was a trend for estrogen levels and overall olfactory sensitivity was determined ($t = 2.085, p = .074$). Two specific odorant thresholds were determined to be significantly related to and predicted by estrogen levels in the blood. Spearmint was found to have a moderately strong negative relationship with estrogen level ($r = -.386, p = .001$). A significant result of $t = -3.359, p = .001$ was also found. A second odorant, muscone, was determined to also have a moderately strong negative relationship ($r = -.318, p = .001$) with estrogen levels significantly predicting the threshold levels of both pregnant and non-pregnant participants ($t = -3.162, p = .002$).

Table 3.2 Estrogen Concentration Levels

| | Minimum (mg/mL) | Maximum (mg/mL) | Mean (mg/mL) | Std. Dev. (mg/mL) |
|--------------------------------------|--------------------|--------------------|-----------------|----------------------|
| Estrogen Concentration Levels | .01 | 21.24 | 4.28 | 4.56 |

Due to lack of significance in the relationship of individual odorant sensitivity and estrogen levels, response bias was taken into account. Firstly, it was found that response biases are moderate to strongly related ($r = .623, p < .001$) to sensitivity with a significantly predictive relationship ($t = 9.141, p < .001$). Response bias per odorant and overall was assessed in comparison with estrogen levels. Interestingly results indicate a significant, moderately strong relationship between estrogen concentrations in the blood and overall response bias

($r = -.404, p < .001$), vanillin response bias ($r = -.307, p = .002$), caraway response bias ($r = -.401, p < .001$), spearmint response bias ($r = .348, p = .001$), and muscone response bias ($r = .304, p < .001$). Thus, when a participant has a response bias to “yes” they tend to also have relatively elevated blood estrogen levels.

Reliability between each threshold administration test half was assessed with the use of Cohen’s kappa. The results of the analyses are presented in Table 3.3.

Table 3.3 Reliability Measure between Test Halves (Cohen’s kappa)

| Odorant | Threshold |
|------------------|------------------|
| Vanillin | 0.727 |
| Caraway | 0.301 |
| Spearmint | 0.643 |
| Muscone | 0.701 |

Oral Contraceptive and Olfaction

A paired sample t-test was used to assess the differences of olfactory ability between participants while taking the active hormone pill of their oral contraceptive and while in menses (i.e., taking the inactive hormone pill). A total of 17 women (27.4%) of the 62 that participated in both assessments were taking oral contraceptives. To be included in analysis the women self-reported being in menses during one of the two assessment times and not in menses during the other. No significant differences were found between the mean threshold values or sensitivities for individual odorants, nor of overall odorant sensitivity.

Olfactory Abilities during Pregnancy

Changes in olfactory ability throughout pregnancy were analyzed with a one-way within-subjects analysis of variance. The means and standard deviations for sensitivity and estimated threshold values are presented in Table 3.3 and Table 3.4 respectively. Vanillin sensitivity ($F(1, 6) = 5.282, p = .049$) with a partial eta of 0.568, vanillin threshold ($F(1, 6) = 6.274, p = .046$) with a partial eta of 0.611, and muscone threshold ($F(1, 6) = 6.681, p = .042$) with a partial eta of 0.627 all significantly changed as women progressed in their pregnancies. This can be seen in Figures 3.2, 3.3, and 3.4. A trending significance was found for spearmint sensitivity ($F(1, 6) = 4.16, p = .078$), which can be seen in Figure 3.5. It should be noted that there is little difference between the mean values of the second and third trimesters. This suggests that olfactory abilities are better in early pregnancy.

Table 3.4 Assessment One and Two Olfactory Sensitivities

| Measure | Assessment 1 | | Assessment 2 | |
|------------------|--------------|-----------|--------------|-----------|
| | Mean | Std. Dev. | Mean | Std. Dev. |
| Overall | 1.16 | 1.14 | .95 | 1.18 |
| Vanillin | 2.19 | 1.61 | 1.17 | 1.06 |
| Caraway | .77 | .53 | .70 | .45 |
| Spearmint | .75 | .56 | .43 | .63 |
| Muscone | 1.09 | 1.19 | .97 | 1.24 |

Table 3.5 Assessment One and Two Estimated Threshold Values

| Measure | Assessment 1 | | Assessment 2 | |
|------------------|--------------|-----------|--------------|-----------|
| | M | Std. Dev. | M | Std. Dev. |
| Vanillin | 2.97 | 2.75 | 10.61 | 7.23 |
| Caraway | 237.94 | 299.63 | 727.39 | 1064.05 |
| Spearmint | 671.46 | 1144.98 | 1122.33 | 1483.54 |
| Muscone | 366.34 | 530.64 | 883.71 | 890.73 |

