THE EFFECT OF ACUTE MODERATE-INTENSITY CONTINUOUS AND HIGH-INTENSITY INTERVAL EXERCISE ON SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR IN RECREATIONALLY TRAINED MALES

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Michael Whitehurst, Department of Exercise Science and Health Promotion, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the College of Education and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

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BDNF is a neurotrophin that enhances neural health and is increased by exercise. PURPOSE: To compare moderate continuous (MCE) and high-intensity interval exercise (HIE) effects on serum BDNF levels, and examine the relationship between BDNF and lactate. METHODS: Seven males completed a VO2peak test and two protocols on separate days, (MCE) 28 min at 60% Workrate max (WRmax) and (HIE) 28 min of intervals at 90%WRmax (10- 1 min intervals separated by 2 min of rest). Serum BDNF and lactate were determined prior, during, and following both protocols. RESULTS: BDNF levels (pg/mL) increased from baseline during HIE and MCE ($p<.05$). The BDNF response to HIE correlated with lactate for area under the curve (AUC) ($r=0.901$; $P<0.05$). CONCLUSION: HIE is an effective alternative to MCE at increasing BDNF. Additionally, lactate may act as a measure of intensity or a mediator of the BDNF response to exercise.

DEDICATION

I would like to dedicate this paper to my parents, who have always supported me in whatever task I chose and are worthy scientists in their own right. In particular, I have been empowered by my father's shared admiration and passion for novelty and knowledge, and strengthened by my mother's constant support and inquiry into my well-being and psyche. I would also like to dedicate this work to my wonderful girlfriend, Maria, for her support throughout some of the most challenging times in my life, whose academic drive I can only hope to model and our shared vision that pushes me to be the greatest version of myself. Finally I would like to dedicate this paper to my grandfather, who showed me what it means to be dedicated yet humble.

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TABLES

FIGURES

I. INTRODUCTION

Endurance exercise has been shown to elicit neuroplasticity and enhance neuroprotection in children, adults, and the elderly (Griffin et al., 2011; Heyman et al., 2012; Penedo & Dahn, 2005). A prominent mechanism underlying exercise-induced neuroplasticity and neuroprotection is an increased expression of the neurotrophin, brain-derived neurotrophic factor (BDNF), which increases in the periphery of humans and brain of animals following extended or intense endurance exercise (Gómez-Pinilla, Ying, Roy, Moletni, & Edgerton, 2008; Griffin et al., 2011; Rasmussen et al., 2009; Vaynman, Ying, & Gómez-Pinilla, 2004).

Mechanistically, BDNF promotes growth and survival of neural tissues (Binder & Scharfman, 2004; Heumann, 1994; Rasmussen et al., 2009, Seifert et al., 2010). The brain is the primary production site of BDNF, with levels fluctuating in response to cognitive activity, disease, and exercise (Castellano & White, 2008; McAllister, Katz, & Lo, 1999). Specifically, as BDNF is able to cross the blood-brain barrier, it is estimated that the brain contributes as much as 70 to 80% of peripheral BDNF during exercise (Colombo et al., 2013). Thus, BDNF accumulation in the periphery is indicative of changes in the brain (Pan, Banks, Fasold, Bluth & Kastin, 1998; Poduslo & Curran, 1996; Rasmussen et al., 2009).

Furthermore, the BDNF response during continuous exercise appears to act in an intensity-dependent manner; in that, exhaustive exercise or sustained moderate-continuous exercise (MCE) have significantly increased peripheral BDNF levels (Ferris,

Williams, & Shen, 2007; Griffin et al., 2011; Heyman et al., 2012; Nofuji et al., 2012). In contrast, shorter moderate or low intensity exercise does not appear to affect the BDNF response (Ferris et al., 2007; Griffin et al., 2011; Heyman et al., 2012; Vega et al., 2006). Although sustained higher intensity exercise can elicit a significant increase in BDNF, little is known regarding the acute effects of high-intensity interval exercise (HIE) on peripheral BDNF kinetics during exercise. Interestingly, Winter et al. (2007) found that two 3 min sprints with a 2 min rest interval resulted in a significant BDNF response; suggesting that short but intense exercise bouts can elicit a BDNF response.

The classic HIE paradigm is based on work/rest intervals at intensities approaching 100% of maximum heart rate (MHR) or VO_{2max} (Laursen & Jenkins, 2002). Although variations in the length of HIE work and rest intervals can be utilized (eg 15 s work with 15 s rest vs 4 min work with 2 min rest), HIE is starkly contrasted by typical moderate continuous exercise (MCE) paradigms (e.g. 30 to 60 min of continuous running at 60 to 70%MHR). Ultimately, HIE and MCE differ significantly in total exercise time and volume with a 40 to 50% savings in exercise time and up to 90% training volume savings for HIE when compared to MCE (Helgerud et al., 2007). Despite the variance in training volume and time, performance and metabolic based outcomes are comparable, with HIE increases VO_{2max}, time to exhaustion, and markers of mitochondrial biogenesis commensurate with MCE (Gibala et al., 2006; Gibala, Little, MacDonald, & Hawley, 2012; Helgerud et al., 2007). Although the acute response of BDNF has been well studied, the BDNF response during exercise is less clear. Specifically, it appears that the BDNF response during exercise has a rising, peaking, and falling phases during exercise as determined by a statistical model and direct

measurement (Schmidt-Kassow et al., 2012; Schmolesky, Webb, & Hansen, 2013). Finally, lactate infusion at rest has been shown to dose-dependently increase BDNF (Schiffer et al., 2011), potentially through increased cerebral lactate metabolism during intense exercise (Bouzmebeur et al., 2010). Given that blood lactate levels rise with exercise intensity (Hurley et al., 1984), it is necessary to investigate the relationship of BDNF and lactate in response to HIE (Ferris et al., 2007; Schiffer et al., 2011; Vega, Hollman, Wahrmann, & Struder, 2012).

In summary, BDNF is a neural growth factor necessary for neuroplasticity and neuroprotection. While relatively intense sustained or exhaustive endurance exercise has been shown to increase BDNF, little is known about the acute effects of HIE. Although HIE requires much less active time, both MCE and HIE activate many of the same metabolic pathways and trigger similar adaptations. Additionally, high-intensity exercise results in substantial lactate accumulation, and lactate potentially mediates a BDNF response. Logically, HIE, like other high-intensity exercise, should promote BDNF expression in conjunction with lactate production. Therefore, the aim of this study was to examine the BDNF response of HIE and MCE in recreationally active males. It was hypothesized that the BDNF response to exercise would not significantly differ between HIE and MCE, but both HIE and MCE would elicit significant elevations in BDNF. Additionally, we also hypothesized that the lactate response to exercise will be correlated with BDNF.

II. REVIEW OF LITERATURE

Among the many benefits of endurance exercise is an increased release of the neurotrophic growth factor, brain derived neurotrophic factor (BDNF). Primarily expressed in the brain, BDNF is critical for neural health and promoting neural networks through synaptic plasticity and neurogenesis. Endurance exercise has specifically been found to elicit a significant BDNF response in the brain and periphery of human and animal models. Furthermore, this BDNF response has been found to be exercise intensity dependent, with moderate or intense exercise increasing BDNF in humans.

