CEREBRAL GLUCOSE UPTAKE AS AN UNDERLYING MECHANISM OF THE EFFECT OF ACUTE PHYSICAL ACTIVITY ON INHIBITORY CONTROL

By

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PUBLIC ABSTRACT

CEREBRAL GLUCOSE UPTAKE AS AN UNDERLYING MECHANISM OF THE EFFECT OF ACUTE PHYSICAL ACTIVITY ON INHIBITORY CONTROL

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Purpose. Acute bouts of exercise have been found to have an influence on cognition. Currently, the published research has yet to narrow down the underlying biological mechanism of "why". In addition to exercise, the delivery of insulin spray up the nose (known as intranasal insulin) has been found to manipulate glucose usage in the brain without affecting peripheral blood glucose (i.e. blood sugar). Therefore, with the use of acute exercise and intranasal insulin, the purpose of this study was to investigate the possible underlying mechanism of cerebral glucose usage and observe its influence on the relationship between exercise and cognition. Additionally, another aim of this investigation was to establish a dose response relationship between intranasal insulin and cognition.

Method. 109 participants (52 exercise, 57 control) were randomized into either an exercise or control (i.e. rest) condition and 1 of 7 dose groups (i.e. 0, 20, 40, 60, 80, 100, 120 IUs). Cognitive performance was assessed before and after the exercise/control condition with the intranasal saline/insulin being administered prior to being on the treadmill.

Results. For behavioral performance (i.e. reaction time and response accuracy) and neural indices of attention (i.e. P3 amplitude) a 2 (Condition) X 7 (Dose) X 2 (Congruency: congruent, incongruent) univariate multi-level model controlling for the random intercept associated with participants was run. The effects of intranasal insulin were limited to a specific part of cognitive performance (i.e. reaction time). Faster reaction time was observed in response to 40 and 80 IU doses of insulin in comparison to the 0 IU placebo dose and 60 IU dose. The 40

IU dose also influenced a faster reaction time in comparison to 120 IU dose. These significant differences were only observed during the control condition. However, a nonsignificant trend was observed for the exercise condition that revealed a relationship of reaction time becoming faster as dose increased in contrast to the relationship that the control condition showed. No significant differences were observed for response accuracy.

Conclusions. With reaction time hitting a threshold for the control condition and a relationship of the change in reaction time continuously becoming faster as dose increased for the exercise condition. This preliminary study provides valuable information about intranasal insulin dose and its possible interactions with exercise for future interventions targeted at cognitive performance.

ABSTRACT

CEREBRAL GLUCOSE UPTAKE AS AN UNDERLYING MECHANISM OF THE EFFECT OF ACUTE PHYSICAL ACTIVITY ON INHIBITORY CONTROL

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Purpose. Acute bouts of exercise have been found to have an influence on cognitive performance. However, the published literature has yet to narrow down the underlying biological mechanism of "why". In addition to exercise, intranasal insulin (without an effect on peripheral glucose levels) has been found to manipulate glucose uptake in the brain. Therefore, with the use of acute exercise and intranasal insulin, the purpose of this study was to investigate the possible underlying mechanism of cerebral glucose uptake and observe its influence on the relationship between exercise and inhibitory control. Additionally, another aim of this investigation was to establish a dose response relationship between intranasal insulin and cognition.

Method. 109 participants (52 exercise, 57 control) were run through a cross-sectional, pre-and post-double-blind design. Each participant was randomized into either an exercise or control (i.e. rest) condition and either a saline control group or an insulin dose group. Inhibitory control performance was evaluated before and after the exercise/rest condition with the intranasal saline/insulin being administered prior to being on the treadmill.

Results. For behavioral performance (i.e. reaction time and response accuracy) and neural indices of attention (i.e. P3 amplitude) a 2 (Condition) X 7 (Dose) X 2 (Congruency: congruent, incongruent) univariate multi-level model controlling for the random intercept associated with participants was run. The effects of intranasal insulin were specific to the behavioral indices of attention with no influence on the P3 amplitude at any dose. Faster reaction time was observed in response to 40 (-46.7 ± 32.4) and 80 (-39.3 ± 23.4) IU doses of insulin in

comparison to the 0 IU (-3.0 ± 14.2) placebo dose (p's ≤ 0.003) and 60 (-5.1 ± 28.3) IU dose (p's ≤ 0.005). The 40 IU dose also influenced a faster reaction in comparison to 120 (-9.4 ± 23.3) IU dose (p = 0.002). These significant differences were only observed during the control condition. However, a nonsignificant trend was observed for the exercise condition that revealed a linear relationship of a shorter reaction time as dose increased in contrast to the curvilinear relationship that the control condition showed. No significant differences were observed for response accuracy.

Conclusions. With a curvilinear relationship of the change in reaction time in response to dose being observed for the control condition and a linear relationship being observed for the change in reaction time in response to dose for the exercise condition. This preliminary study provides valuable information about intranasal insulin dose and its possible interactions with exercise for future interventions targeted at cognitive performance.

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KEY TO ABBREVIATIONS

CNS	Central Nervous System
ERP	Event Related Potential
HHD	Health History Demographic
HR	Heart Rate
PAR-Q	Physical Activity Readiness Questionnaire
RT	Reaction time
VO_2	Aerobic Capacity
VO_{2max}	Maximum Aerobic Capacity
WASI-II	Wechsler Abbreviated Scale of Intelligence – 2nd Edition

CHAPTER 1

Introduction

A growing body of literature has demonstrated that acute exercise enhances cognition (Drollette, Shishido, Pontifex, & Hillman, 2012; Hillman, Pontifex, & Themanson, 2009; Lambourne, Audiffren, & Tomporowski, 2010; Pontifex, Saliba, Raine, Picchietti, & Hillman, 2013). In particular, acute exercise appears disproportionately beneficial for an aspect of cognition known as cognitive control and specifically, inhibitory aspects of cognitive control (Alves et al., 2012; Barenberg, Berse, & Dutke, 2011; Pontifex, Hillman, Fernhall, Thompson, & Valentini, 2009). However, the underlying biological mechanism that drives the influence of acute exercise on inhibitory control is not well understood. One proposed mechanism is that these single bouts of exercise modulate glucose – a main energy source for the brain – in a way that allows for increased glucose uptake and resulting in enhancements of cognitive function (Craft, Murphy, & Wemstrom, 1994; McNay et al., 2010).

In support of such a hypothesis, prior investigations have observed acute exercise induced modulations in glucose uptake in the brain (Adams, 2013; Ide, Horn, & Secher, 1999; Vissing, Andersen, & Diemer, 1996). Additionally, exercise has been found to possibly drive this change in glucose uptake by translocating glucose receptors to the surface of the brain (Bakirtzi et al., 2009). The brain is able to increase its energy uptake due to having more of these glucose receptors available in various brain regions (Bakirtzi et al., 2009; Choeiri, Staines, & Messier, 2002; Messari et al., 1998). However, at present we still have limited understanding of the extent to which such exercise-induced modulations actually relate to the cognitive enhancements following exercise. Accordingly, the aim of this investigation was to assess this relationship by

manipulating glucose uptake in the brain during exercise and examine the extent to which cognition is modulated in response.

One means of manipulating glucose uptake in the brain is through the administration of insulin intranasally. Insulin is a hormone that is produced by the pancreas that controls the uptake and storage of glucose in a variety of tissues in the body (including the brain) (Bakirtzi et al., 2009; Brooks, Fahey, & Baldwin, 2004). This hormone is able to translocate glucose receptors to the surface of muscles to enable them to utilize that source of energy later (Brooks et al., 2004). Just as insulin facilitates this process in the periphery, it also does so in the brain (Bakirtzi et al., 2009; Born et al., 2002).

Administering insulin intranasally allows the hormone to travel the nose to brain pathway to directly affect the brain (Bakirtzi et al., 2009; Born et al., 2002). Additionally, previous research has found that intranasal insulin enhances selective areas of cognition such as cognitive control, visuospatial, and long term memory (Benedict et al., 2004, 2006, 2006; Brünner, Kofoet, Benedict, & Freiherr, 2015; Novak et al., 2014). For instance, in a study conducted by Novak and colleagues (2014), the researchers found an improvement in cognitive performance on their verbal fluency task and visuospatial memory task after acute administration of intranasal insulin in comparison to baseline. Further, as intranasal insulin administration has a direct effect on the brain, it poses little risk for inducing hypoglycemia (Brünner, Benedict, & Freiherr, 2013; Heni et al., 2012; Kern, Born, Schreiber, & Fehm, 1999; Reger et al., 2006, 2008; Zhang et al., 2015). Accordingly, as both an acute bout of exercise and intranasal insulin stimulate glucose uptake (Bakirtzi et al., 2009; Born et al., 2002), establishing a dose-response relationship for the cognitive after-effects will provide initial evidence on the role of glucose uptake as a mechanism underlying acute-exercise induced enhancements in cognition.

Rather than rely only on overt behavioral metrics to quantify changes in cognition, examination of event-related brain potentials (ERPs) provide an additional means of gaining insight into the influence of glucose uptake on distinct cognitive operations. ERPs refer to a class of electroencephalographic activity that occurs in response to, or in preparation for, an event. One such ERP component, which occurs in response to a stimulus, has been observed to modulate both in response to acute exercise and intranasal insulin is the P3 (also known as the P300 or P3b) components. The amplitude of the P3 component is thought to reflect neuronal activity associated with the allocation of attentional resources during stimulus engagement (Polich, 2007). Whereas, P3 timing marked by its peak latency is thought to index stimulus classification and evaluation speed (Verleger, 1997; Duncan-Johnson, 1981). Following acute exercise, the P300 has been observed to have an increased amplitude in comparison to a control condition (Hillman, Snook, & Jerome, 2003; Kamijo et al., 2009; O'Leary, Pontifex, Scudder, Brown, & Hillman, 2011). In contrast, following administration of intranasal insulin, P300 amplitude has been found to decrease (Kern et al 1999). However, this article's results must be taken with caution with the changes to the P300 amplitude not being over the topographic maxima of the centro-parietal region which is where the P300 is typically localized.

Accordingly, the present investigation manipulated glucose uptake in the brain during exercise in order to examine the extent to which both inhibitory control and neuroelectric indices of the allocation of attentional resources are modulated in response. Based upon the existent intranasal insulin and cognition literature, it was hypothesized that as there is an increase in glucose uptake in the brain, inhibition and attention will continue to be enhanced with an eventual plateau in that enhancement. Ultimately, this approach will enable the determination of the dose-response relationship between glucose uptake during exercise and cognition.

CHAPTER 2

Review of Literature

A growing body of literature has established that exercise can enhance our cognitive processes with evidence demonstrating that a 20 minute bout of aerobic exercise can enhance cognitive performance (Hillman et al., 2003; Pontifex et al., 2009). There is evidence of this enhancement across age groups from children to older adults (Hillman, Pontifex, Raine, et al., 2009; Hillman et al., 2003; Kamijo et al., 2009; Pontifex et al., 2013). Acute bouts of exercise have been found to influence a number of aspects of cognition with meta-analyses generally observing a small positive effect on cognition (Chang, Labban, Gapin, & Etnier, 2012; Lambourne et al., 2010; Ludyga, Gerber, Brand, Holsboer-Trachsler, & Pühse, 2016).

Cognitive Control

Despite such global effects across all areas of cognition, evidence has observed that the greatest effects of these acute bouts of exercise appear to be for an aspect of cognition referred to as cognitive control (also known as executive function) (Hillman et al., 2003; Kamijo et al., 2009; O'Leary et al., 2011; Pontifex et al., 2009). Cognitive control refers to a host of cognitive processes that allow us to manipulate and monitor our day-to-day behaviors (Diamond, 2013). One of the prominent theories that was developed to try and explain the inner workings of cognitive control was the dual mechanisms of control (DMC) theory. This model is split up into two main parts (proactive and reactive control). Proactive control involves the person anticipating the event-using goal directed information to respond to the stimuli in a specific manner while reactive control is a modification of a behavior after the stimulus has been presented. These two parts have been linked to different activation patterns in the brain (Braver, Gray, & Burgess, 2007; Braver, 2012).

The current literature supports that there are three main components of cognitive control. These processes are cognitive flexibility, working memory and inhibition. Cognitive flexibility (also known as shifting) is a process that allows an individual to change perspective when demands or rules shift (Diamond, 2013). Next on the list, working memory, allows a person, over a short period of time, to store and manipulate information. This concept is complex with it being most often described using Baddeley and Hitch's model of the working memory system that involves three main components comprised of the phonological loop, the visuospatial sketchpad and the central executive interacting with each other for it to work properly (Baddeley & Hitch, 1974). Lastly, inhibition (also known as inhibitory control) is defined as the ability to block out extraneous stimuli that might interfere with the main activity at the moment as well as response suppression. Concurrent with the discussion above, cognitive control has been defined as distinct constructs that are highly interrelated (especially between working memory and inhibitory control) (Diamond, 2013). However, from a developmental perspective, cognitive control does not start as these distinct processes with it being identified as a unitary concept at a young age (Wiebe, Espy, & Charak, 2008). As children develop, each cognitive control process has been found to improve at different rates and become more distinct with the maturation of the prefrontal cortex (PFC) to eventually form distinct processes in adulthood (Diamond, 2013; Espy, Kaufman, Glisky, & McDiarmid, 2001). This maturation can be observed through researchers having to continuously add more difficulty to cognitive tasks testing cognitive control to elicit errors that were more easily produced at a younger age (Diamond, 2002).

