OBESITY MEASURES PREDICT DIFFERENCE IN OLFACtORY EVENT-RELATED POTENTIAL (OERP) LATENCIES BETWEEN APOLIPOPROTEIN E 4 CARRIERS AND NON-CARRIERS

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The Undersigned Faculty Committee Approves the

Thesis of Melissa R. Cervantez:

Obesity Measures Predict Differences in Olfactory Event-Related Potential (OERP) Latencies Between Apolipoprotein E ε4 Carriers and Non-Carriers

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Alzheimer’s disease (AD) is a neurodegenerative disorder that is characterized by plaques, tangles, and neuronal cell loss. In the earliest stages of AD, tangles develop in brain structures associated with olfactory processing. Previous research has found that along with the current diagnostic measures, the additional use of olfactory measures can increase the correct classification rate for AD. The cause of AD is unknown, however, there are notable risk factors associated with the onset and development of AD. Currently, the strongest genetic risk factor associated with AD is the presence of an ε4 allele of the Apolipoprotein E gene. Other risk factors associated with AD include age, low education, family history, Down syndrome, mild cognitive impairment, head trauma, and cardiovascular health factors, such as high blood pressure, high cholesterol, heart disease, stroke, and diabetes. Increases in adipose tissue, especially in areas around the abdomen, have been linked to increases in cardiovascular health problems. Epidemiological studies suggest a connection between maintaining a healthy weight, exercising, and eating healthier choices and the age of onset of AD such that healthier lifestyles may prolong AD onset by up to 5 years. Therefore, it is important to understand the influence and role of obesity in AD. Olfactory event-related potential latencies were recorded during odor memory encoding and retrieval tasks. Latencies were examined at the N1, P2, N2, and P3 ERP components and the midline electrode sites Fz, Cz, and Pz were analyzed as they produce the cleanest OERP waveform. I investigated correlations between different measures of obesity including body mass index (BMI), waist circumference, and waist to hip ratios and olfactory processing speed for healthy individuals who are genetically at risk for AD (ApoE ε4+) (n= 30) and for those not genetically at risk (ApoE ε4-) (n= 30). Bivariate correlation analyses revealed significant positive correlations between measures of obesity and ERP latencies during odor retrieval for the genetically at risk group (ApoE ε4+). It is important to note that these significant linear relationships occurred for young, middle, and older aged participants. However, the exclusion of older participants from analyses strengthened previous relationships, therefore highlighting the importance of adiposity on brain integrity as early as young and middle age. These findings provide useful information about the negative effects of adiposity, especially among those genetically at risk for AD.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT ............................................................................................................................. iv</td>
</tr>
<tr>
<td>LIST OF TABLES .................................................................................................................. vii</td>
</tr>
<tr>
<td>LIST OF FIGURES .............................................................................................................. viii</td>
</tr>
<tr>
<td>CHAPTER</td>
</tr>
<tr>
<td>1 INTRODUCTION ...............................................................................................................1</td>
</tr>
<tr>
<td>Alzheimer’s Disease ........................................................................................................ 1</td>
</tr>
<tr>
<td>Olfactory Processing ....................................................................................................... 2</td>
</tr>
<tr>
<td>Olfaction in AD ................................................................................................................. 2</td>
</tr>
<tr>
<td>Protective Factors in AD ................................................................................................. 2</td>
</tr>
<tr>
<td>Risk Factors for AD ......................................................................................................... 3</td>
</tr>
<tr>
<td>Obesity ............................................................................................................................. 3</td>
</tr>
<tr>
<td>Obesity and Cognition ..................................................................................................... 4</td>
</tr>
<tr>
<td>Apolipoprotein E ............................................................................................................. 4</td>
</tr>
<tr>
<td>Apoe and Olfaction .......................................................................................................... 5</td>
</tr>
<tr>
<td>Event-Related Potentials (ERPs) ..................................................................................... 5</td>
</tr>
<tr>
<td>Olfactory ERPs ............................................................................................................... 6</td>
</tr>
<tr>
<td>Memory Encoding ............................................................................................................. 6</td>
</tr>
<tr>
<td>Memory Retrieval .............................................................................................................. 7</td>
</tr>
<tr>
<td>Hypotheses ....................................................................................................................... 7</td>
</tr>
<tr>
<td>2 METHODS ....................................................................................................................... 8</td>
</tr>
<tr>
<td>Participants ........................................................................................................................ 8</td>
</tr>
<tr>
<td>Procedure .......................................................................................................................... 8</td>
</tr>
<tr>
<td>Apolipoprotein E Genotyping .......................................................................................... 8</td>
</tr>
<tr>
<td>Olfactory Assessment ....................................................................................................... 8</td>
</tr>
<tr>
<td>Odor Threshold .................................................................................................................. 9</td>
</tr>
<tr>
<td>San Diego Odor Identification Test (SDOIT) ...................................................................... 9</td>
</tr>
<tr>
<td>Dementia Rating Scale (DRS) ........................................................................................... 9</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. MANOVA Results for Odor Threshold, Odor Identification, and DRS Scores for ApoE ε4+ and ε4- Participants ...............................................................................12

Table 2. MANOVA Results for BMI, Waist Circumference, and Waist to Hip Ratio for ApoE ε4+ and ε4- Participants ...............................................................................12

