THE EFFECTS OF ACUTE AEROBIC EXERICSE ON BDNF LEVELS AND COGNITION IN POSTMENOPAUSAL WOMEN

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ABSTRACT

Purpose: The purpose of this study was to examine the peripheral BDNF levels after aerobic exercise in postmenopausal women. It was be hypothesized that exercise would induce greater peripheral BDNF levels and improve choice reaction time.

Methods: The subjects consisted 14 active females (7 premenopausal and 7 postmenopausal). The subjects went through two different trials: an exercise trial and a controlled reading trial. The exercise trial consisted of running on a treadmill at 75% of their VO_2max for 30 minutes. The control trial consisted of a reading session. A Stroop test was given and a blood sample was obtained before, immediately after, and 30 minutes after the exercise and control trial.

Results: The results show a significant difference between the groups over time ($P < .05$). There were interactions between age and FSH with BDNF levels immediately following exercise ($P < .05$). There was a positive correlation between age and Stroop Test time over all time points ($P < .05$).

Conclusion: Within the study, there was not statistical evidence that acute exercise affects BDNF levels nor choice reaction time for the Stroop incongruent test, regardless of menopausal status. There was evidence of a significant interaction between groups (pre and postmenopausal) in the post-exercise time point for BDNF levels but came to result insignificant changes. However, a decline in choice reaction time after menopause was observed within this study. There were observations made that highlight the need for future research within this subject matter.

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CHAPTER I

INDTRODUCTION

Menopause is the point at which women cease to have regular menses and defines the end of reproductive capabilities. It is estimated that 6000 women daily reach menopause (The American Congress of Obstetricians and Gynecologists, 2011). The most notable physiologic changes due to menopause are the lowering in estrogen and progesterone levels and the increase in follicle stimulating and luteinizing hormones. Menopause has been found to play a role in cognitive decline along with the progression of neurodegenerative diseases. There is evidence that menopause accelerates the decline of cognition (Halbreich et. al, 1994). Women appear to be more susceptible to cognitive diseases, such as Alzheimer ’s disease, than men. However, this is likely due to women’s longer life expectancy (Fargo and Bleiler, 2014). Postmenopausal women (65 years or older) are 1.54 times more likely to experience Alzheimer’s disease than men (Roos, 2012). Little research has been done regarding the mechanism behind how exercise could change cognition and help in lowering the postmenopausal women’s risks of getting neurodegenerative diseases. Hormone replacement therapy (HRT) designed to minimize the effects of menopause-induced reductions in estrogen does not come without serious long-term health risks. Therefore, elucidating a mechanism to

relieve cognitive decline that would avoid health risks associated with HRT, and also give additional health benefits alongside an increase in cognition is highly beneficial to postmenopausal women. It is known that exercise can help with improvement in cognition and neural plasticity (Cassilhas, Sergio, and Túlio de Mello, 2015; Dallognol et al., 2016). Recent studies show even a single bout of exercise can have positive effects on cognition (Hötting, Schikert, Röder, and Schmidt-Kassow, 2015). In conjunction with cognitive observations, exercise has also been shown to increase serum BDNF levels both acutely and chronically (Gold et al., 2003; Ferris et al., 2007; Seifert et al., 2010). Brain derived neurotrophic factor (BDNF) levels may act as a potential modulator of this effect (Griffin et al., 2011). BDNF is a protein that has a major role in the plasticity and survivability of the brain (Cassilhas et al., 2015). BDNF interacts with tropomyosin-related kinase B (TrKB) and increases protein binding, which then leads to proper maintenance of neural cells (Cassilhas et al., 2015). Animal models have indicated that BDNF levels within the hippocampus are central to the benefits of exercise for the brain. In the elderly human population however, it remains unclear and is an active area of investigation (Maass et al., 2016). Peripheral levels are measured as a surrogate indicator due to the fact that central BDNF cannot be measured in the hippocampus of human subjects. There is evidence to show that the levels within the periphery correlate positively to central BDNF levels. (Hötting et al., 2015; Plunico et al., 2013). BDNF has other roles within the periphery, such as appetite control, regulation of heart rate, and increase insulin sensitivity (Marosi and Mattson, 2014).

Purpose of the Study

The purpose of this study was to examine the impact of menopausal status on the

relationship between acute aerobic exercise, BDNF and cognition.

Hypothesis

It was hypothesized that aerobic exercise would increase BDNF levels and enhance cognition in postmenopausal women, similar to premenopausal women.

CHAPTER II

LITERATURE REVIEW

Brain Derived Neurotrophic Factor (BDNF)

BDNF is a protein found in the neurotrophic family that is crucial to the development, maintenance, and plasticity of central and peripheral nervous systems. BDNF begins as a pre-proneurotrophin that is cleaved into proBDNF and further processed to mature biologically active mBDNF. Neurons also release proBDNF, which is converted by the tissue plasminogen activator (tPA) plasmin system to mBDNF (Marosi and Mattson, 2014). The expression and release of BDNF are stimulated by excitatory synaptic activity and by particular neuropeptides and hormones (Marosi et al., 2014). Glutamate released from excitatory synapses binds to receptors on the synaptic membrane resulting in the influx of Na+ and Ca2+ through various receptors. Ca2+ activates Ca2+-calmodulin-dependent protein kinases (CaMK), protein kinase C (PKC), and mitogen- activated protein kinases (MAPKs) which, in turn, activate the transcription factors cAMP response element-binding protein (CREB) and nuclear factor kB (NF-kB) to induce BDNF gene transcription (Marosi et al., 2014). BDNF is concentrated in vesicles that are transported into axons/presynaptic terminals and dendrites from which it is released in response to glutamate receptor activation (Marosi et al., 2014). BDNF

mRNA is also located in dendrites where protein translation can be stimulated by synaptic activity. Local BDNF production and release activates its high-affinity receptor TrkB or the low-affinity p75 neurotrophic receptor on synaptic partner neurons and other cells in the immediate vicinity. TrkB is a receptor tyrosine kinase that upon activation leads to activation of transcription factors that regulate the expression of proteins involved in neuronal survival, plasticity, cellular energy balance, and mitochondrial bio-genesis (Cassilhas et al., 2015). Another function of BDNF besides neuroplasticity is its role in controlling energy metabolism. BDNF has been shown to have a role in suppression of appetite, regulation of insulin sensitivity, beta oxidation, and regulation of heart rate (Marosi et al., 2014; Pedersen et al., 2009). Running induces BDNF expression in neurons by stimulating CREB (cAMP response element binding protein) via a Ca2+ influx- and CaMK-mediated mechanism (Marosi et al., 2014). In addition, an exercise-induced muscle protein, FNDC5 (fi- bronectin type III domain containing 5), is induced by exercise in neurons where it mediates upregulation of BDNF.

Estrogen and BDNF

Estrogen is found within the hippocampus and estrogen receptors can be found in pre-synaptic structures that may aid in the proliferation of BDNF and vice versa. Estrogen is known to play a role in neurotrophic and neuroprotective pathways during adulthood (Genazzani, Pluchno, Luisi and Luisi, 2007). It has been observed that both estrogen and BDNF have shown neuroprotective actions within the hippocampus through similar pathways as shown in Figure 1 (Scharfman and MacLusky, 2006; Murpy, Cole, and Segal, 1998).

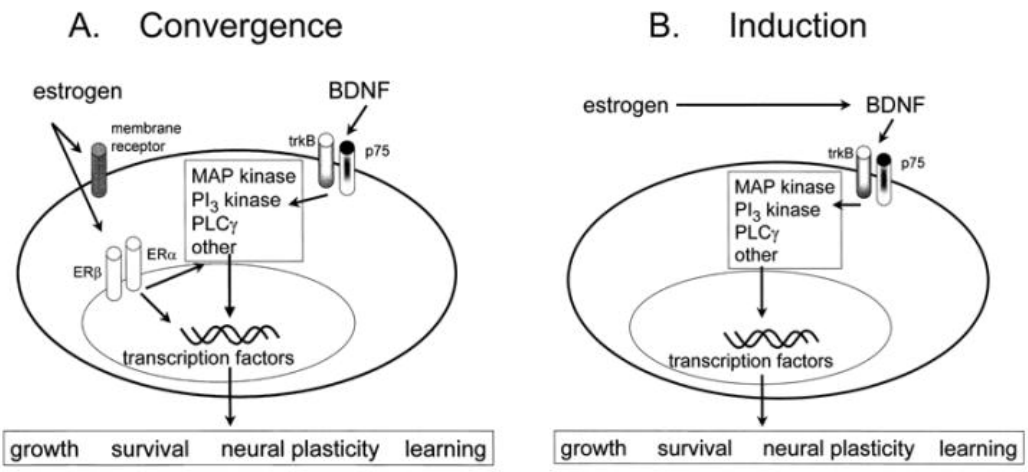

Figure 1. Interactions between estrogen and BDNF (Scharfman and MacLusky, 2006).