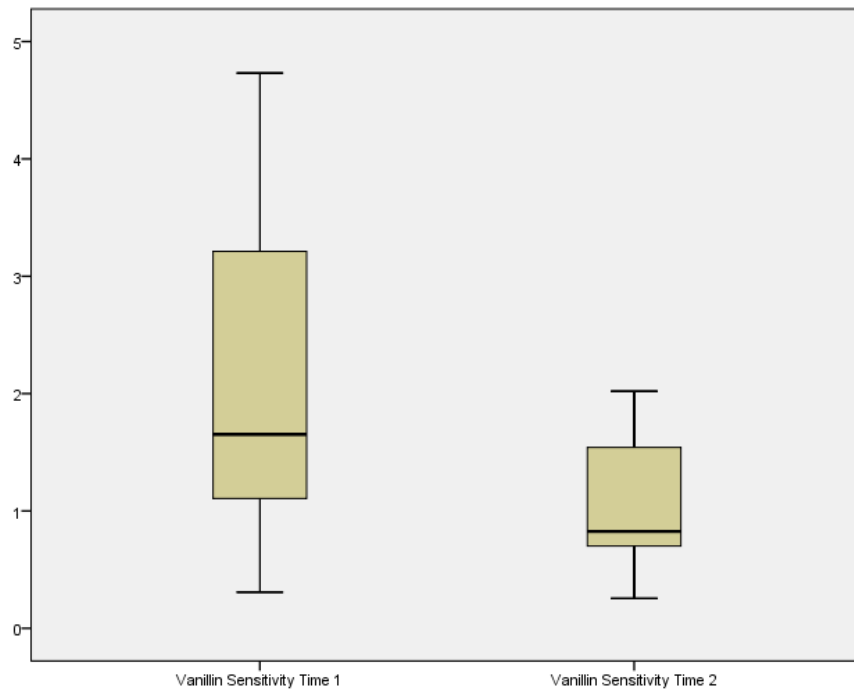


Figure 3.2 Vanillin Sensitivity During Pregnancy

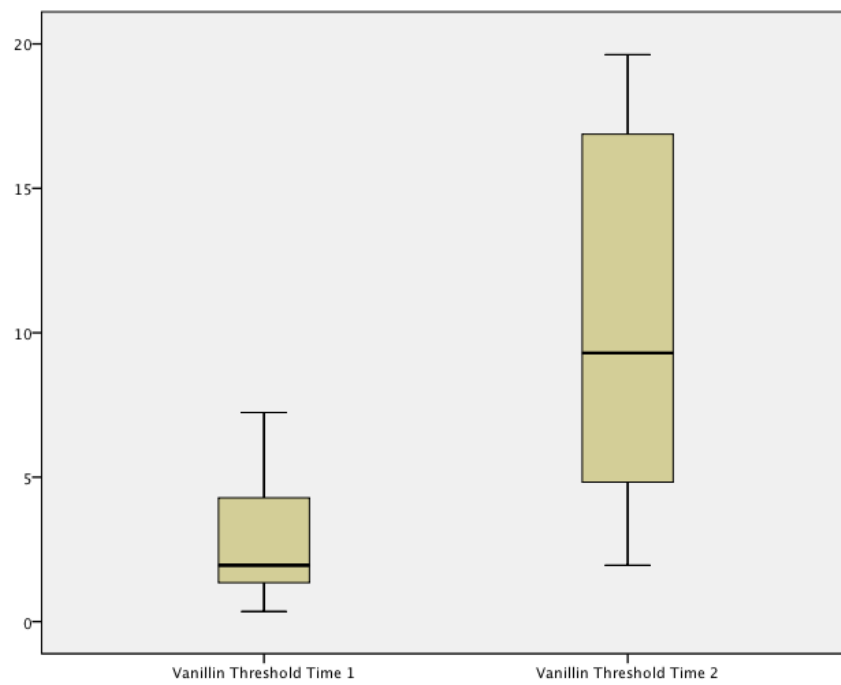


Figure 3.3 Estimated Vanillin Threshold Values During Pregnancy

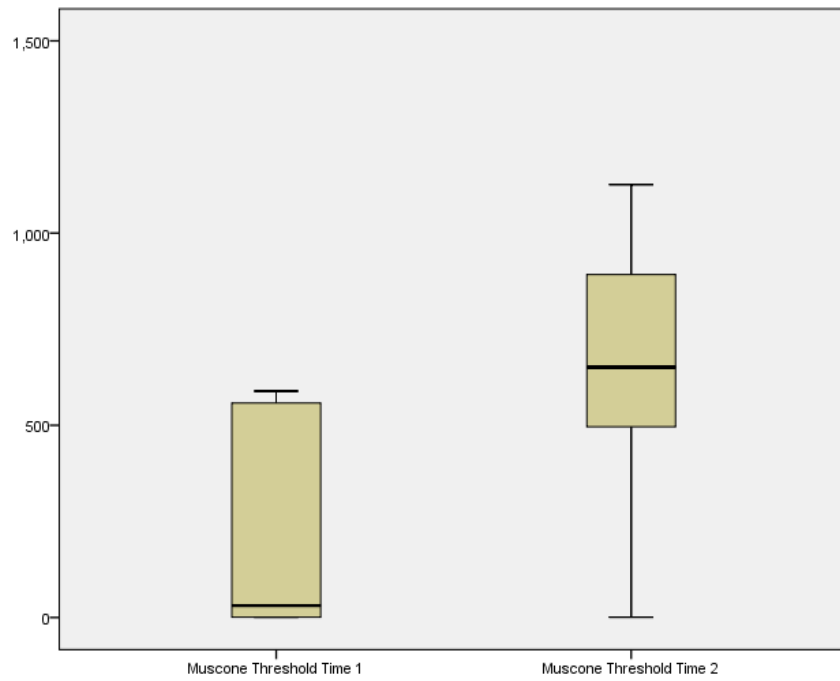


Figure 3.4 Estimated Muscone Threshold Values During Pregnancy

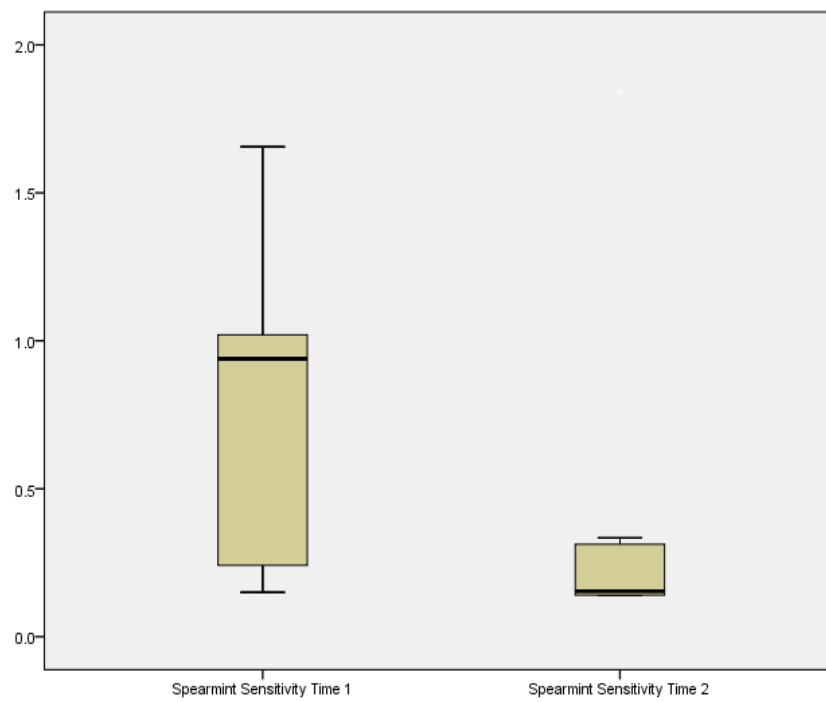


Figure 3.5 Spearmint Sensitivity During Pregnancy

Pregnant Versus Non-Pregnant

An analysis was conducted to determine if olfactory ability was differed for pregnant and non-pregnant individuals. Results indicate that there is a significant difference in the threshold levels of spearmint, with pregnant women having a lower mean estimated threshold ($t = 2.097$, $p = .006$). This difference is also noted with caraway thresholds ($t = 2.029$, $p = .044$). To examine possible explanations a comparison of estrogen levels was made. Overall estrogen levels seem to be similar during pregnancy to the levels experienced by the women on their menstrual cycle, with one exception being that women in the first trimester of pregnancy have slightly higher estrogen levels than non-pregnant participants ($t = 1.981$, $p = .098$)

Demographic Relations

Demographic data were also analyzed to determine if any significant correlations existed between the self-report measure and the olfactory ability measures of sensitivity, threshold and response bias. Caraway sensitivity was found to be weakly related to instances of self-reported chronic headaches ($r = -.249$, $p = .036$) and high blood pressure ($r = -.291$, $p = .037$). Muscone sensitivity was moderately related to a previous history of cancer ($r = -.496$, $p = .001$). Interestingly spearmint sensitivity is the only odorant sensitivity related to self-reported nose problems, including having a broken nose ($r = -.315$, $p = .013$) and a deviated septum ($r = -.281$, $p = .026$). Each relationship discovered with sensitivity is negative in nature; that is to say that sensitivity has a trend of being lower in those individuals that are currently or have previously experienced the aforementioned health related issues.

Overall response bias and response bias for each individual odorant was noted to be significant, but weakly relate ($p < .05$) to a health history of cancer. Individuals that were taking antihistamines at the time of assessment, as well as those that were experiencing some type of infection, including sinus infections, were noted to have moderately strong response bias overall and for each of the four odorants at a significance of $p < .05$ (Table 3.6)