BDNF

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophic factor family that includes nerve growth factor (NGF), NT-3, NT-4, and NT-5. Like other neurotrophins, BDNF is named due to the ability to promote growth and survival of neural tissues. (Binder & Scharfman, 2004). BDNF supports neuroblast cell division, stimulation of glial cell precursor division, neuroprotection from excitotoxic damage, and modulation of peptide expression patterns leading to dendritic sprouting, axonal growth, and receptor proliferation (Heumann, 1994; Rasmussen et al., 2009, Seifert et al., 2010). Like other neurotrophins, BDNF consists of noncovalently linked homodimeric peptides and shares approximately 50% in primary amino acid structure with NGF, NT-3, NT-4, and NT-5 (Mowla et al., 2001).

BDNF synthesis

BDNF is synthesized as pro-BDNF within the endoplasmic reticulum of a neuron and then bound to an intracellular sortilin within the golgi apparatus and folded. The folded pro-BDNF can either be cleaved intracellularly by furin or protein convertases or cleaved extracellularly by plasmin and matrix metalloproteinases (MMP). The intracellularly cleaved mature BDNF or un-cleaved immature proBDNF is then bound to carboxypeptidase E and is sorted into large core vesicles in preparation for secretion or transport to the appropriate dendrites or axons along the regulated secretory pathway. In the absence of the BDNF motif, BDNF is transported along the constitutive secretory pathway (Lu, Pang, & Woo, 2005). BDNF can also be synthesized and secreted from muscle cells, immune cells, and microglial cells, but the brain appears to contribute 70-80% of total levels (Colombo et al., 2013).

Although proBDNF can be cleaved intracellularly into mature BDNF, the majority is secreted in the immature proBDNF form. Therefore, the majority of proBDNF is cleaved extracellularly by plasmin or MMP. Interestingly, mature BDNF (mBDNF) and immature proBDNF have counteractive effects and alternate high-affinity binding sites. There are two possible binding sites for mBDNF, the glycoprotein receptor neurotrophin-p75 (p75NTR) and the tyrosine kinase tropomyosin-sensitive receptor kinase B (trkB). Other neurotrophins can bind to both p75NTR and trkB, but have a higher affinity for trkA and trkC (Heumann, 1994). The two binding sites delegate what effect the ligand (pro-BDNF or mBDNF) will have.

Binding sites

The TrkB binding site is specific to BDNF and neurotrophic factor 4/5. It is the high-affinity binding site for BDNF and consists of an extracellular cysteine-rich cluster, three leucine-rich repeats, a cysteine-rich cluster, and two immunoglobulin-like domains all of which are connected to an intracellular receptor tyrosine kinase (Reichardt, 2006; Ultsch et al., 1999). The trkB binding site leads to several cell-survival pathways, specifically Ras and mitogen-activated protein kinase (MAP kinase), phosphatidylinositol-3 (PI3 kinase) and Akt, or activation of phospholipase C-y1 (PLC-y1) and protein kinase C (PKC) (Reichardt, 2006). The PI3K pathway leads to cell survival, PLC-y leads to synaptic plasticity, and RAS leads to differentiation, survival, and growth of neurons (Nykjaer, Willnow, & Petersen, 2005).

The alternative p75NTR binding site consists of an extracellular domain of four tandemly arranged cysteine-rich motifs, a transmembrane domain, and the intracellular cytoplasmic domain. Binding of pro-BDNF or BDNF occurs at the cysteine-rich extracellular domain. P75NTR is a member of the tumor necrosis receptor superfamily (TNF) (Reichardt, 2006). P75NTR acts as a low-affinity binding site for mBDNF and other mature neurotrophins, but as a high-affinity binding site for pro-BDNF. P75NTR can operate in conjunction with TrkB receptor to further differentiate the effects of BDNF, but in the absence of TrkB, p75NTR is a proapoptotic receptor. The process of how p75NTR leads to apoptosis is not fully understood, but intracellular activation of Jun N-terminal kinase (JNK) is suggested to be the key apoptotic signaling pathway. Downstream p75NTR can also activate nuclear factor-Kappa B (NF-kB) which leads to cell survival, or MAPK which can lead to survival or death (Nykjaer et al., 2005).

Exercise and BDNF

Traditional sustained aerobic exercise has been found to act in a neuroprotective manner, protecting the brain from inflammation, degradation, and age-related decline in volume (Erickson et al., 2010). BDNF levels have also been correlated with the risk of metabolic syndrome, Parkinsons Disease, Alzheimers disease, multiple sclerosis, and depression (Diniz & Teixeira, 2011; Krabbe et al., 2007; Sarchielli, Greco, Stipa, Floridi, & Gallai, 2002; Scalzo, Kümmer, Bretas, Cardoso, & Teixeira, 2010). Specifically, modulation of BDNF and in particular a decrease of BDNF or decreased sensitivity to BDNF is indicative of a diseased state. BDNF is able to rapidly cross the blood brain barrier (BBB) (Pan et al., 1998), and serum BDNF has been found to significantly correlate (r=0.81) to brain-tissue BDNF levels in pig, rat and mice models suggesting that peripherally measured BDNF levels are indicative of brain-specific BDNF (Klein et al., 2011). Additionally, BDNF has been found to be released from the brain during exercise in humans (Rasmussen et al., 2009).

BDNF appears to mediate the effects of exercise on neuroplasticity, dendritic outgrowth, and neuroprotection, as BDNF-inhibition in animal models do not experience exercise-induced changes in cognition or synaptic plasticity (Vaynman et al., 2004). Just as hippocampal volume declines with age, BDNF, which is highly concentrated within the hippocampus, has been found to decrease with age (Erickson et al., 2011). Aerobic exercise has been found to attenuate hippocampal shrinking in elderly patients who were observed for 12 months. Specifically, participants who exercised showed a significant increase in hippocampal volume compared to the control group who showed a decline in hippocampal volume (Erickson et al., 2011). The contrasting volumes of the

hippocampus are indicative of the age-related decline in volume, yet this decline was attenuated and reversed in the exercise intervention group. Similarly, BDNF levels are decreased in MS, depression, PD, and AD, and modulation of BDNF has been suggested as an intervention to improve health in these diseased populations (Scalzo et al., 2010; Karege et al., 2005; Diniz & Teixeira, 2011). Additionally, chronic aerobic exercise has been shown to improve basal BDNF levels in MS to comparable levels in healthy controls (Castellano & White, 2008). Finally, aerobic exercise has also been found to improve cognitive function in dementia and mild cognitive impairment, (Baker et al., 2010), increase BDNF mRNA in the brain of AD-model mice and the hippocampus of healthy rats (Berchtold, Kesslak, Pike, Adlard, & Cotman, 2001; Um et al., 2008), reduce the risk for PD in humans (Chen, Zhang, Schwarzschild, Hernan, & Ascherio, 2005; Xu et al., 2010), reduce depressive symptoms and improve BDNF levels in elderly humans (Laske et al., 2010), and provide protection of overall brain function in aged rats (Cotman, Berchtold, & Christie, 2007).