Table 3. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist to Hip Ratios) ..........................13

Table 4. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist To Hip Ratios) and OERP Latencies at Each Electrode Site for Apoe ε4 Positive Participants, Listed by Odor Memory Session, Response Type, and ERP Component ...................16

Table 5. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist to Hip Ratios) and OERP Latencies at Each Electrode Site for ApoE ε4 Negative Participants, Listed by Odor Memory Session, Response Type, and ERP Component ...................................16

Table 6. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist to Hip Ratios) and OERP Latencies at Each Electrode Site for Young and Middle Aged ApoE ε4 Positive Participants, Listed by Odor Memory Session, Response Type, and ERP Component...........................................................................................................17
LIST OF FIGURES

PAGE

Figure 1. Significant linear relationship between waist circumference and OERP latency during hits at the N1 component for ApoE ε4 positive individuals during odor memory retrieval using odors as cues. .................................................................14

Figure 2. Significant linear relationship between waist to hip ratios and OERP latency during hits at the N1 component for ApoE ε4 positive individuals during odor memory retrieval using odors as cues. .............................................................................15

Figure 3. Significant linear relationship between waist circumference and OERP latency during hits at the N2 component for young and middle age ApoE ε4 positive individuals during odor memory retrieval using odors as cues. ....................21
CHAPTER 1

INTRODUCTION

ALZHEIMER’S DISEASE

Millions of people worldwide have dementia, with Alzheimer’s disease (AD) accounting for the majority of dementia cases. The symptoms often include memory loss, difficulty learning new tasks, and personality changes. The more severe stages of AD can result in the loss of independently caring for oneself. Ferri et al. (2005) estimate that by the year 2040, 8.1 million people will be affected by dementia. By the year 2050, AD will cost the nation an estimated 1.2 trillion dollars. AD is currently the sixth leading cause of death in the United States and there is no way to prevent, treat, or slow the neurodegeneration.

AD is associated with the buildup of amyloid plaques, neurofibrillary tangles, and loss of neurons in the brain. Cell loss, plaques, and tangles have been observed in the entorhinal cortex, hippocampus, prepiriform cortex, anterior olfactory nucleus, and olfactory bulb. The neurofibrillary tangles found in AD first form in the transentorhinal and entorhinal areas and then progress to the hippocampus and other areas in the temporal lobe and then to the frontal cortex (Braak & Braak, 1994). Braak and Braak (1997) noted that a disconnection between the entorhinal and transentorhinal areas from the hippocampus is typically observed in the earliest stages of AD. The disconnection has been associated with a disruption of information communication that results in decreases in memory performance that is a symptom most often observed in AD.

Research has improved AD diagnostic measures with genotyping for risk factors, neuroimaging, and measuring cognitive and sensory decline. However, definitive diagnosis of AD is with post mortem autopsy when the plaques, tangles, and neuronal loss are observed and measured. Current diagnosis is of probable or possible AD by using a combination of neuropsychological tests, clinical examinations, and excluding all other possible disorders. Clinical diagnosis is not always accurate which makes early detection difficult.
OLFACTORY PROCESSING

The primary olfactory cortex, brain structures used in odor processing, consists of the anterior olfactory nucleus, piriform cortex, anterior cortical amygdaloid nucleus, periamygdaloid and entorhinal cortices (Barresi et al., 2012). The piriform cortex is connected to the thalamus, hypothalamus, and orbitofrontal cortex and the entorhinal cortex has fibers that connect to the hippocampus. The right hemisphere which contains the right orbitofrontal cortex and piriform cortex has been linked to odor memory and familiarity ratings while the left hemisphere and left orbitofrontal cortex, left piriform cortex, insula, amygdala, and superior frontal cortex have been associated with emotional response to odors (Royet & Plailly, 2004).

OLFACTION IN AD

The main areas of the brain associated with olfactory processing show neuropathology in the earliest stages of AD such as the olfactory bulbs, anterior olfactory nucleus, and entorhinal cortex. The neuropathology of AD can help to explain the decrease in odor performance that is typically observed in AD patients. Olfactory declines are typically seen in normal aging; however, AD participants are significantly more impaired in tests of odor threshold, odor recognition memory, and odor identification than their healthy peers (Nordin & Murphy, 1996). Morgan and Muphy (2002) found that the additional use of olfactory measures can improve correct classification rate in the assessment of AD. Overall, the more severe degree of dementia is related to the larger odor threshold decline (Murphy, Gilmore, Seery, Salmon, & Lasker, 1990). Demented participants do not differ from normal controls on measures of taste threshold indicating that observed differences in olfactory thresholds are not due to the AD participant’s inability to complete a threshold task. Nordin, Monsch, and Murphy (1995) noted that the impairment in olfactory function that occurs in AD and also in normal aging is usually not obvious to the person affected. This can increase health risks associated with olfactory loss such as exposure to toxic gases or inability to detect smoke.

PROTECTIVE FACTORS IN AD

Research has shown that there are factors that can potentially delay the age of AD onset, referred to as protective factors. Ferrari et al. (2013) found that certain risk factors
delay the age of onset for AD even among genetically at risk subjects. The protective factors include high mental, social, and physical scores, higher education, and the absence of vascular risk factors such as high blood pressure, pre-diabetes and diabetes, stroke, and heart failure. Previous research has also noted that individuals with an ε2 allele of the Apolipoprotein E (ApoE) gene are less likely to develop AD (Corder et al., 1993). The ε2 allele has been referred to as a protective allelic variant in the development of AD.