Table 3.6 Response Bias and Infection

| Response Bias and Infection | Pearson <i>r</i> | p value |
|------------------------------------|-------------------------|----------------|
| Vanillin | .363 | .031 |
| Caraway | .301 | .020 |
| Muscone | .380 | .037 |
| Spearmint | .376 | .042 |
| Overall | .387 | .031 |

Alternatively, estimated thresholds were compared to self-reported infections with spearmint threshold. These threshold levels tended to increase, mean ability to smell decreased, with reported infections. When comparing threshold levels to medications taken at the time of assessment, muscone threshold levels were moderately, positively related to taking antidepressant ($r = .335, p = .004$) and antianxiety ($r = .347, p = .007$) medications.

CHAPTER IV

DISCUSSION

The purpose of the current study was to investigate the relationship of estrogen levels on the olfactory abilities of women. In an attempt to comprehensively understand this possible relationship, two populations were examined: pregnant and non-pregnant women. Each of these populations experiences characteristic changes in hormone levels, particularly estrogen, either during menstruation in non-pregnant women or throughout three trimesters in pregnant participants. In general, it was found that estrogen levels do, in some fashion, influence olfactory abilities.

Olfactory ability, as measured by odorant sensitivity, estimated odorant threshold values and response bias, was seen to change as estrogen levels fluctuated. In fact, a trend of overall olfactory sensitivity increasing as estrogen levels increased was noted. Given that further distinctions were found, this expresses that a woman's ability to sense the presence of an odorant is, in part, influenced by her hormone levels. To understand what other factors could influence sensitivity, response bias, was examined for each participant. As expected, response bias influences an individual's measured sensitivity. Women with a response bias towards "yes" tended to have higher reported olfactory sensitivities, while those with a response bias towards "no" reported a lower sensitivity, or ability, to detect odors.