One of the main effects of menopause is the lowering of estrogen levels which in return could have a negative effect on the CNS (Pardo, Holland, and Cano, 2018). Hormone replacement therapy (HRT) appears to be a simple action for women to take in order to reverse potential decline in the CNS but little research provides evidence that the benefits of HRT are greater than the side effects of this kind of therapy (Genazzani et al., 2007).

To further explore the relationship of estrogen and BDNF, Begliuomini et al. 2007 studied BDNF levels according to women's hormonal status by observing 3 categories of women: normal menstrual cycle, amenorrhoeic, and postmenopausal. Sixty women were used within this study: 20 fertile ovulatory women, 15 amenorrhoeic women, and 25 postmenopausal women. Five out of the 20 fertile had their BDNF levels measured throughout an entire cycle. Ten of the 25 postmenopausal women were given hormone replacement therapy after the initial observation and were reassessed after 6 months. All of the subjects were under an overnight fast and had their blood drawn between 7:00 and 9:00 AM. The fertile women had higher plasma BDNF levels in both

the follicular (541 pg/mL) and luteinizing phases (1186 pg/mL) of their menstrual cycle as compared to amenorrhoeic (150 pg/mL) and postmenopausal women (280 pg/mL). The women who were given hormone replacement therapy experienced a rise in BDNF to the level of the fertile follicular phase women. There was a curvilinear relationship between the BDNF and time of menstrual cycle. BDNF levels peaked at day 24 (1186 pg/mL) but then dropped to similar levels of early follicular levels by day 28. Within the postmenopausal women, chronological age and years after menopause were observed. In both instances, there was a negative relationship with BDNF (Begliuomini et al., 2007).

Aging and Cognition

Literature shows that there is an age related decline in cognition (Rasmussen et al., 2006; Lopez et al., 2018). A decrease in cognitive functions such as processing speed, executive control, and memory all could occur over time as people age. Other consequences such as poor quality of life, decrease in social function, and potential hospitalization could also appear due to a decline in cognitive ability (Rassmessen et al., 2006).

Effects of Exercise on BDNF and Cognition

Aldard et al. (2004) studied the effects of exercise on BDNF mRNA and protein levels following exercise at different time points. The study consisted of 48 Sprague-Dawley male rats that were split into two groups: sedentary (caged without running wheel) and voluntarily active (caged with a running wheel). The sedentary group (N = 12 in total) were caged for the same length of time as the running group. The running group was split into 6 different scenarios: 1, 3, 5, 7, 14, or 28 days. Although it was not a major variable of the study, the Morris water maze was also implemented within the study over

a 6 day period to observe effects of exercise v. sedentary on latencies. The results showed a significant difference on day 28 of BDNF protein levels for the running group over the sedentary groups; there was no significant difference in BDNF protein levels between running and sedentary groups on any of the other timepoints. Likewise, with the BDNF protein levels, BDNF exon expression was found to be significantly different on day 28 with the running group having greater expression than the sedentary group. There was a significant difference in escape latencies on day 2 between exercise and sedentary; exercise having the quicker latency. All other time points did not have any significant differences. Between days 1 and 2, the exercise group was able to significantly decrease their latency time ($P < .05$) whereas the sedentary group did not. The researchers concluded that there was a relationship between time spent exercising and the expression of both BDNF mRNA and protein levels (Adlard, Perreau, Engesser-Cesar, and Cotman, 2004).

A study was done to observe the effects of exercise on mRNA for BDNF and nerve growth factor (NGF) in rodent brains. Thirty-nine Sprague-Dawley male rats were used within the study. They were given a 3-day training period to reduce learning effects before the treatment period. The treatment period consisted of 0 (control), 2, 4, or 7 nights within a cage that had a wheel; the wheel count was monitored on a 24hr period. The results showed a significant increase ($P \leq 0.05$) in mRNA for BDNF and mRNA of NGF. The increase for BDNF was found within the hippocampus and caudal cortex but not in the middle cortex. The increase for NGF was found in both the hippocampus and caudal cortex. The researchers concluded that their data suggested that exercise could help in increasing neurotrophin levels within the brain; which in return could lead to

neuronal survival and maintain cognitive function (Neeper, S., Gomez-Pinilla, F., Choi, J., and Cotman, C.,1996).

Van Praag et al. (1999) observed the effects of voluntary running on synaptic plasticity, learning, and neurogenesis. Thirty-four C57BL/6 mice were assigned to two groups: control (N = 17) and runner (N = 17). The runner group would have a wheel within their cage that gave them free access to exercise; on average the runner group ran 4.78 kilometers per day. Both groups were within their experimental conditions for 2 to 4 months. A spatial learning test was administered to both groups between days 30 and 49 with a second administration between days 54 and 118. The spatial learning test consisted of the Morris water maze. The maze consisted of two different trials: four trials per day and a more challenging two trials per day. The results gave no significant difference in path length, latency, or swim speed for the four trials per day. The results did show a significant difference between the control and runner groups in the more challenging two trials per day. Th runner group had shorter path lengths ($P \leq .04$) and decreased latency ($P < .047$). To determine synaptic plasticity, hippocampal slices were taken and analyzed; specifically within the dentate gyrus. They concluded that the runner group showed an increase in survivability of neuronal cells, increased spatial learning, and a selective increase in long-term potentiation (van Praag, Christie, Sejnowski, and Gage, 1999).

Kim and Sung (2017) examined the effects of exercise on memory in mice that contain a multiple sclerosis (MS)- like disease (experimental autoimmune encephalomyelitis). Thirty mice were used within this study and were randomly divided into 3 separate groups (15 for each group): sham (control), experimental autoimmune encephalomyelitis (EAE), and EAE plus exercise. The EAE plus exercise group ran thirty

minutes a day five times a week for four weeks. A step-down avoidance task was given to measure long term memory. The test consisted of the mouse being placed on a platform and then placing all four paws onto a grid. This gave a latency time which was used as the scale for memory. The latency in the sham group ranged from 119 to 180s, 30 to 91s for EAE, and 39 to 132s for EAE plus exercise. There was a significant difference between EAE and the sham group ($p < 0.001$), indicating there is memory impairment within the EAE group. However, because the EAE plus exercise group was significantly higher than the EAE group, it appeared exercise gave a preventative effect of EAE-impairment (Kim & Sung, 2017). BDNF levels were measured through western blots. BDNF levels within the hippocampus were significantly lower in the EAE group than the sham group ($p < .001$). BDNF levels within the EAE plus exercise group were significantly higher than the EAE group ($p < .015$). The authors concluded that exercise appeared to have a positive effect on memory in their mouse model with EAE. They also concluded that mice with the MS-like disease experienced higher BDNF levels. (Kim & Sung, 2017).

Hötting et al. (2015) examined the effects of acute exercise on memory, peripheral BDNF, and cortisol levels. It was hypothesized that BDNF and cortisol levels would increase after exercise and that there would be a relationship between BDNF and memory consolidation (Hötting et al., 2015). Eighty-one young and healthy university students (40 females, 41 males, mean age = 22) who were German native speakers were used as study subjects in this report. Participants were pseudo randomly assigned to three different groups: high intensity exercise (N = 26), low intensity exercise (N = 27), or a relaxing group (N= 28). Each participant was given 20 Polish-German word pairs (10

nouns and 10 verbs) with the number of words remembered used as the cognitive test (Hötting et al., 2015). The exercise groups then rode a bicycle for 30 minutes at either less than 57% (low intensity) or at 80% (high intensity) of their maximum heart rate. Their maximum heart rates were determined through a reexamination VO_2max test. The relaxing group was asked to sit in a chair for 30 minutes. After the 30 minutes, all participants were asked to watch a silent 20 minute video. Once the video was finished, the participants were given 40 Polish words to listen to (20 new and 20 old). The exercise group recalled less than 4 words whereas the relaxing group recalled over 5 words. However, for the 24-hour retention the scores of day 2 were subtracted from day 1 as a value for memory retention. The high intensity exercise group had a score of 0.2 where as low intensity and relaxing group were scored below 0. BDNF levels were significantly higher in the high intensity exercise group (>24 ng/mL) than both the low intensity (<20 ng/mL) and relaxing group (<20 ng/mL) immediately following exercise/relaxation. The researchers concluded that there is a positive relationship between high intensity exercise and BDNF levels. However, there was not enough evidence to support a relationship between BDNF levels and memory (Hötting et al., 2015).