Although the benefits of exercise on brain health appear to be maximized in diseased or aged populations, where BDNF levels and neural structures are compromised, cognitive benefits and increased BDNF as a result of exercise are still apparent in healthy and young populations (Berchtold, Castello, & Cotman, 2010; Cotman et al., 2007). Hippocampal function, as assessed by a face to name memory task, as well as serum BDNF levels can be improved following 5 weeks of aerobic training in college-aged males. Specifically, 5 but not 3 weeks of aerobic training (30 min at 60% VO_{2max}) is sufficient to elicit a change in the acute response of BDNF to exercise (Griffin et al., 2011). Additionally, Seifert et al. (2010) carried out a 12 week training study in which

twelve sedentary, overweight participants were randomly assigned to either an exercise intervention group (n=7) or control group (n=5). All exercise, including the initial and final VO_{2max} to determine cardiovascular fitness was performed on a cycle ergometer. The exercise group exercised for approximately 60 min per day at approximately 70% HRmax or 65% VO_{2max}. At the end of the three month study, the participants were retested for VO_{2max} and later exercised at 60, 70, 80, 90, and 100% of the participant's VO_{2max} in 4 min intervals with a 6 min rest period in between intervals. Blood sampling was compared between the brachial artery and through the right jugular vein to the bulb of the vein at the base of the skull. This allowed for comparison of peripheral and brain-specific release of BDNF. Peripheral release of BDNF was not changed by 3 months of aerobic training, however BDNF was significantly higher above baseline during exercise. Peripheral release of BDNF was significantly higher than the control during exercise and at baseline following training. Jugular (brain) release of BDNF was not significantly changed after 3 months of aerobic training or in the control, but was significantly higher during exercise and at baseline in the exercise group. At baseline the training group elicited the greatest BDNF response at 70% VO_{2max}, while after 3 months of training the highest response was at 100% VO_{2max}. At baseline in the control group, 60% VO_{2max} elicited the greatest BDNF response, while after the end of the three months the greatest response was seen at 90% VO_{2max}. The large response to a short but high intensity exercise bout suggests that BDNF release may be intensity dependent (Seifert et al., 2010).

The exercise intensity-dependent release of BDNF theory is strengthened by Ferris et al. (2007) who examined the effect of exercise above or below the ventilatory

threshold (VT) on serum BDNF in healthy collegiate participants. Serum BDNF was found to be significantly increased following a VO_{2max} test as well as after exercise 10% above the VT, with BDNF levels increasing 30% and 13% above baseline for VO_{2max} and VT +10% trials, respectively. There was not a significant change in BDNF following exercise at VT -20% trial. A significant correlation was found between blood lactate (La) and serum BDNF (R=0.57) following the VO_{2max} test, further suggesting that a higher exercise intensity and specifically exercise resulting in the accumulation of lactate, may result in elevated BDNF.

BDNF exercise intensity

BDNF levels have also been shown to increase temporarily following as little as 6 min of high-intensity exercise, as well as following VO_{2max} tests or other GXT to exhaustion protocols, ranging from 7 to 16 minutes (Cho et al., 2012; Heyman et al., 2012; Nofuji et al., 2012; Tang, Chu, Hui, Helmeste, & Law, 2008; Vega et al., 2012; Winter et al., 2007). Similarly, extended exercise at moderate to high intensities (>60% VO_{2max}) from 20 to 60 min has also been shown to increase serum BDNF (Castellano & White, 2008; Heyman et al., 2012). However, some extended exercise (20-60 min) and especially low intensity exercise protocols (<60% VO_{2max}) do not significantly increase serum BDNF (Ferris et al., 2007; Nofuji et al., 2012; Rasmussen et al., 2009). Therefore, the BDNF response to exercise is intensity dependent where the necessary intensity threshold is above60% of VO2 max (Schmidt-Kassow et al., 2012).

Exercise intensity, lactate and BDNF

Higher intensity or maximal exercise, specifically where oxygen utilization is not sufficient to convert pyruvate to ATP via oxidative phosphorylation, results in the

conversion of pyruvate to lactate. This accumulation of lactate also results in increased dissociation of hydrogen ions, altering the homeostatic pH and causing acidosis (Schiffer et al., 2011). Alternatively, employing a lactate clamp technique allows for the infusion of lactate while in the resting state. The infusion, thus, mimics intense exercise. Interestingly, the lactate clamp increases blood lactate without a corresponding decrease in pH, a finding contradictory to the metabolic acidosis seen during exercise, yet a significant increase in peripheral BDNF is still observed. Additional research has shown the BDNF response to exercise-induced lactate accumulation is not affected by the infusion of bicarbonate, a pH buffer, during exercise (Vega et al., 2012). These findings demonstrate that the accumulation of lactate, regardless of metabolic acidosis, can result in increased peripheral BDNF (Schiffer et al., 2011; Vega et al., 2012).

Moreover, the suggestion that an increase in BDNF is a result of lactate is strengthened through cerebral metabolism and cell culture studies. Specifically, astrocyte cell lines release BDNF in a dose-dependent fashion to lactate (Coco et al., 2013). Additionally, exercise-induced circulating lactate is preferentially utilized as a brain metabolite during exercise, potentially as a glucose-sparing mechanism (Boumezbeur et al., 2010; Ide, Schmalbruch, Quistorff, Horn, & Secher, 2000; Knudsen, Paulson, & Hertz, 1991). Specifically, the brain-specific utilization of lactate as an energy source corresponds with plasma levels and can provide up to 60% of brain energy during states of highly elevated lactate accumulation (Bouzmebeur et al., 2010). Finally, although not fully understood, it does appear that lactate contributes to the BDNF response during exercise in an intensity dependent manner.

High-intensity interval exercise (HIE)

High intensity interval exercise or HIE relies on relatively short, 15 seconds to 4 minutes, near-maximal work intervals interspersed with either rest or low-intensity intervals (Laursen & Jenkins, 2002). This approach is certainly in contrast to continuous exercise at a sub-maximal intensity or MCE. For example, Burgomaster et al. (2008) compared 4-6 30 second (s) "all-out" efforts on a cycle ergometer (4.5 min rest between) versus 40-60 min cycling at 65% VO2 peak in order to compare the oxidative and metabolic responses to HIE and MCE in young untrained participants. Although there was substantial difference in terms of training duration and intensity, both groups saw significant increases in cardiovascular adaptations as well as markers of mitochondrial biogenesis (Burgomaster et al., 2008). In another example of HIE, Little, Safdar, Wilkin, Tarnopolsky, & Gibala (2010) utilized 8-12 60 s bouts of cycling at peak power followed by 75 s of recovery at 30W to elicit expression of mitochondrial enzymes and regulators of mitochondrial biogenesis in young recreationally trained males. The 60 s bout at peak power is intended to be more manageable than the commonly used "all-out" testing, however increases in mitochondrial biogenesis, enzymes, and transcription factors, as well as improvements in time trial performance were found that were comparable to previous HIE and MCE training (Little et al., 2010). Thus, it appears that both performance and health related HIE benefits result from an upregulation of key regulatory enzymes and transcription factors associated with mitochondrial biogenesis.

Advantages of HIE

It appears that HIE can increase both time to exhaustion and VO_{2max} in as little as two weeks and six total sessions. Interestingly, the HIE and MCE training corresponded

to time commitments over the two weeks of 2.5 hours and 10.5 total hours, respectively. (Gibala & McGee, 2008) Mitochondrial oxidative capacity measured via activity of proliferator-activated receptor-y coactivator 1 alpha (PGC-1a) was similarly increased post exercise when compared to MCE following the 6 weeks of exercise. Therefore, both HIE and MCE operate through similar pathways, yet HIE results in similar oxidative and work capacity gains from much lower volume (Helgerud et al., 2007).