Potential influences of response bias were examined, and, surprisingly, it was found that estrogen levels could play a key role. In fact, individual and overall odorant response biases were

found to have a strong relationship with estrogen levels. This could be a revealing aspect of hormonal influences on factors outside the specified purview of the olfactory system. As estrogen levels fluctuate, response biases countered this trend. That is to say that as a woman's estrogen levels increased, her response bias decreased. If response bias does not remain the same for an individual, it is possible that response bias is a mediating factor of estrogen levels and olfactory sensitivity.

Two odorant thresholds, spearmint and muscone, were found to decrease as estrogen levels increased. This indicates that as more estrogen circulates through the body, like during the follicular and luteal phases of menstruation and during the first trimester of pregnancy, a woman's ability to smell spearmint and muscone is elevated. Due to chiral recognition that occurs with stereoselective receptors, it is not surprising that one of the enantiomer pairs, spearmint, is influenced by estrogen levels while caraway seemingly is not. Estrogen levels are thought to alter the availability of olfactory binding proteins and receptors, but how and in what magnitude these alterations occur is yet unknown. Results indicate that a woman's ability to detect spearmint increases as estrogen levels increase. This could intimate a relationship of estrogen level influence on specific, stereoselective olfactory binding proteins or receptors.

Muscone, being a non-polar molecule, diffuses across the mucus membrane without the assistance of olfactory binding proteins. While changes in hormone levels have been shown to influence the mucus membrane of the uterus, little research has been conducted to investigate if the mucus membrane of the nasal cavity thins and thickens with any regularity. If the thickness of the membrane is not a probable cause of the improvement of a woman's ability to detect muscone as estrogen levels increase, then, from an evolutionary perspective, the ability to smell a social odor, like musk, could be a result of a woman's body preparing for the optimal

fertilization period. Lundstrom, McClintock, and Olsson (2006) found that oral contraceptive users experienced greater olfactory sensitivity when inhaling social odors. Despite there being no relationship between oral contraceptive use and olfactory ability in the current study, it is thought that the relatively high levels of estrogen could have influenced the distinction between social and environmental odors, and therefore resulted in the decreased olfactory thresholds for muscone while estrogen levels were relatively high.

Wohlgemuth, et. al. (2008) found that pregnant women self-reported heightened olfactory sensitivity to multiple odors, including those prominently composed of musk-like components. Little research has been conducted to examine if an objective change in olfactory ability occurs once pregnant, or if it is simply a perceived self-report phenomenon. In the current study it was found that, in fact, there is a difference in olfactory ability as measured by odorant sensitivity and estimated threshold values, for some odorants, between pregnant and non-pregnant participants. In general, it was determined that estrogen levels of the women in their first trimester of pregnancy superseded those of non-pregnant women. These higher level of estrogen indicate the expected lower mean thresholds of spearmint and caraway in the pregnant participants.

Self-report changes in olfactory perception show pregnant women reporting several changes, many of which relate to the odor qualities of spearmint and caraway. The odor of minty toothpaste is one of the most commonly reported stimulant causes of morning sickness during pregnancy (Wohlgemuth, et. al., 2008). Also, pungent and mildew-like odors have been reported to smell more strongly and perceived as more negative in pregnant populations (Ochsenbein-Kölble, et. al., 2007). These self-reported heightened sensitivities to mint and stale type odors could in fact be due to a measureable difference in a pregnant women's ability to smell.

It has been postulated that olfactory ability might be improved for toxic or harmful odors, especially during the first trimester of fetal development, to possibly hinder any negative influences that could cause developmental issues during this rapid growth period. To resolve if olfactory abilities remained heightened throughout pregnancy, participants were assessed at two time points within pregnancy. It was found that during the first trimester vanillin sensitivities and thresholds, as well as muscone thresholds, all were heightened when compared to the second and third trimesters. It has been concluded that while olfactory ability does improve for a short time during pregnancy, the first trimester is the only time that this occurs. It is thought that the olfactory abilities during the second and third trimesters more closely resemble that of non-pregnant women.

Through the examination of demographic data, further relationships of olfactory ability were discovered. It was found that odorant sensitivity is related to numerous health issues. Spearmint sensitivity was found to significantly relate to having a previously broken nose or a deviated septum. This is unsurprising due to the changes the nasal cavity experiences due to these physical alterations. For example, a previously broken nose or deviated septum could result in less air flow across the mucosal membrane, thus preventing an adequate amount of odorant to readily diffuse across the membrane for interpretation. Caraway sensitivity was found to be weakly related to both high blood pressure and reports of chronic headaches. High blood pressure can result in a thickening of the nasal mucosa and a development of persistent or chronic headaches. In addition, chronic headaches can yield symptoms of swelling of nasal epithelium and the thickening of the nasal mucus membrane (Chow, 1993). In both instances, odorants could encounter diffusion or binding problems.

Muscone was noted to have a moderate relationship with cancer, it is unclear why cancer would influence muscone sensitivity specifically. Knowing the type of cancer could shed further light on the relationship, but was outside of the scope of the provided demographic. Estrogen has been found to stimulate breast tissue cell growth and to influence certain blood vessel cancers, vaginal cancer, and melanoma (Ito, Utsunomiya, Yaegashi & Sasano, 2007).

A history of cancer was also related to response bias, as well as those suffering from an infection at the time of assessment. It is thought that individuals suffering from some type of infection, particularly those infections resulting in cold or allergy like symptoms, would, as found, have an increased response bias towards yes. Participants often want to do well on a given assessment, but, when they perceive their abilities to be stunted by environmental factors, like a cold, during a test that is assessing one's ability to smell, many participants seemed to compensate by indicating a "yes" more often than they actually smelled an odorant. Despite this response bias towards responding "yes", spearmint threshold levels still tended to increase in those with self-reported infections.