Griffin et al. (2011) examined the effects of acute exercise and chronic effects on cognition. Forty-seven healthy male students (age = 22 years old) volunteered as participants within the study. All subjects had to be sedentary prior to the study (no regular physical training). The subjects were assigned to either a control group (N = 15) or exercise group (32). The control groups were simply asked to rest for thirty minutes. The exercise groups were then split even further into acute (single bouts of exercise) and chronic exercise groups [three weeks (N = 9) or five weeks (N = 9)]. The acute exercise

groups took a graded exercise test until exhaustion and the chronic exercise groups were given a cycle training session 3 times a week for either 3 weeks or 5 weeks. The Stroop test and blood samples were taken before and after exercise. Although both control and exercise groups improved cognitively, the exercise groups increased at a greater rate in task related performance ($p < .001$). There was no significant difference in performance between control and exercise for the Stroop word-color test. There was a significant difference ($p < .05$) in BDNF levels immediately after acute exercise (1294 pg/ml) as compared to the baseline (974 pg/ml) but not for the other times. There was no significant difference in cognition for the three week chronic exercise group but there was a significant difference in the five week group ($p = .0172$). There was no significant difference in BDNF levels for the chronic three week group but there was a significant difference at thirty minutes after exercise for the chronic five week group (778 pg/ml pre exercise, 1345 pg/ml 90 minutes post exercise). There was not enough evidence to state BDNF levels after exercise have a direct relationship but that exercise can improve cognition and will increase BDNF levels (Griffin et al., 2011).

Maass et al. (2016) examined the effect of chronic exercise on memory, hippocampal perfusion, and volumes in older adults. Forty sedentary healthy older adults (age = 68.4± 4.3 years, 55% females) either trained on a treadmill (training group; N = 21) or performed progressive muscle relaxation/stretch exercises (control group; N = 19). The training group received thirty minute intervals of training on a stationary treadmill for 3 days a week for 3 months. The control group received forty-five minutes of muscle relaxation/stretching training (Maass et al., 2016). There was no significant difference between BDNF levels and the changes in fitness. The training group had a mean serum

BDNF levels of 16.917 ngl/ml and the control group had a mean of 18.471 ngl/ml. Hippocampal perfusion significantly changed from baseline (mean pre = 104; 58.2; post = 97.5; 63.1, respectively) in the exercise group (rCBF p = .014; rCBV p = .040) but not the control. It was concluded that chronic aerobic exercise had no significant effect on BDNF levels in older adults (Maass et al., 2016).

Chang et al., (2017) examined the effects of acute exercise on cognitive function. These authors hypothesized that acute exercise would enhance cognition. Thirty college students from Nation Taiwan Sport University were recruited for this study. All subjects were between the ages of 18 and 30 years (mean = 22.59), right-hand dominant, and reported to have normal vision and color perception. This was a cross-over study where the participants went through a submaximal exercise treatment and a sedentary control treatment. For the exercise treatment, the participants were asked to ride a cycle ergometer for thirty minutes: five-minute warm up followed by a twenty-minute steady state exercise at 60-70% of heart rate reserve (previously calculated on prescreening day) and a five minute cool down. For the control treatment, the participants were asked to read a physical activity-related book for thirty minutes. Blood was drawn after treatments along with the Stroop test. There was a slight difference between accuracy of congruent vs incongruent on the test (99%; 98% respectively). The results did however show significant differences with response times between control and exercise groups. The exercise group had shorter response times for both the congruent and incongruent portions of the test (425 and 463ms respectively). There was no significant difference found between the BDNF levels of the control and the exercise group. The researchers concluded that acute moderate exercise appears to enhance cognitive performance but

does not have any effect on serum BDNF levels.

Chang et al. (2014) examined whether or not fitness level and cognitive task are related to the effects of acute exercise and cognition. Thirty-six college aged adults (25 males; 11 females) from universities around Taoyuan, Taiwan were a part of this experiment. The subjects were each given a maximal exercise test to determine their VO_2max. They were then categorized into three separate groups: low fitness (VO_2max = 35.25 ml/kg/min), moderate fitness (VO_2max = 45.52 ml/kg/min), and high fitness (VO_2max = 56.21 ml/kg/min). The subjects had two sessions with three days in between each session. The first session was an introduction along with prescreening questionnaires. The second session consisted of a thirty-minute bike ride on a cycle ergometer (five minutes warm up, twenty minutes at 65% of VO_2max, and five minutes cool down) and two Stroop tests (Pre exercise and five minutes post exercise). The main result was that for each group, their congruent response times were shorter post exercise than pre exercise. There also appeared to be a small curvilinear relationship between fitness level and Stroop test response times but was ultimately found to be statistically insignificant ($p = .06$). The researchers concluded that there is a positive relationship between acute exercise and cognition and that even a single bout of exercise could help in maintaining cognition.

BDNF, Estrogen, and Exercise

Berchtold et al., 2001 studied the regulation of mRNA and protein levels of BDNF mediated through estrogen after exercise within the rat brain. The study used female Sprague-Dawley rats. Each rat was ovariectomized and then either given a placebo or estrogen supplementation (17 beta estrodial) over a 60 day period. The rats

were then placed into 4 different categories: OVX-st (short term ovariectomy), OVX-lt (long term ovariectomy), ER-st (short term estrogen replacement), and ER-lt (long term estrogen replacement). Each of those 4 categories were then split into 2 groups: sedentary and exercise. The exercise group was given a running wheel within their cage. The results showed that there was a relationship between estrogen deprivation and BDNF expression in both sedentary and exercise groups ($p < .003$; $p < .05$). The researchers concluded that voluntary wheel running elicits an increase in BDNF mRNA expression but is dependent upon time of estrogen deprivation, meaning that the longer the rats were without estrogen the lower the increase in BDNF mRNA expression was observed after exercise (Berchtold et al., 2001).

CHAPTER III

METHODS

Research Design

This study was a correlational study to observe the relationships of menopausal status on the BDNF and cognitive responses to aerobic exercise. The independent variables were aerobic exercise and menopausal status, and the dependent variables were serum BDNF levels and cognitive performance (Stroop Test).

Participants

The study was approved by the Institutional Review Board (IRB) at Cleveland State University. Of the 24 potential participants that showed interest within the study, fourteen women met the requirements to be eligible for the study (inclusion criteria, availability). Fourteen healthy female subjects (7 premenopausal and 7 postmenopausal) were asked to volunteer for the study. They were recruited as a convenience sample through flyers, social media, and word-of-mouth. The postmenopausal subjects were regularly exercising, at least three times a week for at least thirty minutes, and had gone completely through menopause with their last period a minimum of three years prior to participation in the study. The premenopausal subjects were regularly exercising, at least three times a week for at least 30 minutes, that have a regular menstrual cycle for the past

6 months. Before the study, each subject were given an informed consent approved by the IRB. All the subjects were asked to complete the American Heart Association (AHA) and ACSM prescreening questionnaire. Only those that are in the low risk category were chosen for the study. All subjects were free from muscular skeletal injury, cardiovascular disease, mental diseases, and all be non-smokers. Exclusion criteria consisted of any muscular skeletal injury, cardiovascular disease, hormone therapy including HRT or birth control, smoking history, metabolic or endocrine disease, neurological disabilities, color blindness, a percent body fat of > 30% premenopausal and > 35% for postmenopausal, and the inability to run for 30 minutes at a moderately high intensity.

Anthropometry

Height was measured upon arrival to the Human Performance Laboratory via clinically calibrated stadiometer.

Body Composition

Body composition was determined using air displacement plethysmography with the BOD POD system (Life Measurement Instruments; Concord, California).

Menopausal Status

All participants were categorized as pre and post-menopausal. Premenopausal women were defined as having regular menstrual cycle over the last 6 months with a follicle stimulating hormone level of less than 30 mlU/ml. All premenopausal women were tested during the follicular phase of their menstrual cycle. Post menopause were determined by the cessation of menses for at least one year as a result of either surgical or natural menopause and a follicle stimulating hormone level of 30 mlU/ml or greater.

Blood Sampling

Serum BDNF, FSH, and estrodiol levels were determined within this study. Blood samples were taken immediately before, immediately after, and thirty minutes after exercise and the controlled reading. Five mL of venous blood were drawn through the aseptic technique from the antecubital vein for each drawing of blood, with a total of 15mL of blood drawn per session. Only phlebotomy-trained individuals drew blood from the subjects using sterile technique. All the supplies used during the blood sampling were discarded appropriately. Blood was collected in 5 mL serum-separating tubes. After blood collection, the samples were allowed to clot for a minimum of 30 minutes at room temperature, after which samples were spun in a centrifuge, and serum was collected. The serum was stored in a -80^{o} C freezer until analyzed. BDNF levels were measured with the sensitivity of the assays set at 20 pg/mL. (BDNF, R&D Systems*Minneapolis, MN). Follicle stimulating hormone levels were measured with the sensitivity of the assays set at 8 pg/ml. (FSH, Ray Biotech, Nocross, CA). Estrodial E2 levels were measured with the sensitivity of the assays set at 5 pg/mL (Estrodial, Invitrogen, Camarillo, CA). All ELISAs were performed using a Bio-Tek Synergy plate reader.

Cognitive Test

The Stroop test is the most commonly known test for assessing cognitive performance. The Stroop word-color test consisted of 3 different trials (pre, post, and thirty minutes post exercise). The incongruent condition of the Stroop Test was administered which is the most relevant test for measuring executive function. Specifically, this study observed choice reaction time for the incongruent section of the Stroop test. The test was administered via computer program and was the incongruent

style of Stroop Test. The test consisted of three different colors: red, blue, and green. Each subject was asked to name the color of the text rather than the spelling of the word. The subjects were asked to finish the test as quickly and efficiently as possible.