Research in children has particularly focused upon inhibitory aspects of cognitive control given that it relates to academic achievement and the allocation of attention, which are important for the classroom (Hillman, Pontifex, et al., 2012). St. Clair-Thompson and colleagues (2006)

looked at inhibition performance in comparison to achievement on english, math and science standardized tests in 11 and 12-year-old children. They found that inhibition was significantly correlated with achievement in all three areas. Another study found that young children's performance on inhibition was linked to their academic achievement in math and literature (Will, Fidler, Daunhauer, & Gerlach-McDonald, 2016). Inhibition has also been found to be linked to the ability to maintain important relationships and its dysregulation is an key factor in drug abuse (Goldstein & Volkow, 2002; Perry & Carroll, 2008; Wehmeier, Schacht, & Barkley, 2010). Just like working memory, inhibition is also not a unitary concept and can be split up into two different types (which are inhibition of a prepotent response and interference control). Inhibition of a prepotent response is when an individual is trying to block an immediate response to a stimulus. This is commonly measured through tests such as the go/no-go task where the individual is instructed to press a button when one stimulus is shown and refrain from pressing a button when another stimulus is shown. However, interference control involves the process of blocking out extraneous stimuli. The flanker task is often utilized to measure this construct in which individual must filter out the flanking stimuli and respond only to the middle stimulus's direction. The flanking stimuli interfere with the response to the middle stimulus when they face the opposite direction (i.e. <<><< versus <<<<<).

Acute Exercise and Cognition

To date, the vast majority of research on acute exercise and cognitive control has focused on inhibitory aspects of cognitive control. The acute exercise and cognitive control literature support an enhancement in inhibitory control after an acute bout of exercise. It has been observed to have the largest enhancements after an acute bout of exercise in comparison to other cognitive

process with effect sizes ranging from d = 0.59 to 2.7 (Alves et al., 2012; Barenberg et al., 2011; Hillman et al., 2003; Kamijo et al., 2009, 2004; O'Leary et al., 2011).

In particular, a popular task to assess inhibition, in the acute exercise literature, is the Erickson flanker task. This task is comprised of five stimuli aligned across the center of the screen in which the individual must focus and respond to the middle stimulus while ignoring the flanking stimuli. Interference control is activated when the flanking stimuli are different (in the case of letters) or facing the opposite direction (in the case of the arrows and fish) than the middle stimulus (e.g. <<><<). This task has utilized different stimuli within the literature such as letters and arrows with a more child friendly version comprising of cartoon fish. With the ability to change the difficulty level (e.g. speed and having the flanker stimuli show up on the screen first to prime the participant), this task has been used with children and adults as well as special populations such as individuals with ADHD or multiple sclerosis (Hillman et al., 2003; Pontifex et al., 2013; Sandroff, Hillman, Benedict, & Motl, 2016).

A majority of the studies utilizing the modified flanker task have observed an enhancement in inhibition through the decrease of reaction time on the task or specifically, a decrease of reaction time on the incongruent condition, coming closer to the reaction time of the congruent trial that has less interference. Kamijo and colleagues (2007) had college aged adults perform 20 minutes of exercise, at three different intensities, on a bicycle ergometer. After the exercise, they examined inhibitory control performance through the letter stimuli version of the modified Eriksen flanker. In comparison to the baseline condition, they found that reaction time for the behavioral measures was decreased. This decreased reaction time has been supported by a different study where they tested older and younger adults on the modified Eriksen flanker task (with arrows) after performing 20 minutes of light or moderate exercise on a bicycle ergometer

(Kamijo et al., 2009). Furthermore, O'Leary and colleagues (2011) observed a decrease in reaction time interference (i.e. the difference between the reaction time for incongruent and congruent trials of the flanker task) on the modified flanker task after moderate intensity exercise on a treadmill in comparison to seated rest.

Event Related Potentials (ERPs)

Beyond the assessment of overt behavioral responses, event-related brain potentials (ERPs) can provide more a nuanced perspective of acute exercise induced modulations in cognition. ERPs are electrical potentials elicited by our brain in relation to conditions that occur internally or externally. These potentials are generated by pyramidal neurons (in an open field configuration) as postsynaptic potentials that accumulate to create a dipole that can be detected along the scalp. There is a standard naming procedure for stimulus locked ERPs. If it is considered to be negative going, it starts with an "N". If it is positive going, it starts with a "P". So, P300 means that it is a positive-going waveform. The number in the name can indicate ordinal position or the peak latency of the waveform. In the case of the P300, this would indicate that the waveform peaks at 300 milliseconds (ms) but when it is labeled as P3, it indicates that it is the 3rd peak in the waveform. The earlier stimulus locked components (i.e. N1 and P2) have been found to be related to selective attention. On the other hand, later stimulus locked components (i.e. N2 and P3) are related to different facets of cognitive functions such as the allocation of attentional resources, response inhibition and emotion (Hillman, Kamijo, & Pontifex, 2012; Luck, 2012).

Among ERP components, the P3 (also known as the P300 or P3b) has garnered considerable attention in the literature in regard to the effects of acute exercise on changes in cognition. The P3 was discovered in 1965 by Sutton, Braren, Zubin, and John when they ran

participants through a task that provided stimuli of certainty and uncertainty. This stimulus locked ERP is a positive going wave that usually occurs in response to a stimulus within 300-700ms with its topographical maxima centering around the centro-parietal region (Hillman, Kamijo, et al., 2012; Luck, 2012). The P3 is analyzed in terms of its amplitude and latency. Originally, the observed changes in P3 amplitude were considered to be in relation to context updating. Accordingly, after a stimulus is processed, it is compared to a previous stimulus event stored in working memory and only in the case of this new stimulus being different from the previous one is the P3 amplitude changed. However, this theory did not always fit with inconsistent P3 amplitude modulations during stimuli presentations that would expect no change. Due to these inconsistencies, Polich (2007) revised the meaning of the P3 amplitude to be related to the efficiency of the allocation of attentional resources. When the P3 amplitude increases, it is related to the increased efficiency of the allocation of attention processing (Polich, 2007). P3 latency is related to stimulus detection and evaluation time. Evaluation, stimulus and response processing can be identified separately for quick response times but become more difficult as response times slow time (Verleger, 1997). When the latency of the P3 shortens, it is related to the enhancement of stimulus detection and evaluation time (Polich, 2007).

Acute Exercise and Event Related Potentials (ERPs)

To date, relatively few investigations have utilized ERPs to examine the effects of acute exercise on cognition. However, within this small body of literature, the focus has largely been on the P3 ERP component. Consistently, acute exercise has been observed to increase the P3 amplitude with it varying whether the latency is unaffected or decreases. This change of the P3 amplitude and latency has been associated with the enhancement of attention allocation processing and quicker stimulus detection and evaluation time respectively.

Hillman and colleagues (2003) measured twenty college aged young adults (using a within-subjects design) to evaluate the performance and neuroelectric measures in relation to the modified Eriksen flanker task after a thirty-minute bout of submaximal exercise on a treadmill. They found that the P3 had an increased amplitude after this acute bout of exercise. Furthermore, a different study recruited 12 young adults to test performance and neuroelectric measures in relation to the modified Eriksen flanker task after acute bouts of exercise of different intensities on a bicycle ergometer. They, too, found an increase in P3 amplitude (for all intensities except hard) (Kamijo et al., 2007). Additionally, P3 latency was shorter for incongruent trials but not for the neutral trials of the flanker task across exercise intensities providing evidence that tasks requiring more cognitive control are more sensitive to acute exercise than tasks not requiring as much. This supports a previous study executed by Kamijo and colleagues (2004). The researchers recruited 12 young adult males to perform a go/no-go task after 3 intensities of exercise on a bicycle ergometer. However, in this case, while the P3 amplitude did increase after moderate intensity exercise, there was no change for light and a decrease after high intensity. This suggests that the effects of acute exercise might follow the inverted U hypothesis. While the studies mentioned previously focus on the young adult population, modulations in the P3 have been found across the life span. Pre-adolescent children have been observed to have an increased P3 amplitude after an acute bout of physical activity and older adults have been found to have shorter P3 latencies after a moderate bout of exercise (Hillman, Pontifex, Raine, et al., 2009; Kamijo et al., 2009; Pontifex et al., 2013).

Glucose and Cognition

Despite the growing body of literature investigating the effects of acute bouts of exercise on cognition, we still have little understanding of the possible biological mechanisms that could be

driving this relationship. One proposed mechanism is the uptake of cerebral blood glucose. Glucose is the main source of energy for the human brain. This substance is key to fueling the beginning of processes such as neurotransmitter synthesis, the creation of ATP, and neuronal upkeep. Glucose is vital to the proper functioning of the brain, with disruptions in its metabolism implicated in cognitive impairments and neurodegenerative diseases (Gage, Kelly, & Bjorklund, 1984; Mergenthaler, Lindauer, Dienel, & Meisel, 2013).

Due to its role in the brain, investigations have explored glucose utilization and its relation to cognition. Increased cerebral glucose uptake has been observed during the completion of a cognitive task in healthy individuals. Supporting evidence of cerebral glucose uptake affecting cognition has also been found by looking at individuals with differing disorders (Madsen et al., 1995). Specifically, current evidence has shown a decrease in cerebral glucose metabolism in disorders like Alzheimer's and Huntington's (Anchisi et al., 2005; Berent et al., 1988). Researchers have reported similar results about the relationship of cerebral glucose uptake to cognition when investigating spatial working memory in healthy rats and in aging rats (Gage et al., 1984; McNay, Fries, & Gold, 2000). Additionally, researchers have found that as cognitive workload increases the brain requires more energy to execute the task and, therefore, glucose utilization increases (McNay et al., 2000).

Insulin and Cognition

Interestingly, increasing cerebral glucose uptake has also been found to enhance cognition (D. Wang et al., 2012). That is, one way in which to modulate cerebral glucose uptake is through the administration of insulin. This hormone is produced by the pancreas and has the ability to manipulate the uptake and storage of glucose (Brooks et al., 2004). Although predominately thought of for its effects in regulating peripheral glucose levels, insulin also

impacts the central nervous system. Indeed, within the central nervous system, not only does insulin manipulate glucose levels, but it is also involved in neuronal protection through its relationship with low density lipoprotein receptor related protein 1, and learning and memory with its receptors located in different areas of the brain related to cognition (Banks, Owen, & Erickson, 2012; Ma, Wang, & Li, 2015; Werner & LeRoith, 2014). Accordingly, a growing body of research has begun to investigate the utilization of insulin to increase cerebral glucose uptake and enhance cognition.

Most of the insulin found in the brain is not synthesized locally but actually passes from the periphery through the blood brain barrier (BBB) (Banks, 2004). However, even though peripheral administration of insulin can pass into the cerebral spinal fluid and into the brain, the process is slow, and invasive. Additionally, the increase in insulin in the cerebral spinal fluid does not have a linear relationship to the increase of insulin in the periphery through intravenous infusion (Margolis & Altszuler, 1967; Ott, Benedict, Schultes, Born, & Hallschmid, 2012; Wallum et al., 1987). Alternatively, administration of insulin through the nose (i.e., intranasally) does not suffer from such limitations and instead appears to specifically target the central nervous system without impacting peripheral blood glucose (Brünner, Benedict, & Freiherr, 2013; Heni et al., 2012; Kern, Born, Schreiber, & Fehm, 1999; Reger et al., 2006, 2008; Zhang et al., 2015).

While specific details of the mechanism of how insulin enters the brain through intranasal administration are still not yet understood, it is proposed that the insulin takes an extracellular route through the olfactory epithelium, bypassing the blood brain barrier and entering the subarachnoid space in the brain (Born et al., 2002). The insulin then binds to its specified receptor in select areas of the brain (e.g. the frontal cortex and the hippocampus) (Hill, Lesniak,

Pert, & Roth, 1986; J. W. Unger, Livingston, & Moss, 1991) and GLUT4 (an insulin sensitive glucose receptor) is translocated to the membrane surface to increase glucose uptake (Bakirtzi et al., 2009; Born et al., 2002). While researchers are not completely certain on the entire cascade of proteins in the brain that trigger this translocation of the GLUT4 receptor, they do know that the pathway is Phosphatidylinositol 3 Kinase (PI3K) dependent. Grillo, and colleagues (2009) found that translocation of the GLUT4 receptor in the hippocampus of rats was blocked by a PI3K inhibitor. This has also been supported in cultured human neuroblastoma cells when wortmannin (a PI3K inhibitor) blocked the translocation of GLUT4 (Benomar et al., 2006). However, it is thought that the pathway might be similar to the peripheral tissue pathway. Binding of the insulin receptor triggers the phosphorylation of certain proteins that activate PI3K. PI3K then produces phosphatidylinositol (3, 4, 5) triphosphate (PIP3) which goes on to activate phosphoinositide-dependent protein kinase (PDK). Lastly, this leads to the phosphorylation and activation of Akt (Grillo et al., 2009). For the brain pathway, not all the steps have been filled in with researchers reporting that after binding to the insulin receptor and the activation of PIK3, Akt is phosphorylated which then triggers cascade of signals to eventually translocate the GLUT4 receptor (Benomar et al., 2006; Grillo et al., 2009). This process of GLUT4 translocation has been observed to occur within the 5 to 15 minute time range with a peak of action around 10 minutes (Chou et al., 2016). With glucose being the main fuel for the brain, neurons utilize its uptake to help with the process of recycling synaptic vesicles and to sustain neuronal firing (Ashrafi, Wu, Farrell, & Ryan, 2017). Additionally, insulin has also been observed to increase glucose uptake through increasing the gene expression of the GLUT1 receptor in the glial cells (Werner et al., 1989; Wozniak, Rydzewski, Baker, & Raizada, 1993).

