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Effects of Insulin on Brain Functioning in Postmenopausal Women

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Abstract

Insulin has an important role in cognition, in addition to its well-known functions in peripheral glucose metabolism. Impaired insulin signaling has been linked to the cognitive decline seen in type 2 diabetes and Alzheimer's Disease (de la Monte & Wands, 2008). This study examined the effect of insulin on fMRI brain activation during working memory and episodic memory tasks in six postmenopausal women. The goal of this study was to understand how the hormonal change after menopause modifies insulin signaling and how this subsequently impacts fMRI brain activation and cognition. Subjects were tested under conditions of high peripheral insulin levels compared to low levels on two separate study days; these levels were manipulated by ingestion of glucose or water on either study day. We found that high peripheral insulin levels resulted in increased brain activation in both the working memory and episodic memory tasks. Results from this study suggest that insulin functionally changes cognitive processes in the brain in postmenopausal women, however further research is needed in order to understand these changes and their underlying mechanisms.

Introduction

The role of insulin in the brain has become an important area of research over the last several years. While insulin is known for regulating glucose levels in the body, it has been shown to have a variety of functions in the brain as well (Plum et al., 2005). Insulin research has become especially prominent due to the increasing evidence that impaired insulin signaling in the brain is related to the cognitive decline that is seen in both Alzheimer's Disease (AD) (Candeias et al., 2012) and type 2 diabetes (Kim & Feldman, 2015). The importance of understanding mechanisms of insulin action in the brain is vital to our understanding of healthy cognitive processing in the central nervous system (CNS).

Plasma glucose concentration in the periphery is regulated by insulin, which maintains optimal physiological levels of glucose to provide energy for cellular metabolic processes. The role of insulin is to lower blood glucose concentrations. To do this, insulin molecule binds to an insulin receptor on a cell and signals the insulin-sensitive glucose transporter within the cell to move to the membrane of insulin-sensitive peripheral tissue (Saltiel & Kahn, 2001). The presence of the glucose transporter on these insulin-sensitive peripheral tissues allows for glucose uptake to occur from blood into the tissues. In the postprandial state, insulin concentration in the blood increases in order to control a rise in blood glucose levels (Aronoff et al., 2004).

A common test to evaluate the integrity of glucose metabolism and insulin signaling in the periphery is known as the oral glucose tolerance test (OGTT) (Stumvoll et al., 2000). In this test, an individual will have their fasting glucose level measured and they will subsequently drink a solution containing 75 mg of glucose. The physiological

response to this is measured through periodic blood samples measuring glucose and insulin levels over the course of two to three hours. In healthy individuals who complete an OGTT, glucose and insulin levels peak at 60 minutes after drink ingestion (Matsuda & DeFronzo, 1999). The OGTT has been a tool to evaluate for diabetes or impaired insulin sensitivity, which can be seen if blood glucose levels remain elevated throughout the duration of the test.

While the OGTT allows us to observe the direct relationship between insulin and glucose in the periphery, the role of insulin in the brain is more complicated. In fact, studies have shown that insulin has little to do with glucose metabolism in the brain (Seaquist et al., 2001). Early studies assessing the effects of peripheral insulin showed that increasing peripheral insulin levels had no effect on glucose transport across the blood brain barrier (BBB) in both animal models (Hom et al., 1984) and in human (Hasselbalch et al., 1999). However, peripheral insulin concentrations have been correlated with insulin levels in cerebrospinal fluid (CSF), providing evidence that insulin crosses the BBB (Plata-Salaman, 1991) and has significant effects on the CNS (Levin & Sherwin, 2011) even though it does not significantly change glucose metabolism. It has been established that the brain is less insulin-sensitive than peripheral tissues and glucose concentrations in the brain are regulated by other mechanisms (Blazquez et al., 2014).

While insulin may not have a significant impact on glucose metabolism in the CNS as it does in the periphery, it does have many critical roles in the brain that are vital for healthy functioning. Insulin is known to be a potent neuroprotective agent (Blazquez et al., 2014) against apoptosis, oxidative stress, beta amyloid plaques, and ischemia. Many studies have shown that amyloid beta plaques, which are found in high

concentrations in the brains of AD patients and accumulate in the synapse to inhibit neuronal communication (Ferreira & Klein, 2011), bind to hippocampal neurons and trigger the removal of insulin receptors from the plasma membrane (De Felice et al., 2009). The change in insulin signaling in these AD patients may play a key role in cognitive impairment. Insulin also modulates neuronal excitability, including regulation of potassium and calcium dependent ATP channels (Plum et al., 2005). Cumulatively, the neuromodulatory and neuroprotective effects that insulin has on the brain are vital to healthy brain functioning, and disruptions in insulin can cause significant neuronal abnormalities.

Insulin resistance, which is the diminished ability for cells to respond normally to insulin, has been implicated as a possible reason for the cognitive impairments seen in both type 2 diabetes and AD. Longitudinal studies have shown that people with type 2 diabetes, a disease which is characterized by insulin resistance, are at a higher risk to develop AD and vascular dementia (Allen et al., 2004), and that this risk is almost doubled compared to healthy individuals (Yaffe et al., 2004). Alzheimer's disease has come to be known in some realms as a "type 3 diabetes" due to the pathophysiological similarities in impaired insulin signaling in the CNS which has been seen in both diabetes and AD (de la Monte & Wands, 2008). In fact, insulin nasal spray is a novel therapy for AD patients and has been shown to improve performance in attention and memory tasks (Holscher, 2014).

In addition to type 2 diabetes and AD, changes in insulin signaling are also seen during menopause. Menopause is one of the most drastic hormonal changes a woman undergoes in her lifetime, and such a decline in estrogen levels can have significant

effects on both physical and mental health. Menopause is known to cause metabolic changes, including increased central adiposity, dyslipidemia, and metabolic syndrome (MetS) (Gupte et al., 2015). In addition to physical changes, the menopause transition can also play a critical role in cognitive aging for women (Henderson, 2008). Some women experience noticeable changes in memory and other cognitive measures while other women seem to be unaffected. It is important to understand modifiable individual difference factors that contribute to these experiences during and after menopause. One of these factors may be insulin signaling. Jones et al. (2000) studied how estrogen levels in the body affect insulin signaling by studying mice that lack the enzyme to create estrogen, and they showed that these mice developed altered insulin signaling within the first year of life. Studies with human subjects have shown similar results; Pentti et al. (2009) showed that hormone therapy (HT) in postmenopausal women decreased the risk of developing type 2 diabetes. Both of these studies indicate that estrogen likely has a modulatory role in insulin signaling.

One target area in the brain for insulin is the hippocampus. Zhao et al. (1999) showed an increase in expression of insulin receptors in the hippocampus after training in a spatial memory tasks in rodent models. McNay et al. (2010) revealed that insulin is necessary for optimal cognitive functioning after seeing that injection of intrahippocampal insulin in rats acutely improved performance in spatial memory tasks, and intrahippocampal injection of a selective blockade for insulin acutely impaired memory performance in these same tasks. In human models, intranasal insulin has been shown to improve declarative memory performance which is supported by normal hippocampal functioning in healthy subjects (Benedict et al., 2004) as well people

suffering from type 2 diabetes (Novak et al., 2014) and AD (Holscher, 2014). While it is evident that functionality of the hippocampus is regulated to some degree by insulin, insulin is also found in the brain throughout the cerebral cortex and cerebellum (Hopkins & Williams, 1997). Interestingly, the hippocampus has been shown to be an area of the brain that is affected by estrogen and menopause. Maki and Resnick (2000) showed that estrogen therapy (ET) in postmenopausal women over the course of two years was related to increased brain activation in the hippocampus, parahippocampal gyrus, and temporal lobe and better performance on standard neuropsychological memory tests compared to women not taking ET. The link between the role that insulin and estrogen have on the hippocampus is further evidence to suggest the link between these two hormones.

Studies above outline the improvement in cognitive performance seen after insulin administration in a variety of tasks in both healthy and unhealthy animal and human models. Few studies have assessed how these changes in cognitive performance are functionally represented in the brain through imaging techniques. Studies that have utilized brain imaging to assess the effect of insulin on activation and metabolism have mostly been assessing the resting state activity of the brain (Chen et al., 2014) or activity in the hypothalamus after presentation of food –related stimuli (Kullmann et al., 2015). Additionally, functional magnetic resonance imaging (fMRI) to assess brain activation during cognitive tasks has been utilized to examine the acute effects of glucose on cognition (e.g. Schopf et al., 2013) but no study to date has examined the effect of insulin on brain activation when subjects are challenged with a cognitive task. This study explored how insulin affects brain activation during cognitive tasks that assess working

memory and episodic memory. This study was intended to examine the effect of insulin signaling on women after menopause. As previously described, studies have shown estrogen modulates insulin response and sensitivity, and decreased circulating estrogen levels is linked to altered insulin signaling and sensitivity in the periphery (Jones et al., 2000). For this reason, the current study only included postmenopausal women and future studies will assess the same measures in pre-menopausal women.