VO_2max

Before the experimental trial, the subjects were given a treadmill graded exercise test (GXT) to determine their maximum oxygen carrying capacity. The test began with a five-minute warm-up period. Throughout the GXT oxygen consumption and heart rate were monitored with a metabolic cart and three lead ECG respectively (TeleRehab VersaCare, ScottCare Cardiovascular Solutions, Cleveland, OH). The treadmill was then set to a pre-determined speed (established during warm-up). The test continued at the pre-determined speed throughout with an increase in elevation of 3 percent incline every 3 minutes. The test was finished when two of four following criteria are met: leveling off oxygen carrying capacity regardless of workload, a Respiratory Exchange Ration (RER) of 1.15 or higher, volitional fatigue, or age predicted maximum heart rate was achieved. The subjects were told the test can be cancelled at any moment.

Experimental Protocol

The fourteen subjects were assigned for which day they would be exercising first based on when they signed up for the study. The participants with an ID number that was odd would exercise first and the participants with an ID number that was even would exercise on the second day. The volunteers of the study reported to the Human Performance Lab at Cleveland State University on three different occasions. The first occasion, VO_2max was measured, and the participants were given time to get familiarized with the Stroop Test. During the first visit, subjects were given the AHA and ACSM

questionnaires to complete along with the informed consent form. The second visit, the subjects were either given an exercise trial or controlled reading trial. The third visit consisted of either exercise or controlled reading depending upon what the volunteers did on the second visit. Blood samples were taken directly before the first Stroop Test before exercise, immediately after exercise and thirty minutes after exercise in the exercise group. Blood samples were taken for the control at the same points in time but instead of exercise, they were given a controlled book of reading. A second Stroop Test was given immediately after exercise (immediately after reading). The third and final Stroop Test was given thirty minutes after exercise (thirty minutes after reading) as well.

Exercise Test

The subjects were given a single thirty-minute bout of aerobic exercise. The subjects were given 5 minutes of warm-up in which a speed was determined that would allow the subject to work at 75% of their VO_2max. Once the speed was established the subjects then ran on the treadmill for thirty minutes at 75% of VO_2max, as determined by their heart rate response during the VO2max test. Once the thirty minutes was completed, they were then asked to rest for thirty minutes. Heart rate, and rate of perceived exertion were measured throughout the exercise test.

Statistical Analysis

Descriptive statistics were obtained. A repeated measures ANOVA was done to observe changes in BDNF levels and cognitive performance over time and between the two groups. A post hoc ANOVA test was done individually within the groups at each time point in order to further investigate an interaction of BDNF levels between the groups over time. A Spearman Correlation was used to assess the relationships between

BDNF, cognition, and menopausal status. A One-Way ANOVA was done in order to observe any statistical differences between FSH and estrogen levels between the two groups. Following analysis, two postmenopausal women exhibited were determined to be outliers due to exceptionally high estrogen, and were therefore removed from all statistical analysis. SPSS (version 22) was used for all analyses with .05 used as the level of significance.

CHAPTER IV

RESULTS AND DICUSSION

The results of the study are shown in the following tables and figures.

Table 1. Subject Characteristics and Fitness Values

Measure	All	Postmenopausal	Premenopausal
N	14	7	7
Age (yrs)	42.6	57	28
Height (in)	64.3	63	65
Weight (lbs)	127.9	128	128
Body fat (%)	19.9	23	17
$VO2_{MAX}$ (ml/kg/min)	42.0	38.2	46
HR_{MAX} (bpm)	176.4	161	191
75% MHR (bpm)	146.2	134	158

Hormone Levels

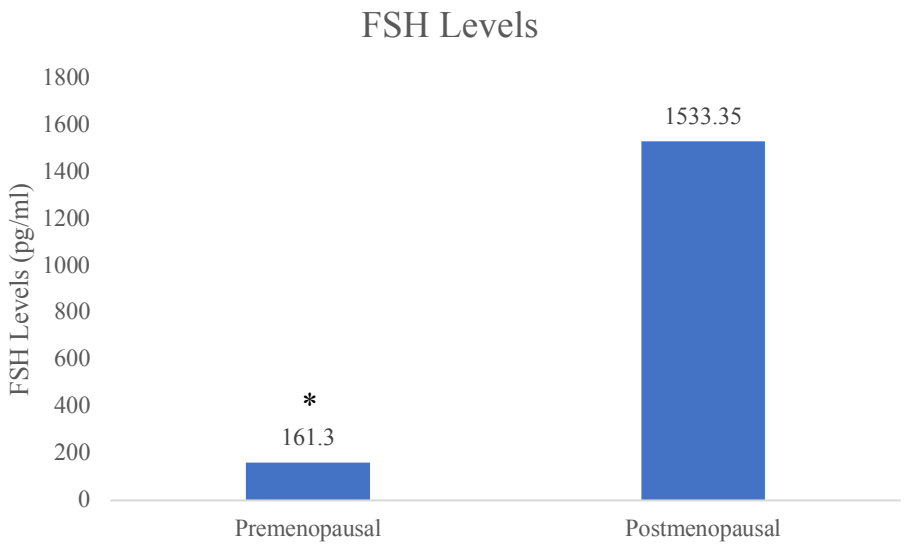

Figure 2. The average FSH (follicle stimulating hormone) levels for pre and postmenopausal groups. (*P < .01)

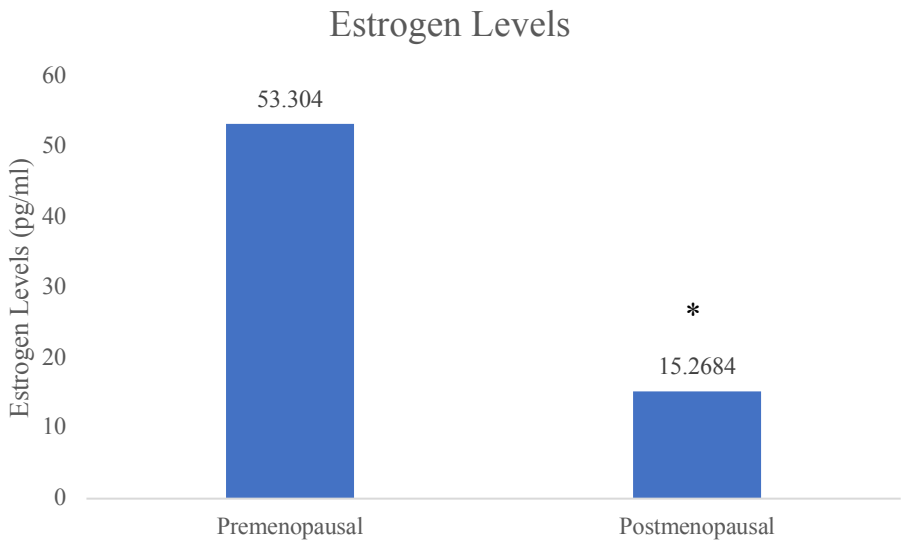

Figure 3. The average Estrogen levels for pre and postmenopausal groups. (*P < .01)

Serum BDNF responses

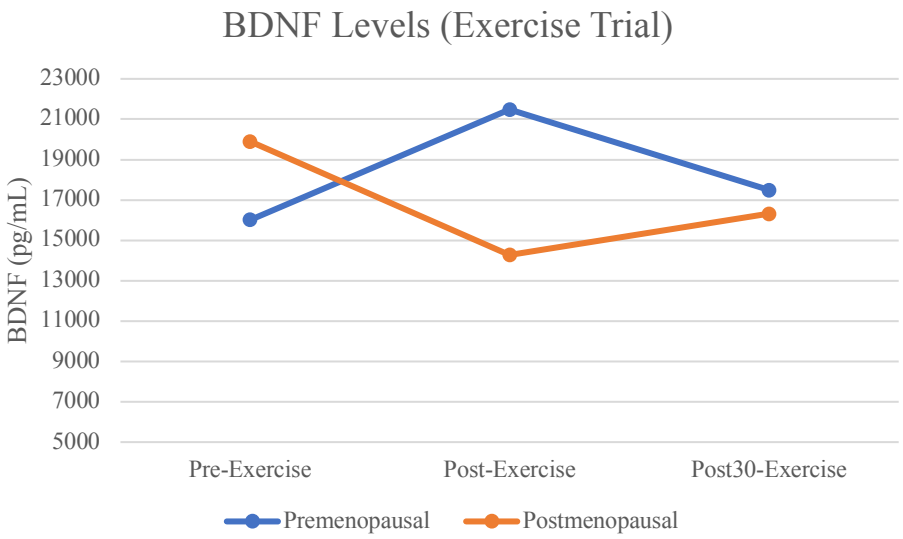

Figure 4. The effects of acute aerobic (75% MHR) on BDNF Levels (pre, post, post30) for exercise for both groups (premenopausal; postmenopausal).