**RISK FACTORS FOR AD**

The strongest risk factor associated with AD is age (Farrer et al., 1995). Other risk factors include low education, family history, having an ε4 allele of the ApoE gene, Down syndrome, and past head trauma. Cardiovascular health problems such as, high blood pressure, high cholesterol, heart disease, stroke and diabetes, have also been shown to increase the risk associated with AD. Being overweight or obese has been linked to increases in the cardiovascular risk factors associated with AD.

**OBESITY**

Obesity can be measured in many different ways; the most common include body mass index ratings (BMI), waist circumference, and waist to hip ratios (Mueller, 1991). Body mass index is calculated by weight in kilograms divided by height in meters squared (k/m²). BMI scores fall on a continuum; however ranges are used to classify health status. The ranges include underweight (BMI < 18.5), normal weight (BMI 18.5 – 25.9), overweight (BMI 26-29.9), and obese (BMI > 30). BMI is typically viewed as a good indicator of measuring adipose tissue; however, studies have shown that BMI is a less reliable indicator as age increases. Waist circumference and waist to hip ratio measurements typically measure adipose tissue and are reliable measurements of central obesity. Waist circumference cutoffs are 102 cm for men and 88 cm for women. Waist to hip ratio scores fall on a continuum with ratings over 1 indicating a higher level of abdominal fat. Herrera et al. (2009) found that the three measures of obesity are most reliable among Whites and less reliable in other ethnic groups (Latin Americans, Hispanics, and non-Hispanic blacks) at detecting coronary heart disease. BMI was the least reliable measure of obesity compared to waist circumference and waist to hip ratio. Zamora, Bartholow, Green, Morgan and Murphy (2012) found that during
a 4 choice odor identification task waist circumference measures correlated with olfactory event-related potential latencies as strong as or possibly stronger than BMI.

**OBESITY AND COGNITION**

Roughly 1 in 3 Americans today are considered obese. In addition to increasing prevalence rates, obesity is a health epidemic that costs the nation an estimated $168.4 billion a year (Cawley & Meyerhoefer, 2012). Obesity is also highly ranked on lists of preventable causes of death. Higher levels of central obesity have been related to increases in other health risks including diabetes, hypertension, and dyslipidemia (Malnick & Knobler, 2006). While the health outcomes related to obesity appear to be physically harmful, there have also been many reports of ways that obesity negatively affects cognition. Gunstad, Lhotsky, Wendell, Ferrucci, and Zonderman (2010) found that performance on global, executive, and memory measures decreases as obesity increases. Additionally, research has found connections between obesity and AD. For example, Whitmer, Sidney, Selby, Johnston, and Yaffe (2005) found that obesity measured at midlife was associated with an increased risk in the development of AD. Additionally, Luchsinger, Cheng, Tang, Schupf, and Mayeux (2012) found that higher waist to hip ratios were associated with increased risk for late onset AD. Lastly, fMRI research has shown decreases in gray matter in frontal, temporal, and parietal areas in obese subjects prior to the official diagnosis of mild cognitive impairment or AD (Driscoll et al., 2012). Due to formation of plaques and tangles near olfactory processing in the earliest stages of AD, it is important to examine the relationship between obesity and olfactory processing to determine if the relationship between obesity and cognition is affected in the earliest stages of AD.

**APOLIPOPROTEIN E**

The Apolipoprotein E (ApoE) gene is located on chromosome 19. It has three different allelic variants ε2, ε3, and ε4. Humans have 2 alleles and 6 different phenotypes exist. Individuals are either homozygous (ε2/ε2, ε3/ε3, or ε4/ε4) or heterozygous (ε2/ε3, ε3/ε4, or ε2/ε4).

Previous literature focuses on the ε4 allele of the Apolipoprotein E as the strongest genetic risk factor associated with AD. The ε4 allele variant has shown younger onset age for AD than the ε3 allele and the protective variant, the ε2 allele (Corder et al., 1993; Farrer et
The presence of an ε4 allele (ε4+) increases the risk of developing AD by 4 times and homozygotes of ε4, who have 2 ε4 alleles, are 15 times more likely to develop AD (Ashford & Mortimer, 2002). Corder et al. (1993) also noted that the average age of late onset for AD decreases from 84 years old to 68 years old when the presence of an ε4 allele is added.

**APOE AND OLFACTION**

ApoE ε4 positive individuals typically exhibit olfactory deficits compared to ε4 negative individuals, where participants without an ε4 allele performed better on olfactory tasks than those with an ε4 allele, even when there were not significant differences in scores for cognitive tasks (Bacon, Bondi, Salmon, and Murphy, 1998; Handley, Morrison, Miles, & Bayer, 2006; Murphy, Bacon, Bondi, & Salmon, 1998).

