ISOMETRIC HANDGRIP EXERCISE AND COGNITIVE FUNCTION IN YOUNG ADULTS

THE EFFECTS OF ACUTE ISOMETRIC HANDGRIP EXERCISE ON COGNITIVE FUNCTION IN YOUNG ADULTS

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LAY ABSTRACT

It is well known that whole-body exercise, such as running, swimming, or lifting weights, improves cognitive function. Cognitive function encompasses our ability to pay attention, remember new information, and make important decisions. We sought to investigate whether isometric handgrip exercise (IHG) could improve cognitive function in young adults, because it may be a new and accessible way to improve cognitive abilities. We also wanted to know if IHG had a different effect on cognitive function in females compared to males. To test cognitive function, participants played computer games that measured how their cognitive abilities were affected by IHG. In particular, we examined how IHG impacted a participant's memory, decision-making, and speed to completion. Our results show that IHG increased blood flow to the brain and made participants feel more alert compared to a control condition, however, IHG did not improve performance on the computer games. Males and females also did not differ in terms of their performance on the cognitive tests. Overall, a single session of IHG did not improve cognitive function in young adults. Although IHG did not improve cognitive function in young adults, it should be investigated in other individuals, such as older adults and people with hypertension, who may stand to gain more from IHG.

ABSTRACT

Acute whole-body exercise transiently improves cognitive function which may be mediated by increased cerebral blood flow (CBF) and arousal. Interestingly, small muscle mass exercise, like isometric handgrip exercise (IHG) may stimulate the same physiological responses as whole body-exercise and improve cognitive function. However, these effects are poorly understood, and whether sex-based differences exist in the cognitive response to IHG is unknown. Therefore, the purpose of this study was to investigate whether acute IHG improves cognitive function in young healthy adults and examine potential sex differences in the cognitive response to IHG. We hypothesized that acute IHG would improve cognitive function compared to a control condition, and that females would have greater improvements in cognitive function due to a lower exercise pressor response. To test this, 30 participants (n=15 females, mean age=23.8±3.3 years; BMI=25.3±4.1 kg/m^2) completed either IHG or a control condition in a randomized-crossover design separated by at least 2 days. IHG consisted of four sets of 2-min unilateral squeezing a handgrip dynamometer at 30% maximal voluntary contraction separated by 3-min of rest. The control condition watched a nature documentary for 20-min. Hemodynamics (systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate) were assessed throughout. Executive function, working memory, and processing speed were assessed via the 4-Choice, Corsi Block, and N-Back tests. Arousal was assessed using the Felt Arousal Scale (FAS). Middle cerebral artery blood velocity (MCAv) was assessed using transcranial Doppler ultrasound. Compared to the control condition, IHG significantly increased MAP (Δ 26 ± 17 mmHg; P<0.001), HR(Δ 18 ± 13 bpm; P<0.001), MCAv (Δ 5.27 ± 19.4 cm/s; P<0.001), cerebrovascular resistance (Δ 0.71 ± 0.69 mmHg/cm/s; P=0.003), and arousal (Δ 2 ± 2 FAS score; P<0.001). Cerebrovascular resistance was calculated as MAP/MCAv. Overall, despite increases in MCAv and arousal, there was no effect of IHG on cognitive performance, and no sex differences were observed in the

cognitive response to IHG. These findings stand in opposition to emerging work and suggests that increased CBF and arousal via acute IHG are an insufficient stimulus to enhance cognitive function in young adults. Furthermore, there seems to be no moderating effect of biological sex on the cognitive response to acute IHG.

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CBF	Cerebral Blood Flow
BP	Blood Pressure
MAP	Mean Arterial Pressure
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
HR	Heart Rate
CVR	Cerebrovascular Resistance
CVC	Cerebrovascular Conductance
MCA	Middle Cerebral Artery
ICA	Internal Carotid Artery
MCA_{CVR}	Middle Cerebral Artery Cerebrovascular Resistance
MCA_{CVC}	Middle Cerebral Artery Cerebrovascular Conductance
ICA_{CVC}	Internal Carotid Artery Cerebrovascular Conductance
TCD	Transcranial Doppler Ultrasound
MCAv	Middle Cerebral Artery Velocity
IHG	Isometric Handgrip Exercise
MVC	Maximal Voluntary Contraction
CO_2	Carbon Dioxide
P_aCO_2	Partial Pressure of Carbon Dioxide
$P_{ET}CO_2$	End-Tidal Carbon Dioxide
FAS	Felt Arousal Scale
PAR-7	Stanford 7-Day Physical Activity Recall
MRI	Magnetic Resonance Imaging
fMRI	Functional Magnetic Resonance Imaging
PET	Positron Emission Tomography
ECG	Electrocardiogram
$\dot{V}O_2$peak	Highest Rate of Oxygen Uptake During a Maximal Aerobic Test
VO_2 max	Maximal Rate of Oxygen Consumption

DECLARATION OF ACADEMIC ACHIEVEMENT

Keegan Nhan's Role:

- Obtained ethics approval with the Hamilton Integrated Sciences Research Board
- Designed study protocol and selected outcome measures to assess
- Developed standard operating procedures for the study
- Set up study materials for data collection
- Trained and supervised undergraduate 3RP3 and 4RR6 research students, who assisted with data collection and analysis
- Led data collection, analysis, and interpretation
- Responsible for manuscript preparation

Role of co-authors:

- JW assisted KN with ethics application
- JW assisted KN with study design and the selection of outcome measures
- JW assisted KN with data interpretation
- JW assisted KN with manuscript writing and editing
- JW obtained study funding

Chapter 1: Literature Review: An Overview of Cognitive Function, Acute Exercise, and Isometric Handgrip Exercise

1.0 Cognitive Function

1.1.1 What is Cognitive Function?

Cognitive function refers to a broad combination of abilities such as attention, problem-solving, and decision-making which make up every day functioning (Fisher et al., 2019). A common method for evaluating cognitive function is the neurocognitive approach, in which cognitive function is divided into unique constructs, and neuropsychological tests are administered to assess cognitive performance in each or several cognitive domains (Harvey, 2022).

1.1.2 Cognitive Domains

The neurocognitive approach can assess many cognitive domains; however, this literature review will focus on the domains of executive function, working memory, and processing speed. Executive functions are an aggregate of skills that help in planning, thought organization, and decision-making (Diamond, 2013). Executive function is composed of three subdomains: inhibitory control; working memory; and cognitive flexibility (Diamond, 2013). Inhibitory control involves resisting urges and temptations in order to make appropriate decisions (Diamond, 2013), working memory involves retaining information in short-term memory that is readily accessible (Cowan, 2014), and cognitive flexibility involves the ability and willingness to switch between different perspectives and strategies depending on the situation (Diamond, 2013).

Processing speed is composed of two components: the speed at which an individual perceives and interprets a stimulus, and the speed at which a response is produced (Anderson et al., 1997). Different aspects of processing speed are responsible for the perception and interpretation of a stimulus, as well as the production of a response following stimulus presentation (Kraft & Woods,

2022). Psychomotor speed refers to an individual's ability to output a response as quickly as possible (Salthouse, 2000). The combination of perceptual and psychomotor speed is ultimately measured as reaction time and represents the integration of perception and action in response to stimulus presentation on a cognitive test (Salthouse, 2000).

Stimuli can be attended to in a bottom-up or top-down manner, depending on the requirements of a task. Bottom-up processing is primarily driven by the stimuli in a reactionary manner (Gençer & Yıldırım, 2022), whereas top-down processing is experience-driven and rules-based, as an individual's previous knowledge and response requirements influences processing (Gençer & Yıldırım, 2022). Basic sensory tasks, such as tests that assess processing speed, would primarily be driven by bottom-up processing, whereas more complex tasks, such as executive function tasks, would primarily be driven by top-down processing (Harvey, 2022).

1.2 The Effect of Acute Exercise on Cognitive Function – An Overview

Several meta-analyses show that acute exercise facilitates transient improvements in cognitive function (Chang et al., 2012; Lambourne & Tomporowski, 2010; Ludyga et al., 2016; Petruzzello et al., 1997; Sibley & Etnier, 2003). Specifically, acute exercise elicits the greatest benefits in the cognitive domains of executive function (Chang et al., 2012) and reaction time (Tomporowski, 2003). In the first meta-analysis of the literature, Petruzzello et al. (1997) showed that acute exercise had a significant small positive effect on cognitive function post-exercise for participants of all ages (Hedges' g = 0.16). Sibley and Etnier (2003) also reported a significant small positive effect on cognitive function post-exercise (Hedges' g = 0.37); however, their study population was limited to young children and adolescents (4-18 years old). Another meta-analysis by Lambourne and Tomporowski (2010) found that cognitive function was only improved ***after*** acute exercise (Cohen's d = 0.20), but not ***during*** exercise in young healthy adults. In fact, acute exercise had a

detrimental effect on cognitive function for studies that employed cognitive testing during exercise (Cohen's d = -0.14) (Lambourne & Tomporowski, 2010). At the time, a meta-analysis by Chang et al. (2012) provided the most comprehensive review of the literature, with participants of all ages (children, young and older adults) being included. Paralleling findings of previous meta-analyses, Chang et al. (2012) found a significant small positive effect of acute exercise on cognitive function post-exercise (Cohen's d = 0.097). Finally, a recent meta-analysis by Ludyga et al. (2016) found a significant positive effect following acute sessions of moderate intensity aerobic exercise on executive function across a range of ages (6 to 50+ years old). However, the majority of previous meta-analyses have solely included studies of aerobic exercise, limiting knowledge of how acute resistance exercise impacts cognitive function. Despite this, a recent meta-analysis by Wilke et al. (2019) reported a medium effect size favoring acute resistance exercise over control conditions for improving cognitive function in young healthy adults (20-72 years old), specifically in the subdomains of inhibitory control (mean standardized difference compared to control: 0.73) and cognitive flexibility (mean standardized difference compared to control: 0.36) (Wilke et al., 2019).

1.3 Moderators of Acute Exercise's Beneficial Effect on Cognitive Function

1.3.1 Exercise Intensity

Exercise intensity may modulate acute exercise's beneficial effects on cognitive function in an inverted-U manner (Chang et al., 2012; Lambourne & Tomporowski, 2010). As exercise intensity increases, increased amounts of catecholamines such as norepinephrine are released via noradrenergic pathways, facilitating the release of the excitatory neurotransmitter glutamate, ultimately reducing the signal-to-noise ratio in the prefrontal cortex (McMorris, 2009). However, higher levels of catecholamine can slow information processing in the brain, which attenuates the cognitive benefits seen at lower arousal levels (Birnbaum et al., 2004). The effect of exercise

intensity is also dependent on when a cognitive test is administered, with lighter intensity exercise being more beneficial for cognitive function when cognitive testing is administered immediately following exercise, and higher intensity exercise being more beneficial when cognitive testing is administered following a delay greater than 1-min (Chang et al., 2012). Emerging evidence suggests that increased cardiovascular fitness may buffer over arousal seen at higher intensities of exercise (Alves et al., 2014; Walsh et al., 2018).

1.3.2 Duration

Exercise duration also follows an inverted-U relationship with post-exercise cognitive function (Chang et al., 2015; Chang et al., 2012). For example, Chang et al. (2015) showed that a moderate duration (20-min) of cycling at 65% of heart rate (HR) reserve improved accuracy and response time on a Stroop Task compared to shorter (10-min) and longer (45-min) durations of cycling in young healthy males. In studies with a delay in cognitive testing administration following exercise, shorter exercise durations (0-10 mins) negatively affected cognitive performance, whereas longer exercise bouts (>20 mins) positively affected cognitive function (Chang et al., 2012). Thus, it appears that longer durations of exercise are ideal for eliciting cognitive benefits either during or after exercise. However, prolonged acute exercise bouts (>45 mins) are associated with dehydration, glycogen depletion, and fatigue, all of which may negatively impact cognitive function (Brisswalter et al., 2002).

1.3.3 Cognitive Test Administered

The type of cognitive test administered also plays a role in determining the effect of acute exercise on cognitive function. Executive functions appear to be the most sensitive to improvements immediately after exercise and following a delay in cognitive testing administration (Chang et al., 2012). Interestingly, the effect size for reaction time is not significantly different from zero despite much of the literature interpreting accelerated reaction time as improved

cognitive function. This is likely due to reaction time being a component of most cognitive tests (Chang et al., 2012).

1.4 Covariates of Participants that may Impact Acute Exercise's Effect on Cognitive Function

1.4.1 Cardiovascular Fitness

Cardiovascular fitness may moderate the effect of acute exercise on cognitive function (Brisswalter et al., 2002; Chang et al., 2012), as higher $\dot{V}O_2$peak is associated with higher scores on visuospatial and verbal cognitive tests in young adults (Åberg et al., 2009), improved executive function and processing speed in older adults (Colcombe & Kramer, 2003), and greater hippocampal volume in older adults (Erickson et al., 2009). Chang et al. (2012) reported that participants with higher fitness improved cognitive performance ***during*** exercise, whereas participants with lower fitness experienced detrimental effects. It is speculated that individuals with lower fitness dedicate more cognitive resources towards maintaining homeostasis during exercise, leaving less cognitive resources available for cognitive testing (Chang et al., 2012).