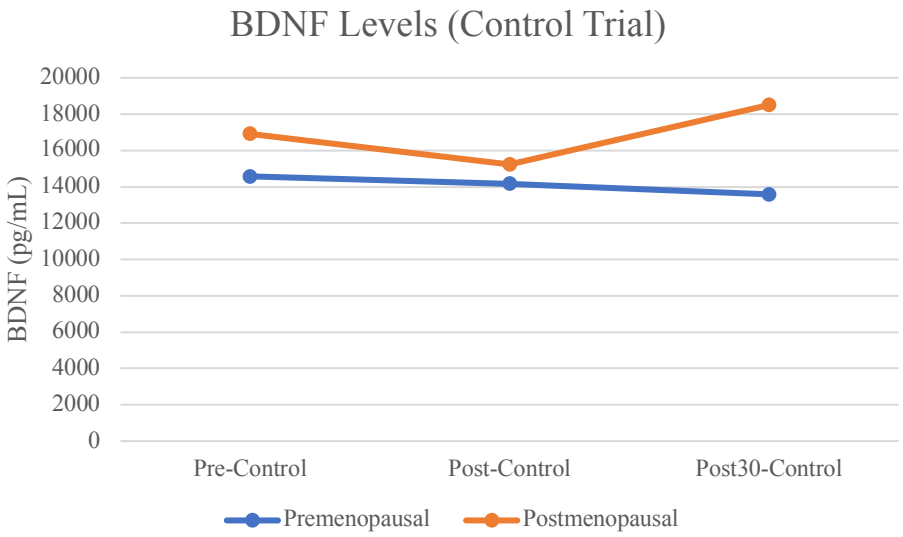

Figure 5. The average BDNF levels during the control trial for both pre and postmenopausal women.

At baseline there was no significant relationship between BDNF levels and choice reaction time for the incongruent Stroop test ($P > .05$). A repeated measures ANOVA was

done in order to observe any changes in BDNF levels due to exercise. There was no statistical difference between BDNF levels over time due to exercise (P = .735). However, there was a statistical interaction (P = .041) between the pre and postmenopausal groups between time points pre and post exercise. The interaction was that premenopausal women exhibited an increase in BDNF from pre to post exercise while postmenopausal women exhibited a drop in BDNF from pre to post exercise. There was a large effect size within this interaction represented with a partial eta squared value of .265. A Post Hoc test was done to further investigate the interaction. There was not enough statistical power (P = .164) to state that there was a difference in BDNF levels (pre to post) but that there appears to be a trend.

Table 2. Correlations (Spearman) of BDNF levels (pre, post, and post30 exercise) with age, FSH, estrogen, and $VO2_{max}$.

		Age	FSH	Estrogen	$VO2_{max}$
Pre-exercise	Correlation Coefficient	-.240	-.371	.105	.175
	Sig. (2-tailed)	.296	.236	.746	.587
Post-exercise	Correlation Coefficient	-.656*	-.615*	.413	.336
	Sig. (2-tailed)	.021	.033	.183	.286
Post30-exericse	Correlation Coefficient	-.356	-.427	-.063	.217
	Sig. (2-tailed)	.256	.167	.846	.499

*Correlation is significant at the .05 level (2-tailed).

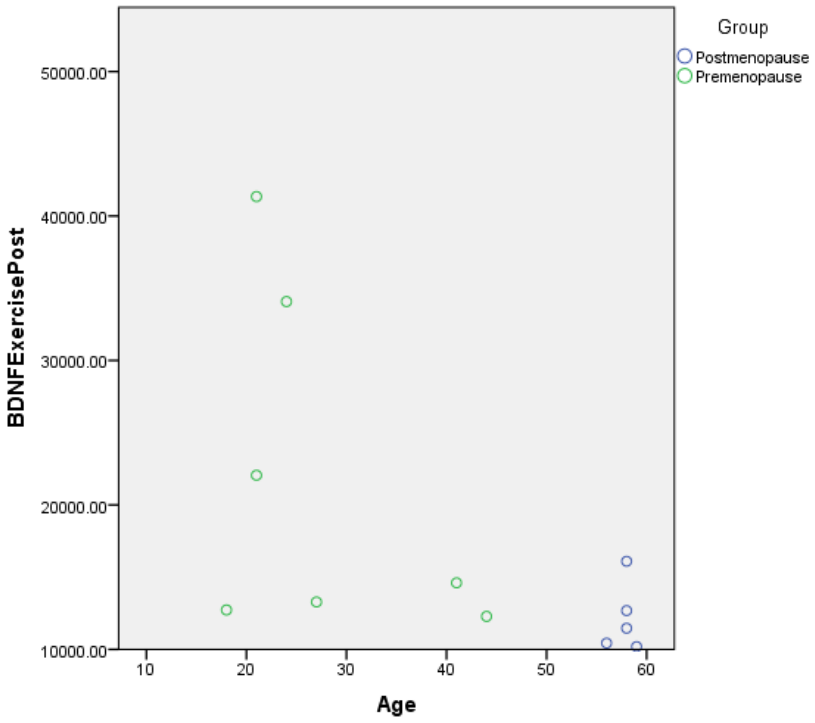

Figure 6. Correlation (Spearman) between age and BDNF levels after exercise (P = .021)

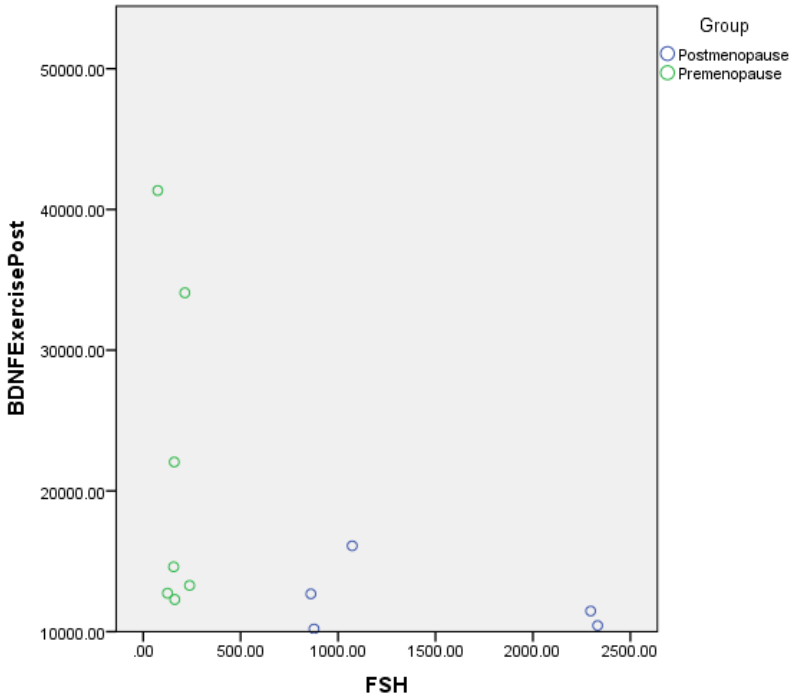

Figure 7. Correlation (Spearman) between FSH and BDNF levels after exercise (P = .033)

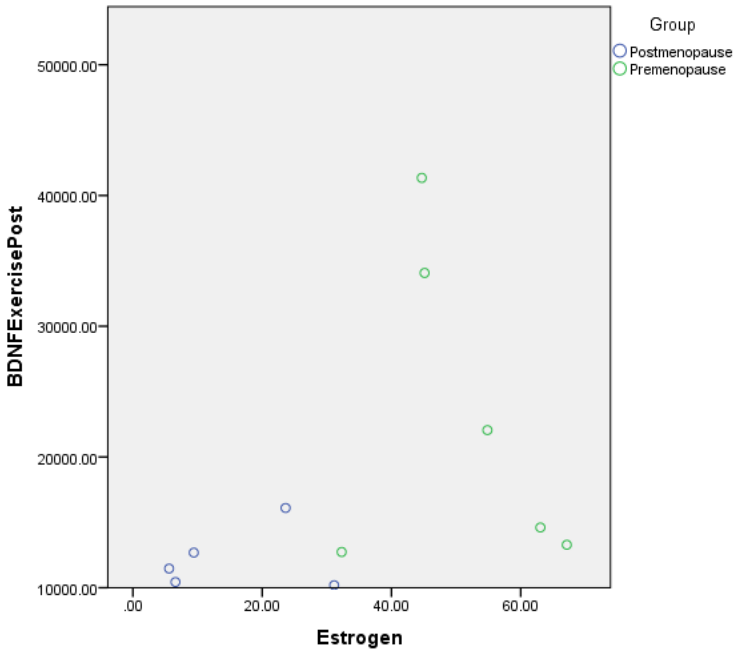

Figure 8. Correlation (Spearman) between estrogen and BDNF levels after exercise (P = .183)

Table 3. Correlations (Spearman) of BDNF levels (pre, post, and post30 control) with age, FSH, estrogen, and $VO2_{max}$.