We manipulated insulin levels in participants by having them ingest a glucose drink (OGTT) compared to an equivalent volume of water (mock OGTT) on two separate study days. We tested the hypothesis that women would have improved performance on working memory and episodic memory tests for the OGTT day compared to the mock OGTT day due to higher levels of insulin in the body. We further hypothesized that we would observe changes in brain activation during fMRI tasks. For the working memory task in this study, decreased activation in the brain, particularly in the prefrontal cortex, has been related to more efficient thinking patterns (Neubauer & Fink, 2009) and thus decreased activation would indicate better performance. We hypothesized decreased frontal activation in the glucose condition compared to the mock condition. For the episodic memory task used in this study, increased activation in the hippocampus was previously associated with improved performance (Kirwan & Stark, 2004), and thus we anticipated increased hippocampal activation in the glucose compared to mock condition.

Methods

Participants

Participants were six cognitively normal postmenopausal women, aged 54-59, *M*(*SD*)= 56(3.0) (see Table 1 for demographic information). Women were recruited by

calling past research subjects in the Clinical Neuroscience Research Unit and by posting advertisements in the community within a 20 mile radius of UVM, as well as using online advertising through UVM. In total, 42 women responded to these advertisements. Women underwent a medical screening over the phone to ensure they were a proper fit for the study. Thirty women were screened over the phone. Inclusion comprised women aged 50-60, postmenopausal (defined by absence of menstruation for at least one year) and without any significant medical conditions. Women were excluded if they were currently taking supplemental estrogen or taking any CNS-acting drug, such as anti-depressants or anti-anxiety medications. Medical exclusion criteria included diabetes or impaired insulin signaling, chronic obstructive pulmonary disease, coronary artery disease, cancer, anxiety, and depression. Medication use by women in this study was as follows: one woman was taking levothyroxine for hypothyroidism, one woman was taking glucosamine for arthritic pain, and one woman was taking naproxen for headaches. Once each woman passed this phone screening, they were scheduled for an in-person screening visit at the University of Vermont (UVM) Clinical Research Center (CRC).

Screening Visit

All participants arrived to the CRC between 7am and 9am having fasted since midnight the night before. After signing informed consent, participants had baseline vital signs (respiratory rate, temperature, blood pressure, and heart rate) taken. Laboratory results were drawn which measured hormone (estradiol, follicule-stimulating hormone, and insulin) and glucose levels. Women were considered postmenopausal if they had not had a menstruation in one year, estradiol levels were less than 50 pg/ml, and FSH levels were greater than >30 IU/L. Insulin resistance was measured using the Homeostatic

Model Assessment (HOMA) scale, which uses fasting glucose and insulin levels to determine how efficiently the body can maintain a normal and steady amount of glucose in the blood (Mojiminiyi & Abdella, 2010). HOMA score values of less than 1.4 were considered acceptable for this study, as is standard for the HOMA-2 calculator that was used to calculate scores (Mojiminiyi & Abdella, 2010). Women were screened for MRI suitability to ensure they had no metal in their body that could cause harm to the patient in the scanner. Seven women were screened for this study and one woman was excluded due to self-reported poor tolerance to fasting. All other women met the above outlined criteria.

Cognitive Screening

Participants were cognitively evaluated using the Mini Mental State Exam (MMSE; (Folstein et al., 1975) and the Brief Cognitive Rating Scale (Reisberg & Ferris, 1988), as well as the Global Deterioration Scale (GDS) which rated the degree of cognitive impairment (Reisburg et al., 1993). On the MMSE a higher score was related to greater global cognitive functioning. On the BCRS and GDS, a higher score was related to a greater degree of cognitive impairment. Participants were required to have a MMSE score greater than or equal to 27 and a GDS score of 1 or 2. All women met these criteria.

Behavioral screening

Behavioral screening involved a partial Structured Clinical Interview for DSM-IV-TR (SCID;(First et al., 2001)) to establish the presence/absence of current or past major depressive disorder (MDD), current mania, or current dysthymia. In addition, participants completed the Beck Depression Inventory (BDI-II) (Beck et al., 1996) and

Beck Anxiety Inventory (BAI) (Beck & Steer, 1990). A cut off score of 10 was used for the BDI and 15 for the BAI. All women met these behavioral screening criteria.

Lifetime Hormone Exposure Questionnaires

Each subject answered a series of questions about lifetime hormone exposure regarding menstrual cycle history, motherhood, menopause and history of HT (Lord et al., 2009). Menstrual cycle history included questions about age at menarche, amenorrhea, and contraceptive hormone use. Information about motherhood regarded questions surrounding number of pregnancies, miscarriages, and length of time spend breastfeeding. Inquiries into the subject's menopause history included age at menopause and whether this transition was natural or surgically induced. Finally the questionnaire assessed hormonal therapy use after menopause, including type, duration, and age of use.

Study Visits

After passing the medical, psychological, and behavioral screening measures, participants came to the UVM CRC for two study days. Upon arriving at the CRC participants had baseline vitals drawn and a plastic catheter was subsequently placed in their arm. Each visit involved an oral glucose tolerance test (OGTT), where the subject ingested a drink containing 75 mg of water, or a mock OGTT where the subject ingested an equivalent volume of water. The study was a single-blind design with the experimenter and nursing staff being blinded to the drink consumption on each study day. A timer was started immediately after the participant finished her drink. Shortly after subjects completed the OGTT or mock drink, they were escorted to the MRI facility in a wheelchair where functional images were taken when subjects completed tasks assessing working memory and episodic memory. Functional tasks in the MRI scanner were timed

so that they would begin 60 minutes after the participant had finished the drink so that insulin response was maximal. After the fMRI session, which took approximately 60 minutes, participants completed two cognitive tasks and a series of questionnaires assessing mood and physical comfort.

fMRI Tasks

N-back Task

The N-back Task was used as a measure of verbal working memory. In this task, the subject viewed a string of consonant letters (except L, W, and Y), one every three seconds. Four conditions were presented: 0-back, 1-back, 2-back and 3-back. In each of the 1-back, 2-back, and 3-back conditions, the task was to decide whether the currently presented letter matched the letter that had been presented 1, 2, or 3-back in the sequence. The subjects were asked to press the "match" button when the letter on the screen matched the letter for the certain conditions, and the "mismatch" button for every other letter. In the 0-back condition, the subject was given a target letter and she made a "match" response when that target appeared. In the one-back condition, the goal was to press the match button when a letter matched the letter that appeared just prior (the letter appearing one item back). In the two-back condition, the match occurred when a letter was identical to the letter two items back. In the three-back condition, a match occurred when a letter was identical to the letter that appeared three items back. Participants were given two trial rounds of each condition on a computer before completing the full N-back task in the MRI scanner In the scanner, the 0-, 1-, 2-, and 3-back conditions were repeated three times in a counterbalanced order so that the same condition wouldn't appear twice in a row. This was a block design task where participants responded to nine

items in a block of 27 seconds, with a rest break following each block with a plus sign (+) fixation for 12 seconds. The total time of this task was 8 minutes and 12 seconds. Accuracy measures and reaction times were automatically recorded for each trial. Different versions of this task were used on separate study days and the sets were counterbalanced across study days.

Participants responded to all items by button press through an MRI compatible fiber optic button response system (Psychology Software Tools, Pittsburgh, PA) to indicate whether the letter was a match or mismatch. Stimuli were delivered through an MR-safe computer monitor. Experimental tasks were programmed using the E-prime software package and presented by PC; the PC recorded participant responses.

Face-Name Encoding Task

Prior to entering the MRI scanner, subjects were shown two unfamiliar pictures with fictional names associated with each picture. The fMRI task used these pictures, which were deemed "familiar" pictures because the participant had been previously introduced to the face-name pair, as well as new face-name associations, which the participant had never seen before. These pictures were part of the "novel" condition, because the participants had not been previously introduced to these stimuli. For each face-name pair, subjects were instructed to press the match button if they believed the fictional name below the photograph "fit" the face. They were told this was a totally subjective decision. They were asked not to make a response if the fictional name did not "fit" with the picture presented. This was a block-design task which lasted approximately six minutes, with alternating 40-second blocks of novel and familiar faces. After the scanning session finished, subjects were given a computer task with the same faces

appearing one at a time on the screen, with two names. Subjects were asked to identify which name was paired with this face during the MRI scan. The computer automatically recorded reaction times and accuracy measures. Different sets of faces were used for each woman's study day and the sets were counterbalanced across days.

fMRI Scan Procedure

All participants were scanned on a Philips 3T Achieva d-Stream scanner and received the following MR sequences as part of the imaging protocol: (1) A sagittal T1-weighted spoiled gradient volumetric sequence oriented perpendicular to the anterior commissure (AC)-posterior commissure (PC) plane using a repetition time (TR) of 9.9 ms, echo time (TE) of 4.6 ms, flip angle of 8 degrees, number signal averages (NSA) 1, field of view (FOV) of 256 mm, 256 X 256 matrix, and 1 mm slice thickness with no gap for 160 contiguous slices. (2) An axial T2-weighted gradient spin echo (GRASE) sequence using the AC-PC line for slice positioning. Twenty-eight contiguous slices 5 mm thick and no gap were acquired using TR 2466 ms, TE 80 ms, NSA 3 and FOV of 230 mm. All images were reviewed by a board-certified neuroradiologist to exclude intracranial pathology. fMRI was performed using EpiBOLD (echoplanar blood oxygenation level dependent) imaging using a single-shot sequence (TR 2500 ms, TE 35 ms, flip angle 90 degrees, 1 NSA for 197 volumes). Resolution was 2.5 mm x 2.8 mm x 4 mm. Thirty-four contiguous slices 4 mm thick with no gap were obtained in the axial oblique plane parallel to the AC-PC plane using a FOV of 240 mm and a matrix size of 128 x 96. Field map correction for magnetic inhomogeneities was accomplished by acquiring images with offset TE at the end of the functional series.