When comparing threshold levels to medications taken at the time of assessment it was found that individuals taking anti-anxiety and anti-depressant medications had higher muscone threshold levels. Areas of the brain that are involved in depression also overlap with areas responsible for olfactory perception (Naudin, El-Hage, Gomes, Gaillard, Belzung, & Atanasova, 2012). Assuming individuals taking anti-depressant medications have been diagnosed with depression, this overlap in brain region could explain why, when depression is present, olfactory abilities are not as acute as they could be.

A final significant relationship was found when examining medication and olfactory abilities. It was found that taking antihistamines related to response bias overall and for each

individual odorant. This can again be attributed to a participants attempt to be perceived as performing well. It stands to reason that an individual taking antihistamines is most likely suffering from allergy or cold-like symptom. If they are experiencing nasal congestion, but want to be perceived as doing well on an olfactory test, their response bias is expected to shift, as it did in the current study. Surprisingly, having seasonal allergies did not show a relationship with olfactory ability. However, because assessment one was conducted in late November and assessment two was conducted in mid-December, very few, if any, seasonal allergies would have been present in the external environment.

CHAPTER V

CONCLUSIONS

Overall, differences were found in the various measures of olfactory ability implemented in the current study, leading to the conclusion that hormone levels, specifically estrogen, do influence olfactory abilities.

Limitations and Directions of Future Research

Assessment Time

While the sample size for non-pregnant participants in the current study is one of the largest known samples, a major limitation to analysis, was the number of times participants were assessed. Each participant, pregnant and non-pregnant alike, was assessed twice. Each pregnant participant was assessed six weeks from time one and time two in the hopes of allowing for two distinct time measurements across two different trimesters of each of their pregnancies. The non-pregnant participants were assessed a total of three weeks apart from time one to time two. While this allowed for a distinction to be made between phases of the menstrual cycle and estrogen levels to naturally differ, some participants were assessed at the same time in their cycle while other participants did not uniformly progress through the menstrual cycle. This led to many individuals changing treatment group in a non-uniform fashion, thus limiting statistical analyses.

In the future, to gain a better understanding of the exact relationship between estrogen level fluctuations during the menstrual cycle and changing olfactory abilities, it would be prudent

to be able to classify each individual by what stage of the menstrual cycle in which they begin the study. Grouping individuals this way would allow for better control and determination of when a second assessment should occur.

Furthermore, both pregnant and non-pregnant women should be assessed a minimum of three times. In the current study, resource and time factors limited the scope to two separate assessments. However, in the future measuring olfactory abilities and estrogen levels during each trimester and in each phase of women's menstrual cycle will allow for a more precise representation of how hormone levels, hormone type, and olfactory ability fluctuate.

Statistical Analysis

With the limited sample of many of the reported demographic selections, the relationships described could be due to chance. The demographic and olfactory ability relationships reported, while significant at an alpha level of .05, could be viewed in a more conservative manner to more accurately assess actual relationships. When looking further into possible relationships between demographic data and olfactory ability, focusing on and increasing the sample size directionally to include people with specifically highlighted previous health histories (e.g., cancer) could be beneficial.

Blood Analysis

Analyzing the blood with the HPLC was a good first step in analysis. However, the peak areas determined from the HPLC output did vary in both retention time and area when compared to the standard solutions. This is most likely due to other small molecules being present and detected. Centrifugation only allows for separation up to a certain size molecule. Estrogen is not

the smallest molecule in the blood, nor is it the only small molecule present in our blood. In addition to other hormones, there are several other possible steroids, proteins and lipids that could be present.

In the future, blood samples should be analyzed as was done in the current study, but the analysis should be lengthened. Due to time and logistic constraints only a single one milliliter sample of blood was analyzed from each tube. For the most representative filtrate mixture of a person's blood contents, three one milliliter samples should be drawn, centrifuged, and then combined and diluted in preparation for HPLC analysis. Once a retention time has been recorded for a sample similar to that of the standard solutions, that sample should be run back through the column and the material corresponding to the peak area measurements should be extracted for further analysis. This further analysis could include mass spectrometry analysis to assess the extracted samples contents by molecular weight.

Also, estrogen was the only hormone examined in this study, yet there are many hormones that are present and fluctuate in the blood during menstruation and pregnancy. Once a procedure for exact analysis has been finalized for estrogen, the project can then be expanded to the analysis of blood estrogen levels to that of progesterone, luteinizing hormone, follicle stimulating hormone, and even gonadotropin releasing hormone.

In addition to examining other hormones, extending the scope of research to investigate disease, infection, and environmental biomarker relationships to and influence on olfactory ability is the next step in this area of research. Biomarkers have been found to be indicative of many biological and psychological conditions (Aronson, 2005). In turn, biomarkers could be employed from the external environment. If bound to specific odorants, biomarkers could be

used as a tracking system, allowing for a better understanding of the specific nuanced differences between how odorants travel within the olfactory system.

Measurement of Olfactory Ability

In the current study olfactory ability was assessed using objective measurements of estimated thresholds, sensitivity, and response bias. While the modified WUTC allows for a comprehensive assessment of olfactory sensitivity, it does not assess the totality of olfactory ability. A measure could be included to assess if odor identification changes in conjunction with olfactory sensitivity and hormone levels. Participants spent on average from one hour to two hours per assessment participating in this research. While adding a lengthy measure of odor identification ability would be cumbersome, and even counterproductive, possibly causing participant fatigue, adding a relatively short measure like the University of Pennsylvania Smell Identification Test (Doty, Shaman, Kimmelman, & Dann, 1984) could be beneficial in yielding a more comprehensive look at the changes experienced in the olfactory system.