**EVENT-RELATED POTENTIALS (ERPS)**

Electroencephalogram (EEG) is used to record electrical activity at the scalp generated by the brain. Event–related potentials (ERPs) measure brain activity evoked by a sensory stimulus or cognitive event with amplitude height relating to the observed cognitive effort and latency relating to the processing speed (Kutas, McCarthy, & Donchin, 1977; Polich, Ehlers, Otis, Mandell, and Bloom, 1986). ERPs are sensitive enough to measure neural activity to the millisecond and have high temporal resolution; however, ERPs tend to have poor spatial resolution (Friedman & Johnson, 2000). EEG noise results from recorded non-stimulus electrical activity, which can interfere with the recorded ERP. The typical method to eliminate ERP noise is by recording and averaging multiple trials. Increases in stimuli presentation trials leads to decreases in EEG noise.

The ERP wave form includes exogenous or sensory components labeled as N1/P2 and an endogenous or cognitive component labeled P3. Overall, P3 latencies increase and amplitudes decrease as participants’ age increases. Previous research has suggested that the degree of dementia measured by the DRS has been positively correlated with longer OERP P3 latencies (Morgan & Murphy, 2002).
**Olfactory ERPs**

Olfactory event-related potentials (OERPs) are evoked potentials produced in response to an odor or cognitive event. OERPs have the sensitivity to detect differences in olfactory processing between normal controls and those with dementia. It is a useful diagnostic tool to measure olfactory processing since it does not require significant cognitive effort from a participant. Therefore, it is especially useful in detecting abnormalities among subjects with dementia, mild cognitive impairment, or Down syndrome. Morgan and Murphy (2002) found that OERPs were better than auditory ERPs at differentiating AD patients from normal controls. When used to discriminate between AD subjects and controls, the OERP P3 latency and the San Diego Odor Identification Test (SDOIT) combined had a correct classification rate of 100%; therefore, suggesting that OERPs have high levels of sensitivity and specificity in detecting waveform abnormalities in AD subjects.

Differences in OERP latencies have been noted among older participants with the presence of an ε4 allele. Those who were ε4 positive typically had longer latencies than ε4 negative individuals. The observed delays in latency were more severe in AD subjects than ε4 positives, suggesting that some ε4 subjects may have been displaying the earliest signs of AD. This difference was not observed among auditory ERPs (Wetter & Murphy, 2001). Morgan and Murphy (2002) found that as Dementia Rating Scale scores decreased OERP latencies increased in AD participants.

**Memory Encoding**

Memory is the brain’s ability to encode, store, and retrieve information. Encoding is the first process or step in memory and plays an important role in the subsequent retrieval stages. Impairment or difficulties with memory encoding often result in deficits in storage and retrieval. Odor encoding uses an odor as a stimulus for brain processing. The disconnection from the entorhinal and transentorhinal areas to the hippocampus in AD is thought to affect memory and olfactory processes so that a test of odor memory that investigates odor encoding and retrieval would be especially sensitive to detecting abnormalities. The plaques and tangles that form at the earliest stages of AD could interfere with odor encoding. Unsuccessful odor encoding would then most likely result in disruptions in odor retrieval and odor label retrieval.
MEMORY RETRIEVAL

This study focuses on two types of retrieval. Odor memory retrieval is the process of trying to recover and recognize previously presented odors and reject new odors. Odor label retrieval uses a cross modality design to try to associate a presented odor label (word) with the previously encoded odors.

ɛ4 positive individuals tend to show odor recognition memory deficits compared to ɛ4 negative individuals. ɛ4 positives are more likely to confuse a new odor (foils) as one of the original odors (targets), resulting in more false positive errors in odor memory tasks than ɛ4 negative individuals (Gilbert & Murphy, 2004).

HYPOTHESES

We hypothesize that measures of obesity such as BMI, waist circumference, and waist to hip ratios will positively correlate with odor memory encoding and retrieval latencies; the higher the measure of obesity the longer the OERP latency. When examined separately, the positive association will be significant among ɛ4 positive individuals but not for ɛ4 negative individuals.
CHAPTER 2

METHODS

This chapter will discuss the design of the current study, including information about participants, procedures, and statistical analyses of the data.

PARTICIPANTS

Participants were recruited from the Lifespan Human Senses Laboratory’s participant pool. Participants all signed San Diego State University consent forms. Participants were in three different age groups: young (18-25 years), middle (45-55), and old (65 and older). Each age group had 20 participants for 60 total participants. In each age group, half of the participants were men and half were women (10 males, 10 females). In each age group, both gender groups consisted of half ε4 positive and ε4 negative individuals (5 ε4+, 5 ε4-).

Participants were given the dementia rating scale (DRS), an odor threshold test, and the San Diego Odor Identification test (SDOIT). Participants with scores indicative of dementia, anosmia, or severe hyposmia (DRS scores lower than 130 out of 144, odor threshold lower than 3 out of 9, and SDOIT scores lower than 3 out of 8) were excluded from this study.

PROCEDURE

Data were collected as part of a larger study focused on differences in olfactory event-related potentials in memory encoding and retrieval between different age groups and ApoE ε4 status.

Apolipoprotein E Genotyping

Participants were genotyped using a polymerase chain reaction based method obtained from a cheek buccal swab to determine the ApoE allele status (Higuchi, 1989).