However, in order for the insulin to traverse this pathway nose to brain pathway, it must be atomized such that the droplets are within a 30 to 100 μm range (Teleflex, 2013). If the intranasal insulin droplet size is too small, the insulin will be deposited in the lungs, whereas if the droplet size is too big the insulin will be deposited in the stomach (Giroux, 2005).

The surface area of the nasal cavity is around 160 cm^2 (Gizurarson, 2012; Heinemann, Pfützner, & Heise, 2001). Of this total surface area, the insulin takes a pathway through the olfactory epithelium, which has been reported to be in the range of 5 to 20 cm^2 (Gizurarson, 2012; Hinchcliffe & Illum, 1999). Due to the route that the insulin takes to the brain, it does not take long for the insulin to act on the central nervous system. Insulin levels in the cerebral spinal fluid (CSF) start to rise within 10 minutes with peak levels reached around thirty minutes after administration without any significant changes in insulin and glucose in plasma blood levels (Born et al., 2002). Only about 1% of the intranasal insulin dose reaches the brain due to the properties of the nasal mucosa (Illum, 2004; Thorne & Frey, 2001). Additionally, the mucous clearance rate of deposited particles is around 15 – 30 minutes (Hinchcliffe & Illum, 1999). This makes it imperative for the participant to clear their nose before administration so that there isn't extra mucous for the insulin droplets to deposit in (which will also be controlled for with the previously mentioned droplet size).

While insulin has a role in glucose metabolism in the CNS, it is important to acknowledge that it has other impacts on the brain as well. Lioutas and colleagues (2015) point out that it has possible anti-inflammatory, antithrombotic, vasodilatory and antiapoptotic properties on the CNS.

For insulin's anti-inflammatory properties, insulin is thought to suppress the production of transcription factors that produce inflammatory proteins (Lioutas et al., 2015). Specifically,

based on the current literature, insulin suppresses the transcription factor NFkB to downregulate transcription of the inflammatory proteins. This ability to suppress NFkB is thought to be due to insulin's stimulation of the release of nitric oxide (Dandona, Aljada, & Mohanty, 2002). Nitric oxide is not believed to be the only way in which insulin does this, as nitric oxide activates the protein IkB which suppresses the translocation of NFkB into the nucleus of the cell, inhibiting its activity (Baldwin, 1996). Lastly, it is also important to account for the enzyme IKKBeta. This enzyme phosphorylates IkB which subsequently degrades the enzyme and allows NFkB to translocate to the nucleus to transcribe inflammatory proteins. While insulin is not known to directly act on IKKBeta, it has been seen to reduce superoxide (O2*) and this superoxide has been seen to activate IKKBeta (Dandona, Chaudhuri, Mohanty, & Ghanim, 2007).

However, among the few studies that have investigated insulin's anti-inflammatory properties, they have focused on the periphery. Aljada and colleagues (2002) found that Egr-1 expression and plasma tissue factor (TF) and plasmogen activator inhibitor-1 (PAI-1) concentrations decreased after four-hour infusion of insulin (2-2.5 IUs an hour). Using the same paradigm, researchers found that NfkB, and vascular endothelial growth factor (VEGF) were inhibited as well with increased concentrations of IkB (Dandona et al., 2001, 2003). Levels of these inflammation proteins were understandably not able to be obtained directly from the brain.

While there is not a literature base for intranasal insulin and its anti-inflammatory effects on the brain, we can turn to the literature that looks at the dysregulation of insulin. It is thought that hyperinsulemia and insulin resistance can increase inflammation in the central nervous system. This dysregulation has been proposed to be linked to cognitive function issues such as dementia (Kullmann et al., 2016). Inducing hyperinsulimia in healthy participants has even been found to raise levels of beta amyloid and other inflammatory markers in the CSF (Fishel et al.,

2005). Overall, the regulation of insulin in the CNS has an important hand in inflammation but whether or not a one-time administration of intranasal insulin would have much of a hand in these mechanisms is uncertain. Of the studies looking at insulin's anti-inflammatory properties, the participants received a constant infusion of insulin over the course of 4 hours. This points to this anti-inflammatory process having a fairly long-time course to take effect. The possibility of these anti-inflammatory properties taking effect during a protocol that looks at the effects of insulin around 40 minutes after nasal administration is unlikely. As for its effect on cognition, we have observed that dysregulation might contribute to diseases like Alzheimer's disease and fixing that problem might alleviate cognitive problems attributed to the disease, but we are viewing this from a broken system point of view. If a healthy individual doesn't have high levels of inflammation in their CNS, downregulating inflammatory proteins is not a large factor involved in someone's cognitive ability.

Insulin has been found to have anti thrombotic (i.e. reduces blood clot formation) and vasodilatory effects as well. Insulin's anti-thrombotic effects is most likely the extension of the mechanism in which it reduces inflammation. Insulin infusion over the course of 4 hours has been observed to decrease production of the proteins TF and PAI-1 that are known to increase fibrinolysis (Aljada et al., 2002). On the other hand, its vasodilatory effects work through a different pathway. Through binding to its insulin receptor, Akt is phosphorylated which increases the activity of endothelial nitrous oxide synthase (eNOS) and therefore, nitrous oxide production. However, eNOS and nitrous oxide has been observed to only have a smaller hand in the mechanism with the release of adenosine and activation of ATP sensitive potassium channels driving most of the reaction to trigger vasodilation (Abbink-Zandbergen et al., 1999; Dimmeler et al., 1999; McKay & Hester, 1996). This vasodilatory effect has been observed after the acute

administration of intranasal insulin in type 2 diabetic and control participants. Novak and colleagues (2014) measured vasoreactivity (vasodilation, vasoconstriction and vasoreactivity rate via the change in cerebral perfusion) and administered different cognitive performance tasks. The researchers found that certain improvements were significantly correlated with perfusion increases and vasodilation in different areas of the brain. While the uptake of glucose is not being measured directly, it is still possible to identify it as the main contributing factor in contrast to vasodilation. Insulin receptors are more highly expressed in neurons than in glial cells (Unger et al., 1989). Glial cells (specifically astrocytes), in comparison to neurons, are largely involved with the control of cerebral blood flow in the brain due to the blood vessels that run through them (Kimelberg, 2010). With insulin receptors being expressed more on neurons, they would not have a major influence on this cascade. Despite all of this, acute administration of insulin has still been reported to stimulate vasodilation. So, there are some important points to make about these articles. Novak and colleagues (2014) correlated changes in cerebral blood flow with certain aspects of cognition but upon closer look, a majority of these correlations were made with non-significant trend changes in vasoreactivity (i.e. the control group without diabetes did not have a significant change in vasoreactivity for any of the studied brain regions). Additionally, the brain regions that have been observed to have increased cerebral blood flow after insulin administration (i.e. insular cortex, left putamen, left caudate nucleus, and inferior frontal gyrus) are known to be involved in motor control, regulation of eating behavior and the integration of sensory. However, with these areas also having involvement in aspects of cognitive functioning, the results of this study will be compared to previously published articles involving glucose ingestion, cognitive function and ERPs to discern between glucose uptake and vasoreactivity stimuli (Bhatia & Marsden, 1994; Chikazoe, Konishi, Asari, Jimura, & Miyashita,

2007; Crutcher & DeLong, 1984; Molnar-Szakacs, Iacoboni, Koski, & Mazziotta, 2005; Nieuwenhuys, 2012; Novak et al., 2014; Schilling et al., 2014). According to one article, ingestion of glucose elicited a shorter latency in the P3 in comparison to the control condition as well as an enhancement in cognitive performance in young adults (Riby et al., 2008). While changes in P3 amplitude have been divided, an increase has been observed after glucose administration in comparison to a control when completing an inhibitory control task (De Pauw et al., 2017). Cognitive behavioral performance results would be hard to compare (due to working with a high functioning population and possible ceiling effects), but if different modulations of the P3 are observed, then it can be inferred that glucose uptake is not the only factor involved in cognitive enhancement after intranasal insulin administration.

Finally, insulin has also been observed to have antiapoptotic effects. According to the literature, insulin binds to its receptor which maintains activation of phosphatidylinositol 3 – kinase (PI-3K) which keeps AKT active by phosphorylation. The active AKT then travels to the cytosol and the nucleus where it phosphorylates and downregulates target proteins that normally would promote apoptosis. Insulin has been found to keep Bcl – 2 protein levels stable (antiapoptotic protein) and downregulates GSK-3Beta (known to increase neuronal apoptosis) (Duarte, Moreira, & Oliveira, 2012; Duarte, Santos, Oliveira, Santos, & Rego, 2008). Unfortunately, there are no intranasal insulin literature base to look at its antiapoptotic effects but there are several studies out there that have utilized animal models and cell cultures to look directly at the extent of insulin's antiapoptotic effects. Researchers have investigated this effect in the protection against apoptosis of retinal neurons (Barber et al., 2001). As stated before with respect to insulin's anti thrombosis effects, these effects had a relatively long timeline with the course of these studies occurring over several hours or a couple days (Duarte et al., 2012; Duarte,

Santos, Oliveira, & Rego, 2005; Duarte et al., 2008). Additionally, with testing healthy neurotypical individuals, providing an antiapoptotic effect would not make a large difference in someone's cognitive function.

Accordingly, intranasal insulin administration in healthy, non-diabetic individuals has been observed to transiently enhance cognition. Specifically, it has been found to enhance memory for visuospatial and odor cued spatial memory (Brünner et al., 2015; Novak et al., 2014). Even though the majority of the literature has focused on acute administration of intranasal insulin's effects on memory, a few studies have touched on its effects on cognitive control. Novak and colleagues (2014) included the trail making tests A and B, digit span and verbal fluency in their cognitive battery. While they did not report results for the first two tasks, they did report that the verbal fluency task was improved by acute intranasal insulin administration in comparison to a baseline cognitive measurement. Another article investigated acute insulin administration and its effects on verbal working memory. Young adults were administered 160 IU of intranasal insulin and then underwent a battery of cognitive tasks with verbal working memory being tested at 20 and 75 minutes after administration. These researchers observed a statistically significant increase in verbal working memory performance, at the 75 minute time point, by the women in their sample (Benedict, Kern, Schultes, Born, & Hallschmid, 2008). However, not all studies have observed enhancements of cognitive control with Reger and colleagues (2006) reporting no change in performance in the Stroop Color Word test or verbal working memory task after administration. It is important to note that the differing results of these studies could be the result in the method of administration of intranasal insulin as well as the number of tasks administered. A portion of the intranasal insulin dose given to the participants might be lost to the lungs or stomach if the droplet size of the insulin is too small or

too big respectively (Giroux, 2005). Additionally, multiple cognitive tasks were administered in these studies. This could add a cognitive workload burden to the participant with the amount of time it takes to complete the tasks and depending on what order the tasks were administered in, that could affect how much influence insulin has on that area of cognition.

Beyond overt behavioral responses, intranasal insulin has also been observed to impact neuroelectric indices of attention. Kern and colleagues (1999) administered 20 IUs of intranasal insulin to 18 healthy adult males every 15 minutes over the course over 90 minutes. Participants completed an auditory oddball paradigm after the intranasal insulin administration while measuring auditory evoked potentials. The investigators found that P3 amplitude decreased and an increase in latency. However, the main effect for amplitude was over the frontal region of the brain rather than the topographic maxima of the centro-parietal region.

Acute Exercise and Glucose in the Peripheral Nervous System

When an individual is engaging in acute exercise, blood glucose levels initially increase (due to the feed forward mechanism to maintain blood glucose homeostasis) and then gradually decline until the exercise stops (Brooks et al., 2004). Blood glucose levels are maintained by this feed forward mechanism during exercise. When exercise begins, skeletal muscles increase their need for the uptake of glucose as an energy resource. In the process, catecholamines rise and insulin falls. This stimulates the production of hepatic glucose production (by the liver) to increase and attempt to maintain glucose homeostasis. However, this feed forward mechanism only keeps glucose levels constant for so long and as the exercise duration increases, glucose levels will start to decrease (Brooks et al., 2004).