		Age	FSH	Estrogen	$VO2_{max}$
Pre-control	Correlation Coefficient	-.127	.112	-.364	-.105
	Sig. (2-tailed)	.485	.729	.245	.746
Post-control	Correlation Coefficient	-.222	-.385	.119	.056
	Sig. (2-tailed)	.488	.217	.713	.863
Post30-control	Correlation Coefficient	.074	-.035	-.126	-.189
	Sig. (2-tailed)	.819	.914	.697	.557

Cognitive Assessment

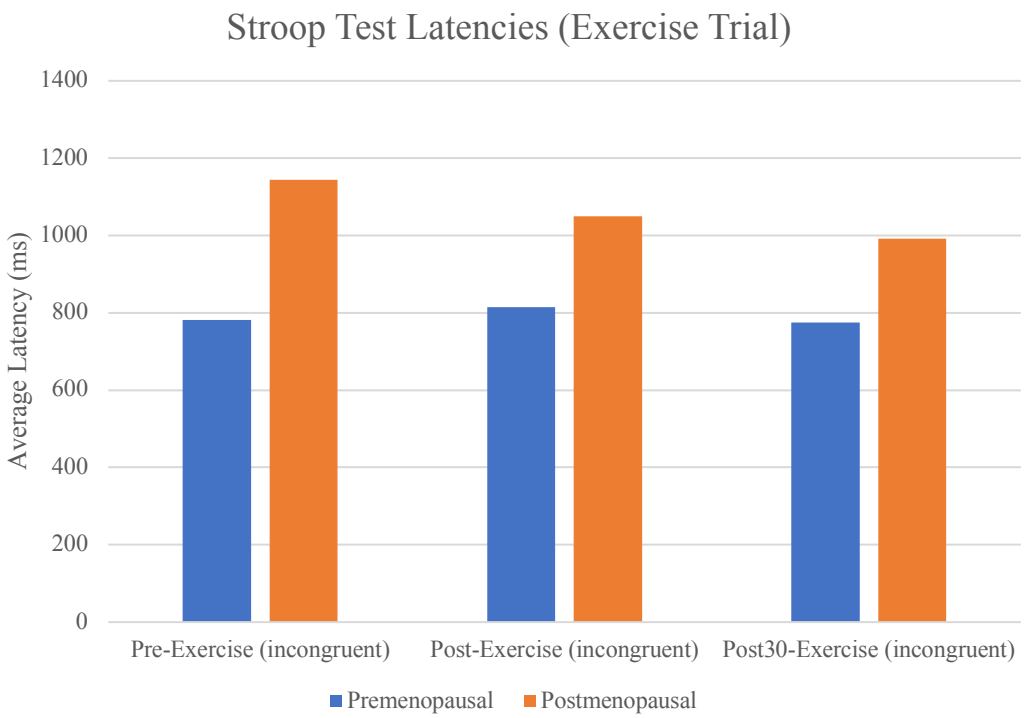

Figure 9. The incongruent Stroop Test results from the exercise trial (pre, post, and post30).

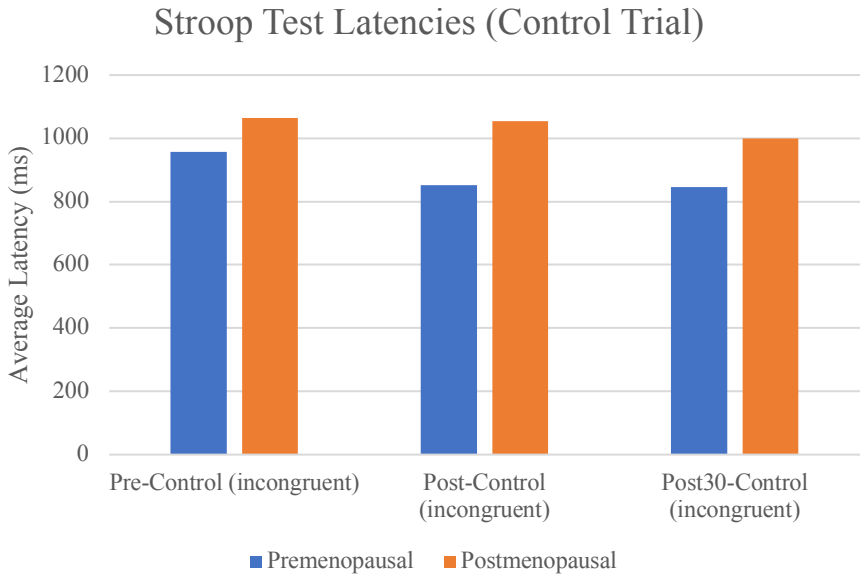

Figure 10. The incongruent Stroop Test results from the control trials (pre, post, post30).

A repeated measures ANOVA was done in order to observe any changes in

cognition due to exercise. There were no statistical differences in the time points (pre, post, post30) due to exercise (P = .235). There was a statistical difference (P = .017) between the means of pre and postmenopausal women at each time point (pre, post, and post30). Postmenopausal women had higher latency scores than the premenopausal women throughout each time point.

Table 4. Correlations (Spearman) of Stroop Test latencies for incongruent (exercise) with age, FSH, estrogen, and $VO2_{max}$.

		Age	FSH	Estrogen	$VO2_{max}$
Incongruent Pre-exercise	Correlation Coefficient	.600*	.713**	-.434	-.427
	Sig. (2-tailed)	.039	.009	.159	.167
Incongruent Post-exercise	Correlation Coefficient	.511	.594*	-.371	-.364
	Sig. (2-tailed)	.089	.042	.236	.245
Incongruent Post30-exercise	Correlation Coefficient	.635*	.699*	-.385	-.524
	Sig. (2-tailed)	.027	.011	.217	.080

*Correlation is significant at the .05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

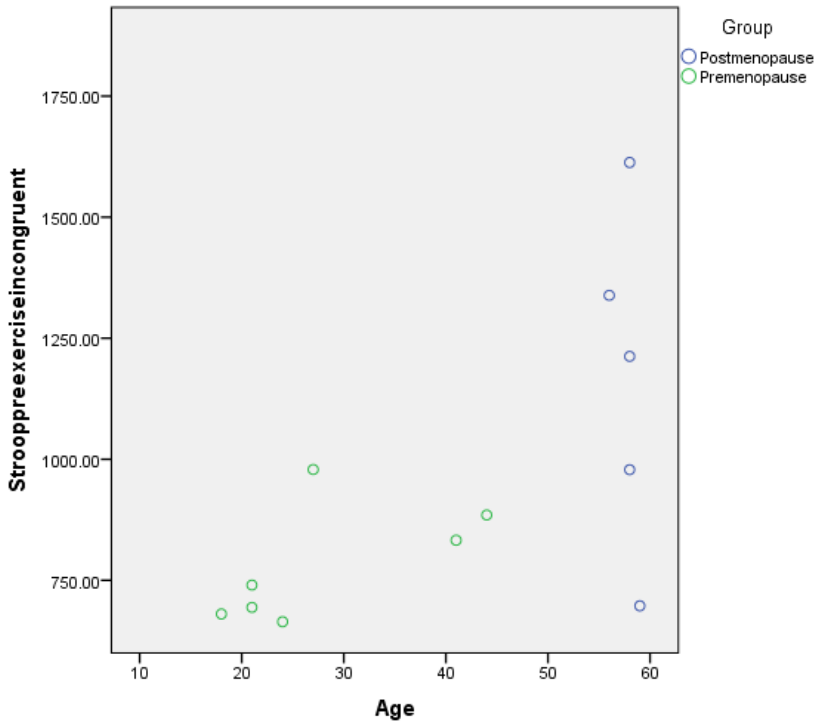

Figure 11. Correlation (Spearman) between age and Stroop Test latencies for the incongruent task (pre-exercise).

Table 5. Correlations (Spearman) of Stroop Test latencies for incongruent (control) with age, FSH, estrogen, and $VO2_{max}$.

		Age	FSH	Estrogen	$VO2_{max}$
Incongruent Pre-reading	Correlation Coefficient	.353	.210	-..035	-.308
	Sig. (2-tailed)	.261	.513	.914	.331
Incongruent Post-reading	Correlation Coefficient	.466	.336	-.140	-.385
	Sig. (2-tailed)	.127	.286	.665	.217
Incongruent Post30-reading	Correlation Coefficient	.564	.364	-.224	-.392
	Sig. (2-tailed)	.056	.245	.484	.208

*Correlation is significant at the .05 level (2-tailed).
**Correlation is significant at the .01 level (2-tailed).