Olfactory Assessment

Participants were genotyped using a polymerase chain reaction based method obtained from a cheek buccal swab to determine the ApoE allele status (Higuchi, 1989).
Odor Threshold

Participants were presented with 2 identical looking bottles with the same amount of clear liquid at the bottom. They were instructed that they were to squeeze and smell the air in both bottles with the same nostril and choose the bottle that smelled strongest. Even if they could not detect a difference, they were told to choose a bottle resulting in a forced choice response. The butanol concentration in the bottles from level 9-0 with level 9 being the weakest concentration. The odorant bottles were presented from weakest to strongest to prevent adaptation of the subject. When the participant chose the blank control bottle the researcher increased the butanol concentration. When the participant chose the correct bottle the researcher presented that same concentration again and once the participant correctly identified the butanol bottle 5 times in a row with the same nostril the odor threshold for that nostril was attained. This was done separately for each nostril and an inter-stimulus interval of 45 seconds for each nostril was enforced to avoid adaptation. Odor threshold scores were averaged between nostrils.

San Diego Odor Identification Test (SDOIT)

In the San Diego Odor Identification Test participants attempted to identify 8 common household odors. Participants were presented with a picture board of 20 common household odors and asked to identify each picture on the board. If the participant incorrectly identified a picture, the researcher was able to tell them the correct response. The participant closed the eyes and an odor was placed under the nose. The opaque jar was then removed and the participant gave a verbal response or pointed to the odor picture on the picture board. Each correct response was recorded by the researcher for a total score of 8.

Dementia Rating Scale (DRS)

The DRS was given to participants using the established testing protocol described by (Mattis, 1976).

Olfactometer Apparatus

Odors were presented to participants using a computer controlled olfactometer similar to the one used by (Kobal, 1981; Morgan & Murphy, 2002). Clean, humidified air was pumped to the subject through tubing. The clean air was heated using insulated tubing to
reach the participant at body temperature (36.5°C). The clean air was also pumped at a rate of .75 L/min and could be adjusted using flow meters located on the olfactometer. To prevent auditory or somatosensory cues affecting the participant, the olfactometer provided constant clean airflow and used valves to replace the amount of clean air with the odorant at the same odor flow rate. To prevent inconsistent airflow in the nasal passageways and eliminate differences in odor presentation timing due to respiration, velopharyngeal closure was used and participants were instructed to breathe through the mouth (Kobal, 1981; Lorig, Elmes, Zald, & Pardo, 1999; Thesen & Murphy, 2001).

**ERP Recording and Processing**

Event-related potentials were recorded using the Compumedics 64-electrode Ag/AgCL sintered Quik-cap. The Synamps 2 amplifiers were used to amplify the waveform and the Neuroscan software package recorded the waveform to a hard disk. Electrode impedances did not exceed 10 kΩ. Electrodes were also placed above and below the left eye region, on both temples, and earlobes. The electrodes placed by the eye were used to measure eye blink activity, electro-ocular activity, which could increase noise on the recorded waveform. Electro-ocular noise activity was excluded from the observed OERP waveform using the Ocular Artifact Reduction (OAR) method from the Neuroscan software. Electrical activity was examined 500 ms pre-stimulus and 1,500 ms post-stimulus. A bandpass filter was applied and each trial was baseline corrected to .01-6 Hz. During the odor and odor label retrieval session, recordings were averaged across the different response types (hits, misses, correct rejections, and false positives) for each individual. If any response was recorded 5 sec post stimulus, the waveform was not included in the individual average. Individual peaks were then included in group peak averages.

**Olfactory Event-Related Potential (OERP) Memory Task**

Participants were told that they would be completing an odor memory task. Session 1 was an odor encoding session. They were instructed to remember the odors that were presented in session 1 for sessions 2 and 3. Participants received 14 odors twice for a total of 28 randomized trials. Odors were presented to the participants using a computer controlled olfactometer. They were instructed to focus on a computer screen that was placed 3 feet
away. During the inter-stimulus interval (ISI) of 30 seconds between odors and during the presentation of odors they focused on a red cross-hair on a black screen. After the stimulus was presented a scale was visible on the screen. The pleasantness rating scale ranged from 0 (least pleasant) to 100 (most pleasant). They gave verbal pleasantness responses ranging from 0-100. After session 1 was completed there was a 5 minute interval for the researcher to instruct the participant. Session 2 was the odor retrieval session. In this session participants were told that they would be presented with new odors and odors from session 1. They had a controller with the option to click “yes” or “no.” They were to choose “yes” if they remembered the odor from session 1 and click “no” if it was a new odor. There were 8 odors from session 1 and 6 new odors that were randomly presented 3 times each for a total of 42 trials. Participants focused on a red cross-hair on a black screen and were not prompted when to respond to the presented odor. They were instructed to respond as quickly as possible when they noticed an odor. A 5 minute break occurred after session 2. Session 3 was considered the odor label retrieval session. Participants were instructed that they would be presented with a word on the computer screen and must use the controller, which records the response using the neuroscan software, to choose “yes” or “no” as quickly as possible. They should choose “yes” if the word on the screen matched an odor they were presented in session 1 and “no” if they did not remember the odor from session 1. Responses from the controller were recorded with the Neuroscan software for session 2 and 3. Session 2 and 3 were randomly counter-balanced so that half of the participants received the olfactory condition first and half received the visual condition first.