Conclusion

In conclusion, BDNF supports neurogenesis and synaptic plasticity. BDNF is found in high quantities within the brain as well as circulation, and the majority of peripheral BDNF is released from the brain. Exercise has been found to elicit BDNF responses as a result of various protocols, but the BDNF response to exercise appears to be intensity-dependent. Similarly, blood lactate accumulation increases with increasing exercise intensity, and lactate accumulation has been found to increase BDNF. Manipulating the dosage of exercise by reducing volume in favor of intensity is the hallmark of the HIE paradigm. However, comparing HIE with the more traditional moderate intensity continuous exercise (MCE) reveals similarities in terms of the metabolic activity and associated adaptations including the activation of oxidative and neuroprotective mechanisms such as BDNF. While little is known about the BDNF response to HIE, HIE does offer advantages in terms of requiring less total time and work than traditional approaches to exercise (e.g. MCE).

III. METHODS

Participants

Seven recreationally active males (demographics listed in table 1) participated in this study. Recreationally active was defined as participation in a minimum of 30 min of moderate-intensity aerobic activity at least 3 sessions/week. All participants completed an informed consent detailing the aim and protocol of the study as well as a health history questionnaire, and were non-smokers, not on any regular medication, and free from any diseases or orthopedic limitation that could impact the outcome of this study. Participants were required to fast at least 3 hours prior to testing and abstain from alcohol intake and intense physical activity for at least 24 hours prior to testing. The study was approved by the Institutional Review Board of Florida Atlantic University.

Participant (n = 7)			**Mean**	**SEM**
Height (cm)			180.1	2.43
Weight (kg)			76.6	4.79
BMI			23.9	1.48
Age (years)			24.6	1.44
VO_{2max} (mL/min)			3736.13	155.5
VO_{2max} (mL/kg/min)			49.4	3.15
WRmax (Watts)			299.6	19.5

Table 1 Participant characteristics All data reported as mean + standard error of the mean (SEM).

Experimental protocol

The experimental protocol consisted of 3 visits, including familiarization and VO_2peak testing, high-intensity interval exercise (HIE), and traditional endurance exercise (MCE). The order of HIE or MCE treatment was counterbalanced for exercise condition. Each participant was tested at approximately the same time of day across treatments with at least 48 hours and up to 2 weeks to complete all 3 visits. The cycle ergometer was adjusted for the dimensions of the participant (seat height, handlebar height and distance from nose of seat to center of handlebars) and the same specifications were used for each visit.

Graded exercise test to exhaustion (visit one)

Visit one was used to obtain informed consent, a medical and exercise history, as well as anthropometric assessment, resting heart rate (HR), resting blood pressure (BP) and allow participants to review all instruments and procedures. Body mass index (BMI) was assessed using the following formula: BMI = mass (kg)/(height (m))^2, and an estimation of body fat % was made through the use of skinfold calipers. Additionally, each participant completed a graded exercise test (GXT) to exhaustion on an electronically-braked cycle ergometer (Lode Excalibur Sport, Model # 909901, Groningen, the Netherlands) designed to elicit peak oxygen uptake (VO_2peak). The protocol for the GXT began with a 10 min warm-up at 50 W, followed by an increase in workload to 100 W and subsequent 25W every 1 min until volitional fatigue. Once participants achieved either an RER of 1.05 or cadence decreased by 10 rpm or more the workload was increased in 10 W increments every 1 min. Participants were allowed to self-select for cadence, but prompted to maintain at least 70-80 rpm. Mean VO_2peak was

determined as the highest 30 s average determined by a ParvoMedics Metabolic Measurement System (ParvoMedics, Sandy, UT). Oxygen uptake (VO_2), carbon dioxide exhalation (VCO_2), and heart rate (HR) were measured every 15 seconds, and RPE once per stage. The criteria for determination of VO_{2peak} was 2 of the following: a plateau of VO2/HR with an increase in workload, a respiratory exchange ratio (RER) ≥1.15, VE/VO2 ratio > 35, or respiratory rate (RR) > 55 breaths/min. Blood lactate was taken immediately post-exercise via a fingertip prick and a YSI lactate analyzer used to determine concentration. Heart rate and blood pressure were measured by telemetry (Polar T31, Polar Electro, Kempele, Finland) and sphygmomanometer, respectively (752M-Mobile Series, American Diagnostic Corporation, Hauppauge, NY).

Max power (Wmax) was determined by the following formula: Wmax = Wcom + (t/T*W), where Wcom is the power in watts of the last completed stage of the VO2peak test, t is the number of seconds into the uncompleted stage, T is stage length in seconds, and W is the increase in power output (watts) of the uncompleted stage (Kuipers et al., 1973).

Endurance or interval exercise

At least 48 hours following the VO2peak testing, participants completed either the MCE or HIE protocol, where the order was randomized across participants. The HIE protocol began with a 10 min warm-up at 50W, followed immediately by 10 repeated 60s efforts of high-intensity cycling at 90% Wmax. Each of the 10 work intervals was separated by 2 min of rest (Little et al., 2010). The MCE protocol began with a 10 min warm-up at 50W, followed immediately by 28 min of continuous exercise at 60% Wmax. Oxygen uptake (VO_2), carbon dioxide exhalation (VCO_2), and heart rate (HR) were

measured every 15 seconds. The average and peak HR and VO2 for each stage, as well as total work output, were determined.

Blood sampling and BDNF determination

Insertion of an indwelling catheter at the antecubital vein and all subsequent blood draws were performed by a trained phlebotomist in accordance with institutional review board standard operating procedure for all three testing sessions. An indwelling catheter was used for all blood draws to accommodate multiple draws during exercise and into recovery. Prior to all blood draws a 1 mL "waste" draw of blood was drawn through the catheter to maintain patency and then discarded. Two 6 mL samples of whole blood were collected from the antecubital vein of each participant at each time point (see Table 2) into either a silica-coated serum separation vacuum blood collection tube or an EDTA K_2 vacutainer for serum and plasma, respectively (BD Nexiva; BD, Franklin Lakes, NJ).

HIE	Pre WU	I 1 1 MIN	I 2 4	I 3 7	I 4 10	I 5 13	I 6 16	I 7 19	I 8 22	I 9 25	I 10 28	P30 58	P60 128
	x	x	x	x	x	x	X	x	x	x	x	x	x
MCE	Pre WU	1 MIN	4	7	10	13	16	19	22	25	28	P30 58	P60 128
	x	x	x	x	x	x	X	x	x	x	x	x	x

Table 2 Timeline of exercise and blood sampling Blood draws (x) will be taken after every interval of HIE and corresponding time points in MCE (every 3 min). Each interval of HIE will be separated by 2 min of rest (total of 3 min per cycle of HIE).

Blood lactate was taken at baseline, at every time interval during exercise, as well as 30 and 60 min into recovery via an aliquot taken from the 6 mL plasma samples for both MCE and HIE. Whole blood lactate was measured with a YSI 2300, which was calibrated prior to each visit.

Serum samples were allowed to clot for 30 min at RT, then centrifuged at 1300G for 15 min at RT, while plasma samples were immediately centrifuged at 3000 RPM for 20 min at RT. Both plasma and serum were decanted and stored at -80°C in cryogenic tubes in 420 μL aliquots for future analysis of BDNF by ELISA kits according to manufacturer instructions (Promega Emax, Wisconsin). The 96 well plate was read with a BioRad iMark microplate reader at an absorbance of 450nm within 30 minutes of the addition of the stop solution.

