The Role of Dopamine on Central Neuromuscular Activation during Passive Hyperthermia

Aiden Scholey, B.Kin (Hons.)

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Health Sciences (Kinesiology)

Supervisor: Stephen S. Cheung, Ph.D.

Faculty of Applied Health Sciences, Brock University
St. Catharines, ON

Abstract

Acute methylphenidate (MPH) (dopamine reuptake inhibitor) ingestion improves cycling time trial performance and power output in hot conditions (30°C), while also allowing for tolerance of higher core temperatures. However, the mechanisms for why this occurs have not been isolated. One potential explanation for this ergogenic benefit is that MPH intake was enhancing neuromuscular activation. Thus, this research project examined the influence of MPH on neuromuscular activation during hyperthermia. Participants ingested either placebo (PLA; 20mg) or MPH (Ritalin; 20mg) 1 hour prior to a passive heating protocol. 6 participants were passively heated until volitional cessation, or after 3 hours of heating had passed. Neuromuscular responses, as indicated by maximal voluntary contraction (MVC) force, and voluntary activation (VA) percentage were assessed prior to drug ingestion, 1 hour after MPH wash-in, throughout the heating protocol and at cessation of heating. A primary non-significant finding of this research project was that participants reached higher rectal temperatures (T_{re}) by ~0.3°C in trials where they ingested MPH ($p = 0.065$). This effect occurred in absence of any differences in thermal comfort or sensation ratings or heating durations. However, while MPH improves thermal tolerance, it was not able to attenuate the decreases in MVC force and VA that occurred during passive heating. Therefore, the aforementioned ergogenic benefits that MPH has in hot conditions are not occurring as a result of enhanced neuromuscular activation.

Acknowledgements

I would like to firstly thank my supervisor, Dr. Stephen Cheung. Your support and guidance have helped me improve not only as a researcher but as a person as well. I am grateful that you gave me the opportunity to design my own thesis project and allowed me to change projects when we ran into COVID-19 restrictions. Your attention to detail and high standards has helped me to improve in many aspects of my life beyond research. It has been a privilege to work with you during my studies.

I was fortunate to have a very supportive and patient advisory committee. Dr. David Gabriel and Dr. Mike Holmes, I would like to thank you for your willingness to answer questions when I had them and challenge me to expand upon my current knowledge. You have both been very willing to adapt when I changed thesis projects and I am very grateful for that.

To the former and current members of the Environmental Ergonomics Laboratory: Gary Hodges, Phil Wallace, Kate Wickham, Scott Steele, Jake Scott and Leed McNabb, thanks for your support. I am especially thankful for Gary Hodges, Rob Kumar and Jake Scott, who assisted with the data collection for this study. Thanks to the three of you for creating an atmosphere where we could enjoy science, it was a privilege to work with you. Additionally, thanks to Matt Mallette who was integral in this projects design and was always willing to support me when I had questions and concerns about the data analysis.

Lastly, thanks to my support system outside of Brock University. To Pam, Scott, Nick, Kiersten and my friends, thank you for providing me with laughs and emotional support. Mom and Dad, you have played a huge role in my journey to accomplish this goal. Your emotional

and financial support have allowed me to get to this point. You have both sacrificed more than I can imagine to allow me to get to this point of my life and I am forever grateful for it.

Table of Contents

Abstract ..... ***II***

Acknowledgements ..... ***III***

List of Tables ..... ***VI***

List of Figures ..... ***VII***

List of Abbreviations ..... ***VIII***

Introduction ***1***

Literature Review ***3***

Efferent Nervous System **3**

Muscle Fatigue **5**

Heat and Performance **8**

The Effects of Dopamine on Performance in the Heat **9**

Objectives and Hypotheses ***12***

Methods ***13***

Experimental Design **13**

Participants **13**

Participant Inclusion Criteria **13**

Participant Exclusion Criteria **13**

Familiarization Session **13**

Experimental Sessions Drug Manipulation **16**

Experimental Protocol **17**

Data Processing **21**

Statistical Analysis **22**

Results ***23***

Thermal Strain **23**

Neuromuscular Function **25**

Discussion ***30***

Limitations **35**

Bibliography ***37***

Appendices ***43***

Appendix A **43**

List of Tables

Table 1. Full Neuromuscular Test Battery 16
Table 2. Changes in additional thermal strain variables at the baseline, post-wash-in and thermal limit time points, in both PLA and MPH conditions. 24
Table 3. Changes in M-Wave and Twitch Characteristics at the baseline, post-wash-in and thermal limit time points, in both PLA and MPH conditions. 29

List of Figures

Figure 1. Example interpolated twitch technique application. Modified from (53). 15
Figure 2. Experimental setup. 19
Figure 3. Forearm setup. 20
Figure 4. Sample experimental protocol. 24
Figure 5. Core temperature at baseline, post-wash-in and thermal limit time points in the PLA (Black) and MPH (White) conditions (n=6). Horizontal bars represent group means. 24
Figure 6. Changes in 3s MVC Force (Top, n=6) and VA (Bottom, n=4) during passive heating, in the PLA (black) and MPH (white) condition. Horizontal bars represent group means. 26
Figure 7. Changes in 45s MVC force (Top, n=6) and VA (Bottom, n=4) during passive heating in both PLA (black) and MPH (white) conditions. Horizontal bars represent group means. 28