Sessions 2 and 3 allowed for 4 different response categories; hits, misses, correct rejections, and false positives. Hits were classified as correctly identifying that an odor was indeed presented in session 1. Misses were classified as indicating that an odor was not presented in session 1 when it was. Correct rejections were classified as correctly identifying that an odor was not presented when it was not presented in session 1. False positives were classified as incorrectly responding that an odor was presented in session 1 when actually it was not presented.
CHAPTER 3

RESULTS

Multivariate analyses of variance (MANOVA) revealed no significant differences in odor threshold, odor identification, or the DRS between ApoE ε4 positive and ε4 negative subjects (Table 1). MANOVA also revealed no significant differences on BMI, waist circumference, and waist to hip ratio between ApoE ε4 positive and ε4 negative subjects (Table 2). Bivariate correlations revealed a significant linear correlation among the three measures of obesity (Table 3).

Table 1. MANOVA Results for Odor Threshold, Odor Identification, and DRS Scores for ApoE ε4+ and ε4- Participants

<table>
<thead>
<tr>
<th></th>
<th>ApoE ε4+</th>
<th>ApoE ε4-</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
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<tr>
<td>Odor threshold</td>
<td>6.5</td>
<td>6.92</td>
<td>0.839</td>
<td>1</td>
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<tr>
<td>Odor ID</td>
<td>6.192</td>
<td>6.52</td>
<td>0.507</td>
<td>1</td>
<td>0.48</td>
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<tr>
<td>DRS</td>
<td>139.692</td>
<td>141.28</td>
<td>1.937</td>
<td>1</td>
<td>0.17</td>
</tr>
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Table 2. MANOVA Results for BMI, Waist Circumference, and Waist to Hip Ratio for ApoE ε4+ and ε4- Participants

<table>
<thead>
<tr>
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<th>ApoE ε4+</th>
<th>ApoE ε4-</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
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<tr>
<td>BMI</td>
<td>27.471</td>
<td>28.983</td>
<td>0.59</td>
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<tr>
<td>Waist circumference</td>
<td>91.75</td>
<td>92.5</td>
<td>0.021</td>
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<tr>
<td>Waist to hip ratio</td>
<td>0.876</td>
<td>0.901</td>
<td>0.871</td>
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Bivariate correlations were used to examine the relationship between OERP latency at the 3-midline electrode sites (Fz, Cz, Pz) with each measure of obesity (BMI, waist circumference, and waist to hip ratio). Separate correlations were examined for each different
Table 3. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist to Hip Ratios)

<table>
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<th>Waist to hip ratio</th>
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<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>BMI</td>
<td>0.601</td>
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<td>Waist circumference</td>
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</tr>
<tr>
<td>Waist to hip ratio</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OERP component (N1, P2, N2, P3) for each response type (hits, misses, correct rejections, and false positives).

**APOE ε4 POSITIVE**

During retrieval of odor memory using odors, there was a significant positive correlation between waist circumference and OERP latency during hits for all three-electrode sites (Fz, Cz, Pz) at the N1 component \[r(30) = .370, p = .044; r(30) = .382, p = .037; r(30) = .376, p = .041\], respectively (See Figure 1). During retrieval of odor memory using odors, there was a significant positive correlation between waist to hip ratio and OERP latency during hits for all three-electrode sites (Fz, Cz, Pz) at the N1 component \[r(30) = .439, p = .015; r(30) = .423, p = .020; r(30) = .425, p = .019\], respectively (See Figure 2). Table 4 shows all additional significant positive correlations between measures of obesity and OERP latencies for ApoE ε4 positive subjects.

**APOE NEGATIVE**

During the retrieval of odor memory using odor labels, there was a significant negative correlation in ApoE ε4 negatives between waist to hip ratio and OERP latency during hits for all three-electrode sites (Fz, Cz, Pz) at the N2 component \[r(28) = -.388, p = .041; r(28) = -.398, p = .036; r(28) = -.423, p = .025\], respectively. Table 5 shows all
Figure 1. Significant linear relationship between waist circumference and OERP latency during hits at the N1 component for ApoE ε4 positive individuals during odor memory retrieval using odors as cues.

additional significant negative correlations between measures of obesity and OERP latencies for ApoE ε4 negative subjects.

**APOE ε4 POSITIVE EXCLUDING OLDER SUBJECTS**

During the retrieval of odor memory using odors, there was a significant positive correlation between BMI and OERP latency during correct rejections for all three-electrode sites at the P2 component \([r(19) = .488, p = .040; r(19) = .515, p = .029; r(19) = .508, p = .031]\), respectively. Additionally, when a more stringent alpha level of \(p = .01\) was enforced
significant correlations remained for the young and middle aged ApoE ε4 positive group. During the retrieval of odor memory using odors, there was a significant positive correlation between BMI and OERP latency during false positives for the electrode site of Pz at the P2 component \([r(19) = .576, p = .01]\). During the odor memory retrieval using odor labels as cues, there was a significant positive correlation between BMI and OERP latency during false positives for the electrode site of Fz at the N1 component \([r(19) = .58, p = .009]\). Lastly, during the retrieval of odor memory using odors, there was a significant positive correlation between waist circumference and OERP latency during Hits for all three-electrode sites at
Table 4. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist To Hip Ratios) and OERP Latencies at Each Electrode Site for ApoE ε4 Positive Participants, Listed by Odor Memory Session, Response Type, and ERP Component