Nature of This Research

This research contributes to the current breadth of knowledge in a profound way. Previous research indicates that this is the first of its kind to compare blood hormone levels to the olfactory abilities of pregnant women. Much of the current knowledge concerning if and how olfaction changes during pregnancy is based in subjective measures of self-report. This has led to findings being heterogeneous and inconclusive. The current study is a first step in the direction of an objective understanding of how olfaction might be influenced throughout pregnancy.

Furthermore, beyond studies that have used self-report survey measures as a means of assessment, this study is one of the largest to assess the non-pregnant female population's olfactory abilities in conjunction with any measure of hormone fluctuation or stage of the menstrual cycle. In addition, the WUTC has allowed for assessment to incorporate and investigate four distinct odorants. Previous research involving threshold and sensitivity measures has been limited to single odorant specifications. By assessing multiple odorants, the current study was able to compare and contrast the possible differences of how each odorant is processed within the olfactory system. In turn, this research has allowed for the finding that estrogen influences some of the fluctuations in olfaction seen in non-pregnant and pregnant populations.

The magnitude and depth of this research has been a result of interdisciplinary cooperation – a component that has only strengthened the level of inquiry possible. Future research should attempt to incorporate multiple disciplines in the discovery, research, and refinement of what is known and unknown regarding the chemical senses in humans.

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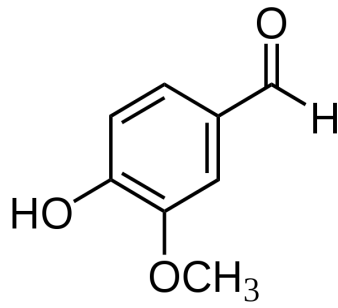
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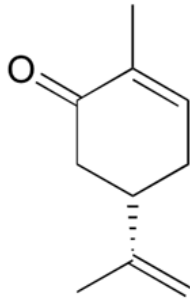
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APPENDIX A
CHEMICAL STRUCTURE OF ODORANTS

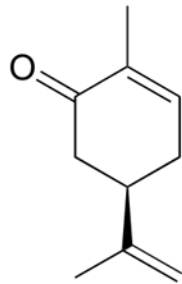
Vanillin:



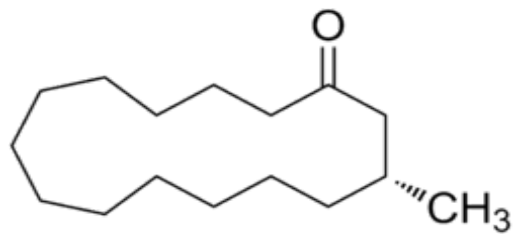
Caraway:



Spearment:



Muscone:



APPENDIX B
DEMOGRAPHIC QUESTIONNAIRE

Demographics Questionnaire

Age (in years): _____

Gender (circle one): Male Female

If female, please also answer the questions located on the last page. ***

Ethnicity (circle one): Caucasian African American Asian Latino

Bi-Racial Other (please indicate): _____

Do you currently smoke (circle one):.....Yes No

If yes, how many cigarettes per day? _____ Cigars per day? _____

What type of cigarettes do you smoke? _____

How many years have you smoked? _____

Do you currently use other tobacco products? (Circle one):.....Yes No

If yes, what quantity per day? _____

What type of tobacco product do you use? _____

How many years have you used tobacco? _____

If not currently smoking/using tobacco products, have you ever used either? (Circle one):...Yes No

If yes, how long ago did you stop? _____

How many cigarettes did you smoke per day? _____; Cigars per day? _____

What type of tobacco product did you use? _____

Did your ability to smell change after you stopped using tobacco? (Circle one):...Yes No

If yes, how? _____

What is your current occupation: _____

Highest grade completed? (Circle only one number):

Grade School: 6 7 8 9 10 11 12

Years of College: 1 2 3 4 5 6+

Please indicate if you have had past history of the following medical illnesses. (Circle Yes or No):

| | | | | | |
|--------------------------|-----|----|----------------------------------|-----|----|
| High blood pressure..... | Yes | No | Diabetes..... | Yes | No |
| Arthritis..... | Yes | No | Heart disease..... | Yes | No |
| Thyroid disorder..... | Yes | No | Chronic Headaches..... | Yes | No |
| Lung trouble..... | Yes | No | Gout..... | Yes | No |
| Epilepsy..... | Yes | No | Circulation problems..... | Yes | No |
| Broken nose..... | Yes | No | Anemia..... | Yes | No |
| Strokes..... | Yes | No | Eye disease (e.g. Glaucoma)..... | Yes | No |
| Asthma..... | Yes | No | Cancer..... | Yes | No |

Please indicate if you have had past history of the following medical illnesses. (Circle Yes or No):

| | | | | | |
|---------------------------------|-----|----|-----------------------------|-----|----|
| Hepatitis..... | Yes | No | Ulcer..... | Yes | No |
| Hiatal hernia..... | Yes | No | Kidney disease..... | Yes | No |
| Sleep Apnea..... | Yes | No | Skin disease..... | Yes | No |
| Prostate problems..... | Yes | No | Infections..... | Yes | No |
| Bleeding/clotting disorder..... | Yes | No | HIV..... | Yes | No |
| TB..... | Yes | No | Neurological disease..... | Yes | No |
| Food allergies..... | Yes | No | Sinus problems..... | Yes | No |
| Medical allergies..... | Yes | No | Deviated septum..... | Yes | No |
| Seasonal allergies..... | Yes | No | Concussion/head trauma..... | Yes | No |