1.4.2 Sex Differences in Cognitive Function following Acute Exercise

Currently, there is no evidence to suggest that there are sex differences in cognitive performance following acute exercise (Herold et al., 2021; Johnson et al., 2019; Johnson & Loprinzi, 2019). To the best of our knowledge, no studies have examined whether sex differences exist in executive function and processing speed following an acute bout of exercise (Herold et al., 2021). However, baseline sex differences may exist in spatial working memory (Voyer et al., 2017) and processing speed (Irwing, 2012), which may impact cognitive performance post-exercise. A recent study provides evidence for this speculation, as college students with lower baseline cognitive performance on a cognitive flexibility task (shifting task) displayed greater improvements in cognitive flexibility, denoted by a reduced switch cost, following 20-min of

moderate-intensity treadmill exercise compared to students with higher baseline cognitive performance who showed no improvement post-exercise (Mou et al., 2023). Conversely, executive function does not seem to display any significant baseline sex differences, with no significant differences in the subdomains of inhibitory control (Hedges' g = -0.01) or cognitive flexibility (Hedges' g = -0.06) being noted in a meta-analysis of 3872 healthy young to middle-aged adults (18-45 years old) (Gaillard et al., 2021).

1.5 Mechanistic Basis for Acute Exercise's Beneficial Effect on Cognitive Function

Presently, the exact mechanisms governing the beneficial effects of acute exercise on cognitive function are controversial (El-Sayes et al., 2019). It is unlikely that a single mechanism is solely responsible for the beneficial cognitive response to acute exercise, but rather interactions between multiple integrated mechanisms drive the improvement in cognitive function following acute exercise (El-Sayes et al., 2019). These mechanisms include: increased cerebral blood flow (CBF) (Paulson, 2002); increased cortical arousal (McMorris, 2009); and increased neurotrophic growth factor expression (Dinoff et al., 2017). For the sake of this literature review, only CBF and arousal will be discussed, but neurotrophic growth factors play an important role in exercise-induced cognitive improvement and neuroplasticity.

1.5.1 Cerebral Blood Flow and Acute Exercise

There is theoretical basis to posit that improvements in cognitive function during acute exercise may be due to increased CBF (Ogoh et al., 2014). Increased CBF would allow for the brain to receive a greater amount of nutrients and oxygen required to meet the metabolic demands of exercise (Querido & Sheel, 2007), which may also alter cognitive function during and after exercise (Ogoh et al., 2014). However, the evidence supporting the link between increased CBF and improved cognitive function in young healthy adults is tenuous at best (Renke et al., 2022). For example, Ogoh et al. (2014) directly manipulated CBF at rest and during 50-min of cycling

exercise using hypercapnic gas in young healthy adults. Despite hypercapnia-induced increases in middle cerebral artery velocity (MCAv), there were no changes in Stroop Task reaction time or accuracy measured at several timepoints during exercise (Ogoh et al., 2014). Interestingly, despite experiencing a reduction in MCAv during exercise, the control group improved their Stroop task performance (Ogoh et al., 2014). Moreover, Shoemaker et al. (2020) investigated whether increased CO_2 and subsequent increases in MCAv during exercise affected visuomotor response, inhibitory control, and cognitive flexibility. Despite increases in MCAv during submaximal and severe exercise intensities, there were no associations between CO_2 or MCAv and cognitive performance (Shoemaker et al., 2020). Furthermore, Shoemaker et al. (2021) reported that decreases in MCAv induced by the drug indomethacin had no effect on cognitive performance in both younger (18-36 years old) and older (50-75 years old) healthy adults compared to pre-indomethacin or post-placebo ingestion. These results suggest that increased CBF is unlikely to affect cognitive function ***during*** exercise, and the beneficial effects of acute exercise on cognitive function are likely due to other mechanisms such as increased arousal (Ogoh et al., 2014). Currently, evidence suggests that the beneficial effects of acute exercise on cognitive function are not strongly related to changes in CBF in young healthy adults.

1.5.2 Arousal and Acute Exercise

One of the mechanisms likely driving acute exercise's beneficial effect on cognitive function is increased arousal (McMorris, 2009). Arousal is defined as the state of physiological and psychological alertness in an individual, with levels ranging on a continuum from extreme drowsiness to wakefulness (Aston-Jones & Cohen, 2005). Arousal and cognitive function are speculated to have an inverted-U relationship, with cognitive function peaking at moderate levels of arousal, and decreasing at high and low levels of arousal (Aston-Jones & Cohen, 2005).

In humans, acute exercise has been shown to increase plasma levels of catecholamines such as norepinephrine (Skriver et al., 2014; Winter et al., 2007). Indeed, both Skriver et al. (2014) and Winter et al. (2007) reported that increased catecholamine concentrations, and presumably increased arousal, were associated with enhanced retention in a motor and vocabulary learning task respectively. It is speculated that during exercise, the hypothalamus stimulates the release of norepinephrine from vesicles of the locus coeruleus located in the brainstem, which is the major site of noradrenaline containing neurons in the brain (McMorris, 2009). Once released, these noradrenergic neurons project to a majority of the cortex and impact excitatory neurotransmitter release (O'Donnell et al., 2012). Exercise also a systemic stressor that stimulates the production of hormones that impact systemic physiology (Mastorakos et al., 2005). Exercise causes the release of corticotropin-releasing hormone from the hypothalamic pituitary axis, which induces the release of cortisol from the adrenal cortex, with cortisol receptors being densely innervated in brain areas such as the hippocampus, the pre-frontal cortex, and the amygdala (Mastorakos et al., 2005). Evidence suggests that moderate increases in cortisol enhance cognitive function (Yuen et al., 2009), whereas excessive concentrations of cortisol impair cognitive function (Tollenaar et al., 2008).

Although arousal can be measured objectively (e.g., pupillometry) (Mathôt, 2018), we will discuss a subjective measure of arousal in this literature review, the Felt Arousal Scale (FAS), originally developed by Svebak and Murgatroyd (1985). The FAS is a visual analog scale anchored on the perceptual prompts 1 = low arousal, and 6 = high arousal, with high convergent validity with the Self-Assessment Manikin, a reputable measure of self-reported arousal (Brito et al., 2022; Thorenz et al., 2023). The FAS induces comparable changes in HR following increasing intensities of resistance exercise (40%, 70%, or 100% of 10-repetition maximal resistance exercise) (Chang

& Etnier, 2009), and intensity-matched increasing durations of aerobic exercise (15-, 30-, or 45-min cycling at 60-70% VO_2 max) (Hacker et al., 2020) in young healthy adults. Interestingly, only Chang and Etnier (2009) reported a decrease in the total time required to complete a post-exercise Stroop Task, with the time to completion decreasing linearly with increasing intensity. In contrast, Hacker et al. (2020) reported no improvement in cognitive performance on a visual recognition memory test following any durations of aerobic exercise. A potential reason for this difference may be due to the different cognitive domains tested, with acute exercise having the greatest beneficial effect on the domains of executive function and processing speed (Chang et al., 2012). Thus, a Stroop Task, which loads primarily on executive function, may be more sensitive to the beneficial effects of acute exercise, whereas a visual recognition memory test which loads primarily on working memory, may display lower sensitivity to acute exercise.

1.6 Summary of the Effect of Acute Exercise on Cognitive Function

Overall, evidence suggests that acute exercise has a small, beneficial effect on cognitive function, particularly in the domains of executive function and reaction time (Chang et al., 2012; Lambourne & Tomporowski, 2010; Ludyga et al., 2016; Petruzzello et al., 1997; Sibley & Etnier, 2003). Several moderators influence acute exercise's ability to enhance cognitive function, namely: the intensity of exercise; the duration of exercise; the timing of cognitive testing (during or post-exercise) (Chang et al., 2012); and cardiovascular fitness (Brisswalter et al., 2002). Baseline sex differences do exist in some cognitive domains, such as spatial working memory (Voyer et al., 2017) and processing speed (Irwing, 2012), however, it is unknown whether this translates into differential cognitive performances post-exercise (Mou et al., 2023). Based on the literature, improvements in cognitive function seen following acute exercise are likely due to an increase in arousal, rather than CBF in young healthy adults (Ogoh et al., 2014). The following section of this literature review will focus on acute handgrip exercise, an exercise paradigm which

may improve cognitive function through similar mechanisms as acute whole-body exercise (Mather et al., 2020; Washio et al., 2021).

2.0 Isometric Handgrip Exercise

2.1 An Introduction to Acute Isometric Handgrip Exercise

Chronic resistance exercise involving large muscle mass is well known to improve muscular strength and size, which can improve physical performance and cognitive function in both younger and older adults (Landrigan et al., 2020). Of interest, isometric handgrip exercise (IHG) is a small muscle mass exercise that requires minimal fitness, expertise, and equipment. An acute IHG protocol typically involves 4 sets of 2-min contractions at a percentage of an individual's maximal voluntary contraction (MVC), with a rest period of 2-3 min in between each set (Yamada et al., 2022). Preliminary evidence suggests that acute IHG may improve cognitive function, possibly through the same mechanisms as acute whole body exercise (Mather et al., 2020; Washio et al., 2021). However, the effect of acute IHG on cognitive function in young healthy adults has been addressed by few studies with conflicting results (Zhu et al., 2022). In addition, previous studies did not examine sex differences despite evidence suggesting that biological sex can influence the effectiveness of exercise interventions on cognitive function (Barha et al., 2019; Herold et al., 2021). Importantly, IHG should not be considered as a substitute for whole-body exercise. Although IHG may increase similar mechanistic moderators of cognitive improvement as whole-body exercise (Mather et al., 2020; Washio et al., 2021), the overall extent of exercise-induced physiological stress is substantially less. Thus, whole-body exercise would be more beneficial for improving cardiovascular fitness as it engages both the lower and upper muscle groups, resulting in greater increases in heart rate and oxygen consumption, compared to IHG which primarily only engages the forearm muscles.

2.2 The Effect of Acute IHG on Hemodynamics in Young Healthy Adults

2.2.1 A Brief Summary of Isometric Resistance Training and Hemodynamics

Given that chronic exercise is the culmination of many bouts of acute exercise, understanding whether hemodynamic changes exist in chronic isometric resistance exercise may elucidate potential relationships regarding dose-response and recovery. Indeed, isometric resistance training reduces systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) in younger and older pre-hypertensive, hypertensive, and normotensive adults (Cornelissen & Smart, 2013; Edwards et al., 2023; Loaiza-Betancur et al., 2020). A recent meta-analysis (n = 15,827 adults) found that isometric resistance training was the best exercise modality for lowering BP (Edwards et al., 2023). However, the moderating effect of biological sex on BP lowering following isometric resistance training is inconclusive, with one meta-analysis denoting similar magnitudes of BP reduction between the sexes (Smart et al., 2019), and two others denoting differential sex-based reductions in BP (Badrov et al., 2013; Inder et al., 2016). Several factors may affect isometric resistance training's BP lowering potential, namely: the duration (Cornelissen & Smart, 2013); intensity (Hess et al., 2016); and frequency of exercise (Badrov et al., 2013), with increased durations, intensities, and frequencies eliciting greater reductions in hemodynamics.

2.2.2 Hemodynamics During and After Acute IHG

Evidence from young normotensive adults shows that acute IHG significantly increases SBP, DBP, MAP, and HR during exercise (Jarvis et al., 2011; Krzemiński et al., 2012; Lalande et al., 2014; Saito et al., 2021; Tarumi et al., 2021; Washio et al., 2021), with values returning to resting levels promptly after exercise (Jarvis et al., 2011; Reid & Conway, 2006; Saito et al., 2021; Washio et al., 2021), 5-min post-exercise (Jatoi et al., 2014; Krzemiński et al., 2012), and 10-min post-exercise (Jatoi et al., 2014). The magnitude of hemodynamic responses to IHG appears to be

intensity-dependent (Krzemiński et al., 2012; Saito et al., 2021; Tarumi et al., 2021; Washio et al., 2021).

Evidence suggests that there may be sex differences in the hemodynamic response to IHG (Jarvis et al., 2011; Wong et al., 2007), as well as during the subsequent recovery period (Teixeira et al., 2018). Overall, women display smaller increases in MAP during acute IHG compared to men (Jarvis et al., 2011; Wong et al., 2007), which was associated with a weaker insular cortex activation, measured using functional magnetic resonance imaging (fMRI) (Wong et al., 2007). Insular cortex activation increases BP and HR (Ichiyama et al., 2004). Following IHG, women may have faster recovery compared to men (Teixeira et al., 2018) due to greater baroreflex sensitivity (Taylor et al., 2015); however, the majority of evidence suggests that both men and women's BP returns to resting values within 5-min cessation from IHG. In contrast, Patel et al. (2015) found no sex differences in hemodynamics immediately following the cessation and 1-hour post rhythmic handgrip exercise in adolescents. However, rhythmic handgrip exercise induces a lower sympathetic stimulus than IHG (Cook et al., 2016), calling into question whether hemodynamics were increased at all during Patel et al. (2015) acute IHG protocol.