<table>
<thead>
<tr>
<th>ApoE ε4 Positive</th>
<th>Fz</th>
<th>Cz</th>
<th>Pz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist Circumference (n = 30)</td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>OS2 Hits N1</td>
<td>0.370</td>
<td>0.044</td>
<td>0.382</td>
</tr>
<tr>
<td>OS2 Hits N2</td>
<td>0.375</td>
<td>0.041</td>
<td>0.378</td>
</tr>
<tr>
<td>Waist to Hip Ratio (n = 30)</td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>OS2 Hits N1</td>
<td>0.439</td>
<td>0.015</td>
<td>0.423</td>
</tr>
<tr>
<td>OS2 Hits P2</td>
<td>0.415</td>
<td>0.022</td>
<td>0.438</td>
</tr>
<tr>
<td>OS2 Hits N2</td>
<td>0.391</td>
<td>0.033</td>
<td>0.415</td>
</tr>
</tbody>
</table>

Note. OS2 refers to odor retrieval using odors as cues.

Table 5. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist to Hip Ratios) and OERP Latencies at Each Electrode Site for ApoE ε4 Negative Participants, Listed by Odor Memory Session, Response Type, and ERP Component

<table>
<thead>
<tr>
<th>ApoE Negative</th>
<th>Fz</th>
<th>Cz</th>
<th>Pz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist to Hip Ratio (n = 28)</td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>OS3 Hits N2</td>
<td>-0.388</td>
<td>0.041</td>
<td>-0.398</td>
</tr>
<tr>
<td>OS3 Hits P3</td>
<td>-0.453</td>
<td>0.015</td>
<td>-0.446</td>
</tr>
<tr>
<td>OS3 CR N2</td>
<td>-0.457</td>
<td>0.017</td>
<td>-0.442</td>
</tr>
</tbody>
</table>

Note. OS2 refers to odor retrieval using odors as cues.
the N2 component \[ r(20) = .62, p = .004; r(20) = .613, p = .004; r(20) = .636, p = .003 \], respectively. Table 6 shows all additional significant correlations between obesity and OERP latencies for young and middle age ApoE ε4 positive subjects.

Table 6. Significant Correlations (r Values) and p-Values Among Measures of Obesity (i.e., BMI, Waist Circumference, and Waist to Hip Ratios) and OERP Latencies at Each Electrode Site for Young and Middle Aged ApoE ε4 Positive Participants, Listed by Odor Memory Session, Response Type, and ERP Component

<table>
<thead>
<tr>
<th>ApoE ε4 Positive</th>
<th>Fz</th>
<th>Cz</th>
<th>Pz</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (n = 19)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS2 CR P2</td>
<td>0.488</td>
<td>0.040</td>
<td>0.515</td>
</tr>
<tr>
<td>OS2 FP P2</td>
<td>0.568</td>
<td>0.011</td>
<td>0.562</td>
</tr>
<tr>
<td>OS3 Hits N1</td>
<td>0.473</td>
<td>0.041</td>
<td>0.516</td>
</tr>
<tr>
<td>OS3 FP N1</td>
<td>0.58</td>
<td>0.009</td>
<td>0.512</td>
</tr>
<tr>
<td><strong>Waist Circumference (n = 20)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS2 Hits N1</td>
<td>0.495</td>
<td>0.027</td>
<td>0.512</td>
</tr>
<tr>
<td>OS2 Hits P2</td>
<td>0.466</td>
<td>0.038</td>
<td>0.487</td>
</tr>
<tr>
<td>OS2 Hits N2</td>
<td>0.62</td>
<td>0.004</td>
<td>0.613</td>
</tr>
<tr>
<td>OS2 CR P2</td>
<td>0.515</td>
<td>0.024</td>
<td>0.519</td>
</tr>
</tbody>
</table>

*Note. OS2 refers to odor retrieval using odors as cues.*
CHAPTER 4

DISCUSSION

Due to the neuropathology in the earliest stages of AD, olfactory processing has become an area of interest in the development and progression of AD. For example, Morgan and Murphy (2002) found that the additional use of olfactory measures increases the correct classification rate in the assessment of AD. While the cause of AD is currently unknown, there are many risk factors and protective factors that have been associated with the development of AD. Some of the health factors that increase the risk for AD include high blood pressure, high cholesterol, stroke and diabetes. Research has also found that obesity in midlife is associated with an increased risk for AD (Luchsinger et al., 2012). This can partially be explained by the co-morbidity between obesity and the health factors that increase AD risk. Additionally, previous research has found that OERPs have the sensitivity and specificity to detect differences between those who are genetically at risk for AD (ApoE ε4 positive) and those who are not at risk (ApoE ε4 negative) before behavioral differences. Therefore, it was important for the current study to examine the relationship between obesity and OERP latencies to assess the effects of obesity on brain integrity among healthy participants who are genetically at risk for AD.