In addition to the rise of catecholamines (epinephrine and norepinephrine) there is also a rise of growth hormone, cortisol, glucagon, and thyroid hormone (T3) plasma concentrations

with exercise. With the increase of these hormone plasma concentrations, the body is moved toward free fatty acid (FFA) metabolism by increasing mobilization of triglycerides from adipose tissue, blocking GLUT1 receptors, stimulating gluconeogenesis and glycogenolysis to spare and create glucose (Brooks et al., 2004; Houston, 2006). Taking all this into consideration, the intensity of exercise is also important to consider when determining the main energy source being utilized by the skeletal muscles. During moderately intense exercise (the intensity that was chosen for this study) plasma glucose levels are spared with FFA metabolism. At this intensity, high levels of citrate inhibit phosphofructokinase (PFK; which is the rate-limiting enzyme for the glycolysis pathway). This would significantly slow down the process that breaks down glucose for energy placing the emphasis on FFA metabolism. As intensity increases, NADH from the glycolysis pathway gradually builds up to the point where the production of lactate is favored (converted from pyruvate) by inhibiting the enzyme pyruvate dehydrogenase. Lactate spills over into the bloodstream and travels to the adipose tissue to shut down free fatty acid mobilization and therefore utilization (Brooks et al., 2004; Houston, 2006). This changes the main energy utilization from free fatty acids to glucose. Therefore, considering this, it is important to set an intensity that elicits the same type of energy source utilization across individuals. Due to lactate's involvement with this switch, one can set a moderate intensity of 60-65% of an individual's maximum heart rate to stay below lactate threshold.

Due to the robustness of this mechanism, whether someone is in a fed or fasted state, it still follows the same pattern of change of plasma glucose levels. Zinker and colleagues (1990) had individuals in either a 36-hour fasted state or not then ride a cycle ergometer at 50% of their VO_2 max until exhaustion. Whether the participant had fasted or not, glucose levels initially, increased and gradually decreased over the course of exercise while only differing at baseline

levels. However, the peripheral blood glucose response can be influenced by training. Kjaer, Farrell, Christensen, & Galbo, (1986) had trained and untrained participants run at 60%, 100% and 110% of their VO2 max. They found that the trained participants had an increase of plasma glucose concentrations at the beginning of exercise with a large increase post exercise during the recovery period. This was in comparison to the untrained individuals having consistent glucose levels during exercise with a smaller increase post exercise during the recovery period.

While plasma glucose levels begin to rise at the beginning of exercise and gradually decrease over time, peripheral insulin levels decrease over the course of acute exercise. This is another plasma glucose sparing mechanism of the body with insulin's role to stimulate the uptake and storage of glucose in various tissues in the body (Brooks et al., 2004). This decrease has been found across exercise intensities. Kjaer and colleagues (1991) found a decrease in insulin plasma concentrations 55% and 82% of VO_2 max with leg and arm exercises. Another study had participants exercise at 85-90% of their VO_2 max on a bicycle ergometer and observed a consistent insulin plasma concentration decrease (Pruett, 1970).

Acute Exercise and Glucose in the Central Nervous System

Acute bouts of exercise also appear to result in modulations in glucose in the central nervous system with increased glucose uptake being observed during exercise (Dalsgaard, 2006; Dalsgaard, Ide, Cai, Quistorff, & Secher, 2002; Ide et al., 1999). Ide and colleagues (1999) recruited 12 healthy young adults to investigate the cerebral carbohydrate uptake in response to submaximal exercise. Using a cannula inserted into the brachial artery of the nondominant arm and internal jugular vein in addition to utilizing NIRS (near infrared spectroscopy) to look at carbohydrate uptake and cerebral oxygenation respectively, the researchers took measurements at rest, and 30% and 60% of the participants' VO_2 max on a cycle ergometer (10 minutes for

each exercise condition on the same day). During submaximal exercise at 60% VO_2 max, carbohydrate uptake increased in the brain indicated by a decrease in the O_2/(glucose + 1/2lactate) ratio. Using a similar technique to look at cerebral carbohydrate uptake, without the NIRS, Dalsgaard and colleagues (2002) had participants cycle at three exercise intensities (light, moderate and exercise to exhaustion). Additionally, they also had a separate cohort exercise at 40% of VO_2 max with and without a neuromuscular blockade. For the first cohort, carbohydrate uptake increased during exercise to exhaustion according to a decrease in the O_2/(glucose + 1/2lactate) ratio and in the second cohort, participants with the neuromuscular blockade during submaximal exercise had an increase in carbohydrate uptake according to the decrease in the previously mentioned ratio.

This phenomenon has also been supported by an animal model. Wistar rats were injected with 2DG to utilize the quantitative 2DG method (uptake and trapping of 2DG in the cells enables the measurement of their metabolic activity) to examine local glucose utilization and total glucose utilization during exercise. The rats either rested or ran for 30 minutes at 85% of maximum oxygen consumption (they were habituated to the treadmill for 7 days before this test so that it was not a novel stimulus). Total glucose utilization increased by 38% during running in comparison to the rest group (Vissing et al., 1996).

Glucose uptake has also been observed to increase after exercise cessation. Healthy college aged individuals completed exercise until exhaustion on a cycle ergometer while measuring glucose uptake with a cannula in the brachial artery of the non-dominant arm and in the internal jugular vein. Glucose uptake increased during the first 5 minutes after exercise according to the O_2/glucose uptake ratio (Ide, Schmalbruch, Quistorff, Horn, & Secher, 2000).

This was also supported by another study that observed, after exhaustive exercise, glucose uptake increased by 30% within the first 5 minutes (Dalsgaard et al., 2002).

Accordingly, given that increased glucose uptake is associated with enhancements in cognition, the support that acute exercise induced increase of glucose uptake during and following the cessation of exercise may serve as a potential mechanism underlying acute exercise induced enhancements in cognition.

While there are some potential limitations to my experimental approach, it sets the stage for future investigations in this area. One limitation is that the current design has a pre and post cross sectional design. Ideally, to fully account for individual differences, one would want to utilize a pre, post within subject design. However, this future direction would not be possible if this proposed study was not conducted due to the participant burden being substantially higher if they are required to come in for four separate sessions. Running this proposed study will obtain the needed information about reasons for participant discontinuation, and how to mitigate that. Additionally, with the novel combination of intranasal insulin and exercise, any possible additional techniques to ensure that the protocol goes as smoothly as possible will be attained before a larger study is launched.

Another limitation to this study is that we are not able to directly measure glucose uptake. For future directions, there are more invasive, expensive and time intensive methods that would allow glucose uptake in the brain to be measured more directly.

To do this with humans, one could use radioactive label tracing with PET to look at cerebral glucose uptake in relation to exercise. 18Fluoro-Deoxyglucose is injected in the blood and is measured by utilizing PET to quantify the uptake of the radioactive tracer into the cerebral tissue (Bingham et al., 2002). Even though the exercise and cerebral glucose uptake literature

hasn't used this method, a participant would be able exercise before being injected with the radioactive tracer and put into the PET machine. However, much like an MRI, there is a certain time period that will lapse before the imaging will be able to begin to measure the uptake and that would need to be accounted for in the analysis.

Researchers have also looked at glucose uptake in the brain via a catheter at the base of the skull, and in the brachial artery of the non-dominant arm. With this technique, the researchers additionally look at oxygen uptake and lactate uptake in order to create the O2/glucose and O2/(glucose + 1/2lactate) ratios. This technique allows data collection during and after exercise. All of the previously mentioned studies cited for cerebral glucose uptake during and after exercise, in humans, used this technique. While the PET and MRI would only allow the viewing of the uptake induced by intranasal insulin and exercise a decent time after the fact, this technique would enable immediate measurement. However, this technique would be difficult to execute in addition to using EEG and cognitive testing due to participant burden. No matter what, there would have to be substantial funding and resources to use any of these techniques in the future.

If the results of the study point more to insulin being a vasodilator and increasing cognitive function through this avenue, perfusion can be looked at through NIRS and arterial spin labeling in the MRI during and after exercise (Murkin & Arango, 2009; Novak et al., 2014).

Currently, this study is focusing on the executive function inhibition for the cognitive measure. However, in the intranasal insulin literature, there is a focus on its influence on memory. There is specially a drive for this with clinical trials currently being conducted on using it as a treatment for Alzheimer's disease. A future direction would be to use a memory cognitive task once a relationship is established from the current study with there being a large

concentration of insulin receptors being located in the hippocampus (J. W. Unger et al., 1991). This would extend the knowledge to observe whether the findings are consistent across different areas of cognition or if they might vary based on brain regions that these areas are linked to.

Purpose

Taking this information into account, intranasal insulin's interaction with glucose in the brain provides a means of investigation into the relationship between acute exercise and cognitive control. Intranasal insulin travels the nose to brain pathway with little to no effect on the periphery; and with insulin production being inhibited during exercise there should be no abnormal decrease in peripheral blood glucose levels with the combination of acute exercise and intranasal insulin. Therefore, this study investigated glucose uptake in the brain as an underlying mechanism of the relationship between a moderately intense bout of acute exercise and inhibitory control and attention by utilizing behavioral, and neuroelectrical measures along with a range of intranasal insulin doses. Every participant's cardiorespiratory fitness were evaluated to ensure that everyone fell into the moderate fitness range (i.e. greater than the 10^{th} percentile and below the 90^{th} percentile based upon their age and sex) and had a pseudo randomized dose of insulin assigned to them to split sex as evenly as possible in addition to covering a wide range of insulin doses.

Rationale

This study provides preliminary data to begin the process of identifying whether cerebral glucose uptake is one of the main driving factors involved in the relationship between acute exercise, and inhibitory control and attention. Providing this data will inform researchers about what biological processes to target when aiming to conduct exercise interventions that focus on enhancing cognitive control.

Hypotheses

My first hypothesis was that acute exercise's enhancement of inhibitory control and neuroelectric indices of the allocation of attentional resources would be replicated. The second hypothesis was that a dose response relationship would be observed for the change in cognitive performance. As dose increased, cognitive performance would increase (as indicated by a shorter reaction time and increase in P3 amplitude). Finally, my third hypothesis stated that exercise-induced enhancement in cognitive performance will increase as intranasal insulin dose increases. This would be indicated by an increase in the P3 amplitude and a shorter reaction time. Such a dose-response relationship provides support for the assertion that glucose uptake is an underlying mechanism, with higher doses of intranasal insulin increasing glucose uptake and allowing for larger enhancements in cognition to manifest. Whereas, should exercise result in relatively stable enhancements in cognition regardless of the intranasal insulin dose, it would suggest there may be a threshold at which increases in glucose uptake does not result in further cognitive enhancements.

CHAPTER 3

Methodology

Participants and Recruitment

A sample of 114 college aged young adults (aged 18 – 30) was recruited from the greater Lansing area (five of them were excluded from analyses due to missing data, totaling out to a sample of 109). The Institutional Review Board at Michigan State University approved all study protocols and methods. Participants completed a consent form, a health and history demographics questionnaire, the Edinburgh Handedness Inventory, a physical activity readiness questionnaire (PARQ), a medical screening questionnaire, and the Wechsler Abbreviated Scale of Intelligence – 2nd Edition (WASI-II; 2-part subtest). Participants were screened for any neurological disorders, physical disabilities and normal or corrected to normal vision. Given the use of intranasal insulin, anyone with type 1 or 2 diabetes, self-reported pregnancy, or having any sinus inflammation due to a cold or allergies at the time of testing was excluded from this study. Participants were compensated with $5 for completing the first session of the study and $15 for completing the second session of the study. Participant demographics are outlined in Table 1. in Appendix C.

Procedure

This study was a cross-sectional, double-blind, pre-and post-design in which participants were randomly assigned to either an exercise or resting condition in addition to either a saline control group or an insulin group receiving a dose of either 20, 40, 60, 80, 100, or 120 IU of insulin. See Table 2. in Appendix C for the breakdown of dose group allocation numbers. This approach allowed for characterizing changes in cognition induced by exercise relative to control

as well as how such changes were modulated by the dose of intranasal solution. Participants came into the lab for 2 sessions. During the first session, the participant completed the necessary paperwork. They were given a set of practice trials of the flanker task to familiarize themselves with the cognitive assessment. At the end of the session, the participant completed a VO_2 max test to assess the participant's level of aerobic fitness. If they fell within the specified fitness range (i.e., greater than the 10^{th} percentile and below the 90^{th} percentile based upon their age and sex) and fit all other inclusion criteria (i.e. free of neurological disorders, physical disabilities, type 1 or 2 diabetes, self-reported pregnancy, or any sinus inflammation due to a cold or allergies at the time of testing and had normal or corrected to normal vision), they were asked to come back for a second session. The fitness range was restricted to 10^{th}-90^{th} percentile (based on normative data from Shvartz & Reibold, 1990) to eliminate individuals on the extreme ends due to the published literature supporting that fitness can have an influence on insulin sensitivity (Clausen et al., 1996; Koch, Britton, & Wisløff, 2012b; Kullmann et al., 2016; Nassis et al., 2005; Wisløff et al., 2005b).

Prior to the second day of testing, participants were asked to not engage in any exercise or intake any caffeine on the day they came into the lab. Participants were also asked to not intake any food at least 3 hours prior to arrival into the lab. Prior to the start of testing, the participant's blood glucose level was assessed via a glucose meter (CVS Health Advanced Blood Glucose Meter, United States). If the participant's blood glucose fell outside the range of 70-115 mg/dL, that person was rescheduled to come in a different day. Participants' electroencephalographic activity was recorded from an EEG cap while performing an inhibitory control task before and after 20 minutes of light to moderate intensity exercise on a treadmill or 20 minutes of sitting on the treadmill. During both conditions, participants watched an emotionally neutral video. The

participant was outfitted with a polar heart rate monitor (Model H7, Polar Electro, Finland) to monitor heart rate throughout the entire session. Researchers administered the specified dose (IU) of intranasal insulin to the participant (in a seated position with their head slightly tilted back with equal amounts squirted into each nostril) before moving on to the exercise on the treadmill or rest condition. Peripheral blood glucose was collected at three different time points throughout the session (before the 1st cognitive test to establish baseline, halfway through the acute bout of exercise, and 5 minutes after the acute bout of exercise) via a glucose meter (CVS Health Advanced Blood Glucose Meter, United States). Lactate was collected with glucose halfway through the bout of acute exercises with a lactate meter (Nova Biomedical Lactate Plus Analyzer, United States). If blood glucose fell below 70 mg/dL, testing was stopped, and proper safety protocols were followed (This occurred for 2 participants). Additionally, information about possible symptoms throughout the session and at the end of the session was collected through a questionnaire given at the beginning (for a baseline measurement) and the end of the session. When collecting peripheral blood glucose, and lactate levels during the exercise session, the participant was asked to step to the side of the treadmill for a moment.