2.3 Cardiovascular Reactivity and Acute IHG

Cardiovascular reactivity to IHG is associated with a greater risk of developing hypertension (Carroll et al., 2012). Cardiovascular reactivity is an individual's hemodynamic response (SBP, DBP, MAP, and HR) to an acute physical or psychological stressor (Hughes & Lü, 2013). Current evidence suggest that individuals with greater cardiovascular reactivity prior to engaging in an IHG training program appear to benefit the most from IHG training (Somani et al., 2018), suggesting that cardiovascular reactivity may help predict potential response patterns to IHG training; however, more work is needed to establish this phenomenon. To date, no studies

have examined sex differences in cardiovascular reactivity to acute IHG. However, evidence from studies employing other stress tasks including the cold pressor test (Keller-Ross et al., 2020) and a social stress task (Whited & Larkin, 2009) suggests that there are no sex differences in the cardiovascular reactivity to non-exercise stressors; however, investigation into potential sex differences in cardiovascular reactivity to an exercise stressor are warranted.

3.1 The Effect of Acute IHG on Cerebral Blood Flow in Young Healthy Adults

3.1.1 The Regulation of Cerebral Blood Flow

A constant supply of CBF is needed to maintain brain function, with insufficient levels of CBF leading to unconsciousness whereas excessive perfusion pressure leads to neurological damage (Bisson et al., 2016). The regulation of CBF is complex, with many regulatory mechanisms working in tandem to ensure adequate perfusion, optimal oxygen, and nutrient delivery (Willie et al., 2014). CBF is primarily modulated by the partial pressure of arterial carbon dioxide ($PaCO_2$), arterial blood pressure (MAP), and cerebral metabolism (Perry & Lucas, 2021; Willie et al., 2014). At rest, $PaCO_2$ levels are the most influential factor regulating CBF (Willie et al., 2014). Reductions in $PaCO_2$ (hypocapnia) leads to the vasoconstriction of cerebral arteries, increasing cerebrovascular resistance (CVR), thus reducing CBF (Perry & Lucas, 2021; Willie et al., 2014). Conversely, increases in $PaCO_2$ (hypercapnia) leads to vasodilation of the cerebral arteries, decreasing CVR, thus increasing CBF (Perry & Lucas, 2021; Willie et al., 2014). The sensitivity of the cerebrovasculature to $PaCO_2$ stems from maintaining pH levels. For example, reduced breathing increases the accumulation of $PaCO_2$, thereby increasing the amount of hydrogen ions in the blood through the formation of carbonic acid, causing acidemia (Carr et al., 2021). Chemoreceptors detect this change and induce breathing to washout excess levels of $PaCO_2$, returning pH to homeostatic levels (Carr et al., 2021). CBF is also tightly coupled to cerebral

metabolism, with increases in neural activity leading to a concurrent increase in CBF (i.e., neurovascular coupling) (Iadecola, 2017). Neurovascular coupling is the close spatial and temporal response of increased CBF matching increased neuronal activity in the brain (Iadecola, 2017).

CBF was thought to be strictly maintained over a range of 60-150 mmHg (Lassen, 1959), known as cerebral autoregulation. However, recent evidence suggests a more pressure-passive relationship in which changes in MAP result in concordant changes in CBF (Willie et al., 2014). Moreover, cerebral autoregulation is better adapted to adjusting the cerebrovasculature in response to acute hypertension (i.e., vasoconstriction) rather than hypotension (i.e., vasodilation), a phenomenon known as hysteresis (Willie et al., 2014). Therefore, cerebral autoregulation is effective at buffering the large transient increases in BP commonly seen during resistance exercise (Perry & Lucas, 2021).

One method for measuring CBF is Transcranial Doppler Ultrasound (TCD). TCD involves placing an ultrasound probe on certain regions of the skull, termed “acoustic windows”, to estimate changes in cerebral blood velocity as a surrogate for CBF. The probe emits sound waves (approximately 2 megahertz) that are reflected by the red blood cells located within cerebral vessels. The difference between emitted and reflected waves is proportional to the speed of moving red blood cells, termed the Doppler shift frequency, which is used to determine blood velocity. Advantages of TCD include it’s feasibility and high temporal resolution, however, TCD provides poor spatial resolution compared to other methods of estimating CBF. Furthermore, TCD assumes a constant diameter of the insonated vessel given that blood velocity is proportional to blood vessel radius (Willie et al., 2011).

3.2 Acute IHG increases CBF in Young Healthy Adults

Acute IHG is a significant sympathetic stimulus, inducing large increases in BP during exercise. Indeed, significant increases in MCAv occur with increased BP in response to acute IHG (Fernandes et al., 2016; Tarumi et al., 2021; Washio et al., 2017; Washio et al., 2021). Washio et al. (2021) found a significant increase in MCAv using TCD during acute IHG, which was similar to previous work by Washio et al. (2017). Tarumi et al. (2021) reported significant increases in CBF in the internal carotid artery (ICA), the vertebral artery, the internal jugular vein, and the total artery using MRI following acute rhythmic handgrip exercise. Fernandes et al. (2016) had participants undergo 2 bouts of acute IHG either before (control) or 170-min post prazosin ingestion. Prazosin is an α_1-adrenergic receptor antagonist that causes smooth muscle relaxation, thus decreasing BP and vascular resistance (Basquez & Pippin, 2023). The control condition displayed increased ICA CBF, measured by Doppler ultrasound, which is in line with the rest of the literature (Tarumi et al., 2021; Washio et al., 2017; Washio et al., 2021). However, following prazosin ingestion, ICA CBF was increased to a higher degree compared to the control condition, which was accompanied by an attenuated decrease in ICA_{CVC}. CVC decreasing during acute IHG denotes vasoconstriction of the cerebrovasculature to buffer increased BP during IHG, and is reported in the control condition of Fernandes et al. (2016), as well as in Washio et al. (2017). Thus, prazosin attenuated the protective effect of vasoconstriction seen in the control condition, allowing for a greater increase in CBF (Fernandes et al., 2016). Therefore, autoregulatory factors are blunting the effect of IHG-induced increases in BP in young healthy adults. For all the above-mentioned studies, CBF returned to baseline levels following the cessation of IHG (Fernandes et al., 2016; Tarumi et al., 2021; Washio et al., 2017; Washio et al., 2021).

3.3 Sex Differences in the Cerebral Blood Flow Response to Acute IHG in Young Healthy Adults

To date, few studies have examined whether sex differences exist in the CBF response to acute IHG. Of the above-mentioned studies, only Tarumi et al. (2021) included sex-differences analyses and found no sex differences in CBF following acute rhythmic handgrip exercise. Similarly, Joshi and Edgell (2019) reported that young healthy men and women displayed similar increases in MCAv following acute IHG, despite females having an attenuated exercise pressor reflex compared to males. Joshi and Edgell (2019) also found that women had an attenuated ventilatory response during IHG compared to men. This could potentially lead to smaller changes in CO_2, which could impact CBF; however, this is likely explained by male participant's higher levels of baseline of end-tidal CO_2 (Joshi & Edgell, 2019). Sex differences in CBF may also exist at rest, as one study found that females tended to have higher CBF compared to males, as measured by PET scans (Aanerud et al., 2017).

4.1 Acute IHG Modulates Arousal in Young Healthy Adults

In young healthy normotensive adults, acute IHG increases arousal (Bachman et al., 2023; Mather et al., 2020; Saito et al., 2021). Mather et al. (2020) found that tonic arousal, assessed using pupil diameter, increased during acute IHG in young normotensive women. Importantly, phasic pupil responses increased during a subsequent oddball task corresponded with a faster detection of oddball tones, whereas tonic arousal decreased (Mather et al., 2020). This suggests that tonic arousal is increased during IHG, whereas phasic arousal is increased following IHG in response to a cognitive challenge (Mather et al., 2020). Tonic arousal refers to an individual's overall level of alertness regardless of external stimuli, whereas phasic arousal refers to short-term changes in alertness that occur in response to an external stimuli (Wass et al., 2015). Using a similar protocol, Bachman et al. (2023) observed heightened tonic arousal during IHG; however, there were no changes in post-IHG tonic arousal during a subsequent working memory test in which young

participants displayed improved reaction time. Therefore, it is unknown whether acute IHG's beneficial effects on cognitive function are due to increased tonic arousal during IHG or increased phasic arousal following IHG. Similarly, Saito et al. (2021) reported elevated levels of arousal, measured using the FAS following acute rhythmic handgrip exercise in young adults. However, this increase in arousal did not improve cognitive performance on a subsequent Go/No-Go or memory recognition task (Saito et al., 2021).

Although sex differences were not assessed in previous studies that examined the effect of acute IHG on arousal, arousal may be mediated differently between men and women. Indeed, females may be more sensitive to increased arousal given a higher concentration of noradrenergic neurons in the locus coeruleus compared to men (Bangasser et al., 2019). Moreover, estradiol augments the effects of norepinephrine in the locus coeruleus via estrogen receptor signaling (Bangasser et al., 2019). Furthermore, the use of oral contraceptives has been shown to blunt arousal responses, assessed using salivary cortisol, towards a cold pressor test compared to naturally cycling women (18-35 years old) (Nielsen et al., 2013). Future work should assess the influence of contraceptive status in the context of acute IHG-induced arousal.

To summarize, the literature regarding the effect of acute IHG on arousal in young healthy adults is conflicting. Based on the results of Mather et al. (2020) and Bachman et al. (2023), arousal seems to modulate improvements in post-exercise cognitive function; however, whether improvements in cognitive function are due to decreased tonic and increased phasic arousal post-IHG ***or*** increased tonic arousal during IHG is unknown. Furthermore, arousal, as a mechanism itself, may not underlie improvements in cognitive function, as increased arousal post-IHG measured by the FAS did not improve cognitive function (Saito et al., 2021).

5.1 The Effect of Acute IHG on Cognitive Function in Young Healthy Adults

Preliminary evidence suggests that acute IHG may improve processing speed in young healthy adults (Bachman et al., 2023; Mather et al., 2020; Washio et al., 2021). Washio et al. (2021), Mather et al. (2020), and Bachman et al. (2023) reported decreased reaction times on a Go/No-Go, oddball, and n-back task post-IHG, respectively. The improvement in reaction time seen in Mather et al. (2020) may be associated with an increase in functional connectivity of the frontoparietal network, assessed using locus coeruleus MRI contrast. The frontoparietal network is involved in executive function, and greater functional connectivity leads to increased processing of salient stimuli and decreased processing of non-salient stimuli (Lee et al., 2018). Interestingly, these studies used a short-duration high intensity protocol (Bachman et al., 2023; Mather et al., 2020), suggesting that cognitive function and underlying brain function can be rapidly modulated by a small stimulus. By comparison, a study that used the IHG protocol recommended by the American Heart Association for BP management (Whelton et al., 2018), found improved reaction time on a post-exercise Go/No-Go task (Washio et al., 2021). Although not measured by Washio et al. (2021), the improvement in reaction time on the post-exercise Go/No-Go task may be due to increased arousal. Indeed, increases in MCAv and MAP did not persist at the time of post-exercise cognitive assessment (Washio et al., 2021). Therefore, Washio et al. (2021) IHG protocol may have induced a significant increase in arousal, consequently enhancing the secretion of catecholamines, thereby improving processing speed (McMorris, 2009).

In contrast, some studies show no effect of acute IHG on cognitive function (Brown & Bray, 2015; Saito et al., 2021; Yamada et al., 2021). For example, Saito et al. (2021) reported no improvements in reaction time on a post-exercise Go/No-Go task, despite using the same participants as Washio et al. (2021). The discrepant findings in cognitive performance are likely due to a less intense IHG protocol (Saito et al., 2021). Indeed, Saito et al. (2021) IHG protocol

induced a significantly smaller MAP response (Δ 10 ± 10 mmHg) compared to that of Washio et al. (2021) (Δ 26 ± 14 mmHg). Therefore, a less intense IHG protocol may not provide enough sympathetic stimulus to improve cognitive function (Saito et al., 2021). Similarly, Yamada et al. (2021) found no improvement in Stroop task performance following acute IHG. A potential reason for a lack of cognitive improvement may be due to insufficient rest periods between contractions. For example, Washio et al. (2021), despite using a lower intensity (25% MVC), had longer recovery periods (3-min vs 1-min) in between sets. Only Brown and Bray (2015) used a volitional exhaustion IHG protocol in which participants were randomly allocated to one of four conditions: low intensity (30% MVC); moderate intensity (50% MVC); high intensity (70% MVC); or a control condition. All conditions, including the control, showed a significant improvement in response speed on the Stroop Task (Brown & Bray, 2015). In contrast, the high-intensity group showed no changes in error frequency post-exercise (Brown & Bray, 2015). Therefore, engaging in higher intensities of IHG until exhaustion may attenuate an individual's ability to detect errors on the Stroop task (Brown & Bray, 2015).