Episodic Memory Buschke Selective Reminding Test (SRT)

After the MRI, participants completed the Buschke SRT as a measure of episodic memory (Buschke & Fuld, 1974). The SRT is a multi-trial verbal list-learning task allowing the examination of acquisition, encoding, and retrieval. This standard test offers measures of storage into and retrieval from memory. For this task, a list of 16 unrelated words was read aloud to the subject. The subject recalled as many words as possible. Then, the person administering the task would selectively remind the subject of the words that she did not recall, and asked her to try to recall the list of 16 words again in any order. There were eight trials in this task. In addition, there was one delayed recall trial that was administered about 20 to 30 minutes after the end of the eighth trial. The total recall was the total amount of words the subject recalled from all eight trials. Consistency occurred when the subject remembered a word in succession for two trials. Intrusions were words the subject recalled not on the specified list. Recall failure occurred when the subject failed to remember a word on two consecutive trials. Totals among these variables in all trials were added to give total recall, total consistency, total recall failure, total intrusions, and delayed recall.

Letter Number Sequencing Task

The Letter-Number Sequencing task (Wechsler, 1987) is a measure of verbal working memory. In this task, a series of letters and numbers is read aloud to the subject and they are asked to repeat them back by saying the numbers first in order and then the letters in alphabetical order. The subject can attempt to complete three trials of equal difficulty. If the subject repeats back one of these trials accurately, the experimenter moves on to the next sequence which is of greater difficulty. If the subject fails to

accurately repeat back any of the three sequences of equal difficulty correctly, the task is finished. Difficulty is characterized by the number of letters or numbers in each trial, with more items representing a greater working memory load.

Mood and Physical Symptoms

After the cognitive battery was completed, participants completed the Profile of Mood States (POMS) (McNair et al., 1971) as well as the Stanford Sleepiness Scale (Hoddes et al., 1973), Subjective Visual Analogue Scale (SVAS; (Newhouse et al., 1994)), and a Physical Symptom Checklist (PSCL). The experimenter completed the Brief Psychiatric Rating Scale (BPRS; (Overall & Gorham, 1962)) and Objective Visual Analogue Scale (OVAS; (Newhouse et al., 1994)). The IV catheter was removed after the 120' blood sample was drawn, and the participants were given lunch prior to leaving. Participants left the CRC after the nurses ensured that they felt physically at baseline.

fMRI Analyses

For the N-back task, statistical analyses were performed using a 2(Drink: Glucose, Water) X 2(Working Memory Load: 0- back 2-back) random effects ANOVA using standard ANOVA procedures in Brain Voyager (Brain Voyager QX, The Netherlands). In an effort to correct for multiple comparisons we used a cluster-level correction by utilizing the cluster-level statistical threshold estimator from Brain Voyager QX to estimate a minimum cluster size threshold based on the approach of Forman and colleagues (Forman et al., 1995).This procedure estimated a minimum cluster size of 27 voxels in functional space (3x3x3) at an alpha level of 0.05 for the fMRI analyses.

For the Face-Name Encoding task, statistical analyses were performed using a 2(Drink: Glucose, Water) by 2(Recognition: Novel, Familiar) random effects ANOVA

using standard ANOVA procedures in Brain Voyager (Brain Boyager, QX, The Netherlands). The same cluster-level correction described above was used to correct for multiple comparisons in this task. This procedure estimated a minimum cluster size of 29 voxels in functional space (3x3x3) at an alpha level of 0.05 for the fMRI analyses.

Results

Activation Data: Working Memory

First, we examined working-memory related brain activation in the N-back task to demonstrate the expected task effects on the mock day (Figure 1). As expected, greater activation was seen in the bilateral frontal, parietal, and cerebellar regions with greater working memory load (the 2-back condition minus the 0-back condition) on the mock day (Cohen et al., 1997).

Second, we examined the effect of increased working memory load on brain activation in the OGTT compared to the mock day. This contrast examined the effect of high physiological levels of insulin on working memory and brain activation compared to low insulin levels. We specifically examined the effect of the OGTT minus the mock day in the 2-back minus 0-back condition, as is commonly done using this task (Cohen et al., 1997) to assess the difference between activation for greater working memory loads on the two separate study days. For this comparison, greater activation was seen for the OGTT compared to the mock drink (Figure 2). This increased activation was specifically seen in the inferior parietal lobe bilaterally (BA 40) (Table 2).

Activation Data: Episodic Memory

First, we examined episodic-memory related brain activation in the Face-Name encoding task to demonstrate the expected task effects on the mock day (Figure 3). As

expected, bilateral hippocampal activation was seen in the novel compared to the familiar faces condition on the mock day (R. Sperling et al., 2003) along with a diffuse episodic encoding network.

Second, we assessed activation in the novel versus familiar facial presentations on the OGTT day compared to the mock day to examine the effect of insulin on brain activation during the episodic memory task. We specifically examined the effect of OGTT minus the mock day in the novel condition minus the familiar condition and found increased activation in the brain (Figure 4). There were many regions that showed increased activation in this task (Table 3) mostly on the left side of the brain, as well as the bilateral cingulate gyrus, medial temporal gyrus, and frontal gyrus.

Working Memory Performance

Data were analyzed with a 2(Drink: Glucose, Water) X 2(Working Memory Load: 0-back, 2-back) mixed model ANOVA for the proportion correct and proportion of false alarms (Figures 5 and 6). Drink and working memory load were within-subject factors. There was a significant effect on working memory load on proportion correct ($F(1,5)=30.625$, $p=0.003$) and proportion of false alarms ($F(3,15)=.14.45$, $p<0.001$). There was no main effect or interaction involving the glucose or water condition OGTT or mock condition on N-back performance.

Episodic Memory Performance

Reaction time and accuracy measures were analyzed for the face-name encoding task (Table 4). There were no significant differences between these measures for the OGTT day compared to the mock day for either accuracy or RT ($ps>0.12$).

Behavioral Measures

On each study day, participants completed the POMS, the PSCL, Stanford Sleepiness Scale, and the SVAS as subjective measures of current mood and physical symptoms. The experimenter completed the BPRS and OVAS as objective psychiatric and physical symptom measures. There were no significant differences between any subjective measures between study days (*p*s>0.08, smallest *p* value for the alertness measure on the SVAS) (Tables 5-7). There were no significant differences between any objective measures between study days (*ps*>0.11, smallest *p* value for the sweat measure in the OVAS) (Table 8).

Cognitive Measures

For the Buschke SRT, there was a significant difference in the total recall failure measure of the task which showed greater total recall failure on the OGTT day compared to the mock OGTT day ($t(5)=2.59$, $p=0.048$). This was consistent with the pattern of means from other measures of the Buschke SRT, where performance on the OGTT day was worse compared to the mock OGTT (Table 9) however these results were not significant (*p*s>0.12). There was no significant difference in performance between study days for the letter-number sequencing task (Table 9) (*p*>.50).

Glucose and Insulin Measures

Blood samples measuring glucose and insulin values were drawn at four time points throughout the study days: 0' 30', 60', and 120' minutes. Data were analyzed with a 2(Drink: OGTT or mock drink) X 4 (Time: 0, 30 , 60, 120 minutes) mixed model ANOVA for glucose levels. Drink and time were within subject factors. There was no main effect of drink on glucose levels ($F(1,5)=1.681$, $p=0.251$). There was no main effect

of time on glucose ($F(3,15)= 1.245$, $p=0.329$), nor was there an interaction between drink and time over the course 120 minutes ($F(3,15)=0.621$, $p=0.612$) (Figure 7).

Insulin levels were analyzed with a 2 (Drink: OGTT or mock) X 4(Time: 0, 30, 60, 120 minutes) mixed model ANOVA. Drink and time were both within subject factors. There was a main effect of drink ($F(1,5)=18.4$, $p=0.008$) and time ($F(3,15)=8.443$, $p=0.002$). There was a significant interaction between drink and time ($F(3,15)=7.554$, $p=0.003$). To further probe this interaction, we assessed differences for each time point on both study days. Insulin levels were significantly greater at the 30' ($t(5)=4.96$, $p=0.004$), 60' ($t(5)=3.41$, $p=0.018$), and 120' ($t(5)=2.93$, $p=0.03$) time points on the OGTT day compered to the mock day. As expected, insulin levels were not significantly different at the zero minutes, which was prior to drink ingestion ($t(5)=1.48$, $p=0.20$) (Figure 8).