Statistical analysis

The serum BDNF response was assessed by a two-way repeated-measures analysis of variance (rANOVA) with main effects examined for protocol (HIE and MCE) and time (pre, 5 time points during exercise, post-ex, 30 min post-ex, 60 min post-ex). Post hoc pair wise comparisons were conducted using Bonferroni's test. To better analyze the repeated measures overload release of lactate and BDNF in response to exercise, the area under the curve with respect to ground (AUCg) was calculated using the following formula: $\sum_{i=1}^{n-1}\left(\frac{(m(i+1)+mi)*ti}{2}\right)$ with "t_i denoting the individual time distance between measurements, m_i denoting the individual measurement, and *n* the total number of measures (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003)." The relationship of BDNF AUCg and lactate AUCg across all times points was analyzed via Pearson's correlation. All data were presented as mean ± SEM and significance was set at 0.05. All statistical analysis was completed in SPSS version 20.0.

IV. RESULTS

The current study compared the effect of two exercise conditions on serum BDNF expression. Our primary hypothesis was that HIE would elicit a BDNF response similar to MCE. Additionally, we hypothesized that the peak BDNF response would not be significantly difference between the exercise conditions. Finally, we also expected to observe a positive relationship between lactate and BDNF during exercise.

Serum BDNF response

To increase statistical power, the total number of analyzed data points during exercise were reduced from 10 to 5 by averaging adjacent time points, as indicated in Table 1. The time points used for data analysis for pre as well as 30 min post and 60 min post exercise were unchanged.

T1	**T2**	**T3**	**T4**	**T5**
1 – 4 min	7 – 10 min	13 – 16 min	19 – 22 min	25 – 28 min

Table 3 Timeline for BDNF analysis Data for BDNF analysis were averaged and results in 5 data points (T1, T2, T3, T4, and T5).

Mauchly's test indicated that the assumption of sphericity had been violated for the main effect of time $X^2(14)= 32.311$, $p < 0.01$, but not for the main effect of condition or the time by condition interaction. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($E = 0.519$) for the main effect of time.

There was a significant main effect for time on serum BDNF levels, $F(7,14.782) = 13.404$, $p = 0.011$. However, for BDNF, there was no main effect for condition or a condition by time interaction ($p > 0.05$). Finally, BDNF was not different between exercise conditions at baseline, within exercise, as well as at 30 min and 60 min of recovery.

The results of the one-way ANOVA revealed that both MCE and HIE elicited a significant BDNF response, $F(7,30)=15.36$, $p < 0.01$ and $F(7,112.5)=13.33$, $p < 0.01$. Post-hoc analysis by Bonferroni revealed that BDNF was significantly elevated above baseline at T4 and T5 for both HIE and MCE, while p60 was significantly higher than baseline for MCE as indicated in Figure 1.

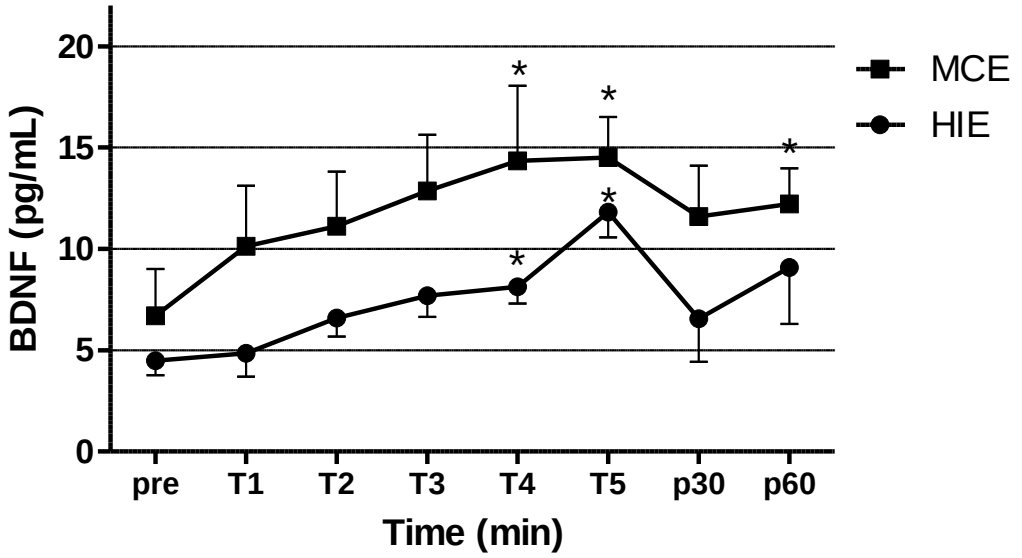

Figure 1 BDNF response to exercise Serum BDNF response as a function of time for both MCE and HIE during exercise and into recovery. Data points within exercise were collapsed (e.g. T1 = 1-4 min, T2 = 7-10 min, T3 = 13-16 min, T4 = 19-22 min, T5 = 25-28). Error bars report standard error of the mean and significant differences from baseline are indicated with * ($p < .05$)

Lactate response

Interestingly, as revealed by Pearson's correlation (Figure 2), there was a strong positive relationship between lactate AUCg and BDNF AUCg for HIE ($p<0.01$, $r = .909$) while there was no significant relationship for MCE ($p= .22$).

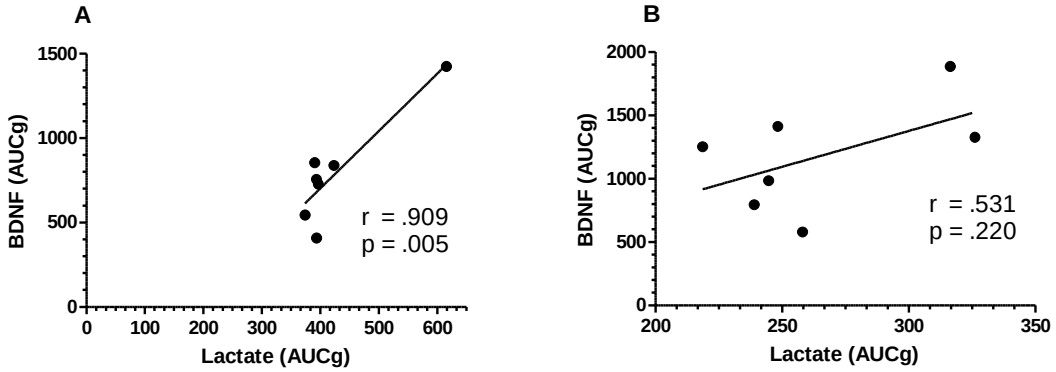

Figure 2 Correlation of BDNF and lactate Pearson's correlation of Lactate AUCg and BDNF AUCg response during exercise and into recovery. A = HIE B= MCE

Total work output and exercise conditions

A significant difference between HIE and MCE was found in work completed ($t[6]= 21.244$, $p < 0.001$) and power ($t[6]= 22.697$, $p < 0.001$). Exercise conditions are reported in table 4.