Overall, the differing cognitive performances following acute IHG are likely due to differences in exercise protocol including whether exercise was performed to volitional exhaustion versus repeated sets (holding an MVC interspersed with recovery periods), the intensity (% MVC), and volume (Zhu et al., 2022). Moreover, differential cognitive responses may be due to investigations of different cognitive domains. For example, post-exercise improvements were seen in processing speed (Bachman et al., 2023; Mather et al., 2020; Washio et al., 2021), but not in executive function (Brown & Bray, 2015; Yamada et al., 2021)

5.1.1 Sex Differences in Cardiovascular Reactivity and Cognitive Function Following Acute IHG

To date, no study has directly examined the impact of biological sex on cognitive function following acute IHG. Preliminary evidence suggests that young healthy adults with higher cardiovascular reactivity have lower exercise-induced improvements in processing speed on the Go/No-Go task. Given that young healthy females tend to have lower cardiovascular reactivity during (Wong et al., 2007) and after acute IHG (Teixeira et al., 2018), it is plausible that women may experience greater IHG-induced improvements in cognitive function compared to men. While the mechanisms are not fully elucidated, Washio and Ogoh (2023) speculate that higher cardiovascular reactivity may reflect un-optimal increases in brain catecholamine concentrations, , thereby increasing neural noise and attenuating cognitive function. Although the traditional view is that higher cardiovascular reactivity is associated with worse cognitive function, some evidence suggests that greater cardiovascular reactivity in young adults is associated with improved performance on the Digit Symbol Substitution and Stroop Task (Yano et al., 2016). Therefore, the moderating effect of age should be considered in this relationship.

6.1 Summary of the Effects of Acute IHG on Hemodynamics, CBF, Arousal, and Cognitive Function

To summarize, acute IHG may improve cognitive function (Bachman et al., 2023; Mather et al., 2020; Washio et al., 2021) through increases in CBF (Washio et al., 2021) and arousal (Bachman et al., 2023; Mather et al., 2020; Saito et al., 2021), due to the hemodynamic stress of acute IHG (Washio & Ogoh, 2023). In terms of sex differences, mechanistic evidence suggests that biological sex impacts systemic hemodynamics (Smith et al., 2019), resting CBF (Aanerud et al., 2017), and arousal due to stress reactivity (Bangasser et al., 2019), which may result in differential cognitive responses between men and women following acute IHG. However, no

studies have directly evaluated the impact of biological sex on cognitive function following an acute bout of IHG.

6.1.1 Purpose and Hypothesis for the Study

The purpose of this study is to investigate the effect of an acute bout of IHG on cognitive function in young normotensive adults, and to determine whether any potential sex differences in cognitive function exist following an acute bout of IHG. We hypothesized that acute IHG would improve cognitive function through an increase in arousal (McMorris, 2009), and that women would have greater exercise-induced improvements in cognitive function due to a lower exercise pressor reflex (Teixeira et al., 2018; Wong et al., 2007). Furthermore, we also hypothesized that IHG would increase CBF, however, we do not expect changes in CBF to be associated with improvements in cognitive function based on previous literature (Ogoh et al., 2014; Shoemaker et al., 2021; Shoemaker et al., 2020). Finally, based on the findings of Washio et al. (2021), we hypothesized that individuals with lower cardiovascular reactivity during IHG would have greater improvements in cognitive function.

Chapter 2: The Effect of Acute Isometric Handgrip Exercise on Cognitive Function in Young Healthy Adults

2.0 Introduction

Whole-body exercise training (aerobic or resistance) has myriad benefits for cognitive function, brain structure, and brain function across multiple age and health states (Erickson et al., 2019; Stillman et al., 2020). Interestingly, some of the beneficial effects of exercise occur rapidly, as a single bout of moderate intensity aerobic exercise transiently enhances cognitive functions such as processing speed, inhibitory control, and selective attention in healthy adults (Pontifex et al., 2019). The exact mechanisms underlying the beneficial effects of acute exercise on cognitive function remain elusive; however, hypothesized mechanisms include an increase in cerebral blood flow (CBF) (Paulson, 2002), arousal (McMorris, 2009), and neurotrophic growth factors (Basso & Suzuki, 2017). Ultimately, the beneficial effect of acute exercise on cognitive function likely stems from an integration of the above-mentioned mechanisms (El-Sayes et al., 2019). However, conventional whole-body exercise is not easily accessible for everyone due to mobility and health limitations, which are especially prevalent among older adults (Brahms et al., 2021). These limitations are further exacerbated by chronic diseases such as heart disease, hypertension, and osteoarthritis, which increase the risk for future mobility limitations (Daley & Spinks, 2000). Another more accessible exercise option is IHG, in which an individual squeezes a portable hand-held device for a sustained period of time, separated by short rest periods (Zhu et al., 2022). A typical IHG protocol involves four sets of 2-min sustained contractions at a percentage of an individual's MVC, with a rest period of 2-3 min in between each sets (Yamada et al., 2022). Previous studies have shown that both chronic (Inder et al., 2016) and acute IHG (Millar et al., 2010; Souza et al., 2018) decrease resting BP in normotensive and hypertensive populations, with national governing bodies such as Hypertension Canada, endorsing IHG as a form of hypertension

management (Wood et al., 2020). Moreover, a recent meta-analysis (n = 15827 participants) found that isometric exercise training was the best exercise modality for decreasing SBP and DBP compared to dynamic resistance, aerobic and high-intensity interval training (Edwards et al., 2023). Critically, IHG is simple, cost-effective, time efficient, and is a highly tolerable form of resistance exercise that requires minimal fitness, expertise, or equipment (Zhu et al., 2022).

Preliminary evidence suggests that acute IHG may improve cognitive function through similar mechanisms as acute whole-body exercise (Mather et al., 2020; Washio et al., 2021). For example, a single bout of IHG improved reaction time on a Go/No-Go task, indicating faster processing speed, in young healthy adults (Washio et al., 2021). Other studies report that acute IHG elevates CBF in young healthy adults (Tarumi et al., 2021; Washio et al., 2021), which may support enhanced cognitive function (Paulson, 2002). Moreover, acute IHG increases arousal, which corresponded with improved reaction time on a subsequent working memory task in young healthy women (Mather et al., 2020). However, despite the ability of acute IHG to potentiate physiological function, the reported effects of acute IHG on cognitive function are inconsistent (Brown & Bray, 2015; Saito et al., 2021; Yamada et al., 2021), as well as the hypothesized mechanisms underlying the potential cognitive benefits of IHG (Bachman et al., 2023; Nielsen & Mather, 2015).

Furthermore, prior research yet to investigate potential sex differences in cognitive function following acute IHG. Evidence suggests that women have a lower exercise pressor response to acute IHG compared to men (Teixeira et al., 2018; Wong et al., 2007). It has been hypothesized that biological sex may influence the magnitude of cognitive improvement following acute (Herold et al., 2021) and chronic exercise (Barha et al., 2019), potentially through these differential sex BP responses which modulate CBF (Bond et al., 2012) and arousal (Critchley &

Mathias, 2003) ultimately impacting cognitive function. Given that women tend to have lower exercise pressor responses compared to men (Smith et al., 2019; Teixeira et al., 2018; Wong et al., 2007), it is plausible that women may experience different outcomes in cognitive improvement following acute IHG. Interestingly, Washio et al. (2021) reported that people with lower cardiovascular reactivity during acute IHG exhibited greater improvements in processing speed during the post-exercise cognitive testing period. Accordingly, differences between males and females in BP responses to IHG may differentially impact cognitive outcomes. However, to the best of our knowledge, no studies have directly explored the influence of biological sex on cognitive function and the potential underlying mechanisms following acute IHG. Therefore, there is a need for a study examining the effect of acute IHG on cognitive function in young healthy adult and whether there are sex-based differences in this response.

The purposes of this study were to investigate the effect of acute IHG on cognitive function in normotensive adults, and to investigate the effect of biological sex on cognitive performance following acute IHG. Based on previous research, we hypothesized that cognitive function would be enhanced following an acute bout of IHG compared to a control condition, and that improvements in cognitive function would be facilitated by increased arousal (McMorris, 2009), rather than CBF (Ogoh et al., 2014; Shoemaker et al., 2021; Shoemaker et al., 2020). Furthermore, we hypothesized that women would have greater exercise-induced improvements in cognitive function due to a lower exercise pressor reflex compared to men (Smith et al., 2019; Teixeira et al., 2018; Wong et al., 2007). Finally, we hypothesized that individuals with lower cardiovascular reactivity during IHG would have greater improvements in cognitive function.

2.1 Methods

2.1.1 Participants

Thirty male and female adults (n = 15 females; age = 23.8 ± 3.3 years) from the McMaster University and Hamilton communities volunteered to participate. Inclusion criteria included: 1) being between the ages of 18-35 years old; 2) having normal blood pressure (≤ 120/80 mmHg); 3) no diagnosis of high blood pressure (pre-hypertension or hypertension); 4) no diagnosis of cardiovascular diseases; and 5) not being a competitive endurance athlete (i.e., training specifically for cycling, triathlon or distance running, and compete at a high level). Exclusion criteria included: 1) having a history of previous cardiovascular events that required hospitalization (e.g., heart attack, stroke); and 2) being diagnosed with Type 1 or 2 diabetes. The protocol was approved by the Hamilton Integrated Research Ethics Board (HiREB project number 14058) (refer to appendix D for the HiREB approval letter), and each participant provided written informed consent prior to participation.

2.1.2 Study Design

This study employed a randomized crossover design and involved a total of 3 visits to the Brain Exercise and Enhancement Laboratory at McMaster University. Prior to the start of the experiment, the total number of participants (30) were divided into two separate groups, group 1 and 2. Group 1 would perform exercise followed by control, and group 2 would perform control followed by exercise. Participants were randomly allocated to one of the two groups using computer generated randomization. When group 1 reached the maximum number of participants (15), the remaining participants (2) were allocated to group 2 to ensure counterbalance. Individuals interested in participating were invited to the lab for a familiarization visit. After reading and signing the study consent form, a researcher recorded height, weight, dominant hand, and years of education for each participant. Sleep and physical activity over a 7-day period was recorded using

the Seven-Day Physical Activity Recall (PAR-7) (see appendix A for a copy of the PAR-7). Total daily energy expenditure (kcal/day) was calculated from the PAR-7. Participants were also familiarized with the cognitive testing battery and the IHG protocol. Female participants were asked to report whether they were taking oral contraceptives using a menstrual cycle questionnaire (see appendix B for a copy of the menstrual cycle questionnaire). For women experiencing natural menstrual cycles, we tested during the early follicular phase, which was defined as days 2-7, with the onset of menses representing day 1. For participants using oral contraceptives, we tested during days 2-7 of the low hormone phase (placebo pill) to match the naturally cycling group. All eligible participants were instructed to refrain from vigorous physical activity for > 24 hours, alcohol and caffeine > 8 hours, and food > 2 hours prior to each of the two subsequent visits.

2.1.3 Experimental Protocol

In a randomized order, participants completed either IHG (exercise) or a control condition separated by a minimum of 2 days following their familiarization visit **(Figure 1)**. Upon arrival of the lab, participants rested quietly in a seated position while they were instrumented with physiological monitoring equipment which included an electrocardiogram (ECG) to measure HR, a finger cuff to continuously measure BP, a TCD to measure MCAv, an automatic BP cuff to measure BP at the brachial artery and calibrate the finger cuff, and a mouthpiece to measure expired gases (CO_2). Following instrumentation, participants completed either the IHG or control condition. IHG consisted of four sets of 2-min dominant hand unilateral squeezing a handgrip dynamometer (BMS-G200 Handgrip Dynamometer, Biometrics Ltd, Richmond Hill, Ontario) at 30% MVC separated by 3-min of rest. To determine MVC, participants performed 3 MVC's for 2 sec in duration, each separated by 1-min. The highest force generated from these three trials was set as each participant's 100% MVC, and 30% of a participant's MVC was calculated and

displayed on a monitor to provide visual feedback using the LabChart 8 software (AD instruments). The control condition watched a nature documentary for 20-min.

Cognitive function was assessed immediately before (pre-intervention) and after (post-intervention) exercise or control using 3 cognitive tests that collectively assessed the cognitive domains of processing speed, working memory, and executive function. Arousal was measured using the subjective 6-point FAS (Svebak & Murgatroyd, 1985) at time-matched points during each intervention (before the start of pre- or post-cognitive testing and at the end of each set of IHG).