Discussion

We found that there was an interaction between the two groups in BDNF levels over time (P .041). That is, that premenopausal women will have a rise in BDNF levels after exercise and that postmenopausal women will have a decrease in BDNF levels after exercise. These findings are similar to that which was found in Berchtold et al., 2001 where BDNF levels dropped/did not change after exercise in both active and sedentary mice. The findings found within the present study because revealed a decrease in BDNF levels after exercise for postmenopausal women but not for premenopausal women. These results could give evidence of similarities between the interaction of BDNF and estrogen in animal and human subjects. A lowering of BDNF level proliferation was found only in mice that were estrogen deprived for an extended period of time and for postmenopausal women who have been "estrogen deprived" for at least 12 months.

Hotting et al., 2015 observed the effects of exercise on memory both immediately after exercise and twenty-four hours after exercise. The researchers concluded that immediately after exercise, there was not an increase in memory retention as compared to sedentary or relaxing groups. This lack of increase in cognition align with our results in that we did not see an apparent increase in choice reaction time performance immediately following exercise. Chang et al., 2017 mentions that there was a curvilinear relationship (P = .06) between fitness level and cognitive performance after exercise. Especially within our premenopausal group, our test subjects had fairly high fitness levels (VO_2max = 46 kg/ml/min) and therefore could be the potential reason behind not seeing an increase in cognitive performance immediately after exercise. We did however see correlations between age and cognitive performance over each time point. The postmenopausal group

was significantly slower in pre and post30 for both exercise and control trials ($P < .05$). This gave evidence to a decline in incongruent choice reaction time due to age. There was no significant relationship between BDNF levels and choice reaction time for the incongruent Stroop test ($P > .05$).

CHAPTER V

SUMMARY AND CONCLUSION

Limitations

All participants in the study were regularly active females. Activity level could play a role in BDNF levels and cognitive performance. Within this study running on a treadmill was the only form of aerobic exercise used. Other forms of aerobic exercise (ex. swimming, biking) could elicit a different response both in BDNF levels and cognitive performance. A reason for the lack of improvement in choice reaction time due to exercise could be the cognitive test used within the study. Only female subjects participated within the study, there could be gender differences in BDNF levels and cognitive performance after exercise. The study had a small sample size and although there were observations, there was a lack of statistical power behind many of these observations due to low sample sizes. Although it was controlled to the best of the researchers ability, the lab at which the participants exercised and took the Stroop Test has a constantly changing environment (ex. people coming in and out, movement of equipment, temperature) that could have had an effect on the results.

Future Research

Suggestions for future research would be having a larger sample size; as stated

before there were a few significant observations within the study but there was limited statistical support for many other observations. Different modes of aerobic exercise could reveal different results, An example would be swimming or biking as opposed to a treadmill run. Though it may be difficult to find qualified participants in the postmenopausal category, increasing the intensity of the aerobic exercise of the study may elicit a different response of BDNF and show an improvement on cognitive performance. Comparing sedentary to regularly active individuals could elicit different BDNF levels after exercise. There is also a substantial need for further exploration of the mechanistic associations of BDNF to estrogen and the exercise hormonal milieu.

Conclusion

The purpose of this study was to examine the impact of menopausal status on the relationship between acute aerobic exercise, BDNF and cognition. We saw correlations between age and cognitive performance that gives evidence to a slowing of choice reaction time due to age. It was hypothesized that acute aerobic exercise would increase serum BDNF levels and increase cognitive performance. We were not able to show statistical evidence in any differences in choice reaction time performance after acute aerobic exercise. However, there was a divergent response in BDNF in response to exercise, with premenopausal women showing a rise in BDNF, and postmenopausal women exhibiting a drop in BDNF after exercise. It is unclear what the long-term implications of this finding are in terms of neuronal health, but it may be that postmenopausal women have a different timeline for post-exercise BDNF responses that warrants further exploration. Therefore, we reject the hypothesis that pre and postmenopausal women would have similar responses in BDNF but accept the hypothesis

that pre and postmenopausal women would have similar responses in incongruent choice reaction time after exercise. That being said, we did have observations that support the need for further investigate this area of research.

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APPENDIX A: INFORMED CONSENT

INFORMED CONSENT FOR PARTICIPATION

Effect of an acute aerobic exercise bout on cognition and brain-derived neurotrophic factor (BDNF) in postmenopausal women

Introduction

Thank you for considering to be a part of this study. My name is Ryan Wiet and I am working on my Master's Thesis at Cleveland State University. This study will be lead under the supervision of Dr. Emily Kullman, Associate Professor of Exercise Science in the Health and Human Performance Department.

The purpose of this study is to observe the cognitive response to exercise in postmenopausal women. We will specifically look at BDNF levels and cognition. BDNF is a protein that helps protect neurons. This study will provide information on how aerobic exercise influences BDNF levels and cognition. The research study will be conducted in the Human Performance Laboratory (HPL) at Cleveland State University (CSU).

Procedures

BDNF will be measured through a blood draw from your arm. Cognition will be measured using the Stroop test. For this test, you will be shown color words in a different ink than the actual text. You will name the color of the ink of each word as quickly and accurately as possible. We will recruit 40 healthy female subjects, who regularly exercise. Subjects will complete an AHA/ACSM pre-screening questionnaire to determine medical history. The information submitted will be used to determine eligibility for the study.

You will be asked to come to the HPL 3 separate times. Each session will last about 1.5 - 2 hrs and will require a 10 hour fast prior to testing. The total study time commitment is around 5 – 6 hrs spread over 1 – 2 weeks. During session one, basic measurements will be taken. These include: height, weight, BMI, body composition, resting heart rate, blood pressure, and an exercise test to determine VO_2 max. ECG will be used to determine max heart rate. A metabolic cart will be used to determine max VO_2. You will be allowed to practice the Stroop test. We will also measure body composition by using a BodPod. This procedure takes around 10 minutes to complete, and requires you to be dressed in a bathing suit, or form-fitting clothes. A cognitive screening questionnaire will be administerd as well (TICS – M).

The order of the last two sessions will be randomized. One session will be a 30-minute run at 75% max HR. The other session will be a reading session. A blood sample and Stroop test will be given before, immediately after, and 30 minutes after each trial. There will be a total of 3, 5 mL blood draws for each exercise session for a study total of 30 mL. All storage and analysis of blood will be done at the HPL by study personnel. Each session will be performed during the same time of day. Each session will be performed in an overnight fasted state (10 hours). Premenopausal women will be tested within 8 days of starting their menstrual cycle.

Risks
Risks of these tests are minimal and do not exceed those of normal exercise. Risks associated with this study include: muscle soreness, shortness of breath, fatigue, heart attack, or acute injuries resulting from the exercise. This risk would be the same experienced from a normal training routine. In regards to drawing blood, the needle stick may hurt. There is also a small risk of bruising, a rare risk of infection, or lightheadedness. Every effort will be made to minimize these risks. Understand that the laboratory is equipped with an AED. All lab personnel are certified in CPR and First Aid. Emergency procedures include calling EMS (x911) stating to the dispatcher: "We have a medical emergency in the Human Performance Laboratory PE Building-Room B60". CPR/First aid will be administered until EMS arrives. Understand that one can voluntarily stop at any time during the protocol.

Benefits
I understand that there are no direct benefits for participating in the study other than engaging in an exercise session. The results of this study will be beneficial to the general population who are seeking to maintain and or improve their brain health through exercise.

Confidentiality
To protect privacy, any data and information obtained during participation will be confidential. It will not be disclosed to anyone without consent. A number will be assigned to each subject in place of a name. The information, however, may be used for a statistical or scientific purpose with the right of privacy retained. Dr. Emily Kullman and Ryan Wiet will be the only witnesses of the information being presented. Data will be stored in the Human Performance Lab PED60B in a locked filing cabinet.

Participation
I understand that participation in this project is by choice. I have the right to withdraw at any time with no consequences. I attest and verify that I have no known health problems that could prevent me from successfully participating in the testing. If I have any questions about the procedures, I can contact Dr. Emily Kullman at (216) 687-4854 or Ryan Wiet at (614) 949-6116.

I understand that if I have any questions about my rights as a participant, I can contact Cleveland State University's Review Board at (216) 687-3630.

Participant Acknowledgement
The procedures, purposes, known discomforts and risks, possible benefits to me and to others have been explained to me. I have read the consent form or it has been read to me, and I understand it. I also understand that all data, even data collected to determine eligibility for the study will be stored in a secured file in the HPL for at least 3 years then shredded.

I agree to participate in this study. I am at least 18 years of age.

I have been given a copy of this consent form.

Signature:_____ **Date:** _____

Name Printed:_____

Witness:_____ **Date:**_____

Menopausal Status
Please check the box that most accurately describes your current menopausal status.

- ☐ I have experienced normal, monthly menstrual cycles during the previous six months.

- ☐ I have not experienced a menstrual cycle in the previous 12 months as a result of surgical or naturally occurring menopause.
- ☐ Other
  Please explain: _____

APPENDIX B: AHA/ACSM PRESCREENING QUESTIONNAIRE

Name _____ Date _____

AHA/ACSM Pre-participation Screening Questionnaire

Assess Your Health Needs by Marking all ***true*** statements

History

You have had:

- ☐ A heart attack
- ☐ Heart Surgery
- ☐ Cardiac Catheterization
- ☐ Coronary angioplasty (PTCA)
- ☐ Pacemaker/implantable cardiac
- ☐ Defibrillator/rhythm disturbance
- ☐ Heart valve disease
- ☐ Heart failure
- ☐ Heart transplantation
- ☐ Congenital heart disease

Recommendations:

If you marked any of the statements in this section, consult your healthcare provider before engaging in exercise. You may need to use a facility with a medically qualified staff.