	Work Rate (Watts)		Average $\%VO_2$ (Average Peak for HIE)	
	Mean	**SEM**	**Mean**	**SEM**
HIE	269.6*	17.5	76.3*	1.27
MCE	179.5	11.9	70.4	0.995

Table 4 Exercise conditions Work rate and $\%VO_2$ for high-intensity interval (HIE) and moderate continuous exercise (MCE). All data reported as mean + standard error of the mean (SEM). Significant difference between workrates and VO_2 noted with * ($p < .05$).

V. DISCUSSION

The present study examined the effect of two acute exercise conditions (HIE and MCE) on serum BDNF response. Specifically, a more traditional continuous cycling protocol (MCE- 28 min at 60% PP) was compared with a novel high intensity interval exercise protocol (HIE- 10 – 1 min intervals at 90% PP, each interval separated by 1 min of rest) (Ferris et al., 2007; Gold et al., 2003; Seifert et al., 2010).

The results of the present study revealed that both MCE and HIE can elicit a significant BDNF response, and that the BDNF response is not significantly different between protocols. Moreover, the MCE protocol utilized in the current study elicited 70.6% VO2peak, which again is in agreement with previous work showing substantial increases in BDNF within an acute 30 minute continuous exercise bout between 60-70% VO_{2max}. Conversely, the HIE protocol, like previous GXT protocols to exhaustion and repeated bouts of sprinting exercise is very effective at eliciting a BDNF response; thus, further illustrating the exercise intensity dependent nature of BDNF expression (Ferris et al., 2007; Gustafsson et al., 2009; Laske et al., 2010; Nofuji et al., 2012; Vega et al., 2006; Vega et al., 2012; Winter et al., 2007).

We modified a HIE protocol from Little et al. (2010), resulting in a total of 10 min of high intensity exercise separated by a total of 18 min of rest. The 10 min of total HIE is in stark contrast to the 28 min of total and continuous cycling employed in the

present study, as well as the typical longer MCE protocols (Ferris et al., 2007; Goekint et al., 2008; Schulz et al., 2004; Winter et al., 2007), Yet HIE required only 53% of the total work output of the MCE condition.. Interestingly, the HIE power output and average peak VO2 per bout were significantly higher than in the MCE condition. Therefore, given that there was no significant difference between the HIE and MCE BDNF response, HIE is a more efficient exercise model at elevating BDNF as measured by both exercise time and work completed. Finally, this finding not only supports an intensity-dependent BDNF response to exercise, but highlights the efficacy of HIE as a novel and potentially beneficial form of exercise (Ferris et al., 2007; Gibala et al., 2012; Vega et al., 2006).

In this study, along with elevated BDNF, lactate production increased in response to exercise, a finding in agreement with previous investigations (Ferris et al., 2007; Schiffer et al., 2011; Vega et al., 2012). Interestingly, the correlation of BDNF and lactate only occurs following maximal exercise and has yet to be significantly correlated for continuous exercise (Ferris et al., 2007). The current study supports previous research with a strong positive correlation between lactate and BDNF for HIE. Although pH and blood gases were not measured in the current study, lactate appears to correlate with BDNF response independent of acidosis as the accumulation of lactate either by exercise or lactate clamp coupled with the addition of a pH buffer still results in a BDNF response (Schiffer et al., 2011; Vega et al., 2012). The mechanism by which exercise-induced lactate could cause a BDNF response is as of yet unknown, but lactate may act as a pseudo-hormone on the brain by altering brain metabolism (Boumezbeur et al., 2010; Schiffer et al., 2011).

The BDNF response to HIE seen in this study may be explained in part by rising lactate levels. That is, as lactate production increased with exercise, BDNF also rose. Given that lactate is able to cross the BBB, it may be utilized as a cell signaling device by both astrocytes and neurons triggering an increased production of BDNF with a subsequent release to the periphery (Boumezbeur et al., 2010; Knudsen et al., 1991). For example, Coco et al. (2013), using an astrocyte cell line, found that when L-lactate was introduced BDNF was released in a dose-dependent relationship. Similarly, Schiffer et al. (2011) employed the lactate clamp technique to determine whether lactate infusion at rest might influence BDNF concentrations in the periphery. Results indicated that BDNF significantly and dose-dependently increased along with lactate infusion, and BDNF levels quickly returned to baseline values following the removal of lactate. These data suggest that blood lactate is involved in the regulation of BDNF blood concentrations and represents a potential central mediator of the peripheral BDNF response to high-intensity exercise (Ide et al., 2000). Additionally, although lactate correlates well with the BDNF response to HIE, other within-exercise effects may account for the increase in BDNF, especially within MCE, and causality cannot be determined. Interestingly, a vast majority of BDNF is sequestered in platelets in circulation (Fujimura et al., 2002). Therefore, an increase in sheer stress or changes in blood gases may cause a release of BDNF from platelets, and a resulting increase in measured circulating BDNF, although serum sampling should result in the release of all if not the majority of BDNF from the platelets sampled.

In the present study, HIE required less total exercise time and total work when compared to MCE. Yet, while having a transient effect, HIE still promoted BDNF

elevation in the periphery commensurate with MCE. Thus, HIE appears to be a more efficient exercise model than MCE. However, beyond a simple time savings, HIE may be an effective tool to modulate BDNF expression in other populations (Castellano & White, 2008; Gibala et al., 2012; Winter et al., 2007). Specifically, it has been reported that HIE increases exercise adherence, promotes greater enjoyment than more traditional exercise paradigms and has been shown to be more effective than continuous exercise interventions in diseased populations and in the elderly (Gibala et al., 2012; Rognmo, Hetland, Helgerud, Hoff, & Slørdahl, 2004; Tjønna et al., 2008). Similarly, aged rats who perform HIE bouts of treadmill running showed significant improvements in learning and memory in conjunction with hippocampal plasticity as mediated by increased BDNF expression (Aguiar et al., 2011). Finally, the fact that HIE and MCE are similarly effective in increasing peripheral BDNF supports HIE as an effective alternative to MCE particularly among young recreationally trained males and warrants further study across a wider range of participants (Rognmo et al., 2004; Rasmussen et al., 2009).

Limitations of this study include a small sample size and peripheral measure of BDNF rather than directly from the brain. It is important to note that although BDNF was measured only in the periphery, peripheral BDNF levels have been found to correlate strongly ($r = 0.81$) with cortex BDNF in rats, and more than 75% of BDNF contributions to the periphery originate from the brain via efficient transport across the BBB (Karege et al., 2005; Klein et al., 2011; Pan et al., 1998). Additionally, the release of BDNF from the brain as a result of exercise has also been confirmed in humans (Krabbe et al., 2007; Rasmussen et al., 2009).

Given that this is a healthy population, future research should focus on utilization of the protocol in a population such as multiple sclerosis or in aging, where BDNF levels are decreased. Additionally, future study is necessary to connect lactate metabolism and BDNF release from the brain during exercise. Finally, training studies are warranted utilizing the novel HIE protocol to determine if chronic administration of interval exercise can elicit beneficial changes in BDNF and brain health.

In conclusion, HIE, a novel high-intensity interval exercise protocol elicited a significant BDNF effect comparable to the more traditional MCE condition. The HIE condition required less exercise time and work output than MCE, resulting in a more efficient intervention for eliciting changes in BDNF. The strong correlation between lactate and BDNF response during and following HIE warrants further investigation, but the specific mechanism of exercise-induced BDNF cannot be determined here. However, the correlation of lactate and BDNF does strongly support the intensity-dependent nature of an exercise-induced BDNF response. Ultimately, HIE is not only an efficient form of exercise, it may also be a promising exercise intervention in promoting neural health across a wide population.