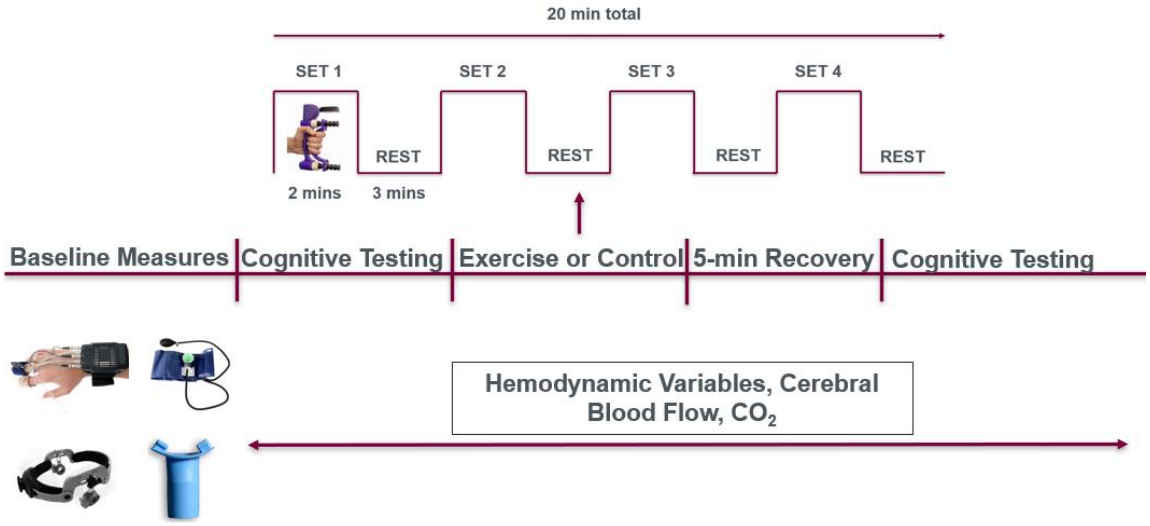

Figure 1. Overview of experimental protocol. Hemodynamics, MCAv, and expired gases (CO_2) were continuously monitored following baseline instrumentation. Participants reported their arousal levels at time-matched points in both interventions: before pre- and post-cognitive testing, and at the end of each set of IHG/time-matched control. Cognitive testing was conducted in a randomized order for all three visits.

2.1.4 Systemic Hemodynamic Assessment

Arterial BP and heart rate (HR) were continuously monitored during the protocol using a non-invasive finger cuff (Human NIBP Nano System, model INL382, AD Instruments, Colorado Springs, CO, USA). The Human NIBP finger cuff utilizes photoplethysmography to determine changes in BP, which assumes that changes in blood volume in a microvascular bed (e.g., finger capillaries) can be detected by changes in light absorbency. An increase in blood volume results in

a decrease in light absorption in a vascular region, and this change in the ratio of volume to light is used by the finger cuff to calculate changes in BP (Laulkar & Daimiwal, 2012).

NIBP finger cuff values were calibrated to BP measured at the brachial artery to ensure changes in blood volume accurately reflected changes in BP. An automatic BP sphygmomanometer (OMRON Healthcare Co. Ltd, Kyoto, Japan) was used to take 3 BP measurements at the brachial artery in succession with 30 secs in between each measurement and the median value was used. Continuously measured hemodynamic variables were averaged using the last 15 seconds of data points at resting baseline, during exercise (at the end of each set of IHG), and recovery (20-min post-exercise).

Cardiovascular reactivity in response to IHG was assessed to determine whether changes in cardiovascular reactivity were related to post-exercise cognitive performance. Cardiovascular reactivity was calculated as the change in hemodynamics from baseline to the end of the fourth set of IHG exercise. To assess correlations of cardiovascular reactivity with cognitive performance, cognitive change scores were calculated by subtracting the post-exercise/control cognitive score from the pre-exercise/control cognitive score for all measures of cognitive performance.

2.1.5 Cerebrovascular Assessment

TCD was used to assess blood velocity in the right MCA. A TCD probe (Multigon Ultrasound System with Robotic Headband, Elmsford, NY, USA) was affixed to a participant's right temporal window (between the temple and ear) via a specialized headband to continuously measure MCAv, a surrogate for CBF. MCAv was continuously recorded in LabChart and the MCAv outcomes were obtained from the peak envelope waveform. MCA_{CVR} was calculated by dividing MAP by MCAv. Cerebrovascular measures were averaged using the last 15 seconds of

data points at resting baseline, during exercise (at the end of each set of IHG), and recovery (20-min post-exercise).

2.1.6 Expired Gas Assessment

End tidal carbon dioxide ($P_{ET}CO_2$) was obtained from participant's expired CO_2, given that changes in CO_2 are a major contributor to cerebrovascular tone. Participants wore a mouthpiece that connected to a gas analyzer (Respiratory Gas Analyzer, ML206, AD Instruments, Colorado Springs, CO, USA) to continuously measure expired gases (CO_2). $P_{ET}CO_2$ was determined on a breath-by breath basis using the Cyclic Measurement function built into LabChart. The end of each participant's expiration (i.e., the highest point in the CO_2 waveform) was used to calculate $P_{ET}CO_2$ between breaths. $P_{ET}CO_2$ was averaged using the last 15 seconds of data points at resting baseline, during exercise (at the end of each set of IHG), and recovery (20-min post-exercise). To assess correlations of MCAv with $P_{ET}CO_2$, change scores in MCAv and $P_{ET}CO_2$ were calculated by subtracting the value of MCAv or $P_{ET}CO_2$ during the fourth set of IHG exercise from baseline.

2.1.7 Cognitive Function Assessment

Cognitive function was assessed using a battery of three psychometrically valid tests on a laptop computer using cognitive testing software (Millisecond Inquisit Lab Version 6.5.2, LLC). The cognitive testing battery was comprised of three tests, the N-Back test (1- and 2-Back), the Corsi Block Forward test, and the 4-Choice Reaction Time test.

N-Back: The N-Back test primarily assesses the cognitive domain of working memory, although other aspects of executive function such as inhibitory control are also tested (Soveri et al., 2017). Participants were shown a sequence of letter stimuli (white letters on a black background) and were asked to indicate whether the current presented stimulus was the same as the N-back number. For example, during a 1-back, participants pressed the "A" key each time the current letter was the same as the letter presented 1 position before. During a 2-back, participants

pressed the "A" key each time the current letter was the same as the letter presented 2 positions before. If the letters were different in both cases, the participant was instructed not to respond. Participants completed three rounds of the 1-back and three rounds of the 2-back in a randomized order. To assess cognitive performance, we measured 1-back reaction time and 2-Back reaction time in milliseconds (ms), proportion correct, and d-prime (hit rate/false alarm rate), a measure of sensitivity.

Corsi Block Forward: The Corsi Block Forward test primarily assesses the cognitive domain of spatial working memory (van Asselen et al., 2006). Participants were presented with a screen of 9 boxes. The boxes would light up in a pre-determined sequence and participants were asked to click the boxes in the same order that they appeared. The sequence length would start at 3 boxes and would increase up to 8 boxes. Participants were allowed 2 attempts for each sequence length. If one of the sequences was entered correctly, the next sequence would commence. Participants completed the Corsi Block test until failure or until they reached the maximum sequence length of 8 boxes. To assess cognitive performance, a composite score – "Block Total" – was computed by multiplying a participant's "blockspan" (the length of the last correctly recalled sequence, max 8) by "nCorrectTotal" (the number of recalled sequences across the whole task, max = 17).

4-Choice: The 4-Choice Reaction Time test primarily assesses the cognitive domain of processing speed (Burke et al., 2017). Participants were presented with 4 gray boxes in a horizontal array (1, 2, 3, and 4, with 1 being the left most box). The boxes would turn red in a random fashion and participants were required to press the corresponding response key: either D, F, J, or K respectively, as quickly and accurately as possible. To assess cognitive performance, mean reaction time (ms) and proportion correct were measured.

2.1.8 Felt Arousal Scale

Arousal was assessed using the 6-point FAS throughout the intervention (6 timepoints per intervention). The FAS is a visual analog scale that is anchored on the perceptual prompts 1 = low arousal and 6 = high arousal. Evidence suggests that the FAS demonstrates high convergent validity with the Self-Assessment Manikin (SAM), with scores on the FAS being highly correlated with scores on the SAM (Brito et al., 2022; Thorenz et al., 2023). In terms of objective measures, the FAS is moderately correlated with changes in HR (Chang & Etnier, 2009), but no study has compared FAS arousal with pupillometry. The FAS was chosen for this study due to its ease of administration and comprehension. Please refer to appendix C for a copy of the FAS.

Participants were asked to rate their arousal levels immediately before the start of each cognitive testing period, as well as immediately after the completion of each set of IHG. In the control condition, participants were asked to rate their arousal during the nature documentary at time-matched points.

2.1.9 Sample Size Determination

The first objective of this study was to determine whether acute IHG impacted cognitive function in young healthy adults. Based on the effects observed in examining the impact of acute IHG in young healthy adults (Washio et al., 2021), we anticipated small-sized effects (partial η^2 = 0.065) for acute changes in cognitive function. Based on this, a sample size of 22 is needed to detect a 5% cognitive improvement, assuming 80% power with an alpha level of 0.05 (two-tailed) calculated using G*Power v3.1 (refer to appendix E for this sample size calculation). The second objective of this study was to detect sex differences in cognitive function. We performed a 2-way ANOVA of exercise and reaction time on the 2-back test between males and females in our previous pilot data. Based on the effects observed in our pilot data examining the impact of acute IHG in young healthy adults, we anticipated small-sized effects (partial η^2 = 0.042). We also ran

a Pearson's correlation between pre-intervention 2-back reaction time in the control condition and post-intervention 2-back reaction time in the control condition (r = 0.664, p=0.001). The Pearson's r = 0.664 was used as the correlation among repeated measures. Based on this, a sample size of 24 was needed to detect a significant sex difference, assuming 80% power with an alpha level of 0.05 (two-tailed) calculated using G*Power v3.1 (refer to appendix F for this sample size calculation). To preserve power and account for potential issues with data quality or participant attrition, 30 participants were recruited.

2.1.10 Statistical Analyses

Statistical analyses were performed using the JASP software for Windows (version 0.14.1). Q-Q plots and Shapiro-Wilk tests were used to assess normality and skewness. Data that failed to meet assumptions of normality were log-transformed. All values are expressed as mean ± SD. A linear mixed-effects model with fixed effects of condition (exercise vs control), time (baseline, exercise/time-matched control point, and recovery), and sex (male vs female), and participants as a random effect was used to compare all cardiovascular, cerebrovascular, and cognitive variables followed by Bonferroni post-hoc tests. Pearson correlations were run to assess the relationship between MCAv and MAP, MCAv and $P_{ET}CO_2$, and hemodynamics (SBP, DBP and MAP) with N-Back reaction time (1- and 2-back reaction time) overall proportion correct, Corsi Block total, and 4-Choice mean reaction time and proportion correct. For all statistical tests, significance was set at $\alpha = 0.05$, and sex differences were considered to be present if a there was a significant condition * time * sex interaction.

2.2 Results

2.2.1 Participant Characteristics

Thirty young healthy normotensive participants (n = 15 females) volunteered to participate in this study. Overall, there were no differences in age (P=0.967), BMI (P=0.059), proportion of left hand dominant (P=0.334), years of education (P=0.535), resting MAP (P=0.060), and average

moderate-to-vigorous physical activity per week (P=0.898) between males and females. Conversely, height (P<0.001), weight (P<0.001), and total daily energy expenditure (P=0.0017) were significantly different between males and females. Participant characteristics are displayed in **Table 1**.

Table 1: Participant Characteristics

	Total Sample (n = 30)	Males (n= 15)	Females (n=15)
Age	23.77 ± 3.32	24.53 ± 4.00	23.00 ± 2.36
Height (cm)	169.22 ± 10.66	177.31 ± 7.96	161.13 ± 5.63*
Weight (kg)	73.18 ± 17.16	84.39 ± 16.13	61.98 ± 8.97*
BMI (kg/m^2)	25.32 ± 4.08	26.76 ± 4.32	23.87 ± 3.35
Resting MAP (mmHg)	87 ± 8	90 ± 8	84 ± 7
Proportion of left-hand dominant	6/30	2/15	4/15
Years of Education	17.2 ± 1.56	17.3 ± 1.80	17.1 ± 1.33
Total Daily Energy Expenditure (kcal/day)	896 ± 334	1087 ± 345	705 ± 182*
Average Moderate-to-Vigorous Physical Activity (min/week)	362 ± 207	367 ± 197	357 ± 224

Data are mean ±SD. * indicates significant difference compared to Males; P < 0.05.

2.2.2 Effect of Acute IHG on Hemodynamics

Acute IHG significantly increased SBP (Δ 36.52 ± 25.29 mmHg; time * condition interaction, P<0.001), DBP (Δ 26.93 ± 15.66 mmHg; time * condition interaction, P<0.001), MAP (Δ 26 ± 17 mmHg; time * condition interaction, P<0.001) **(Figure 2)**, and HR (Δ 18 ± 13 bpm; time * condition interaction, P<0.001). Following IHG, all hemodynamic variables returned to baseline levels (all P<0.001). There was a significant effect of sex for SBP (P=0.027) such that males experienced larger increases in SBP during IHG compared to females (Δ 46.20 mmHg vs. 26.84 mmHg, respectively). There were no observed sex-based differences for MAP (P=0.063),

DBP (P=0.086), or HR (P=0.324). Table 2 summarizes the observed hemodynamic responses to IHG and control.