Other health issues:

- ☐ You have musculoskeletal problems. *(Specify on back)**
- ☐ You have concerns about the safety of exercise. *(Specify on back)**
- ☐ You take prescription medication (s). *(specify on back)**
- ☐ You are pregnant

Symptoms

- ☐ You experience chest discomfort with exertion.
- ☐ You experience unreasonable breathlessness.
- ☐ You experience dizziness, fainting, blackouts
- ☐ You take heart medications.

Cardiovascular risk factors

- ☐ You are a man older than 45 years.
- ☐ You are a woman older than 55 years or you have had a hysterectomy or you are postmenopausal.
- ☐ You smoke.
- ☐ Your blood pressure is greater than 140/90 mm Hg.
- ☐ You don't know your blood pressure.
- ☐ You take blood pressure medication.
- ☐ You don't know your cholesterol level.
- ☐ You have a blood cholesterol >240 mg/dl.
- ☐ You have a blood relative who had a heart attack before age 55 (*father/brother)* or 65 *(mother/sister).*
- ☐ You are diabetic or take medicine to control your blood sugar.
- ☐ You are physically inactive *(i.e., you get less than 30 minutes of physical activity on at least3 days/week).*
- ☐ You are more than 20 pounds overweight.
- ☐ None of the above is true.

If you marked two or more of the statements in this section, you should consult your healthcare provider before engaging in exercise. You might benefit by using a facility with a professionally qualified staff to guide your exercise program.

You should be able to exercise safely without consultation of your healthcare provider in almost any facility that meets you needs.

- Proceed with test if musculoskeletal problems are minor, concerns about safety of exercise are normal, and prescription medications are not for cardiac, pulmonary, or metabolic disease.

Risk Status (Low, Moderate, High): _____

APPENDIX C: TICS-M QUESTIONNAIRE

TICS-M

Orientation Score '1' for each correct answer and '0' if incorrect

1. (i) What day of the week is it? ___Day of the week
   (ii) What is today's date? ___Day ___Month ___Year
   (iii) What season are we in? ___Season
2. What is your age? ___Age
3. What is your telephone number? ___Telephone Number

Registration/Free Recall

4. I'm going to read you a list of 10 words. Please listen carefully and try to remember them. When I'm done, tell me as many as you can in any order. Ready? Now tell me all the words you remember.

___Cabin	___Watch
___Pipe	___Whip
___Elephant	___Pillow
___Chest	___Giant
___Silk	___Theatre

Attention/Calculation

5. Please take 7 away from 100. Now continue to take 7 away from what you have left over until I ask you to stop.

___93	___72
___86	___65
___79	

6. Please count backwards from 20 to 1 ___No Mistakes

Comprehension, Semantic, and Recent Memory

7. What do people usually use to cut paper? ___Scissors
8. What is the prickly green plant found in the desert? ___Cactus
9. Who is the president now? ___Correct Name
10. Who is the vice president now? ___Correct Name
11. What is the opposite of east? ___West

Language/Repetition

12. Pleas say this: "Methodist Episcopal" ___Exactly Right

Delayed Recall

13. Please repeat the list of 10 words I read earlier

___Cabin	___Theatre
___Pipe	___Watch
___Elephant	___Whip
___Chest	___Pillow
___Silk	___Giant

ID_____ Date_____ ___Maximum of 39

VOLUNTEERS WANTED FOR AN EXERCISE STUDY

Cleveland State University

If you are an active female between 18 – 70 years old and regularly exercise you may qualify

Participants will receive free aerobic fitness and body composition testing

Volunteers will be asked to come to the lab on 3 separate occasions for exercise assessments; each visit will last 1.5 to 2 hours

Do you run on a regular basis?

Are you interested in learning more about your physical fitness?

The CSU Human Performance Lab is recruiting for a study observing cognition and hormones after exercise according to menstrual status.

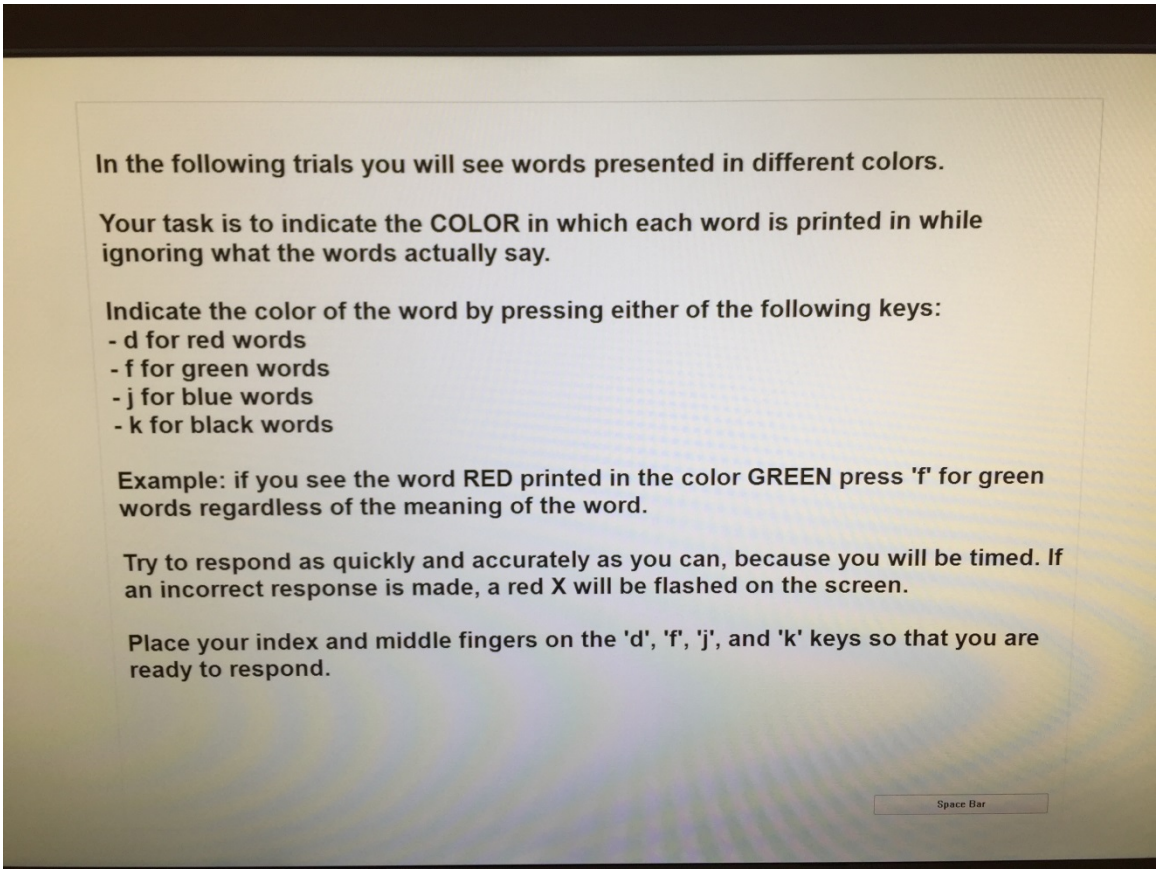
Interested in finding out more contact:

Ryan Wiet
614.949.6116
rtwiet@gmail.com

or

Dr. Emily Kullman
216.687.4854
e.kullman@csuohio.edu

APPENDIX E: STROOP TEST



APPENDIX F: INSITUTIONAL REVIEW BOARD APPROVAL LETTER

Sep 25, 2017

Dear Emily Kullman,

RE: IRB-FY2018-44
The effects of acute aerobic exercise on BDNF levels and cognition in postmenopausal women

The IRB has reviewed and approved your application for the above named project, under the category noted below. Approval for use of human subjects in this research is for a one-year period as noted below. If your study extends beyond this approval period, *you must contact this office to initiate an annual review of this research.*

Approval Category: Expedited Category 2B
Approval Date: Sep 25, 2017
Expiration Date: Sep 24, 2018

By accepting this decision, you agree to notify the IRB of: (1) any additions to or changes in procedures for your study that modify the subjects' risk in any way; and (2) any events that affect that safety or well-being of subjects. Notify the IRB of any revisions to the protocol, including the addition of researchers, prior to implementation.

Thank you for your efforts to maintain compliance with the federal regulations for the protection of human subjects. Please let me know if you have any questions.

Sincerely,

Mary Jane Karpinski
IRB Analyst
Cleveland State University
Sponsored Programs and Research Services
(216) 687-3624
m.karpinski2@csuohio.edu