Specific Allergy(ies): _____

Other medical illness(es): _____

Please indicate if you are currently taking any of the following types of medications or vitamins. (Circle Yes or No):

| | | | | | |
|--------------------------------------|-----|----|-----------------------------|-----|----|
| Antibiotics..... | Yes | No | Antidepressants..... | Yes | No |
| Lithium..... | Yes | No | Antianxiety..... | Yes | No |
| Blood Pressure..... | Yes | No | Hormone replacements..... | Yes | No |
| Antihistamines..... | Yes | No | Pre-Natal Vitamins/DHA..... | Yes | No |
| Anti-inflammatory [†] | Yes | No | | | |

[†]Including ibuprofen

Antineoplastic^{††}..... Yes No

^{††}Examples of Antineoplastics are *Elspar* (asparaginase), *Alkeran* (melphalan), floxuridine, lomustine, procarbazine, thioguanine, thiotepa

Stimulant medications^{†††}..... Yes No

^{†††}Examples of Stimulant medications are *Adderall* and *Vyvanse*

*****Females (optional, but each question is VERY BENEFICIAL to answering research questions)**

If **FEMALE**: What is the date of the first day of your most recent period? _____

If **FEMALE**: What is the date of the last day of your most recent period (use today's date if you are currently menstruating)? _____

If **FEMALE**: Are you currently taking birth control? (Circle one):..... Yes No

If yes, what type (e.g. oral contraception, IED, patch, etc.)? _____

If yes, what brand of birth control are you using (e.g. Yaz, Apri, etc.)? _____

If yes, how long have you been taking birth control? _____

If **FEMALE**: Are you currently pregnant? (Circle one):..... Yes No

If yes, how many weeks pregnant are you? _____

If known, what was your conception date? _____

If known, what is your expected due date? _____

If known, what sex is your child? _____

If **FEMALE**: Have you had a previous pregnancy? (Circle one):..... Yes No

If yes, how many previous pregnancies have you had? _____

Of that/those, how many pregnancies resulted in a live birth? _____

APPENDIX C
INSTITUTIONAL REVIEW BOARD APPROVAL

Institutional Review Board
Dept. 4915
615 McCallie Avenue
Chattanooga, TN 374 03-2598
Phone: (423) 425-5867
Fax: (423) 425-4052
instrb@utc.edu
<http://www.utc.edu/irb>

MEMORANDUM

TO: Carrie LeMay **IRB # 13-139**

FROM: Lindsay Pardue, Director of Research Integrity
Dr. Bart Weathington, IRB Committee Chair

DATE: October 8, 2013

SUBJECT: IRB #13-139: Hormones Rule the Roost: Fluctuations of Olfactory Functioning
Throughout the Menstrual Cycle and During Pregnancy

The IRB Committee Chair has reviewed and approved your application and assigned you the IRB number listed above. You must include the following approval statement on research materials seen by participants and used in research reports:

The Institutional Review Board of the University of Tennessee at Chattanooga (FWA00004149) has approved this research project # 13-139 .

Please remember that you must complete a Certification for Changes, Annual Review, or Project Termination/Completion Form when the project is completed or provide an annual report if the project takes over one year to complete. The IRB Committee will make every effort to remind you prior to your anniversary date; however, it is your responsibility to ensure that this additional step is satisfied.

Please remember to contact the IRB Committee immediately and submit a new project proposal for review if significant changes occur in your research design or in any instruments used in conducting the study. You should also contact the IRB Committee immediately if you encounter any adverse effects during your project that pose a risk to your subjects.

For any additional information, please consult our web page <http://www.utc.edu/irb> or email instrb@utc.edu

Best wishes for a successful research project.

APPENDIX D

MODIFIED WUTC THRESHOLD TEST INFORMED CONSENT

INFORMED CONSENT FORM (Threshold Test)

Odor Sensitivity and Hormone Levels

Please read this consent document carefully before you decide to participate in this study. The Institutional Review Board of the University of Tennessee at Chattanooga (FWA00004149) has approved this research project #13-139.

Purpose of the research study:

The purpose of this study is to measure odor sensitivity in healthy female, pregnant and non-pregnant, adults.

What you will be asked to do in the study:

You will initially be asked to complete a brief demographics page. A researcher will then begin the threshold test by presenting a tube filled with clear liquid beneath your nose for 5 seconds. After these 5 seconds have passed, you will be given 10 seconds to tell the researcher “yes” (you did detect an odor) or “no” (you did not detect an odor). The test contains 54 tubes with various odors and concentrations, although not all of the tubes will contain odors.

Time required:

~ 45 minutes

Risks and Benefits:

You may experience some nasal dryness from prolonged smelling. We do not anticipate any direct benefit from the study, but we do appreciate your participation as this will add to a growing body of research that will benefit others in the future.

Compensation:

A nontransferable gift card will be issued to you during the second portion of your participation in this study.

Confidentiality:

Your identity will be kept confidential to the extent provided by law. Your information will be assigned a code number. The list connecting your name to this number will be kept in a locked file in my research office to which only I and my other research team members have access. When the study is completed and the data have been analyzed, the list will be destroyed. Your name will not be used in any report.

Voluntary participation:

Your participation in this study is completely voluntary. There is no penalty for not participating.