Power Analysis

Based upon an *a priori* power analysis, computed using G*Power 3.1.2 (Faul, Erdfelder, Lang, & Buchner, 2007) assuming alpha at 0.05 and beta at 0.2 (i.e., 80% power), a sample of 114 participants provided a sufficient sample to detect medium effect sizes or larger (see Table 3.). Such effects sizes are consistent with those observed in the acute exercise and cognition literature as well as those within the intranasal insulin and cognition literature. Specifically, medium to large effect sizes ranging from d = 0.59 to 2.7 have been calculated from articles assessing the effect of acute exercise on inhibition and attention (Hillman et al., 2003; Kamijo et

al., 2009, 2004; O'Leary et al., 2011). Large effect sizes (ranging from d = 1.14 to 2.85) have been calculated from investigations on the effects of intranasal insulin on cognition (Benedict et al., 2008; Brünner et al., 2015). Further, this sample size allowed for some participant attrition and the ability to account for a large number of potential confounding variables without completely sacrificing statistical sensitivity as observed with only being able to analyze 109 participants (Table 3.; Appendix C)

Inhibitory Control Task

The modified Eriksen flanker task was utilized to assess inhibitory control. In this version of the task, capital letters were utilized (specifically the pairs of M and N, U and V, E and F, T and I). For 80 practice trials, D and B were used. The goal of the task was for the participant to indicate, by pressing the button assigned to the letter, what letter is present in the middle of a line of letters in the center of the screen (e.g. MMMMM or NNNNN). There are congruent trials where the four flanking letters are the same (e.g. EEEEE) and incongruent trials where the four flanking letters are different (e.g. EEFEE). For the incongruent trials, the participant needed to inhibit the prepotent response to the flanking letters to respond correctly to the middle letter. Participants completed 320 trials total (160 pre-exercise and 160-post exercise) with the flanking letters displayed on the screen 300ms before the middle letter with a total presentation time of 400ms with a response window of 1000ms and a variable ITI equally distributed at 2300, 2400, 2500, 2600, and 2700.

Cardiorespiratory Fitness Assessment

A VO_2 max test was utilized to assess the maximal rate at which the individual's body can deliver and consume oxygen (American College of Sports Medicine, 2014). A computerized indirect calorimetry system (Parvomedics True Max 2400) was utilized to determine relative peak oxygen consumption (ml/kg/min). Participants ran or walked at a constant speed on a motor driven treadmill in which the grade increases 2.5% at 2-minute intervals until the participant is not able to continue at that intensity (American College of Sports Medicine, 2014). The main criterion that determined that VO_2 max was achieved was evidenced by a plateau of oxygen consumption (an increase that is ≤ 2 ml/kg/min after an increase in workload). Including plateau of oxygen consumption, they met at least two of the four criteria (other criteria listed here): 1. Maximal heart rate adjusted for age (220-age) 2. A respiratory exchange ratio (RER) that is ≥ 1.1 or 3. Relative perceived exertion (RPE) that is > 7. Cardiorespiratory fitness percentiles were established utilizing normative data produced by Shvartz & Reibold, (1990).

Exercise and Rest Assessment

Participants randomly assigned to the exercise condition walked on the treadmill for 20 minutes at a moderate intensity. So that all participants stayed within the same main energy utilization system, intensity was assigned as 60-65% of the maximum heart rate obtained from the VO_2 max test completed during the first session. Glucose and lactate blood levels were obtained at the 10-minute mark, which represented the midpoint of the experimental condition, of the moderate exercise or rest condition. Every two minutes the feeling scale (FS; out of the range of -5 to 5) and rated perceived exertion (RPE; out of a range from 0 to 10) were obtained from the participant. If participants were randomized into the rest condition, they sat on the

treadmill for 20 minutes. All participants, regardless of exercise or resting conditions, watched an emotionally neutral video (specifically, minutes 65–85 from Wonders of the Universe) (*Wonders of the Universe*, 2011) during this 20 minute period to make certain that the outcomes from these two conditions were not a result of experimenter interactions or non-exercise. RPE, and FS were evaluated every two minutes during the rest condition.

Neuroelectric Assessment

EEG activity was recorded from 32 electrode sites arranged in an extended montage based on the International 10-10 system (Chatrian, Lettich, & Nelson, 1985) using a Neuroscan Quik-Cap (Compumedics, Inc., Charlotte, NC). Recordings were referenced to averaged mastoids (M1, M2), with AFz serving as the ground electrode and impedance less than 10 kΩ. In addition, electrodes were placed above and below the left orbit and on the outer canthus of both eyes to monitor electrooculographic (EOG) activity with a bipolar recording. Continuous data was digitized at a sampling rate of 1000Hz and amplified 500 times with a DC to 70Hz filter using a Neuroscan SynAmps RT amplifier. The EEG data were then imported into EEGLAB (Delorme & Makeig, 2004) and prepared for temporal ICA decomposition. Data more than 2 s prior to the first event marker and 2 s after the final event marker were removed to restrict computation of ICA components to task-related activity. The continuous data were then filtered using a 0.05Hz high-pass 2nd order Butterworth IIR filter to remove slow drifts (Mognon, Jovicich, Bruzzone, & Buiatti, 2011), and the mastoid electrodes was removed prior to ICA decomposition. ICA decompositions were performed using the extended infomax algorithm to extract sub-Gaussian components using the default settings called in MATLAB implementation of this function in EEGLAB with the block size heuristic drawn from MNE-Python (Gramfort et al., 2013). Following the ICA decomposition, the eyeblink artifact components were identified

and removed. Identification of the eyeblink artifact components was performed using the icablinkmetrics function (Pontifex, Miskovic, & Laszlo, 2016). Following removal of the eye blink components, stimulus-locked epochs were created for correct trials from −100-1000msec around the stimulus, baseline corrected using the −100 to 0msec prestimulus period, and filtered using a zero-phase shift low-pass filter at 30Hz (24 dB/oct). Trials with artifact exceeding ±100 μV were rejected. The P3 component was evaluated as the mean amplitude within a 50ms interval surrounding the largest positive going peak within a 300-700msec latency window. Due to the topographic maxima of the P3, amplitude was averaged across a 9-electrode site 'hot-spot' in the centro-parietal region (C1, Cz, C2, CP1, CPz, CP2, P1, Pz, P2).

Intranasal Insulin

Intranasal insulin and/or saline was administered using the MAD Nasal Atomizer into alternating nostrils with a total administration volume of 1.2mL (6 doses of 0.2mL of solution). Participants were randomly assigned to either a saline control group or an insulin group with the insulin group receiving either 20, 40, 60, 80, 100, or 120 IUs of intranasal insulin (Insulin Aspart). For all participants, the total volume of solution intranasally administered was kept constant. Participants in the saline control group were administered 6 doses of 0.2mL of saline. Participants in the 20 IU insulin group were administered 1 dose of 0.2mL of insulin and 5 doses of 0.2mL of saline. Participants in the 40 IU insulin group were administered 2 doses of 0.2mL of insulin and 4 doses of 0.2mL of saline. Participants in the 60 IU insulin group were administered 3 doses of 0.2mL of insulin and 3 doses of 0.2mL of saline. Participants in the 80 IU insulin group were administered 4 doses of 0.2mL of insulin and 2 doses of 0.2mL of saline. Participants in the 100 IU insulin group were administered 5 doses of 0.2mL of insulin and 1 dose of 0.2mL of saline. Finally, participants in the 120 IU insulin group were administered 6

doses of 0.2mL of insulin. The researcher and participants were blinded to the specific dose administered to prevent any possible bias about possible outcomes of the study (i.e. the saline and Insulin Aspart vial labels were covered by a member separated from study data collection). Prior to administration, the participant was asked to blow their nose to clear the nasal passageway. They were then seated with their head slightly tilted back. Following each dose of solution, participants were instructed to sniff following administration to facilitate the transport of the solution into the nasal cavity with approximately 30 seconds between each dose.

Statistical Analysis

All data analyses were R version 3.6.1 utilizing a family wise alpha of p=0.05. Prior to analysis, all study variables were screened for homoscedasticity and normality. For behavioral performance (i.e. reaction time and response accuracy) and for neural indices of attention (i.e. P3 amplitude) a 2 (Condition: control, exercise) X 7 (Dose: 0, 20, 40, 60, 80, 100, or 120 IUs) X 2 (Congruency: congruent, incongruent) univariate multi-level model controlling for the random intercept associated with participants was utilized (using the lme4 (Bates& Sarkar, 2006), lmerTest (Kuznetsova, Brockhoff, & Bojesen, 2017), and emmeans (Lenth, Love, & Herve, 2017) packages).

CHAPTER 4

Results

Participant Characteristics

See Table 4. in Appendix D for mean blood glucose (at three time points throughout the session), heart rate and lactate during the session between conditions. Blood glucose and lactate are also represented visually in Figure 2 and 3 respectively in Appendix E. Symptoms reported throughout the session and at the end are mapped in Figure 4 and 5 in Appendix E.

Behavioral Performance

Reaction time.

There was a main effect of Dose, $F(6, 95) = 2.3$, $p = 0.043$, $f^2 = 1.15$ [95% CI: 0.70 to 1.92], that was superseded by an interaction of Condition x Dose, $F(6, 95) = 2.5$, $p = 0.028$, $f^2 = 1.26$ [95% CI: 0.78 to 2.10]. Post-hoc decomposition of the Condition x Dose interaction was conducted by examining the effect of Dose within each Condition. For the control condition, the 40 IU (-46.7 ± 32.4) dose and the 80 IU (-39.3 ± 23.4) dose were associated with a larger reduction in reaction time from the pre to posttest relative to the 0 IU (-3.0 ± 14.2) placebo dose, t's$(50) \geq 3.1$, p's ≤ 0.003, d_s's ≥ 1.55 [95% CI: 0.52 to 2.88]; and the 60 IU (-5.1 ± 28.3) dose, t's$(50) \geq 2.9$, p's ≤ 0.005, d_s's ≥ 1.46 [95% CI: 0.43 to 2.78]. Additionally, the 40 IU (-46.7 ± 32.4) dose was associated with a larger reduction in reaction time from the pre to posttest relative to 120 IU (-9.4 ± 23.3) dose, $t(50) = 3.3$, $p = 0.002$, $ds = 1.60$ [95% CI: 0.59 to 2.59]. For the exercise condition, analysis revealed no main effect of Dose, $F(6, 97) = 1.9$, $p = 0.09$, $f^2 = 0.11$ [95% CI: 0.00 to 0.33] (Figure 6; Appendix E). While there was no significant interaction between the exercise condition and dose, a borderline significant trend of reaction time becoming quicker as dose increased was observed ($p = 0.09$). The effect sizes of the change of reactiontime

were visually inspected to obtain a better understanding of the emerging patterns between the two conditions. In the control condition, a curvilinear relationship was observed with a threshold at which the uptake of glucose shortens reaction time (Figure 7; Appendix E). In the exercise condition, while subdued, a linear relationship that as dose increases, reaction time continues to become faster was observed (Figure 8; Appendix E).

Response accuracy.

Analyses revealed that there was a main effect of Congruency, $F(1, 95) = 4.9$, $p = 0.029$, $f^2 = 0.56$ [95% CI: 0.28 to 0.99]. Post hoc comparisons showed, the difference between Congruent (-0.9 ± 6.1) and Incongruent (0.5 ± 6.2) was statistically significant; $t(95) = 2.2$, $p = 0.029$, drm = 0.22 [95% CI: 0.02 to 0.41]. Change in response accuracy across doses by condition is displayed within Figure 9 in Appendix E ($p > 0.05$).

Neuroelectric Activity

P3 Amplitude.

Analyses revealed a main effect of Congruency, $F(1, 93) = 6.8$, $p = 0.011$, $f^2 = 0.59$ [95% CI: 0.30 to 1.04]. Post hoc comparisons exposed that the difference between Congruent (0.1 ± 1.4) and Incongruent (-0.2 ± 1.5) was statistically significant; $t(93) = 2.6$, $p = 0.011$, drm = 0.20 [95% CI: 0.05 to 0.35]. Change in P3 amplitude across doses by condition is displayed within Figure 10 in Appendix E ($p > 0.05$).