Vital Signs

There were no significant differences in systolic blood pressure ($t(4)=1.88$ $p=0.13$), diastolic blood pressure ($t(4)=1.5$, $p=0.21$), respiratory rate ($t(4)=0.61$, $p=0.57$), pulse ($t(4)=0.80$, $p=0.46$), or temperature ($t(4)=0.14$, $p=.89$) between study days (Table 10).

Discussion

The current study was the first to examine the effect of peripheral insulin levels on brain activation during cognitive tasks. Results showed that varying insulin levels does change brain activation in both the N-back task and the face-name encoding task, which assessed working and episodic memory, respectively. We tested the hypothesis that peripheral insulin levels would change fMRI activation for working memory and

episodic memory tasks in postmenopausal women. For the N-back, decreased activation was expected in the prefrontal cortex with improved performance on the tasks. For the face-name encoding task, we hypothesized that increased hippocampal activation would be observed as well as better recognition memory performance after the scan. Our data did not support these hypotheses. In the N-back task, greater activation on the OGTT day compared to the mock day was seen in the inferior parietal lobe bilaterally for the 2-back condition compared to the 0-back condition. The inferior parietal lobe has been implicated in retrieval during working memory tasks (Berryhill & Olson, 2008). As previously discussed, prior neuroimaging studies have shown that increased activation in areas implicated in working memory is associated with less efficient thinking patterns (Neubauer & Fink, 2009). This was consistent with N-back performance data trends; participants had the poorest performance measures on the 2-back compared to other conditions on the OGTT day compared to the mock day (see Figures 5 and 6). Both the imaging and performance data indicate a negative effect of high peripheral insulin on the brain and cognition. It should be noted that we did not compare the 3-back minus the 0-back condition due to prior literature suggesting that performance is affected by mental fatigue in tasks which involve a high degree of working memory load, and that this mental fatigue is greater in the 3-back compared to the 2-back condition (Guastello et al., 2015).

Similar findings were seen with the face-name encoding task activation patterns. Studies have shown that successful encoding of face-name pairs is associated with greater activation in the brain, however this activation pattern is typically seen in the hippocampus (Kirwan & Stark 2004; Sperling et al., 2001). In this study, greater

activation on the OGTT day compared to the mock day was seen in the bilateral middle temporal gyri and many other regions of the brain (see Figure 4 and Table 3). The middle temporal gyri are areas of the brain that have been implicated in encoding tasks (Bernstein et al., 2002). However, increased activation on the OGTT day compared to the mock day was seen diffusely throughout the brain, more so in the frontal lobe of the left hemisphere compared to the right hemisphere.

Similar to the N-back task where greater activation is associated with decreased neural efficiency (Neubauer & Fink, 2009), diffuse activation for encoding tasks has been negatively associated with task performance and positively associated with increased insulin resistance (Baker et al., 2011). Baker et al. (2011) showed that individuals with pre-diabetes or type 2 diabetes had greater and more diffuse activation on positron emission tomography (PET) imaging. Findings from Baker et al. (2011) suggest that increased peripheral insulin levels may impair cognition and increase brain activation compared to normal insulin levels, and these findings are consistent for both the N-back task and the face-name encoding task.

It should be noted that greater activation in the left hemisphere for encoding processes is consistent with prior literature. Typically, different regions in the brain are implicated in memory encoding and retrieval processes. The hemispheric encoding/retrieval asymmetry (HERA) model has shown through a large set of functional neuroimaging studies that encoding processes typically occur in the left hemisphere and retrieval processes in the right hemisphere (Habib et al., 2003). The greater left-sided hemispheric activation seen on the OGTT day compared to the mock day in the encoding task is consistent with this HERA model, which suggests that peripheral insulin has a

modulatory role in encoding processes and activates brain regions involved in the episodic memory network.

Ultimately, our results did not show anticipated activation patterns for either the working memory or the episodic memory task. Performance measures for working memory tasks were numerically worse on the OGTT day compared to the mock day. Performance measures for the episodic memory task were improved on the OGTT day compared to the mock day, however these measures are completed after the scan and represent retrieval success rather than encoding success.

Other cognitive measures in this study included the Buschke SRT (a measure of episodic memory) and the letter-number sequencing task (a measure of working memory) (see Table 9). While there were no significant differences in performance between study days for the letter-number task, there was a significantly greater recall failure for the OGTT day compared to the mock day. This result was consistent with other performance measures on the Buschke SRT such as the total recall and total consistency; both measures showed decreased performance on the OGTT day compared to the mock day, however these results were not significant. These trends do not align with previous data supporting improved cognitive performance on working memory tasks with higher physiological levels of insulin (Reger et al., 2008), however these improvements are typically seen with intranasal delivery rather than peripheral delivery of insulin.

Studies showing cognitive improvement with intranasal insulin have not stratified results by gender, which may have a large role on insulin signaling in the brain. The role of gender in the context of circulating gonadal steroid levels is an important factor to consider in these studies that assess how cognition intranasal insulin sprays. The goal of

this study is to understand how estrogen modulates insulin signaling and cognition. Thus far, the only study to date examining the differential effect of intranasal insulin between men and women has assessed body fat, showing that intranasal insulin reduces body fat in men over the course of eight weeks, but not in women (all of whom were taking oral contraception) (Hallschmid et al., 2004). This study shows that intranasal insulin has different influences in men and women and we therefore may not be able to expect to see the same cognitive improvements in a sample of postmenopausal women that have been previously seen in both women and men.

The postmenopausal status of these women should be taken into account when considering the results from this study. Low circulating estradiol levels in postmenopausal women likely have an impact on these results. Additionally, based on responses from lifetime estrogen exposure questionnaire, only two women used HT after menopause and only continued it for a maximum of two weeks. Based on prior research suggesting that estrogen is key for normal insulin signaling (Jones et al., 2000), one hypothesis for the activation data seen for both memory tasks is that there is not enough estrogen in the body to regulate even the higher physiologic doses of insulin seen in the OGTT day compared to the mock day. Insulin may not be able to carry out optimal functioning in the brain due to low estrogen levels.

Another hypothesis for our results is that perhaps the peripheral insulin increase was not large enough to have any significant cognitive implications on performance. While all peripheral insulin levels were significantly greater for the OGTT study day compared to the mock OGTT day, peripheral insulin levels are not always correlated with CNS levels (Havrankova et al., 1979) and these levels likely have different effects on

cognition, with high CNS levels providing cognitive improvement which can be seen based on the effectiveness of intranasal insulin spray in the context of AD (Holscher, 2014) and high peripheral levels relating to cognitive impairment seen in the context of insulin resistance (Pavlik et al., 2013). While none of the women who participated in this study were insulin resistant based on their HOMA-IR scores, perhaps the presence of higher than baseline insulin levels is enough to decrease cognitive performance measures. We hypothesize that this increased peripheral insulin may be another factor contributing to the negative effect that the brain activation in the OGTT compared to the mock day represents.

The OGTT itself has acute effects in the body that may be detrimental to cognition. Choi et al. (2013) showed that glucose ingestion after an OGTT significantly increased peripheral inflammatory markers. Furthermore, acute peripheral inflammation decreases glucose metabolism particularly in the medial temporal lobe of the brain and significantly impairs performance on cognitive tasks, including spatial memory performance (Harrison et al., 2014). These studies suggest that there may be confounding factors related to manipulating insulin through the OGTT. The hippocampus plays a major role in both spatial and episodic memory (Burgess et al., 2002). Perhaps the increase in peripheral inflammation and decrease in glucose metabolism in the hippocampus causes suboptimal hippocampal functioning. This is one hypothesis for why there is no change in hippocampal activation for the face-name encoding task between study days. Furthermore, decreased hippocampal functioning could induce a compensatory response in the brain (Stern, 2002) which may lead to the diffuse increased

activation in the face-name encoding task seen on the OGTT day compared to the mock day.

In conclusion, there are a variety of variables that may have influenced data gathered in this study. Our results indicate that high peripheral insulin levels in postmenopausal women negatively impacts fMRI brain activation during episodic memory and working memory tasks. This may be due to altered insulin signaling in these postmenopausal women because of low estrogen levels. It may also be a result of differences in insulin levels in the periphery versus the CNS, or the acute inflammation that has been shown to occur during OGTTs.

Limitations

There are several limitations in this study to consider. One limitation in this study is the sample size was a total of six women. A larger sample-size is needed to confirm the results of this study that they are not due to chance. With a larger sample size, the statistical power would be greater.

A second limitation was the use of OGTT to increase peripheral insulin levels. An increase in insulin levels is a secondary effect of glucose ingestion, and other physiologic factors might affect rate and amount of insulin release for each participant. Insulin levels could be better controlled and monitored if participants were given an insulin infusion. Furthermore, peripheral insulin may not have a significant effect on CNS insulin and a more direct route of insulin administration to the CNS, such as intranasally, would be a more effective way to assess insulin response in the brain. There are some adverse effects to intranasal delivery, such as mild rhinitis, nosebleeds, and the risk of allergic reaction.

Thus, in order to minimize risk and assess how baseline physiologic insulin levels change cognition, the OGTT method was utilized in this study.