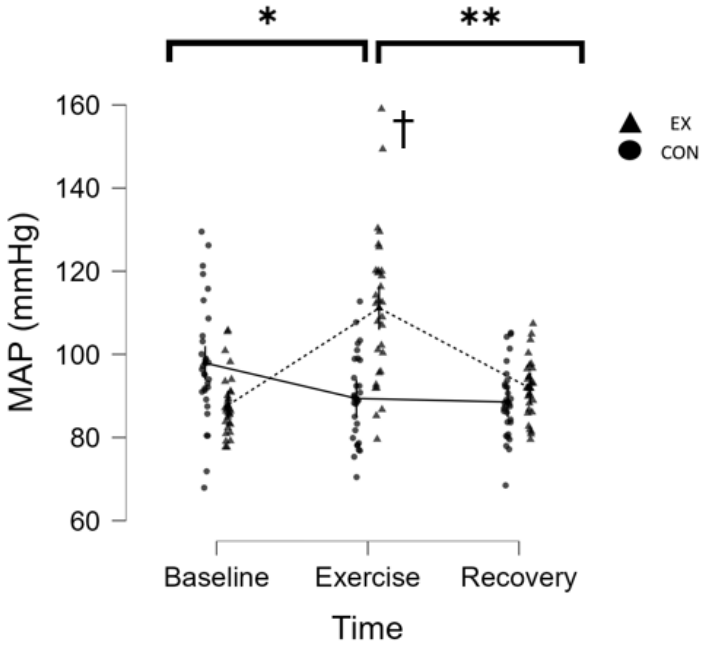

Figure 2. MAP response to baseline, exercise (corresponding to the end of the fourth set of exercise), and recovery. Triangles represent the exercise condition and circles represent the control condition. *Different from Baseline, **Different from Recovery. † Different from Recovery. † Different from control. A time * condition interaction was observed in the exercise condition (P<0.001).

2.2.3 Effect of Acute IHG on Cerebrovascular Measures

Acute IHG significantly increased MCAv (Δ 5.27 ± 19.4 cm/s; time * condition interaction, P<0.001; **Figure 3**) and MCA_{CVR} (Δ 0.71 ± 0.69 mmHg/cm/s; time * condition interaction, P=0.003; **Figure 4**). However, post-hoc analyses revealed that MCAv (P=0.082) and MCA_{CVR} (P=0.125) during IHG were not significantly different from the time matched point in the control condition. Following acute IHG, MCAv and MCA_{CVR} returned to baseline levels. No sex difference was observed for MCAv (P=0.176). A sex difference was observed for MCA_{CVR} (P= 0.042), with males displaying a larger increase in MCA_{CVR} (Δ 0.63 mmHg/cm/s vs. Δ 0.16 mmHg/cm/s, respectively) during IHG compared to females.

Given that CO_2 is an important regular of CBF, we measured $P_{ET}CO_2$ levels to account for its potential influence on MCAv. Both acute IHG and the control condition significantly decreased $P_{ET}CO_2$ (time effect, P<0.001) during IHG compared to baseline (Δ-1.2 in exercise vs Δ -0.93 in control; **Figure 5**). Following either exercise or control, $P_{ET}CO_2$ returned to baseline levels. No differences between conditions were observed (P=0.340), and no sex differences in $P_{ET}CO_2$ was observed (*P*=0.326).

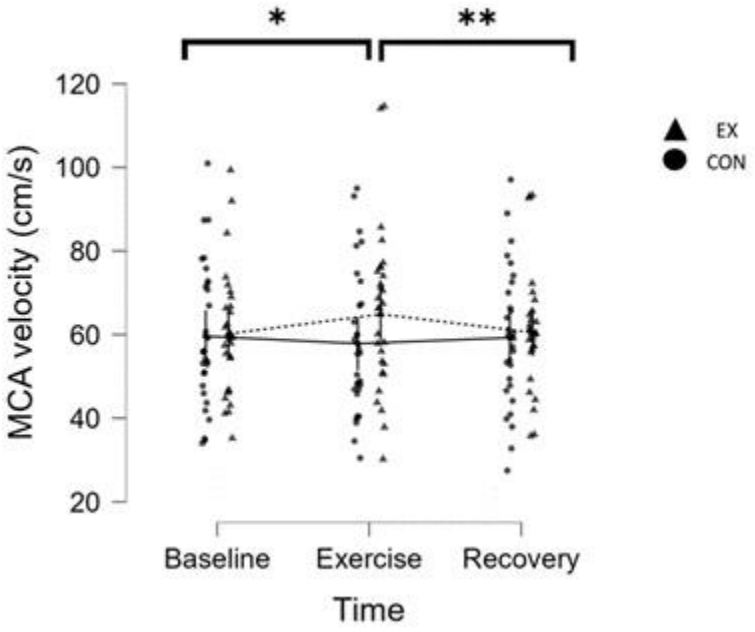

Figure 3. MCAv response (cm/s) to baseline, exercise (corresponding to the end of the fourth set of exercise), and recovery. Triangles represent the exercise condition and circles represent the control condition. *Different from Baseline, **Different from Recovery. A time * condition interaction was observed in the exercise condition (P<0.001).

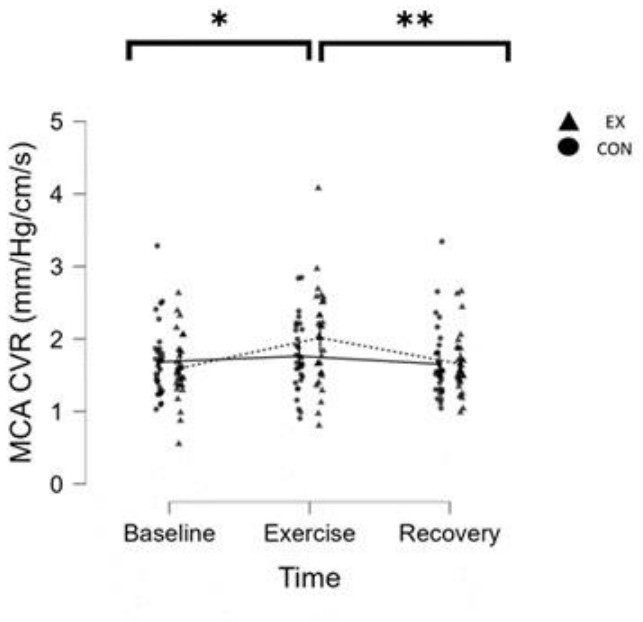

Figure 4. MCA_{CVR} response (mmHg/cm/s) to baseline, exercise (corresponding to the end of the fourth set of exercise), and recovery. Triangles represent the exercise condition and circles represent the control condition. *Different from Baseline, **Different from Baseline, **Different from Recovery. A time * condition interaction was observed in the exercise condition (P=0.003).

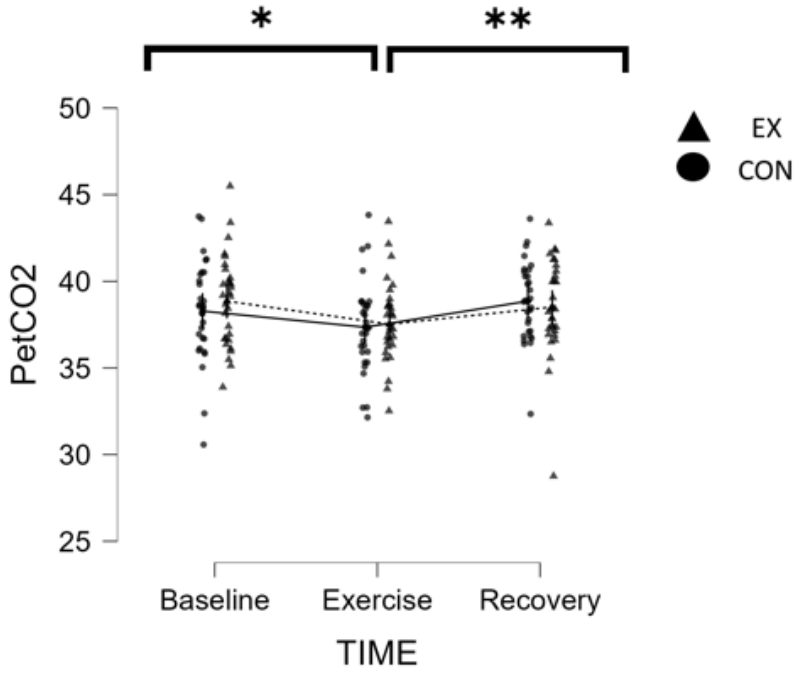

Figure 5. $P_{ET}CO_2$ (mmHg) response to baseline, exercise (corresponding to the end of the fourth set of exercise), and recovery. Triangles represent the exercise condition and circles represent the control condition. *Different from Baseline, **Different from Recovery. A time effect was seen in both conditions (P<0.001).

2.2.4 Effect of Acute IHG on Arousal

Acute IHG significantly increased arousal (Δ 2 ± 2 FAS score; time * condition interaction, $P<0.001$) compared to the control condition (**Figure 6**). Following IHG, arousal was elevated compared to the control condition. Post-hoc analyses revealed that the arousal remained elevated during recovery following IHG compared to control ($P<0.001$). There were no observed sex differences in the arousal response to IHG ($P=0.536$).

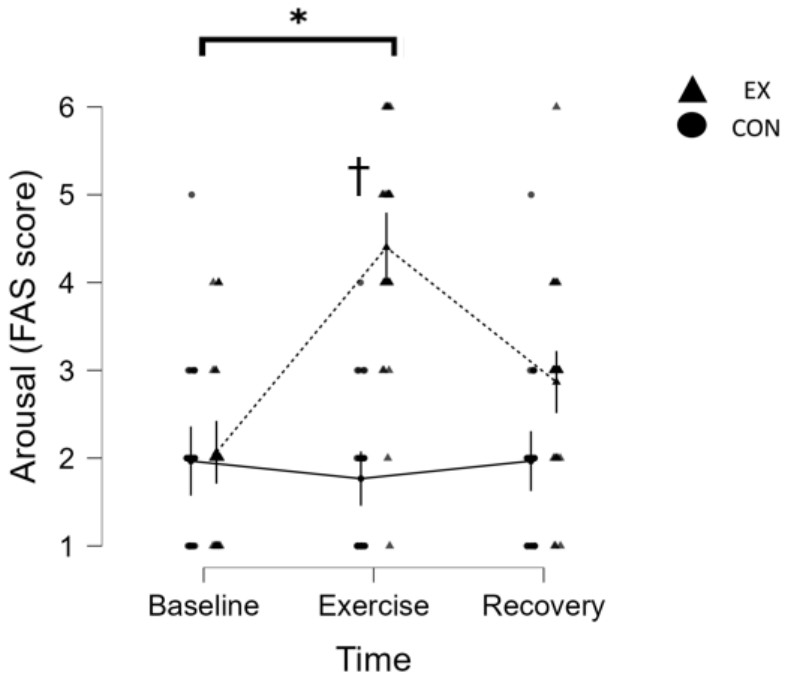

Figure 6. Arousal response (FAS score) to baseline, exercise (corresponding to the end of the fourth set of exercise), and recovery. Triangles represent the exercise condition and circles represent the control condition. *Different from Baseline, **Different from Recovery. † Different from control. A time * condition interaction was observed in the exercise condition (P<0.001).

Table 2: Cardiovascular, Cerebrovascular, Arousal, and CO_2 responses to IHG

	IHG Condition			Control Condition		
	Baseline	IHG	Recovery	Baseline	Control	Recovery
SBP (mmHg)	116.33 ± 12.26	152.85 ± 25.29*	118.53 ± 11.43#	117.81 ± 14.39	119.42 ± 14.29	113.69 ± 11.14
DBP (mmHg)	74.99 ± 6.78	101.92 ± 15.66*	78.89 ± 7.73#	74.93 ± 9.78	76.42 ± 9.70	74.80 ± 8.97

MAP (mmHg)	87 ± 8	113 ± 17*	92 ± 7#	98 ± 15	89 ± 10	89 ± 9
HR (bpm)	69 ±8	86 ± 13*	68 ±8#	69 ± 9	65 ± 9	67 ± 9
MCAv (cm/s)	60.19 ± 14.70	65.46 ± 19.36 *	61.04 ± 14.30#	59.60 ± 17.00	57.84 ± 17.21	59.25 ± 16.40
MCA_{CVR} (mmHg/cm/s)	1.60 ± 0.45	2.00 ± 0.69*	1.64 ± 0.41#	1.68 ± 0.50	1.76 ± 0.49	1.65 ± 0.50
$P_{ET}CO_2$ (mmHg)	38.86 ± 2.63	37.68 ± 2.40*	38.51 ± 2.81#	38.28 ± 2.90	37.35 ± 2.67*	38.85 ± 2.30#
Arousal (FAS score)	2 ± 1	4 ± 1*	3 ± 1#	2 ± 1	2 ± 1	2 ± 1

Data are mean ± SD. * $P < 0.05$ vs. Rest, # $P < 0.05$ vs. Exercise/Control.