Right to withdraw from the study:

You have the right to withdraw from the study at any time without consequence.

Whom to contact if you have questions about the study:

Carrie LeMay (344 Holt Hall, 423-506-5987 and carrie.lemay@utc.edu)

William Tewalt (344 Holt Hall, wtewalt@gmail.com)

Dr. Nicky Ozbek (350 Holt Hall, 425-4285 and Nicky-Ozbek@utc.edu).

Agreement:

I have read the procedure described above. I voluntarily agree to participate in the procedure and I have received a copy of this description.

Participant Signature: _____ Date: _____

Printed Name: _____

If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact Dr. Bart Weathington, Chair of the Institutional Review Board, at 423-425-4289. Additional contact information is available at www.utc.edu/irb

APPENDIX E
BLOOD DRAW INFORMED CONSENT

INFORMED CONSENT FORM (Blood Donation)

Odor Sensitivity and Hormone Levels

Please read this consent document carefully before you decide to participate in this study. The Institutional Review Board of the University of Tennessee at Chattanooga (FWA00004149) has approved this research project #13-139.

Purpose of the research study:

The purpose is to determine various hormone levels in the blood samples.

What you will be asked to do in the study:

You will be asked to provide a sample of blood that will be stored for later analysis in the determination of hormone levels.

Time required:

~30 minutes

Risks and Benefits:

Donors will be screened for previously diagnosed allergic response to mild volatile chemicals. Subjects will be exposed to hypodermic needles.

Compensation:

No direct compensation will be given.

Confidentiality:

Your identity will be kept confidential to the extent provided by law. Your information will be assigned a code number. The list connecting your name to this number will be kept in a locked file in my office to which only I and my other research team members have access. When the study is completed and the data have been analyzed, the list will be destroyed. Your name will not be used in any report.

Voluntary participation:

Your participation in this study is completely voluntary. There is no penalty for not participating.

Right to withdraw from the study:

You have the right to withdraw from the study at any time without consequence.

Whom to contact if you have questions about the study:

Carrie LeMay (344 Holt Hall, 423-506-5987 and carrie-lemay@utc.edu)

Dr. Manuel F. Santiago (615 McCallie Avenue, 425-5364 and Manuel-Santiago@utc.edu).

Agreement:

I have read the procedure described above. I voluntarily agree to participate in the procedure and I have received a copy of this description.

Participant Signature: _____ Date: _____

Printed Name: _____

If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact Dr. Bart Weathington, Chair of the Institutional Review Board, at 423-425-4289. Additional contact information is available at www.utc.edu/irb

APPENDIX F

BLOOD ASSURANCE OF CHATTANOOGA INFORMED CONSENT



Phlebotomy educational material and consent

Thank you for allowing us to collect your blood today. We would like to explain the risks of donating tubes of blood and allow you to ask any questions. Donating blood is not risk free. If you do not feel well, then notify an employee.

What are the possible adverse effects of blood donation?

Pain, redness, swelling and bruising and rarely phlebitis, vascular injury, local infection, muscle or tissue damage, and scarring. Anxiety, fever, headache, lightheadedness, paleness, sweating, chills, nausea, vomiting, dizziness, excessive tiredness, weakness, hyperventilation, itching, hives, low blood pressure and fainting. Fainting or loss of consciousness following donation can cause the donor to fall which can lead to physical injuries with long-term complications including death. Other rare and severe symptoms can include seizures, incontinence, chest pain, respiratory problems including shortness of breath, difficulty breathing, a severe allergic reaction, tetany and cardiac arrhythmia.

What should I do after donating the tubes of blood?

- Eat and drink in the recovery area. Sit for 15 minutes before leaving.
- Leave the adhesive bandage around the arm for 1 hour and the Band-Aid on for 4 hours. If the needle site bleeds, apply firm pressure over the bandage and raise the arm for 5-10 minutes.
- Avoid strenuous activities such as participating in team sports, lifting, pushing or picking up heavy objects for 4-5 hours after donating.
- Apply ice if a bruised area appears on the arm. The ice should be applied periodically for 10-15 minutes for the first 24 hours following donation. In subsequent days, periodically apply warm moist heat to the area. The area may be discolored for 10 days or more.
- If you feel dizzy or lightheaded, do not drive. Sit down and lower the head or lie down, keeping the head lower than the rest of the body if you feel dizzy.
- Call us at 1-800-962-0628 or 423-756-0966 if dizziness persists, or if any other problems occur after donation.

I understand that I am about to have blood drawn from my arm (phlebotomy). My blood will be used for research purposes. I have been given the opportunity to ask questions and can withdraw my consent at any time.

Donor Signature: _____ Date: _____

Print:

First Name: _____ Print MI _____ Last _____ Suffix _____

VITA

Carrie LeMay is a Tennessee native, born in Memphis, TN, to the parents of William and Debra LeMay. Carrie attended the University of Mississippi and completed her Bachelor of Science degree in Biological Sciences and her Bachelor of Arts degree in Psychology with a minor in Chemistry. After accepting a graduate position at the University of Tennessee at Chattanooga, Carrie was a part of multiple research teams. Her main focus centered around neurological ties to behavior, specifically olfactory functioning, cultural competency of health care professionals, response-shift bias within education, and executive function as investigated through a cognitive aging lens. She graduated with a Masters of Science degree in Psychology in May 2014. Carrie is continuing her education by pursuing a Ph.D. in Clinical Psychology at East Tennessee State University with the end goal of working as a clinician in an integrative healthcare setting.