Future Studies

This was a study that examined the interactive effect of estrogen and insulin on cognition. A similar protocol should be completed in the future with premenopausal women in order to further understand the role of insulin in the brain and how estrogen may modulate its effects. Additionally, other studies in the future assessing memory in the context of more controlled peripheral and CNS insulin manipulation may provide greater further insight into the role of insulin on cognition in postmenopausal women.

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Table 1. Demographic data and baseline fasting insulin/glucose levels (means and standard deviations) for all study participants (n=6).

Age (years)	56 (3.0)
BMI	25.01 (3.0)
Education (years)	17.66(2.88)
Fasting Glucose Levels (mg/dl)	89.89 (6.26)
Fasting Insulin Levels (uU/mL)	3.99 (0.86)
HOMA-IR Score	0.50 (0.14)

Table 2. Effects of the OGTT compared to the mock drink in the 2-back minus the 0-back condition including Talairach coordinates, cluster size, region descriptions (Brodmann's areas, BA), *t* values, and uncorrected voxel-level *p* values.

Contrast	Coordinates (x,y,z)	Cluster Extent	Region Description	T-value	P-value
OGTT-MOCK 2-BACK- 0-BACK	42, -32, 44	986	Right Inferior Parietal Lobule (BA 40)	-3.98	0.0012
	-37, -46, 41	757	Left Inferior Parietal Lobule (BA 40)	-3.03	0.0084

Table 3. Effect of the OGTT compared to the mock drink in the novel faces minus the familiar faces including Talairach coordinates, cluster size, region descriptions (Brodmann's areas, BA), *t* values, and uncorrected voxel-level *p* values.

Contrast	Coordinates (x,y,z)	Cluster Extent	Region Description	T-value	P-value
OGTT- MOCK NOVEL-FAMILIAR	48, -53, 5	9792	Right Middle Temporal Gyrus (BA 39)	13.29	0.0004
	37, 19, -0.39	2392	Right Insula (BA 13)	10.61	0.0001
	15, -58, 14	1851	Right Posterior Cingulate (BA 30)	6.15	0.0016
	1, 41, 37	788	Right Medial Frontal Gyrus (BA 8)	5.32	0.0031
	0.04, -59, 14	1868	Left Precuneus (BA 7)	5.75	0.0022
	-4, 24., 39	2321	Left Cingulate Gyrus (BA 32)	6.38	0.0014
	-9, -69, 15	1212	Left Cuneus (BA 18)	6.82	0.0010
	-23, 51, -14	1216	Left Cerebellar Culmen (BA N/A)	5.19	0.0035
	-21, -78, -12	860	Left Fusiform Gyrus (BA 19)	6.39	0.0014
	-26, -3., -12	1249	Left Middle Frontal Gyrus (BA 6)	4.61	0.0058
	-29, -72, 32	824	Left Precuneus (BA 19)	4.90	0.0044
	-41, 20, -4.	2924	Left Inferior Frontal gyrus (BA 47)	5.71	0.0023
	-48, 4, 35	784	Left Precentral Gyrus (BA 6)	7.22	0.0007
	-55, -54, 6.	5289	Left Middle Temporal Gyrus (BA 39)	8.30	0.0004

Table 4. Accuracy (proportion correct) and reaction time (ms) (mean and standard deviations) for the recognition memory of faces and names seen during the MRI task. There were no significant differences among these measures between study days (*p*s>0.12).

	Mock	**OGTT**
Accuracy	0.70 (0.062)	0.80(.080)
Reaction Time	3430.54(933.74)	2776.84(564.76)

Table 5. Scores for POMS measures (means and standard deviations) for each study day. There were no significant differences among these measures between study days (*p*s>0.19, smallest p for the anger measure).

	Mock	**OGTT**
Tension	1 (0.89)	1.5 (1.38)
Depression	0(0)	0.83(2.04)
Anger	0(0)	1.67(2.73)
Vigor	20.83(7.46)	18 (7.77)
Fatigue	1.83(2.64)	2 (2.60)
Confusion	3.5 (2.59)	2.16(0.75)
Total Mood Disturbance	-14.5 (10.29)	-9.83(13.81)

Table 6. SVAS measurements (mm) of physical symptom and mood (means and standard deviations) for each study day. There were no significant differences among these measures between study days (*p*s>0.08, smallest p value for the alertness measure).

	Mock	**OGTT**
Anxiety	2.33 (1.99)	8.75(9.57)
Mood	77.83 (6.66)	79.91 (14)
Alertness	80.58 (15.27)	69 (24.80)
Comfort	29.33 (30.52)	37.75 (40.31)
Fear	3.58 (3.90)	3.83 (1.94)
Irritability	9.5 (8.54)	28.33 (39.97)
Hunger	55.33 (19.94)	38.33 (17.78)
Interest	82.41 (15.46)	85.5(9.46)

Table 7. Scores of the PSCL and Stanford Sleepiness Questionnaires (means and standard deviations) for each study day. There were no significant differences among these measures between study days (*p*s>0.24).

	Mock	**OGTT**
PSCL	5 (3.46)	3.17 (2.04)
Stanford Sleepiness	1.83 (0.98)	1.83 (0.98)

Table 8. OVAS measurements (mm) and BPRS objective scores (means and standard deviations). There were no significant differences among these measures between study days (*p*s>0.11, smallest *p* value for the sweat measure).

	Mock	**OGTT**
Drowsiness	16.33(15.78)	18(8.34)
Restlessness	0.66(1.17)	3.59(8.05)
Disorientation	1.17 (3.86)	0.16(0.41)
Speech Impairment	1.16(2.16)	0.33(0.81)
Euphoria	1 (2.44)	6 (14.21)
Irritability	6.25 (2.23)	15.17(13.34)
Sweating	0.83 (0.25)	0.58(0.80)
GI Distress	0.17(0.25)	0.42(0.66)
Incoordination	1.08(1.50)	2.33(5.71)
Fatigue	32.75 (11.56)	26 (22.18)
Depression	1.83(2.99)	4.91(5.29)
Anxiety	15 (14.95)	24.16(24.28)
Alertness	71.75 (8.80)	70.33 (7.43)
BPRS score	24 (0)	24(0)

Table 9. Buschke SRT and the Letter-Number Sequencing scores (means and standard deviations) between study days. There was a significant difference in total recall failure between study days ($t(5)=2.59$, $p=0.048$). There were no significant differences among other measures between study days ($p>0.55$).

	Mock	**OGTT**
Total Recall	84.67(19.74)	81.67(12.84)
Total Consistency	49.67 (24.94)	46.5 (17.34)
Total Recall Failure	10.83 (10.62)	13.83 (9.33)
Total Delayed Recall	9.67 (3.88)	10.67(3.67)
Total Correct Letter-Number	11.83(3.19)	12.5(1.97)

Table 10. Vital Signs measures (means and standard deviations) taken at the beginning of each study visit. There were no significant differences among these measures between study days (*p*s>0.21, smallest p for diastolic blood pressure).

	Mock	**OGTT**
Systolic BP (mm/Hg)	115.2 (18.69)	120.8(18.77)
Diastolic BP (mm/Hg)	62.4 (9.31)	67.2(9.09)
Pulse (beats/minute)	57.2 (8.07)	59 (13.36)
Respiratory Rate (breaths/minute)	14.8(2.68)	16 (2.83)
Temperature (C)	36.22 (0.18)	36.2(0.41)

Figure Legends

Figure 1. Activation map for the N-back task comparing the 2-back minus the 0-back on the mock day only.

Figure 2. Activation map for the N-back task comparing the 2-back minus the 0-back minus on the OGTT day compared to the 2-back minus 0-back condition on the mock day.

Figure 3. Activation map for the Face-Name encoding task comparing the novel minus familiar faces condition for the mock day only.

Figure 4. Activation map for the Face-Name encoding task comparing the novel minus familiar faces condition on the OGTT day compared to the novel minus familiar condition on the mock day.

Figure 5. Performance measures (proportion of correct hits) for each N-back condition. There was a main effect of working memory on performance ($F(3,15)=8.474$, $p=0.002$). There was no main effect of drink (OGTT or mock) on performance ($F(1,5)=1.404$, $p=0.289$); or interaction between drink and working memory on performance ($F(3,15)=0.272$, $p=0.845$).

Figure 6. Proportion of false alarms for each N-back condition in the OGTT compared to the mock day. There was a significant effect of working memory load on performance ($F(3,15)=14.45$, $p<.0010$). There was no main effect of drink on performance ($F(1,5)=0$, $p=.996$). There was no interaction between drink and working memory load on number false alarms ($F(3,15)=0.692$, $p=0.571$).

Figure 7. Serum glucose levels (mg/dl) measured at four time points during the OGTT day compared to the mock day. There was no main effect of drink on glucose level

($F(1,5)=1.681$, $p=0.251$). There was no main effect of time on glucose level($F(3,15)=1.245$, $p=0.329$), nor was there an interaction between drink and time over the course 120 minutes ($F(3,15)=0.621$, $p=0.612$).

Figure 8. Serum insulin levels (uu/mL) at four time points during the OGTT study day compared to the mock day. There is a main effect of drink ($F(1,5)=18.4$, $p=0.008$) and time (F $(3,15)=8.443$, $p=0.002$). There was a significant interaction between drink and time ($F=7.554$, $p=0.003$).