2.2.5 Effect of Acute IHG on N-Back Performance

Acute IHG did not affect overall proportion correct (time * condition interaction, P=0.471), N1 d-prime (time * condition interaction, P=0.254), or N2 d-prime (time * condition interaction, P=0.764). A significant time * condition interaction (P=0.050) was observed for overall reaction time, with the control condition displaying a larger decrease in reaction time compared to the exercise group (Δ -4.13 ± 136.02 ms vs Δ -33.40 ± 104.03 ms, respectively). When separated by difficulty level (1-back or 2-back), no significant time * condition interactions were observed for either 1-back reaction time (P=0.338) or 2-back reaction time (P=0.061). No sex differences in overall reaction time (P=0.433), 1-back reaction time (P=0.432), 2-back reaction time (P=0.880), overall proportion correct (P=0.425), 1-back d-prime (P=0.588), or 2-back d-prime (P=0.149) were observed.

2.2.6 Effect of Acute IHG on Corsi Block Performance

Acute IHG did not improve block total on the Corsi Block forward test compared to a control condition (time * condition interaction, P=0.070). No sex differences in block total were observed (P=0.327).

2.2.7 Effect of Acute IHG on 4-Choice Performance

Acute IHG did not improve reaction time (time * condition interaction, P=0.266) or proportion correct (time * condition interaction, P=0.881) on the 4-Choice Reaction Time test. A significant effect of time (P=0.005) was observed for 4-Choice reaction time, with both the EX and CON group decreasing their reaction times from pre- to post-intervention. No sex differences in reaction time (P=0.449) or proportion correct were observed (P=0.119).

Table 3: Cognitive Responses to IHG

	Exercise		**Control**	
N-Back	*Pre*	*Post*	*Pre*	*Post*
Overall Reaction Time (ms)	562.86 ± 120.08	558.74 ± 136.02	580.28 ± 128.35	546.88 ± 104.03
Overall Proportion Correct (%)	0.97 ± 0.031	0.96 ± 0.063	0.97 ± 0.031	0.97 ± 0.040
N1 Reaction Time (ms)	528.70 ± 108.52	516.92 ± 115.23	540.74 ± 111.94	513.36 ± 102.53
N2 Reaction Time (ms)	596.04 ± 153.48	604.14 ± 188.74	622.00 ± 159.43	582.91 ± 115.44
N1 D-Prime	4.61 ± 0.75	4.50 ± 1.10	4.41 ± 0.84	4.54 ± 0.83
N2 D-Prime	4.05 ± 1.09	3.88 ± 1.31	3.87 ± 1.08	3.89 ± 1.05
Corsi Block				
Block Total	76.97 ± 22.04	78.63 ± 26.91	71.70 ± 26.91	83.67 ± 30.30
4-Choice				
Reaction Time (ms)	400.55 ± 43.98	389.82 ± 40.38	398.88 ± 43.16	393.94 ± 40.95
Proportion Correct (%)	0.95 ± 0.037	0.93 ± 0.033	0.94 ± 0.038	0.93 ± 0.043

Data are mean ± SD.

2.2.8 Exploratory Correlations

We assessed whether changes in CO_2 were associated with changes in CBF. Changes in MCAv during exercise were significantly correlated with changes in $P_{ET}CO_2$ (r = 0.376, *P*=0.049). MAP is also known to influence CBF, therefore, we sought to determine whether changes in MAP would be significantly correlated with changes in CBF. We found that changes in MAP during exercise were not correlated with changes in MCAv (r = 0.094, *P*=0.627). We evaluated whether cardiovascular reactivity was correlated with changes in cognitive function. For the N-back test, the change in 1-back reaction time was significantly correlated with MAP reactivity to IHG (r = -0.421, *P*=0.023) **(Figure 7)**. However, this relationship was not present for changes in 2-back condition and MAP reactivity (r = 0.071, *P*=0.713). There were no relationships between changes in hemodynamics and performance on the other cognitive tests.

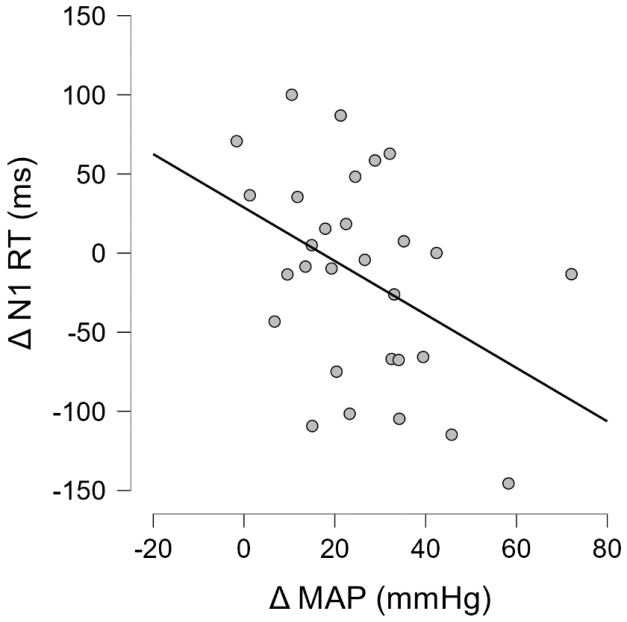

Figure 7. Relationship between changes in MAP (mmHg) and 1-Back reaction time during IHG. Pearson's r = -0.421, *P*=0.023

2.3 Discussion

The purpose of this study was to determine whether an acute bout of IHG improved aspects of cognitive function in normotensive adults. We also investigated whether cognitive responses to IHG were differentially impacted by biological sex. We hypothesized that compared to the control condition, IHG would: 1) improve cognitive function; 2) that women would have greater improvements in cognitive function following acute IHG compared to men; and 3) individuals with lower cardiovascular reactivity to IHG would have greater improvements in cognitive function. Contrary to our hypothesis, acute IHG did not affect cognitive function despite increasing arousal and CBF. Our results also suggest that there are no sex differences in cognitive performance after acute IHG, despite observed sex differences in SBP and MCA_{CVR} responses. Further, we found that higher cardiovascular reactivity was associated with improved cognitive performance on a post-exercise N-Back test, despite no overall cognitive improvement. Overall, our findings suggest that acute IHG did not improve cognitive function in young healthy adults, despite increases in potential mechanistic moderators of cognitive improvement.

Contrary to previous literature, we found no cognitive improvement following acute IHG (Bachman et al., 2023; Mather et al., 2020; Washio et al., 2021). A potential reason for this may be due to differences in exercise protocol, namely, the intensity (% of MVC) the modality (rhythmic vs IHG), and the duration (acute vs chronic) of exercise. Washio et al. (2021) reported an improvement in reaction time on a Go/No-Go task following acute IHG using a similar intensity (25% MVC). The slightly higher intensity used in our study may have contributed to a higher degree of fatigue, potentially impacting post-exercise cognitive performance. Indeed, high-intensity IHG attenuates cognitive improvement, as performing 70% MVC until volitional exhaustion resulted in minimal improvement on a Stroop Task compared to lower intensities of IHG (Brown & Bray, 2015). However, it is unlikely that the 30% MVC intensity used in our study

impacted fatigue, as participants underwent a 5-min recovery. Furthermore, prior to post-exercise cognitive testing, we confirmed that participant's BP and HR variables were within 5 mmHg/bpm of their baseline levels, ensuring a full recovery from exercise. A recovery of 5-min was chosen given that the largest effect sizes for post-exercise cognitive enhancement exist following a delay of at least 5 min (Chang et al., 2012). Moreover, evidence shows that females have better fatigue resistance during IHG compared to males (Hicks et al., 2001), which may have mitigated the detrimental effects of fatigue on post-exercise cognitive function. However, given that we did not observe any sex differences in cognitive performance nor did IHG improve cognitive function in general, we have confidence that IHG was not effective at improving cognitive function in our sample. Our results conflict previous work by Mather et al. (2020) and Bachman et al. (2023), who reported improved reaction times on an oddball and n-back task respectively following rhythmic handgrip exercise. Rhythmic handgrip exercise induces lower sympathetic stimulation than IHG (Cook et al., 2016), which may have modulated arousal levels to a more optimal point for cognitive benefit, in line with the inverted-U curve hypothesis (McMorris, 2009). Our results align with Saito et al. (2021) and Yamada et al. (2021), who reported no cognitive improvement on a Go/No-Go and Stroop Task respectively following acute IHG. The lack of cognitive improvement seen in Saito et al. (2021) may be due to a low-intensity IHG protocol, resulting in low sympathetic stimulation, whereas Yamada et al. (2021) protocol may have been too intense, with participants resting only 1-min between contractions. Moreover, a single bout of IHG may not be sufficient to induce improvements in cognitive function, as opposed to multiple repeated bouts of IHG. Indeed, preliminary evidence from Dempster et al. (2018) shows that 8-weeks of IHG training significantly improved the time to completion on the Trail Making Test Part A in older hypertensive adults. Although the mechanisms of cognitive improvement following chronic IHG are not well

understood, evidence suggests that reductions in BP and the long-term enhancement of CBF to support neural function may play a role (Dempster et al., 2018; El-Sayes et al., 2019). In contrast, a single bout of acute IHG is unlikely to induce any significant structural or molecular change within the brain, with enhancements in cognitive function likely being attributed to a state of increased arousal, which is transient and temporary (Bachman et al., 2023; Mather et al., 2020; Saito et al., 2021). Furthermore, although evidence suggests that individuals with higher cardiovascular fitness may experience greater benefits following acute exercise (Brisswalter et al., 2002; Chang et al., 2012), we did not observe an improvement in cognitive function following acute IHG despite our population being seemingly very fit (high levels of moderate-to-vigorous physical activity on the PAR-7). Therefore, it is possible that participants may have demonstrated a ceiling effect, in which they were close to their cognitive plateau due to their high levels of physical activity.

We explored the previously described relationship between lower cardiovascular reactivity and cognitive improvement post-IHG (Washio et al., 2021). Washio et al. (2021) found that participants with lower cardiovascular reactivity during IHG displayed greater improvements in reaction time on a Go/No-Go task post-exercise. In contrast, we observed that higher cardiovascular reactivity was associated with improved 1-back reaction time post-exercise. A potential reason for the differing relationships between cognitive function and cardiovascular reactivity may be due to differences in the cognitive domain tested. Indeed, lower cardiovascular reactivity is associated with worse processing speed (Ginty et al., 2011), the main cognitive domain of the Go/No-Go task (Gomez et al., 2007). Although the mechanisms are not well understood, lower cardiovascular reactivity may reflect lower neural activity in the prefrontal cortex leading to

less activation of the prefrontal cortex, an area of brain important for cognitive function (Carroll et al., 2017).

Improvements in cognitive function following exercise have been purported to be facilitated by increased CBF (Paulson, 2002), although this relationship is tenuous and not well supported (Renke et al., 2022). Increased CBF would provide greater nutrients and oxygen delivery to the brain to support performance of cognitively demanding tasks (Ogoh et al., 2014). However, previous studies have failed to show that changes in CBF are associated with changes in cognitive function (Ogoh et al., 2014; Shoemaker et al., 2021; Shoemaker et al., 2020). Our study aligns with this trend, as we did not observe any changes in cognitive function post-exercise despite an increase in MCAv during IHG. The increase in MCAv was accompanied by a corresponding increase in MCA_{CVR}, which may seem contradictory as MCA_{CVR} actively resists blood flow via vasoconstriction. However, in the context of IHG, this vasoconstriction is likely an autoregulatory response to blunt the potentially damaging effects of high BP (Perry & Lucas, 2021). Another hypothesized mechanism by which IHG improves cognitive function is arousal (McMorris, 2009). Arousal and cognitive function are speculated to have an inverted-U relationship, with cognitive function peaking at moderate levels of arousal and decreasing at excessively high and low levels of arousal (Aston-Jones & Cohen, 2005). We found that IHG increased arousal levels compared to the control condition and remained elevated during the recovery period. This finding aligns with Mather et al. (2020), who reported increased arousal during a post-exercise oddball task, corresponding to improved reaction time. Thus, increased phasic arousal following exercise may be responsible for acute IHG's beneficial effects on cognitive function, rather than decreased tonic arousal. Overall, in line with our hypothesis, acute

IHG significantly increased CBF and arousal compared to a control, however, increases in these mechanistic moderators of cognitive improvement did not lead to enhanced cognitive function.

We assessed whether there were sex differences in the effect of IHG on cognitive function, given that biological sex may influence cognitive responses to exercise (Barha et al., 2019; Herold et al., 2021). We did not find any differences between males and females on any of the cognitive tests used in this trial. We did observe sex differences in the SBP and MCA_{CVR} response to exercise; however, these differential responses had no impact on cognitive performance. Although the data regarding sex differences in post-exercise cognitive function is sparse, our findings align with previous research, which noted no effect of biological sex on episodic memory following acute exercise (Johnson et al., 2019; Johnson & Loprinzi, 2019). Interestingly, we found that males displayed greater changes in SBP during IHG compared to females. A potential explanation is that women display a lower metaboreflex compared to males due to a lower accumulation of metabolites (Ettinger et al., 1996; Smith et al., 2019), and lower sympathetic sensitivity to IHG (Hogarth et al., 2007). The differences in SBP likely contributed to the observation that males displayed higher MCA_{CVR} during IHG compared to females. This stems from two reasons, how MCA_{CVR} is calculated and increased vasoconstriction. MCA_{CVR} was calculated as MAP/MCAv. Therefore, an increase in SBP, which is used in the determination of MAP (1/3 SBP * 2/3 DBP), would increase MAP, thus increasing MCA_{CVR}. Recent evidence suggests that BP and CBF may have a pressure-passive relationship, whereby changes in BP result in proportional changes to CBF (Willie et al., 2014). In line with the pressure-passive relationship, we observed a concomitant elevation of MCA_{CVR} alongside SBP increase, which denotes a protective mechanism to safeguard the brain against excessive BP (Perry & Lucas, 2021). Thus, greater increases in SBP would evoke increased vasoconstriction to counteract the impact of elevated SBP on the brain.