CHAPTER 5

Discussion

It was first hypothesized that acute exercise's enhancement of cognition would be replicated. This was not replicated as there were no significant differences for the change in reaction time or accuracy observed between conditions. These results contrasted with the current literature in an adult population after an acute bout of exercise (Kamijo et al., 2009, 2007; Pontifex, Parks, Henning, & Kamijo, 2015). This might be due to different restrictions in this study concerning exercise intensity. While previous articles chose wider percentage ranges of heart rate maximum, this study restricted the range to 60-65% of heart rate max which is toward the lighter intensity side of the moderate intensity range which controlled for lactate's involvement with energy source utilization in the body and also for safety reasons (Brooks et al., 2004; Houston, 2006). This study tested the feasibility of being able to combine exercise and intranasal insulin. While it is well documented that intranasal insulin does not affect peripheral blood glucose, one can't be too cautious when testing a new protocol (Schmid et al., 2018). The symptom across conditions that was reported the most was burning/tingling in the nose during the intranasal insulin spray. In the talking with the participants during the session, this symptom was short lived and only lasted for the duration of the spray and a few moments after. This protocol was deemed feasible and safe as none of the symptoms reported during the session or at the end were severe across doses and conditions.

The second hypothesis was that there would be a dose response relationship between the change in cognitive performance and intranasal insulin dose that as dose increased, so would cognitive performance. While the results did not support this linear dose response relationship, there was a trend of a curvilinear dose response relationship in the control condition for reaction

time with several doses leading to a faster reaction time when compared to each other (See Figure 7; Appendix E).

With a high concentration of GLUT4 receptors in the cerebellum, this might explain the significant change in reaction time in response to these doses (Choeiri et al., 2002). The cerebellum is responsible for the coordination of muscle movements along with the motor cortex (Manto et al., 2012). So, with an increase of glucose uptake, more resources could possibly be utilized to increase motor movement speed. However, future investigations would need to take a more direct measurement approach to fully elucidate GLUT4's response to intranasal insulin in humans as most studies have been done in animal models (Alquier, Leloup, Lorsignol, & Pénicaud, 2006). Again, with the population tested for this study, a ceiling effect was most likely observed for accuracy on the flanker task consistent with the published literature (Hillman et al., 2003; Pontifex et al., 2019, 2015).

Finally, the third hypothesis stated that exercise-induced enhancement in cognitive performance would increase as intranasal insulin dose increased indicated by an increase in the P3 amplitude as well as a decrease in reaction time. Results revealed that a significant interaction of dose and condition for reaction time. In contrast to the hypothesis, post hoc comparisons revealed that this interaction was only found between specific doses within the control condition. Nevertheless, a borderline significant linear trend ($p = 0.09$) was observed for the exercise condition that as dose increased, cognitive performance increased. This pattern being observed in the exercise condition provides preliminary evidence that intranasal insulin and exercise build upon each other in terms of influencing cognitive performance. The way that glucose is being influenced by exercise and the intranasal insulin doses may be different from what the original hypothesis predicted. Reaction time in the control condition, when graphed, displayed a

curvilinear dose response relationship that indicates that there is a possible threshold for higher doses of insulin. In contrast, the exercise condition displayed the hypothesized linear dose relationship. By adding the exercise variable, glucose availability may be increasing in the CSF and therefore, allowing the insulin to pull from a greater pool of resources in the brain. In addition to increasing glucose uptake, even though the literature is not as abundant, exercise has been observed to increase availability of cerebral glucose (Béquet, Gomez-Merino, Berthelot, & Guezennec, 2001; Béquet et al., 2000). Contrarily, in the control condition, there is less glucose availability in the CSF and therefore, a threshold in performance is reached with higher doses, as they are depleting the resource too much. Yet, it is important to keep in mind that this is a hypothesis as this study used change in cognitive performance as an indirect measure of glucose uptake and was not able to measure availability versus uptake directly.

Furthermore, this study was able to control for the baseline availability of cerebral glucose by requiring participants to fast three hours before coming in for the second session. If this restriction was not put in a place, a participant coming in having a full meal in comparison to someone who hadn't eaten all day would have different levels of glucose resources in the cerebrospinal fluid (CSF) (Wang & Jia, 2018). This would create a baseline difference in the pool of glucose available in the CSF for the insulin and exercise to increase uptake from and could bias the results. Additionally, as stated before, ingestion of glucose can enhance cognitive performance and without controlling for food/drink sugar consumption, results would not be able to be attributed fully to the session without questioning if it was something the participant had previously ingested (Riby, 2004; Riby et al., 2008; Scholey, Sünram-Lea, Greer, Elliott, & Kennedy, 2008).

The P3 amplitude results of this study did not replicate the current literature as it has shown an increase of the amplitude of the P3 after exercise (Hillman et al., 2003; Kamijo et al., 2004, 2007). These results might be representative of the light to moderate intensity physical activity bout that the participants were restricted to. Additionally, P3 amplitude was not modulated in response to any of the intranasal insulin doses. As there is limited intranasal insulin literature that looks at neuroelectric data, it is hard to draw any solid conclusions from this finding. As stated previously, Kern and colleagues (1999) found that the P3 amplitude decreased following administration of intranasal insulin but the changes were not located over the topographic maxima of centro-parietal region. Participants in this study were administered intranasal insulin throughout the study as they completed an auditory oddball task. In comparison to this study, one conclusion we may draw is that our findings could be the result of the timing of the action of the insulin. Insulin Aspart was chosen specifically for a short action time that would fit within the appropriate window for proper cognitive performance testing within the confines of the study protocol (Novo Nordisk, 2005). Born and colleagues (2002) stated that peak levels are reached in the CSF around 30 minutes but with it also taking time to translocate GLUT4 receptors to the surface and begin functioning, the actual time frame of action may be longer than originally thought. Additionally, with the observation of reaction time being significantly influenced by dose, the insulin might be having a faster course of action in the cerebellum than in the area of the brain that is linked to inhibitory control. It is possible that this is due to a high concentration of GLUT4 receptors in the cerebellum (Choeiri et al., 2002). While other intranasal insulin studies have not looked at the P3, the time window in which they administered cognitive tasks has ranged from 20-75 mins after administration (Benedict et al., 2008; Brünner et al., 2015; Novak et al., 2014). The window in which the participants completed the inhibitory

control task might have been right before the insulin was at its full effect for any possible change to be observed for the neuroelectric measure. Additionally, while this study focused on intranasal insulin as a tool to manipulate cerebral glucose uptake, as stated previously in the literature review, insulin roles beyond glucose metabolism in the CNS. Insulin has been found to have anti-inflammatory properties, vasodilatory, anti-thrombosis and anti-apoptotic effects in the brain (Lioutas et al., 2015). Based on the current literature, most of these effects take a long-time course to occur with a majority of the studies showing effects over multiple hours or several day time window (Abbink-Zandbergen et al., 1999; Dandona et al., 2002; Dandona et al., 2007; Dimmeler et al., 1999; Duarte et al., 2012, 2008; McKay & Hester, 1996). Even effects that have been shown to take a shorter amount of time (i.e. vasodilatory effects), seem to still follow a longer timeline than the time window for cognitive testing utilized in this study (Novak et al., 2014). With previous studies have such a heterogeneity of when the cognitive tasks were administered, it is hard to piece apart whether this glucose uptake produced the magnitude of cognitive performance change observed in combination or in isolation from these other possible effects. This could be why the magnitude of the change of performance, for both behavioral and neuroelectric measures, is different in this study in comparison to what has been previously published.

Limitations & Future Directions

While this study has many strengths, it is still important to highlight a few limitations. Consistent with previous studies, there were no significant results for accuracy for the inhibitory control task. The population recruited for this study was a high functioning college aged population and it is possible that they were hitting a ceiling for accuracy on this task (Hillman et al., 2003; Pontifex et al., 2019, 2015).

Lactate threshold can vary across individuals with different aerobic fitness levels. This threshold is lower in individuals who fall within the lower fitness percentiles than ones higher within the range. Even set at a moderate intensity tailored to each participant, utilizing a range from 10th to 90th percentile, participants on either end of the spectrum could have significant differences in their lactate threshold levels (Bosquet, Léger, & Legros, 2002). Restricting the intensity to a small percentage of maximum heart rate was intended to control for main energy system utilization (Brooks et al., 2004; Houston, 2006). However, with varying lactate threshold levels, participants may have not been receiving as homogenous exercise intensity as intended. Future investigations may be able to account for more participant variability through utilizing heart rate reserve which would account for differing resting heart rates among individuals across the percentile range which in turn could account for differing lactate thresholds.

As previously discussed, intranasal insulin has been shown to increase glucose uptake in the brain (Bakirtzi et al., 2009; Born et al., 2002; J. W. Unger et al., 1991). Additionally, it has been published that insulin can have other effects on the CNS but the literature published in this arena focusing on intranasal insulin is scarce (Lioutas et al., 2015). Using this study design, we were able to tease out that glucose uptake was involved but with limited published literature currently in this area; one can't fully eliminate the possibility of alternative effects.

Looking at glucose uptake in the brain via behavioral and neuroelectric measures is one method by which one can answer the question of whether or not glucose uptake has an influence on the relationship between exercise and cognition control. However, it is an indirect measure. More direct measures such as radioactive labeling with PET involves high participant burden and cost which was not feasible for conducting this study. Nonetheless, with the preliminary evidence that this study has provided, future directions could involve more complex techniques

and measures that are able to take a more direct approach (possibly fully eliminating alternative effects of intranasal insulin).

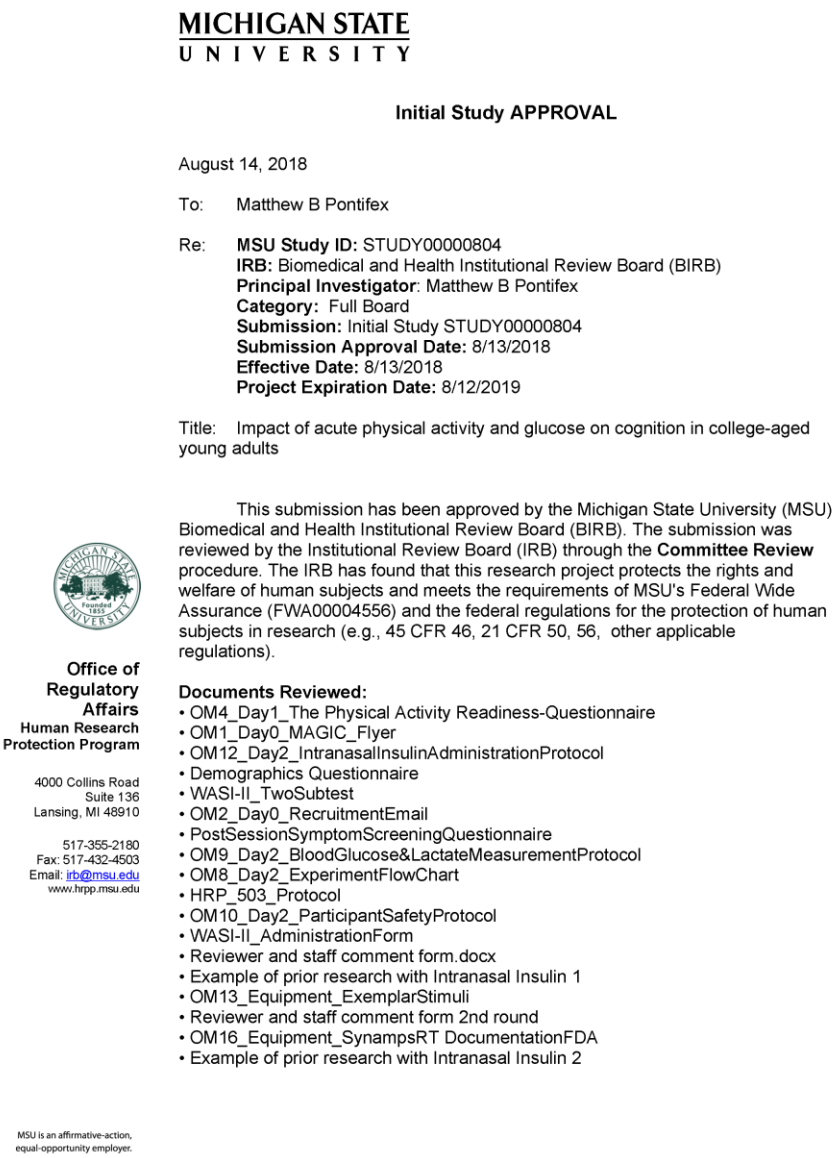
Further, the results of this study can help inform researchers and practitioners for targeted interventions aimed at improving quality of life for individuals across the lifespan whether it is to set up children for success or improving older adults' functioning.

Conclusion

Reaction time became significantly faster in the control condition in response to different doses of intranasal insulin with no statistically significant differences between doses for the exercise condition. Additionally, there was no significant change in the P3 amplitude between conditions or in response to intranasal insulin doses. While no differences between the exercise and control conditions are in contrast with the literature, this may be the result of the light to moderate exercise intensity range that was chosen for this study. However, a trend was observed in the exercise condition for reaction time becoming faster as dose increased. Visual inspection of effect size plots for reaction time change in response to dose for the exercise and control conditions led to the conclusion that exercise might be increasing glucose availability to complement intranasal insulin's increase in uptake. Even with this study's limitation, this preliminary data sets the basis for the dose response of intranasal insulin and its interactions with exercise. This could assist in future guidelines for interventions aimed at enhancing cognitive function.