Figure 1.

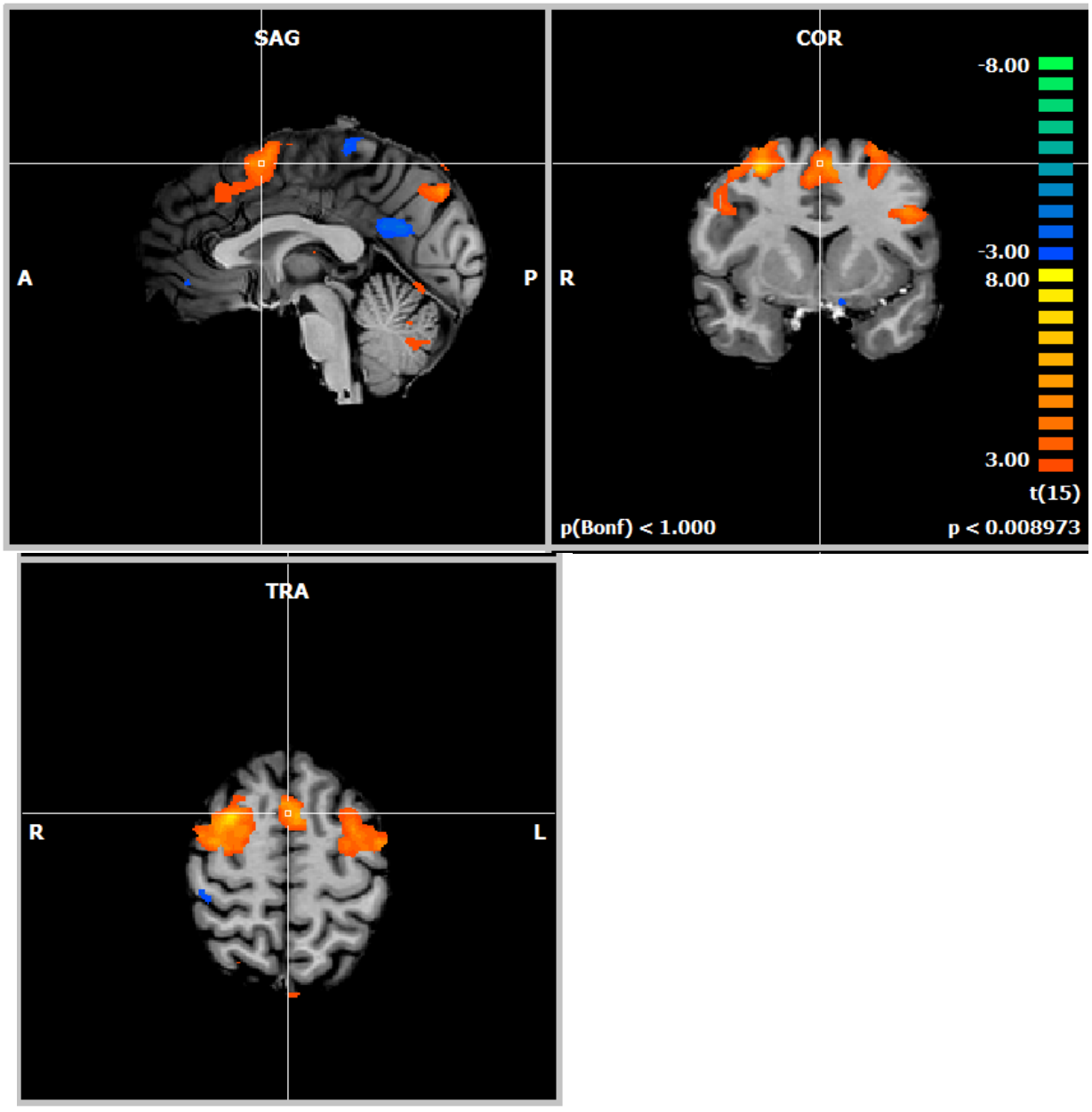


Figure 2.

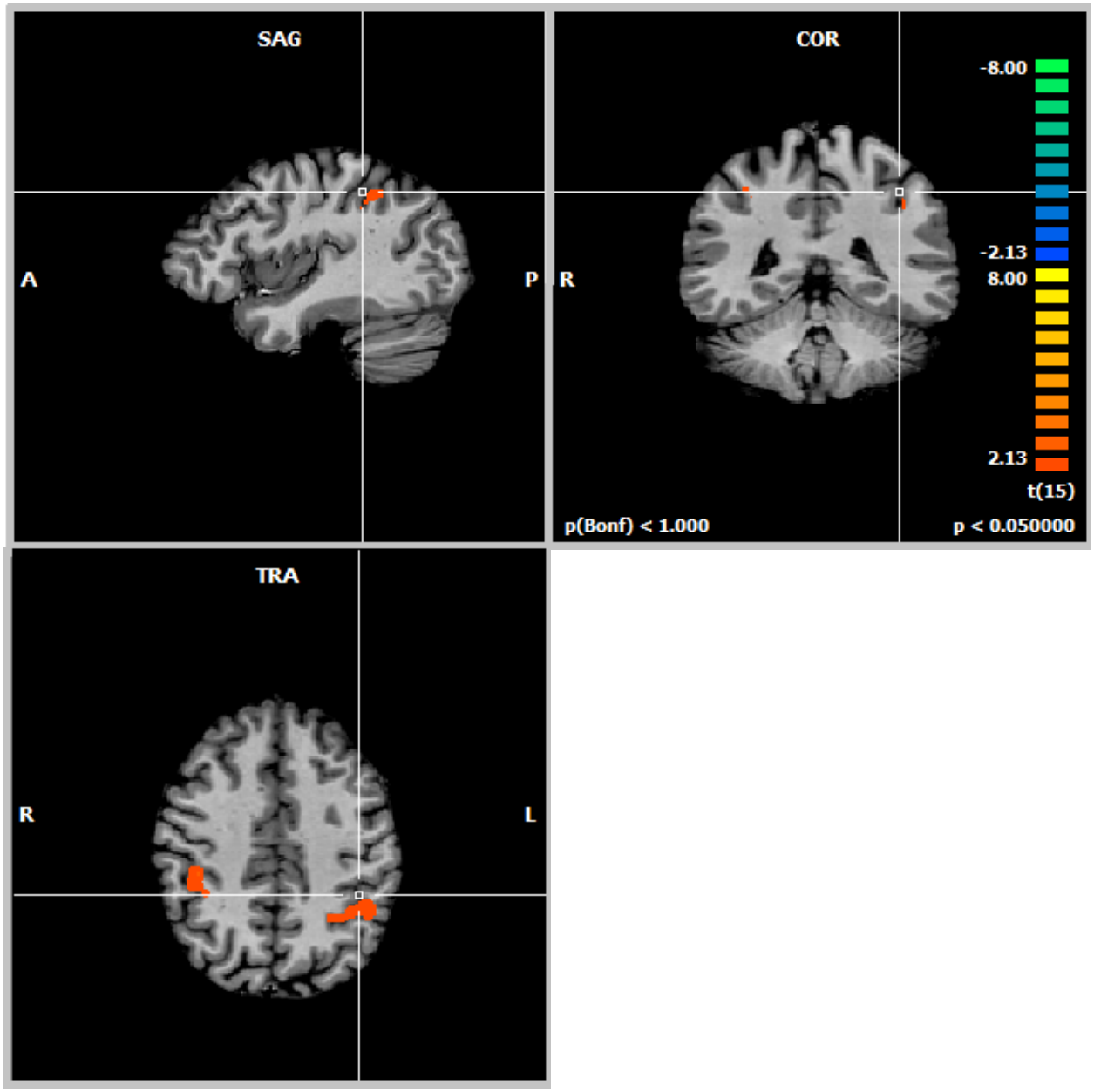


Figure 3.