2.3.1 Limitations and Future Directions:

Our study had limitations worthy of acknowledgement. Firstly, TCD assumes a constant diameter of the insonated blood vessel to provide a stable surrogate for CBF (Willie et al., 2011). However, MCA diameter has been shown to change in response to stimuli present in this study (changes in MAP and $P_{ET}CO_2$). Furthermore, the majority of our participants were right-handed (80%), therefore only 20% of our MCAv measurements were contralateral to the exercising hand, given that we always measured the right MCA. A previous study reported that the cross-sectional area of the contralateral MCA constricted in response to rhythmic handgrip exercise, resulting in an attenuated increase in CBF (Verbree et al., 2017). Therefore, we cannot conclude that increased MCAv would simultaneously occur in both hemispheres during IHG. However, a sensitivity analysis revealed that the absolute (P=0.470) and percent change (P=0.531) in MCAv from baseline to IHG for both left- and right-handed participants was not significantly different. Secondly, our population consisted of young healthy adults. Although we did not find any improvements in cognitive function following acute IHG, our findings cannot be generalized to older adults and/or people with hypertension. Given that IHG is the most effective method of lowering BP compared to other exercise modalities (Edwards et al., 2023; Wood et al., 2020), people with hypertension may stand to gain more from IHG. Furthermore, acute IHG may be insufficient to induce transient cognitive changes, and rather chronic IHG may be required to induce cognitive benefits via BP reduction and long-term CBF enhancement. Future studies should consider using a chronic IHG paradigm in older individuals with or without hypertension. Thirdly, our population was seemingly very fit, denoted by the high amounts of moderate to vigorous physical activity per week on the PAR-7. A potential explanation for this may be due to the nature of the PAR-7, which classifies walking to and from various locations as moderate intensity exercise. Given that our participant demographic was primarily comprised of university students,

a group who commutes daily to class by walking compared to other populations (e.g., office workers who drive to work), this may have led to an overestimation in moderate intensity domain of physical activity. For example, other physical activity recalls may classify walking as light intensity exercise as opposed to moderate intensity exercise. Evidence suggests that individuals with high cardiovascular fitness experience greater cognitive improvement following acute exercise (Chang et al., 2012), however, we observed no improvement in cognitive function. All PAR-7 reporting was done in an interview fashion to reduce the amount of recall and social desirability bias. Finally, there were significant variations in cardiovascular reactivity among participants during IHG, with some individuals displaying a decrease in MAP during the fourth set of IHG compared to the first set of IHG. A potential explanation for this may be that participants did not achieve their true MVC during the calibration phase, thus lowering the intensity of their subsequent IHG exercise. However, we are confident that participants performed their true MVC as they were coached on how to properly perform MVC's during a familiarization visit and were verbally encouraged during the experimental visit to maximally perform 3 MVC's, in which the highest was taken to calibrate the subsequent IHG exercise.

2.3.2 Conclusion:

Despite increases in physiological factors that may increase cognitive abilities, acute IHG did not affect cognitive function, and there were no sex differences in cognitive outcomes. Our findings stand in opposition to recent work and suggest that increased CBF and arousal do not contribute to cognitive function enhancement in young healthy adults. Therefore, acute IHG may not improve executive function, working memory, or processing speed in young adults. Future research should focus on utilizing IHG as a chronic paradigm in older adults and people with hypertension and examine whether biological sex-based differences in cognitive function exist in the above-mentioned populations.

2.4 References

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Appendices

Appendix A – 7-Day Physical Activity Recall

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The Seven-Day Recall

PAR#: 1 2 3 4 5 6 7 Participant_____

Interviewer_____Today is_____. Today's Date_____

1. Were you employed in the last seven days? 0. **No** (Skip to **Q#4**) 1. **Yes**
2. How many days of the last seven did you work? ____ days
3. How many total hours did you work in the last seven days? ____ hours last week
4. What two days do you consider your weekend days? _____ _____
   (mark days below with a squiggle)

WORKSHEET

		DAYS						
	SLEEP	1 __	2 __	3 __	4 __	5 __	6 __	7 __
MORNING	Moderate							
	Hard							
	Very Hard							
AFTERNOON	Moderate							
	Hard							
	Very Hard							
EVENING	Moderate							
	Hard							
	Very Hard							
Total Min Per Day	Strength:	____	____	____	____	____	____	____
	Flexibility:							

4a. Compared to your physical activity over the past three months, was last week's physical activity more, less or about the same?
1. More
2. Less
3. About the same

Worksheet Key:
An asterisk (*) denotes a work-related activity.
A squiggly line through a column (day) denotes a weekend day.

Rounding: 10-22 min.=.25 1:08-1:22 hr/min.=1.25
23-37 min.=.50
38-52 min.=.75
53-1:07 hr/min. =1.0

Appendix B – Menstrual Cycle Questionnaire

Menstrual Cycle Questionnaire

Handgrip and Cognition Study

Please read through the following questions and answer as best as possible.

1. When was your last period? _____

2. How long do your periods last? _____

3. If you are not currently having a menstrual cycle, when was the last time you menstruated?

_____

4. Are you taking oral contraceptive pills (birth control pills) or other hormones?

_____

a. If so, how long have you been taking them? _____

Appendix C – Felt Arousal Scale (FAS)

FELT AROUSAL SCALE

Estimate here how aroused you actually feel. Do this by circling or pointing to the appropriate number. By "arousal" here is meant how "worked-up" you feel. You might experience high arousal in one of a variety of ways, for example, excitement or anxiety or anger. Low arousal might also be experienced by you in one of a number of different ways, for example, as relaxation or boredom or calmness.

6 HIGH AROUSAL

5

4

3

2

1 LOW AROUSAL

Appendix D – HiREB Approval Letter

HiREB
Hamilton Integrated Research Ethics Board

Oct-13-2022

Project Number: 14058

Project Title: The effect of acute isometric handgrip exercise on cerebral blood flow and cognitive function in people with hypertension

Principal Investigator: Dr Jeremy Walsh

The Hamilton Integrated Research Ethics Board (HiREB) has reviewed and approved the abovementioned study.

The following documents have been approved on both ethical and scientific grounds:

Document Name	Document Date	Document Version
GRIP_deidentifier key	Jul-06-2022	1
GRIP_Study Budget	Jul-06-2022	1
GRIP_Study_DCS_V1	Jul-06-2022	1
GRIP_Study_Phone_Email_Correspondance_Script_V1docx	Jul-06-2022	1
GRIP_Study_Recruitment_Poster_V2_CLEAN	Aug-12-2022	2
GRIP_Study_Protocol_V2_CLEAN	Aug-12-2022	2
MoCA_Test_GRIP_Study	Aug-12-2022	1
GRIP Study_Cognitive Testing Script	Aug-12-2022	1
Consent_Form_GRIP_V2_Clean	Oct-12-2022	2

The following documents have been acknowledged:

Document Name	Document Date	Document Version
Walsh_tcps2 certificate	Jul-06-2022	1
HIREB_Response_to_Reviewers_14058	Aug-24-2022	1

In light of the current COVID-19 pandemic, while HiREB has reviewed and approved this application, the research must be conducted in accordance with institutional and/or public health requirements.

Please Note: All consent forms and recruitment materials used in this study must be copies of the above referenced documents.

We are pleased to issue final approval for the above-named study for a period of 12 months from the date of the HiREB meeting on August 3, 2022. Continuation beyond that date will require further review and renewal of HiREB approval. Any changes or revisions to the original submission must be submitted on a HiREB amendment form for review and approval by the Hamilton Integrated Research Ethics Board.

PLEASE QUOTE THE ABOVE REFERENCED PROJECT NUMBER ON ALL FUTURE CORRESPONDENCE

Sincerely,

Frederick A Spencer

Dr. Frederick A. Spencer, MD
Chair, Hamilton Integrated Research Ethics Board

The Hamilton Integrated Research Ethics Board (HiREB) represents the institutions of Hamilton Health Sciences, St. Joseph's Healthcare Hamilton, Research St. Joseph's-Hamilton, the Faculty of Health Sciences at McMaster University, and Niagara Health and operates in compliance with and is constituted in accordance with the requirements of: The Tri-Council Policy Statement on Ethical Conduct of Research Involving Humans; The International Conference on Harmonization of Good

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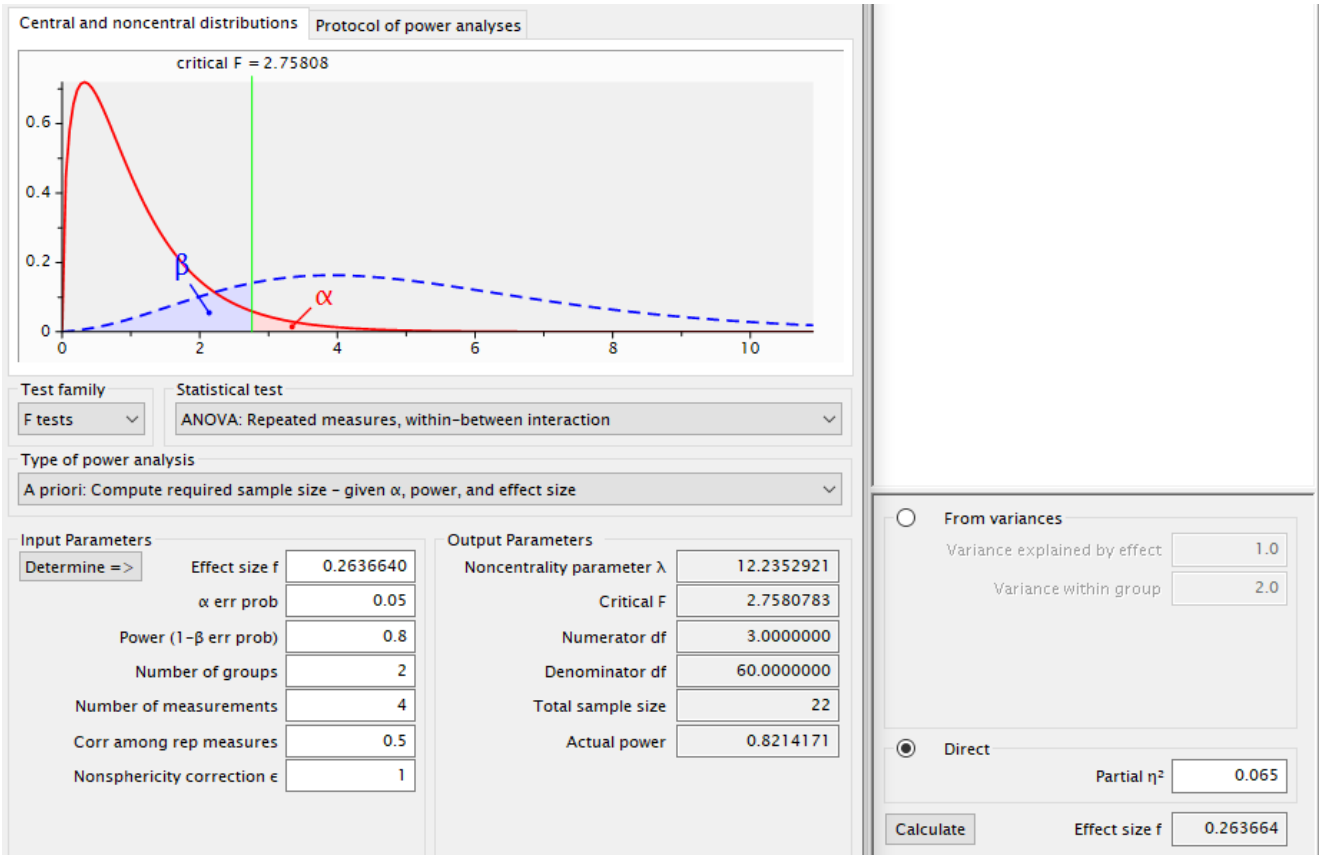
Clinical Practice Guideline (ICH GCP); Part C Division 5 of the Food and Drug Regulations of Health Canada, Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act 2004 and its applicable Regulations. For studies conducted at St. Joseph's Healthcare Hamilton, HiREB complies with the Health Ethics Guide of the Catholic Alliance of Canada.

Appendix E – Sample Size Calculation to Detect Cognitive Effects Following IHG



Appendix F – Sample Size Calculation to Detect Sex Differences in Cognitive Function