APPENDICES

Appendix A: IRB Approval Letter



MICHIGAN STATE
UNIVERSITY

Initial Study APPROVAL

August 14, 2018

To: Matthew B Pontifex

Re: **MSU Study ID:** STUDY00000804
IRB: Biomedical and Health Institutional Review Board (BIRB)
Principal Investigator: Matthew B Pontifex
Category: Full Board
Submission: Initial Study STUDY00000804
Submission Approval Date: 8/13/2018
Effective Date: 8/13/2018
Project Expiration Date: 8/12/2019

Title: Impact of acute physical activity and glucose on cognition in college-aged young adults

This submission has been approved by the Michigan State University (MSU) Biomedical and Health Institutional Review Board (BIRB). The submission was reviewed by the Institutional Review Board (IRB) through the **Committee Review** procedure. The IRB has found that this research project protects the rights and welfare of human subjects and meets the requirements of MSU's Federal Wide Assurance (FWA00004556) and the federal regulations for the protection of human subjects in research (e.g., 45 CFR 46, 21 CFR 50, 56, other applicable regulations).

Documents Reviewed:
- OM4_Day1_The Physical Activity Readiness-Questionnaire
- OM1_Day0_MAGIC_Flyer
- OM12_Day2_IntranasalInsulinAdministrationProtocol
- Demographics Questionnaire
- WASI-II_TwoSubtest
- OM2_Day0_RecruitmentEmail
- PostSessionSymptomScreeningQuestionnaire
- OM9_Day2_BloodGlucose&LactateMeasurementProtocol
- OM8_Day2_ExperimentFlowChart
- HRP_503_Protocol
- OM10_Day2_ParticipantSafetyProtocol
- WASI-II_AdministrationForm
- Reviewer and staff comment form.docx
- Example of prior research with Intranasal Insulin 1
- OM13_Equipment_ExemplarStimuli
- Reviewer and staff comment form 2nd round
- OM16_Equipment_SynampsRT DocumentationFDA
- Example of prior research with Intranasal Insulin 2

Office of Regulatory Affairs
Human Research Protection Program

4000 Collins Road
Suite 136
Lansing, MI 48910

517-355-2180
Fax: 517-432-4503
Email: irb@msu.edu
www.hrpp.msu.edu

MSU is an affirmative-action, equal-opportunity employer.

Figure 1. Copy of IRB approval letter.

Figure 1 (cont'd).

• Jeffrey Richard Kovan CV
• Kovan Letter
• OM5_Day1_Edinburgh Handedness Inventory
• IND Exemption 21 CFR 312.2b1_PI Documentation
• FDA Exemption
• OM15_Equipment_Polar_Compliance_Documentation
• Recent publication on the safety of intranasal insulin
• Figure Illustrating Dosing
• NovologInsert
• OM11_Day2_MADAtomizationNasalSprayProcedure
• OM14_Equipment_GlucometerInstructions&FDAApproval
• FDA Exemption
• MAGIC_InformedConsent_Adult
• OM7_Day1_Wechsler Abbreviated Scale of Intelligence 2 WASI-II assessment
• OM3_Day1_MedicalScreeningQuestionnaire

Continuing Review: IRB approval is valid until the expiration date listed above. If the research continues to involve human subjects, you must submit a Continuing Review request at least one month before expiration.

Modifications: Any proposed change or modification with certain limited exceptions discussed below must be reviewed and approved by the IRB prior to implementation of the change. Please submit a Modification request to have the changes reviewed. If changes are made at the time of continuing review, please submit a Modification and Continuing Review request.

Immediate Change to Eliminate a Hazard: When an immediate change in a research protocol is necessary to eliminate a hazard to subjects, the proposed change need not be reviewed by the IRB prior to its implementation. In such situations, however, investigators must report the change in protocol to the IRB immediately thereafter.

Reportable Events: Certain events require reporting to the IRB. These include:

- Potential unanticipated problems that may involve risks to subjects or others
- Potential noncompliance
- Subject complaints
- Protocol deviations or violations
- Unapproved change in protocol to eliminate a hazard to subjects
- Premature suspension or termination of research
- Audit or inspection by a federal or state agency
- New potential conflict of interest of a study team member
- Written reports of study monitors
- Emergency use of investigational drugs or devices
- Any activities or circumstances that affect the rights and welfare of research subjects
- Any information that could increase the risk to subjects

2

Figure 1 (cont'd).

Please report new information through the project's workspace and contact the IRB office with any urgent events. Please visit the Human Research Protection Program (HRPP) website to obtain more information, including reporting timelines.

Prisoner Research: If a human subject involved in ongoing research becomes a prisoner during the course of the study and the relevant research proposal was not reviewed and approved by the IRB in accordance with the requirements for research involving prisoners under subpart C of 45 CFR part 46, the investigator must promptly notify the IRB.

Site Visits: The HRPP Compliance office conducts post approval site visits for certain IRB approved projects. If this project is selected for a site visit, you will be contacted by the HRPP Compliance office to schedule the site visit.

For Projects that Involve Consent, Parental Permission, or Assent Form(s):

Use of IRB Approved Form: Investigators must use the form(s) approved by the IRB and must typically use the form with the IRB watermark.

Copy Provided to Subjects: A copy of the form(s) must be provided to the individual signing the form. In some instances, that individual must be provided with a copy of the signed form (e.g. projects following ICH-GCP E6 requirements). Assent forms should be provided as required by the IRB.

Record Retention: All records relating to the research must be appropriately managed and retained. This includes records under the investigator's control, such as the informed consent document. Investigators must retain copies of signed forms or oral consent records (e.g., logs). Investigators must retain all pages of the form, not just the signature page. Investigators may not attempt to de-identify the form; it must be retained with all original information. The PI must maintain these records for a minimum of three years after the IRB has closed the research and a longer retention period may be required by law, contract, funding agency, university requirement or other requirements for certain projects, such as those that are sponsored or FDA regulated research. See HRPP Manual Section 4-7-A, Recordkeeping for Investigators, for more information.

Closure: If the research activities no longer involve human subjects, please submit a Continuing Review request, through which project closure may be requested. Human subject research activities are complete if data collection is complete and there is no further interaction or intervention with human subjects, and analysis of identifiable private information is complete.

For More Information: See the HRPP Manual (available at https://hrpp.msu.edu/msu-hrpp-manual-table-contents-expanded).

Contact Information: If we can be of further assistance or if you have questions, please contact us at 517-355-2180 or via email at IRB@ora.msu.edu. Please visit hrpp.msu.edu to access the HRPP Manual, templates, etc.

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Appendix B: Dissertation Funding Sources

Dissertation Funding Sources

1. **University Dissertation Completion Fellowship – Awarded December 2018**
   From Department of Kinesiology, Michigan State University
   Funded - $7,000
   Use: Partial release from teaching to be able to focus on recruitment and data collection for my dissertation during the Spring 2019 semester

Appendix C: Method Section Tables

Table 1.

Participant demographics (Mean ± SD).

Measure	
N	109 (68 Females)
Age (years)	20.8 ± 2.6
White/Caucasian (%)	82%
WASI-II (IQ)	107.8 ± 9.6
Fitness (Percentile)	46.9 ± 24.7
# of Biological Siblings	3 ± 1
Mother's Education	1 Missing
	6 H.S. Not Complete
	14 H.S.
	3 Some College
	8 A.D.
	55 B.S.
	22 Advanced or Terminal Degree
Gross Household Income	1 Missing
	1 < $10,000
	22 $10,000 - $60,000
	45 $61,000 - $100,000
	40 > $100,000

Note: Wechsler Abbreviated Scale of Intelligence 2nd Edition two-part subtest composite (WASI-II), High School Degree (H.S.), Associates Degree (A.D.), Bachelors of Science Degree (B.S.), Greater than (>), Less than (<).

Table 2.

Participant dose group breakdown.

		Condition	
		Exercise	Control
Dose	0	8	8
	20	8	7
	40	8	9
	60	7	8
	80	6	8
	100	8	9
	120	7	8

Table 3.

Number of subjects needed to observe an increase in R^2.

		Effect Size		
		Small ($f^2 = 0.02$)	Medium ($f^2 = 0.15$)	Large ($f^2 = 0.35$)
Number of Predictors	2	485	68	31
	3	550	77	36
	4	602	85	40
	5	647	92	43
	6	688	98	46
	7	725	103	49
	8	759	109	52
	9	791	114	54
	10	822	118	57

Appendix D: Results Section Tables

Table 4.

Participant heart rate, blood glucose and lactate (Mean ± SD).

	Condition	
Measure	Exercise	Control
HR (bpm)	117.2 ± 9.2	73.3 ± 12.2
Glucose Baseline (mg/dL)	93.4 ± 9.3	95.8 ± 7.9
Glucose Midway through Experimental Condition (mg/dL)	86.2 ±7.3	93.6 ± 8.3
Glucose 5 minutes Post Experimental Condition (mg/dL)	88.9 ± 9.4	93.2 ± 9.0
Lactate (mmol/L)	1.72 ± 0.76	1.36 ± 0.5

Note: Heart rate (HR), Beats per minute (bpm), milligrams per deciliter (mg/dL), millimoles per liter (mmol/L).

Appendix E: Results Section Figures

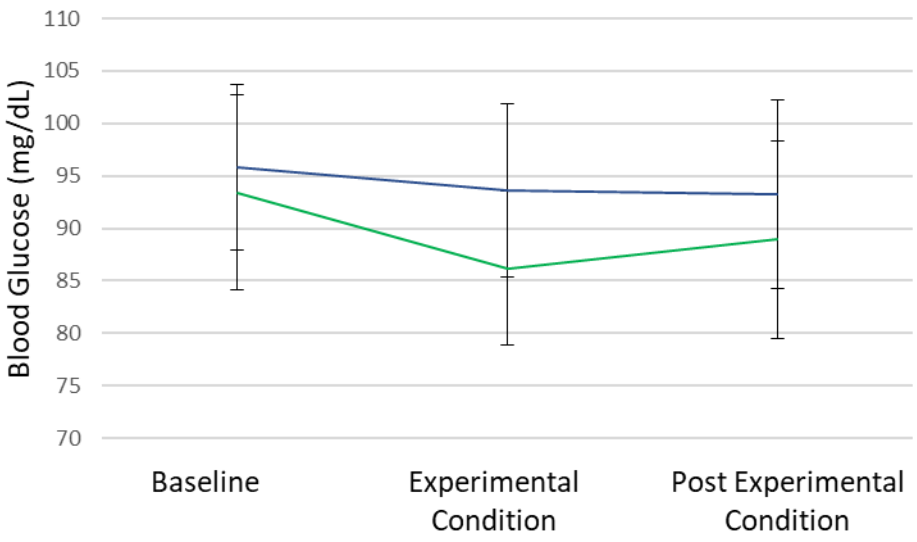

Figure 2. Blood glucose levels during the session. *Error bars represent the standard deviation of the means. Conditions are represented by the different colored lines (i.e. exercise is green and control is blue).*

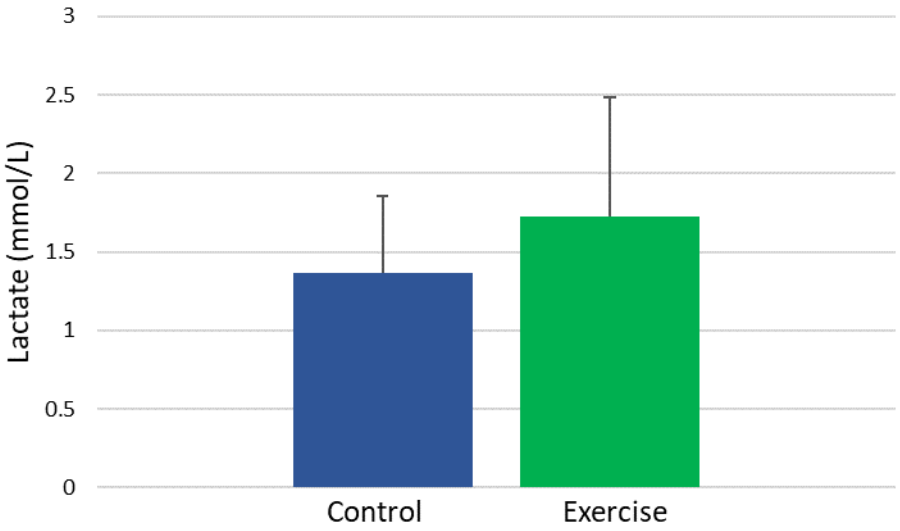

Figure 3. Average blood lactate levels between conditions. *Error bars represent the standard deviation of the means.*

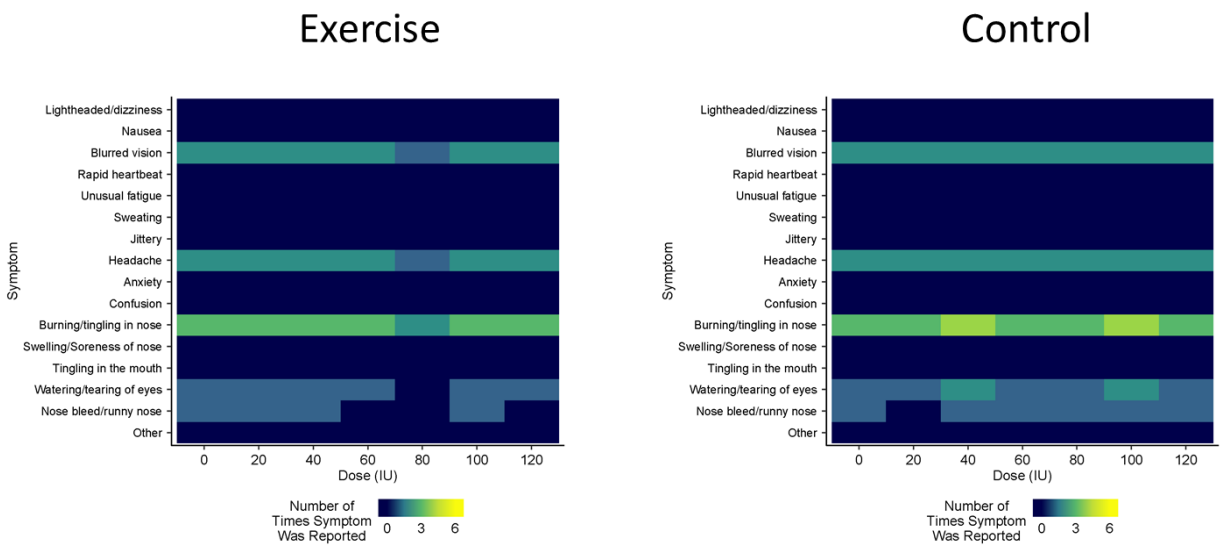

Figure 4. Symptoms reported during the session.