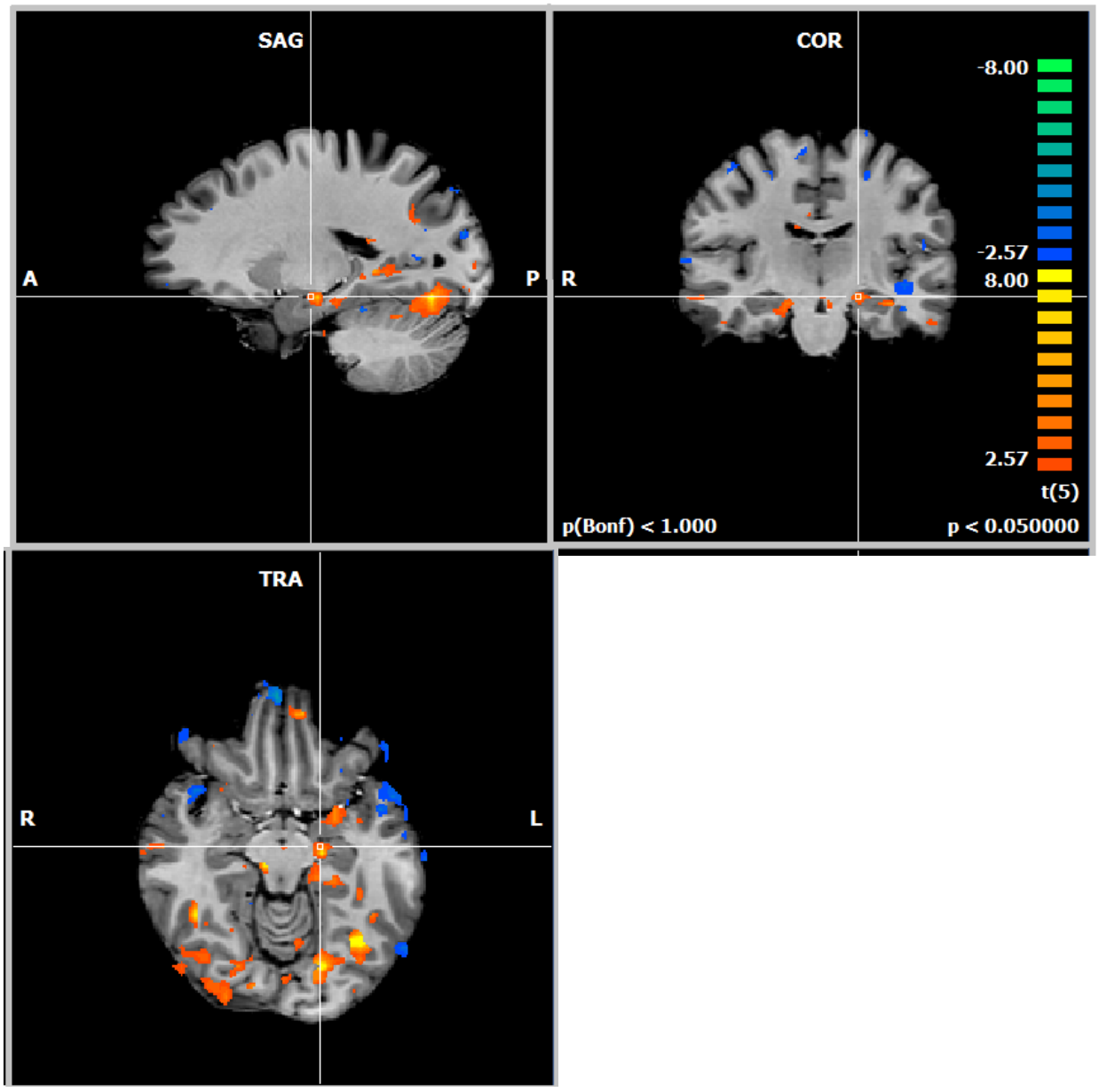


Figure 4.

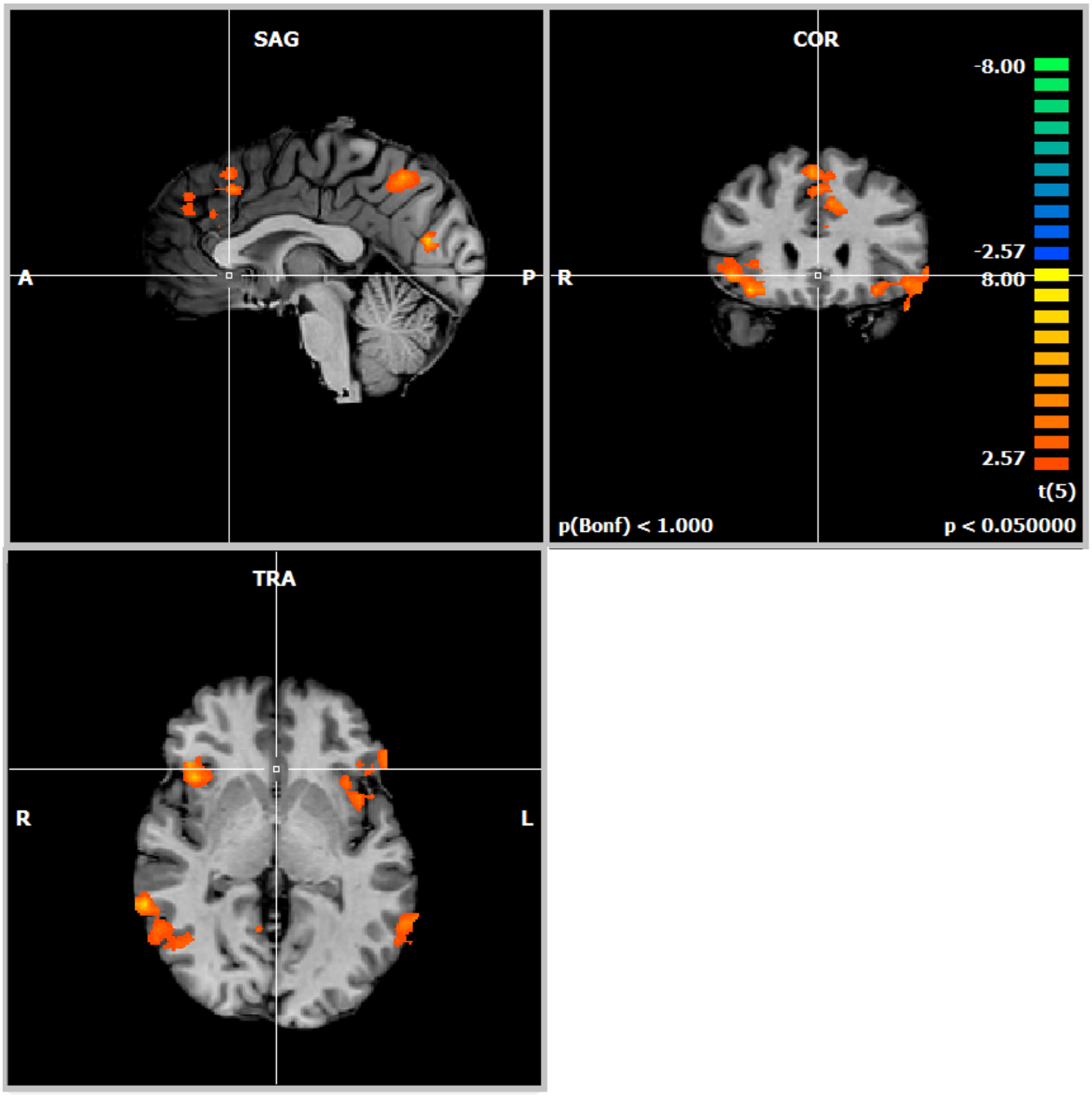


Figure 5.

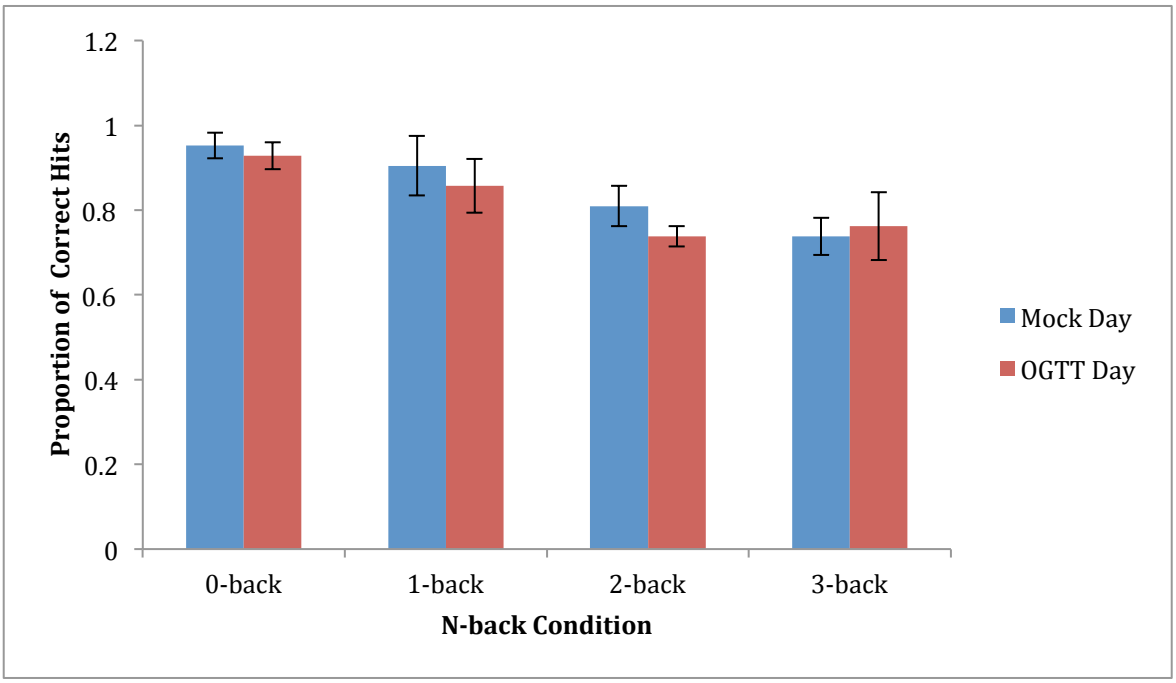

1.2
1
0.8
0.6
0.4
0.2
0
Proportion of Correct Hits
0-back
1-back
2-back
3-back
N-back Condition
Mock Day
OGTT Day

Figure 6.