OBESITY

Currently, about 1 in 3 Americans are considered obese with increasing prevalence rates. BMI, waist circumference, and waist to hip ratios have been used to measure obesity with the current literature divided on which one is the best indicator of obesity. For the current study, all three measures were examined and BMI, waist circumference, and waist to hip ratios were significantly positively correlated. Previous research has found that higher levels of central obesity lead to an increased risk for AD. For example, Luchsinger et al. (2012) found that waist to hip ratios were associated with higher late onset AD risk, however, this association was not found with BMI and waist circumference measurements. The current study found significant linear relationships between obesity and OERP latencies for
ApoE ε4 positive individuals when using measures of central obesity including waist circumference and waist to hip ratios (Figures 1 & 2). This relationship could be due to the co-morbidity of other diseases that are usually found in those with higher levels of abdominal fat such as heart disease, stroke, and diabetes. Additionally, those co-morbidities are considered health risk factors associated with the development of AD. Therefore, the current results support previous research that higher levels of central obesity have negative effects on brain cognition.

**Odor Retrieval**

Previous research has found a significant linear relationship between adiposity and OERP latency for ApoE ε4 positive individuals during a cross-modal odor identification task (Zamora et al., 2012). The cross-modal OERP design used odors as the initial stimuli and words as the retrieval stimuli, which uses visual stimuli to retrieve the encoded odor memories. Previous research using odors as the stimuli for encoding and retrieval found that those who are genetically at risk for AD (ApoE ε4 positive) had significantly longer OERP latencies than those who are genetically not at risk (ApoE ε4 negative) (Green, Cervantez, Graves, Morgan & Murphy, 2013). The current study expands upon previous research by examining the relationship between obesity measures and OERP latencies for ApoE ε4 positive individuals during an odor memory task that uses odors as the encoding and retrieval stimuli. Study results indicate that higher waist circumference and waist to hip ratio measurements significantly correlated with longer odor retrieval brain processing latencies for ApoE ε4 positive participants when they correctly recognized an odor from the encoding session (hits). While those genetically at risk for AD (ApoE ε4 positive) typically have longer OERP latencies than those who are not genetically at risk (ApoE ε4 negative) the current findings suggest that measures of obesity are significantly related to OERP latencies during odor retrieval among the genetically at risk group, where the health implications of having higher levels of abdominal fat are related to longer odor memory brain processing speeds. I speculate that the results suggest that higher measures of obesity may be more detrimental to brain integrity for those who are genetically at risk for AD and support the notion that maintaining a healthy weight is a protective factor that can delay the age of onset for AD.
ERP COMPONENTS
The early ERP components of N1 and P2 are typically referred to as the sensory components of the ERP waveform while P3 has been established as the cognitive component (Bennington & Polich, 1999; Kotchoubey, 2005; Morgan & Murphy, 2010). The significant positive correlations between obesity and OERP latency in the present study all occurred at the earlier sensory ERP components of N1, P2, and at N2. Therefore, the results suggest that using odors as the encoding and retrieval stimuli in an odor memory paradigm produce more significant effects at the sensory components of the OERP waveform rather than at the P3 cognitive component. This is particularly interesting, since previous studies report significant P3 findings when the task involves odor identification and the retrieval stimuli are visual and stimulate semantic networks of memory (Zamora et al., 2012).

EXPLORATORY ANALYSES
While the current study found significant relationships between OERP latencies with the measures of central obesity, no significant relationships were established with BMI. This could be attributed to a few different reasons. BMI uses height and weight to compute a score that falls on a continuum, which does not take fat distribution into account and centrally located abdominal fat is usually associated with increases in health problems. Also, previous research has established that BMI is not the best measure of obesity among older aged participants due to the lack of muscle mass that is typical with healthy aging. The current sample included participants from three different age groups, young, middle, and old age. Therefore, the older participants could have influenced the lack of significant correlations between BMI and OERP latency. To examine this further, exploratory analyses were conducted to exclude older aged participants, which produced significant relationships between BMI and OERP latencies while strengthening previous relationships.

When a more stringent alpha level of $p = .01$ was enforced, correlations for the young and middle-aged ApoE ε4 positive group produced significant relationships (Figure 3). The significant relationship between obesity and OERP latency for the young and middle aged ApoE ε4 positive group supports the notion that obesity measures are less reliable in older age. Also, research has noted that sometimes weight and other obesity measures decrease in old age and weight decline during the progression of AD has been reported. Loss of smell in
Figure 3. Significant linear relationship between waist circumference and OERP latency during hits at the N2 component for young and middle age ApoE ε4 positive individuals during odor memory retrieval using odors as cues.

the earliest stages of AD can make food appear less appetizing or simply people with AD sometimes forget to eat, especially among those without caregivers. Additionally, older participants overall have longer OERP latencies than young and middle aged participants. Therefore, it would be interesting to systematically examine differences in the relationships among age, ApoE ε4 status, and obesity with OERP latencies in future work.
CONCLUSION

The current findings expand on previous research about the health implications and role obesity plays on cognition. Importantly, it provides novel information about the relationships between obesity measures and OERP latencies for ApoE ε4 positive individuals during an odor memory encoding and retrieval task using odors as cues. The findings support the hypothesis that obesity is detrimental to brain integrity among those who are already genetically at risk for AD. Additionally, it supports the notion that OERPs can be used to detect differences between ApoE ε4 positive and ε4 negative groups. Lastly, the results support the notion that lifestyle risk factors such as obesity are associated with dementia risk.
REFERENCES