REFERENCES

Aguiar Jr, A. S., Castro, A. A., Moreira, E. L., Glaser, V., Santos, A. R., Tasca, C. I., & Prediger, R. D. (2011). Short bouts of mild-intensity physical exercise improve spatial learning and memory in aging rats: involvement of hippocampal plasticity via AKT, CREB and BDNF signaling. *Mechanisms of Ageing and Development*, *32*(11), 560-567.

Baker, L. D., Frank, L. L., Foster-Schubert, K., Green, P. S., Wilkinson, C. W., McTiernan, A., & Craft, S. (2010). Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Archives of Neurology*, *67*(1), 71-79.

Berchtold, N. C., Castello, N., & Cotman, C. W. (2010). Exercise and time-dependent benefits to learning and memory. *Neuroscience*, *167*(3), 588-597.

Berchtold, N. C., Kesslak, J. P., Pike, C. J., Adlard, P. A., & Cotman, C. W. (2001). Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. *European Journal of Neuroscience*, *14*(12), 1992-2002.

Binder, D. K., & Scharfman, H. E. (2004). Mini review. *Growth Factors*, *22*(3), 123-131.

Boumezbeur, F., Petersen, K. F., Cline, G. W., Mason, G. F., Behar, K. L., Shulman, G. I., & Rothman, D. L. (2010). The contribution of blood lactate to brain energy

.

metabolism in humans measured by dynamic 13C nuclear magnetic resonance spectroscopy. *The Journal of Neuroscience*, *30*(42), 13983-13991.

Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., MacDonald, M. J., McGee, S. L., & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low-volume sprint interval and traditional endurance training in humans. *The Journal of Physiology*, *586*(1), 151-160.

Castellano, V., & White, L. J. (2008). Serum brain-derived neurotrophic factor response to aerobic exercise in multiple sclerosis. *Journal of the Neurological Sciences*, *269*(1), 85-91.

Chen, H., Zhang, S. M., Schwarzschild, M. A., Hernan, M. A., & Ascherio, A. (2005). Physical activity and the risk of Parkinson disease. *Neurology*, *64*(4), 664-669.

Cho, H. C., Kim, J., Kim, S., Son, Y. H., Lee, N., & Jung, S. H. (2012). The concentrations of serum, plasma and platelet BDNF are all increased by treadmill VO2max performance in healthy college men. *Neuroscience Letters*, *519*(1), 78-83.

Coco, M., Caggia, S., Musumeci, G., Perciavalle, V., Graziano, A. C., Pannuzzo, G., & Cardile, V. (2013). Sodium L-lactate differently affects brain-derived neurothrophic factor, inducible nitric oxide synthase, and heat shock protein 70 kDa production in human astrocytes and SH-SY5Y cultures. *Journal of Neuroscience Research*, *91*(2), 313-320.

Colombo, E., Bedogni, F., Lorenzetti, I., Landsberger, N., Previtali, S. C., & Farina, C. (2013). Autocrine and immune cell-derived BDNF in human skeletal muscle:

Implications for myogenesis and tissue regeneration. *The Journal of Pathology*, *231*(2), 190-198.

Cotman, C. W., Berchtold, N. C., & Christie, L. A. (2007). Exercise builds brain health: Key roles of growth factor cascades and inflammation. *Trends in Neurosciences*, *30*(9), 464-472.

Diniz, B. S., & Teixeira, A. L. (2011). Brain-derived neurotrophic factor and Alzheimer's disease: Physiopathology and beyond. *Neuromolecular Medicine*, *13*(4), 217-222.

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., Kim, J. & Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences*, *108*(7), 3017-3022.

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Heo, S., McLaren, M., Pence, B.D., Martin, S.A., Vieira, V.J., Woods, J.A., McAuley, E., & Kramer, A. F. (2010). Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. *The Journal of Neuroscience*, *30*(15), 5368-5375.

Ferris, L. T., Williams, J. S., & Shen, C. L. (2007). The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Medicine and Science in Sports and Exercise*, *39*(4), 728-734.

Fujimura, H., Altar, C. A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J. I. & Tandon, N. N. (2002). Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thrombosis and Haemostasis-Stuttgart*, *87*(4), 728-734.

Gibala, M. J., Little, J. P., MacDonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *The Journal of Physiology, 590*(5), 1077-1084.

Gibala, M. J., Little, J. P., Van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A. & Tarnopolsky, M. A. (2006). Short-term sprint interval versus traditional endurance training: Similar initial adaptations in human skeletal muscle and exercise performance. *The Journal of Physiology, 575*(3), 901-911.

Gibala, M. J., & McGee, S. L. (2008). Metabolic adaptations to short-term high-intensity interval training: A little pain for a lot of gain?. *Exercise and Sport Sciences Reviews*, *36*(2), 58-63.

Goekint, M., Heyman, E., Roelands, B., Njemini, R., Bautmans, I., Mets, T., & Meeusen, R. (2008). No influence of noradrenaline manipulation on acute exercise-induced increase of brain-derived neurotrophic factor. *Medicine and Science in Sports and Exercise*, *40*(11), 1990-6.

Gold, S. M., Schulz, K. H., Hartmann, S., Mladek, M., Lang, U. E., Hellweg, R. & Heesen, C. (2003). Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *Journal of Neuroimmunology*, *138*(1), 99-105.

Gómez-Pinilla, F., Ying, Z., Roy, R. R., Molteni, R., & Edgerton, V. R. (2002). Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *Journal of Neurophysiology*, *88*(5), 2187-2195.

Griffin, É. W., Mullally, S., Foley, C., Warmington, S. A., O'Mara, S. M., & Kelly, Á. M. (2011). Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. *Physiology & Behavior*, *104*(5), 934-941.

Gustafsson, G., Lira, C. M., Johansson, J., Wisén, A., Wohlfart, B., Ekman, R., & Westrin, Å. (2009). The acute response of plasma brain-derived neurotrophic factor as a result of exercise in major depressive disorder. *Psychiatry Research*, *169*(3), 244-248.

Helgerud, J., Hoydal, K., Wang, E., Karlsen, T., Berg, P., Bjerkaas, M. & Hoff, J. (2007). Aerobic high-intensity intervals improve VO2max more than moderate training. *Medicine and Science in Sports and Exercise*, *39*(4), 665.

Heumann, R. (1994). Neurotrophin signalling. *Current Opinion in Neurobiology*, *4*(5), 668-679.

Heyman, E., Gamelin, F. X., Goekint, M., Piscitelli, F., Roelands, B., Leclair, E. & Meeusen, R. (2012). Intense exercise increases circulating endocannabinoid and BDNF levels in humans—Possible implications for reward and depression. *Psychoneuroendocrinology*, *37*(6), 844-851.

Hurley, B. F., Hagberg, J. M., Allen, W. K., Seals, D. R., Young, J. C., Cuddihee, R. W., & Holloszy, J. O. (1984). Effect of training on blood lactate levels during submaximal exercise. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, *56*, 1260-1264.

Ide, K., Schmalbruch, I. K., Quistorff, B., Horn, A., & Secher, N. H. (2000). Lactate, glucose and O2 uptake in human brain during recovery from maximal exercise. *The Journal of Physiology*, *522*(1), 159-164.

Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J. M., & Bertschy, G. (2005). Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biological Psychiatry*, *57*(9), 1068-1072.

Klein, A. B., Williamson, R., Santini, M. A., Clemmensen, C., Ettrup, A., Rios, M., & Aznar, S. (2011). Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *The International Journal of Neuropsychopharmacology*, *14*(03), 347-353.

Knudsen, G. M., Paulson, O. B., & Hertz, M. M. (1991). Kinetic analysis of the human blood-brain barrier transport of lactate and its influence by hypercapnia. *Journal of Cerebral Blood Flow & Metabolism*, *11*(4), 581-586.

Krabbe, K. S., Nielsen, A. R., Krogh-Madsen, R., Plomgaard, P., Rasmussen, P., Erikstrup, C., & Pedersen, B. K. (2007). Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia*, *50*(2), 431-438.

Laske, C., Banschbach, S., Stransky, E., Bosch, S., Straten, G., Machann, J., & Eschweiler, G. W. (2010). Exercise-induced normalization of decreased BDNF serum concentration in elderly women with remitted major depression. *The International Journal of Neuropsychopharmacology*, *13*(05), 595-602.

Laursen, P. B., & Jenkins, D. G. (2002). The scientific basis for high-intensity interval training. *Sports Medicine*, *32*(1), 53-73.

Little, J. P., Safdar, A., Wilkin, G. P., Tarnopolsky, M. A., & Gibala, M. J. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: Potential mechanisms. *The Journal of Physiology*, *588*(6), 1011-1022.

Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature Reviews Neuroscience*, *6*(8), 603-614.

McAllister, A. K., Katz, L. C., & Lo, D. C. (1999). Neurotrophins and synaptic plasticity. *Annual Review of Neuroscience*, *22*(1), 295-318.

Mowla, S. J., Farhadi, H. F., Pareek, S., Atwal, J. K., Morris, S. J., Seidah, N. G., & Murphy, R. A. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *Journal of Biological Chemistry*, *276*(16), 12660-12666.

Nofuji, Y., Suwa, M., Sasaki, H., Ichimiya, A., Nishichi, R., & Kumagai, S. (2012). Different circulating brain-derived neurotrophic factor responses to acute exercise between physically active and sedentary subjects. *Journal of Sports Science & Medicine*, *11*(1), 83.

Nykjaer, A., Willnow, T. E., & Petersen, C. M. (2005). p75 NTR–live or let die. *Current Opinion in Neurobiology*, *15*(1), 49-57.

Pan, W., Banks, W. A., Fasold, M. B., Bluth, J., & Kastin, A. J. (1998). Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology, 37*(12), 1553-1561.

Penedo, F. J., & Dahn, J. R. (2005). Exercise and well-being: A review of mental and physical health benefits associated with physical activity. *Current Opinion in Psychiatry*, *18*(2), 189-193.

Poduslo, J. F., & Curran, G. L. (1996). Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. *Molecular Brain Research*, *36*(2), 280-286.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28*(7), 916-931.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M. V., Leick, L., Hart, E., Secher, N., Pedersen, K., & Pilegaard, H. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology*, *94*(10), 1062-1069.

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society: Biological Sciences, 361*(1473), 1545-1564.

Rognmo, Ø., Hetland, E., Helgerud, J., Hoff, J., & Slørdahl, S. A. (2004). High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing

aerobic capacity in patients with coronary artery disease. *European Journal of Cardiovascular Prevention & Rehabilitation, 11*(3), 216-222.

Sarchielli, P., Greco, L., Stipa, A., Floridi, A., & Gallai, V. (2002). Brain-derived neurotrophic factor in patients with multiple sclerosis. *Journal of Neuroimmunology, 132*(1), 180-188.

Scalzo, P., Kümmer, A., Bretas, T. L., Cardoso, F., & Teixeira, A. L. (2010). Serum levels of brain-derived neurotrophic factor correlate with motor impairment in Parkinson's disease. *Journal of Neurology*, *257*(4), 540-545.

Schiffer, T., Schulte, S., Sperlich, B., Achtzehn, S., Fricke, H., & Strüder, H. K. (2011). Lactate infusion at rest increases BDNF blood concentration in humans. *Neuroscience letters*, *488*(3), 234-237.

Schmidt-Kassow, M., Schädle, S., Otterbein, S., Thiel, C., Doehring, A., Lötsch, J., & Kaiser, J. (2012). Kinetics of serum brain-derived neurotrophic factor following low-intensity versus high-intensity exercise in men and women. *Neuroreport*, *23*(15), 889-893.

Schmolesky, M. T., Webb, D. L., & Hansen, R. A. (2013). The effects of aerobic exercise intensity and duration on levels of brain-derived neurotrophic factor in healthy men. *Journal of Sports Science & Medicine*, *12*(3), 502.

Schulz, K. H., Gold, S. M., Witte, J., Bartsch, K., Lang, U. E., Hellweg, R., & Heesen, C. (2004). Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. *Journal of the Neurological Sciences, 225*(1), 11-18.

Seifert, T., Brassard, P., Wissenberg, M., Rasmussen, P., Nordby, P., Stallknecht, B., & Secher, N. H. (2010). Endurance training enhances BDNF release from the human brain. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *298*(2), R372-R377.

Tang, S. W., Chu, E., Hui, T., Helmeste, D., & Law, C. (2008). Influence of exercise on serum brain-derived neurotrophic factor concentrations in healthy human subjects. *Neuroscience Letters*, *431*(1), 62-65.

Tjønna, A. E., Lee, S. J., Rognmo, Ø., Stølen, T. O., Bye, A., Haram, P. M., & Wisløff, U. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: A pilot study. *Circulation*, *118*(4), 346-354.

Ultsch, M. H., Wiesmann, C., Simmons, L. C., Henrich, J., Yang, M., Reilly, D., & de Vos, A. M. (1999). Crystal structures of the neurotrophin-binding domain of TrkA, TrkB and TrkC. *Journal of Molecular Biology*, *290*(1), 149-159.

Um, H. S., Kang, E. B., Leem, Y. H., Cho, I. H., Yang, C. H., Chae, K. R., & Cho, J. Y. (2008). Exercise training acts as a therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. *International Journal of Molecular Medicine*, *22*(4), 529.

Vaynman, S., Ying, Z., & Gómez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience*, *20*(10), 2580-2590.

Vega, S. R., Hollmann, W., Wahrmann, B. V., & Strüder, H. K. (2012). pH buffering does not influence BDNF responses to exercise. *International Journal of Sports Medicine*, *33*(01), 8-12.

Vega, S. R., Strüder, H. K., Vera Wahrmann, B., Schmidt, A., Bloch, W., & Hollmann, W. (2006). Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Research*, *1121*(1), 59-65.

Winter, B., Breitenstein, C., Mooren, F. C., Voelker, K., Fobker, M., Lechtermann, A., & Knecht, S. (2007). High impact running improves learning. *Neurobiology of Learning and Memory*, *87*(4), 597-609.

Xu, Q., Park, Y., Huang, X., Hollenbeck, A., Blair, A., Schatzkin, A., & Chen, H. (2010). Physical activities and future risk of Parkinson disease. *Neurology*, *75*(4), 341-348.