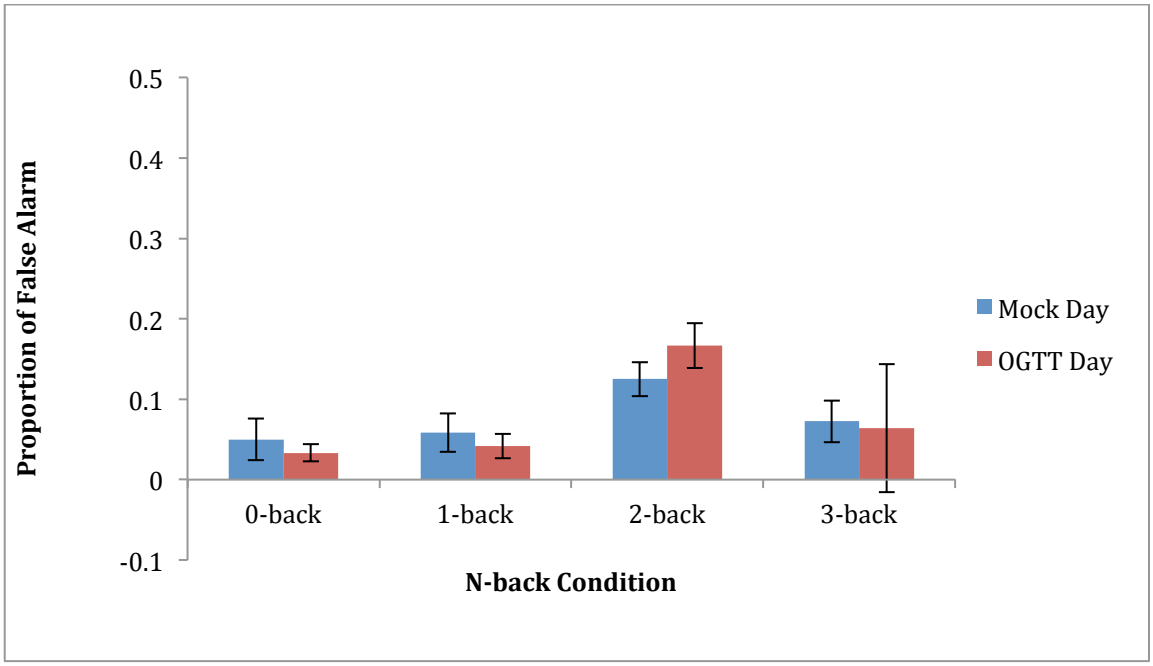

0.5
0.4
0.3
0.2
0.1
0
-0.1
Proportion of False Alarm
0-back
1-back
2-back
3-back
N-back Condition
Mock Day
OGTT Day

Figure 7.

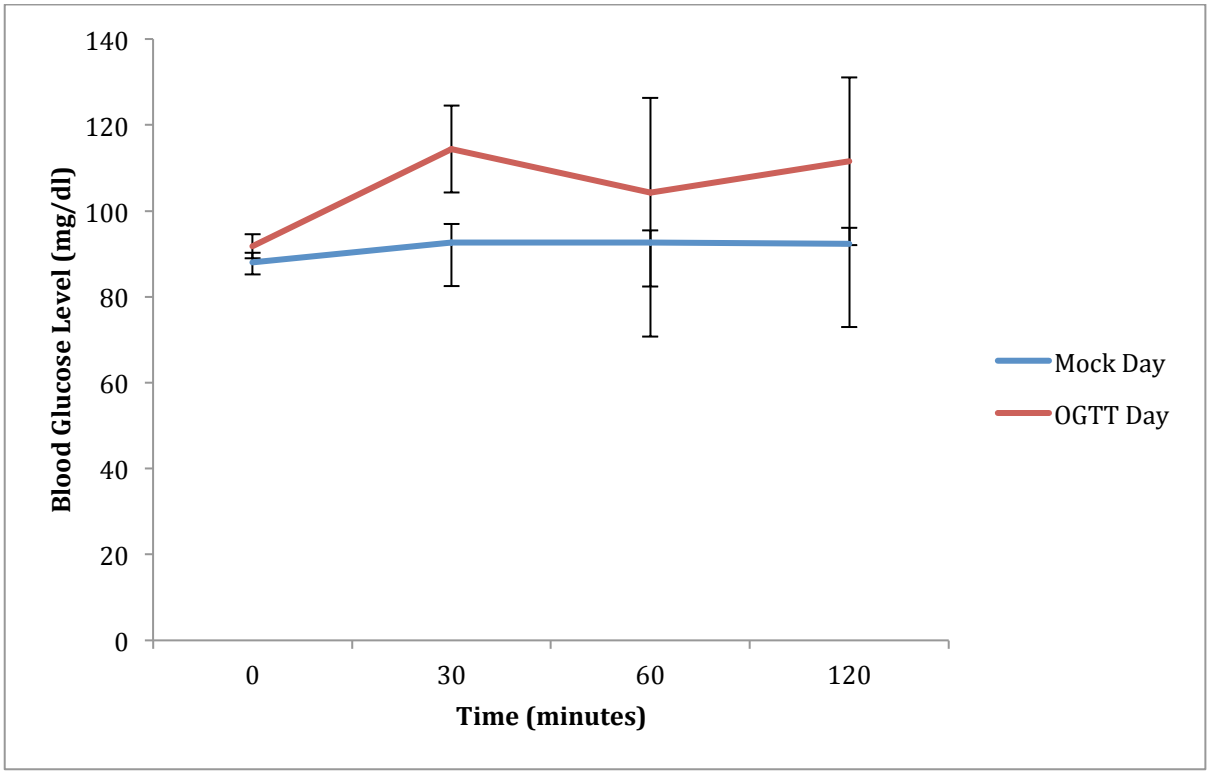
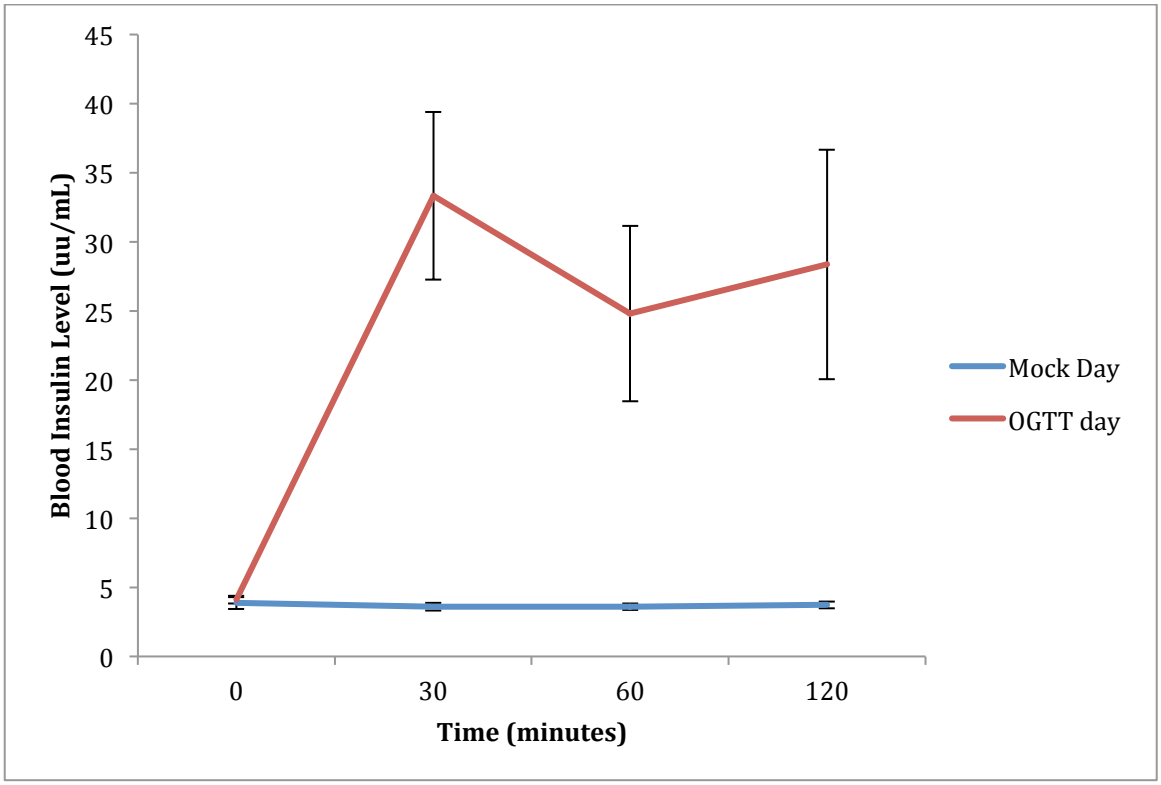

140
120
100
80
60
40
20
0
Blood Glucose Level (mg/dl)
0
30
60
120
Time (minutes)
Mock Day
OGTT Day

Figure 8.


45
40
35
30
25
20
15
10
5
0
Blood Insulin Level (uu/mL)
0
30
60
120
Time (minutes)
Mock Day
OGTT day