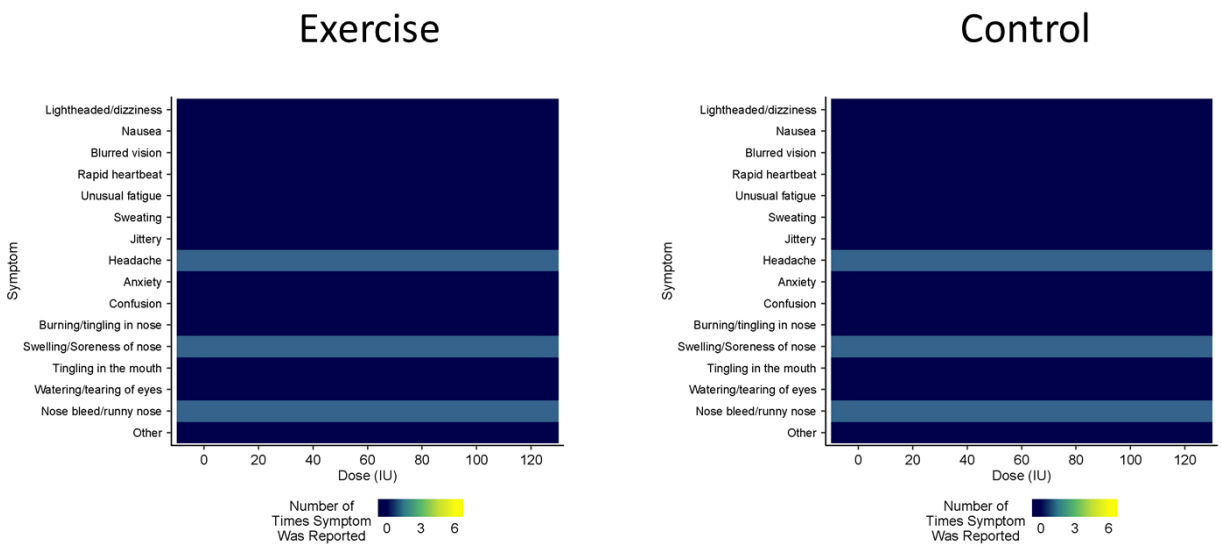

Figure 5. Symptoms reported at the end of the session.

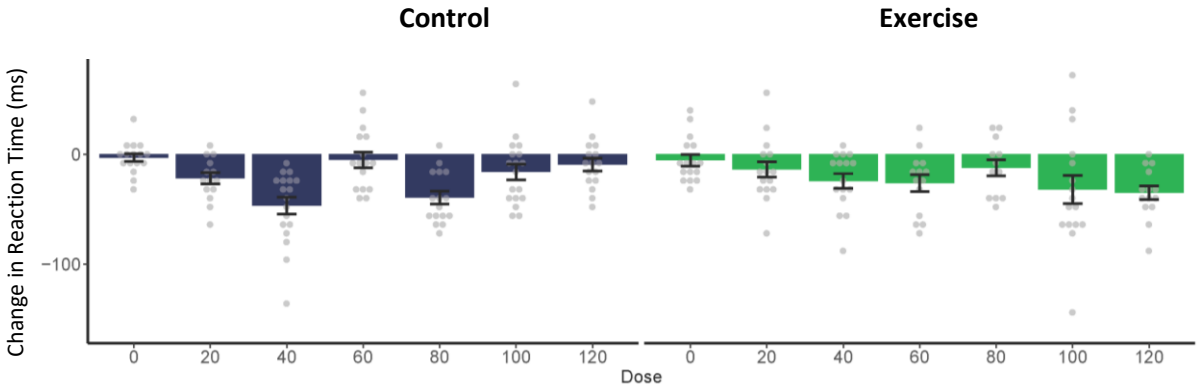

Figure 6. Change in reaction time dose response by condition.

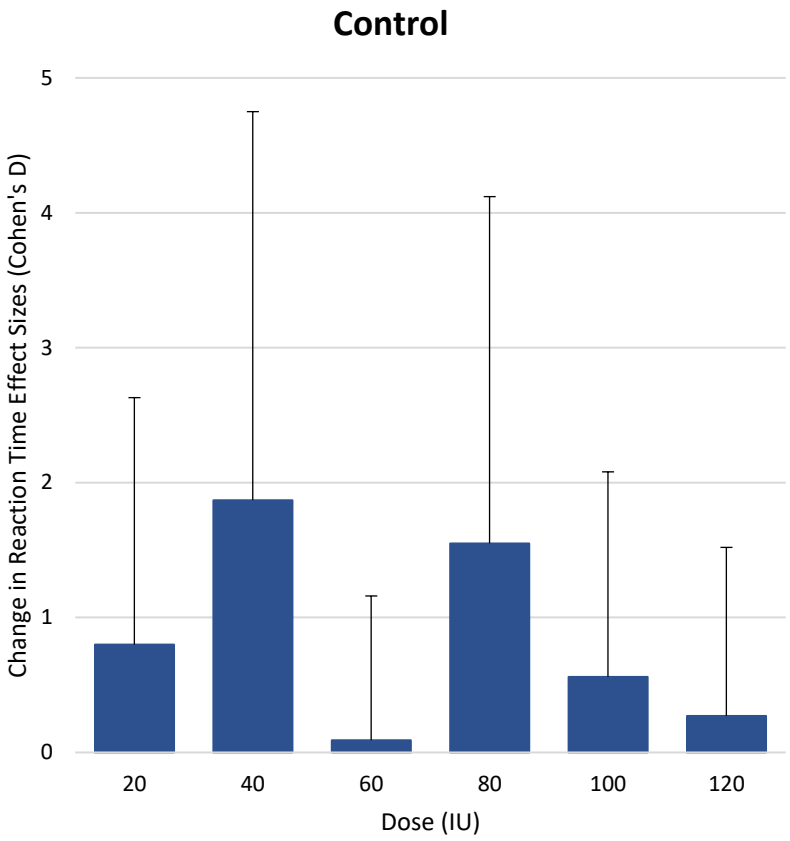

Figure 7. Effect sizes for change in reaction time: control condition. *Each bar within the figure represents effect sizes from each dose and its comparison with 0 IU placebo dose. Error bars represent the positive end of the calculated 95% confidence interval for each dose group comparison to the 0 IU placebo dose.*

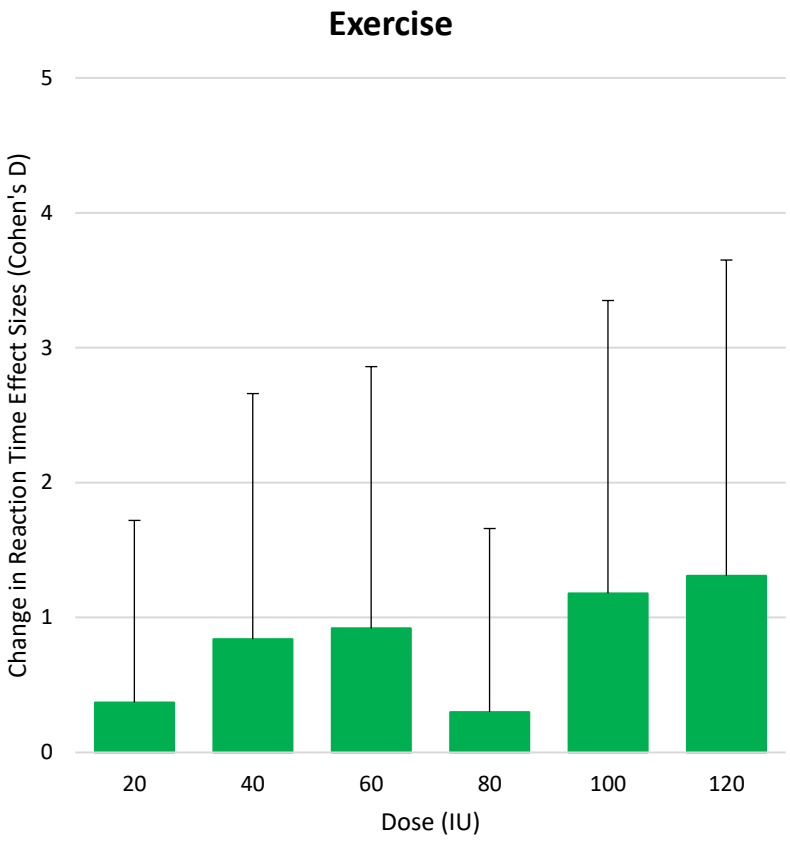

Figure 8. Effect sizes for change in reaction time: exercise condition. *Each bar within the figure represents effect sizes from each dose and its comparison with 0 IU placebo dose. Error bars represent the positive end of the calculated 95% confidence interval for each dose group comparison to the 0 IU placebo dose.*

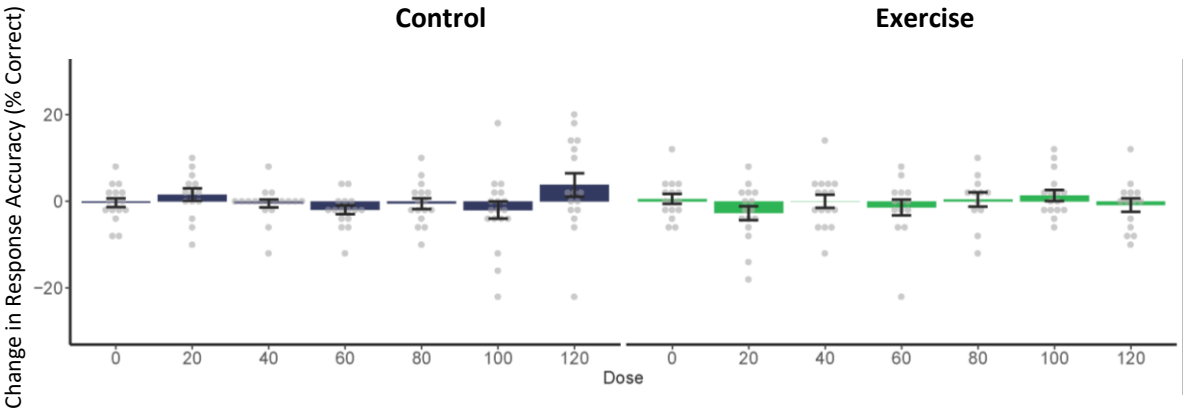

Figure 9. Change in response accuracy dose response by condition.

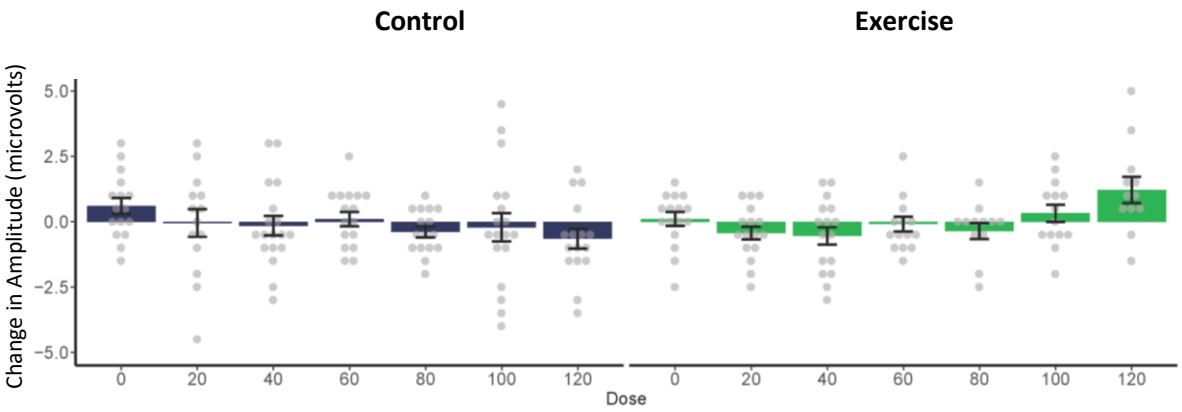

Figure 10. Change in P3 amplitude by condition.

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