List of Abbreviations

ANOVA	Analysis of Variance
CNS	Central Nervous System
T_{core}	Core Temperature
ECG	Electrocardiogram
EMG	Electromyography
FCR	Flexor Carpi Radialis
$T_{forearm}$	Forearm Temperature
ITT	Interpolated Twitch Technique
MVC	Maximal Voluntary Contraction
MPH	Methylphenidate
NE	Norepinephrine
PL	Palmaris Longus
PLA	Placebo
PO/AH	Pre-Optic Anterior Hypothalamus
sEMG	Surface Electromyography
TS	Thermal Sensation
TC	Thermal Comfort
TMS	Transcranial Magnetic Stimulation
T_{re}	Rectal Temperature
T_{sk}	Skin Temperature
VA	Voluntary Activation

Introduction

Hyperthermia, or elevated body temperatures, reduces exercise capacity and causes premature fatigue (1). Fatigue, as described by the taxonomy of Kluger et al (2) is a concept with two distinct attributes: 1) performance fatigability - the decline in an objective measure of performance over a discrete period, and 2) perceived fatigability - changes in the sensations that regulate the integrity of the performer. Thus, fatigue is a "disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability" (3). This definition integrates the possibility that fatigue can be caused by psychological changes alongside physiological changes. This is often displayed with higher ratings of perceived exertion during exercise at a constant workload in the heat (4).

A common result of hyperthermic fatigue is a failure of the neuromuscular system to produce muscle force at desired levels. This neuromuscular impairment has been reported directly from passive hyperthermia, independent of fatigue from exercise (5–9). When examining the literature, it becomes clear that high core temperatures (T_{core}) progressively reduces voluntary isometric force production and central voluntary muscle activation (5, 6, 8), an effect independent of peripheral temperature (6), and fitness (7). This suggests that changes within the central nervous system (CNS) with hyperthermia strongly influences neuromuscular impairment.

Neurotransmitter activity may have a role in this hyperthermic neuromuscular impairment. Of particular interest is dopamine, a monoamine catecholamine in the brain. Dopamine levels are often manipulated using methylphenidate (MPH, common name Ritalin®)

a dopamine reuptake inhibitor. When MPH is ingested prior to exercising in the heat, it provides ergogenic benefits, specifically improving cycling time trial performance through increasing wattage output (10). The mechanisms explaining how this occurs are not yet known, however, it may be a result of enhanced neuromuscular activation. Only one study exists examining neuromuscular responses to dopamine manipulation in humans, which reported no change in central fatigue following thermoneutral exercise (11). However, it is not known how MPH ingestion will affect the impairment in central drive that is seen during hyperthermia, and this is the gap in the literature that this research project will fill.

Literature Review

Efferent Nervous System

Movements are executed via the efferent portion of the CNS. This process begins in the brain, and specifically in the motor cortex which includes multiple areas, the premotor area, supplementary motor area and primary motor cortex, all of which appear to have different roles in movement (12). The premotor area is involved in coupling cues with motor acts, whereas the supplementary appears to have a role in the planning of movement and the primary motor cortex has been implicated in control of muscle force (12). Movement related information is carried away from the brain to the spinal cord via the corticospinal tract, which plays a vital role in voluntary control of distal movements (13, 14). One of the major connections the corticospinal tract makes is to alpha motor neurons, which are directly responsible for causing muscle contractions. Alpha motor neurons are responsible for activating groups of skeletal muscle fibres, a grouping that is often referred to as a motor unit. Barring any pathology, if an action potential appears in a motor neuron, all muscle fibres innervated by it will activate roughly simultaneously (15), producing a muscle contraction.

Skeletal muscle has a highly structured organization (16). Single muscle cells are divided into myofibrils that are formed from a series of axially arranged sarcomeres, each consisting of interdigitating systems of axial filaments – the thick and thin filaments (16). Thin filaments extend from Z-disks and contain a double-helical strand of actin along with troponin-tropomyosin complexes that are involved in the regulation of contraction (16). Thick filaments consist of myosin which extend radially (16). Initial reports in 1954 (17, 18) first described that striated muscle shortens as a result of the sliding between actin and myosin, a process which

has since been termed the sliding filament theory. This sliding process consists of actin and myosin forming cross-bridges which use chemical energy derived from adenosine triphosphate (ATP) hydrolysis to drive an axial sliding motion between thick and thin filaments (16). This process can occur following electrical activation of the cell, which leads to opening of calcium channels, and subsequent calcium release from the sarcoplasmic reticulum into the myofibril (16). When muscle is relaxed, the troponin-tropomyosin complex blocks myosin-binding sites on the thin filament (16). However, when calcium enters the myofibril, it binds with the troponin-tropomyosin complex, causing a conformational changed that reveals the binding sites and allows for cross-bridge formation (16). After binding, cross-bridges release energy derived from ATP hydrolysis and generate force, driving muscle contraction (16). Reuptake of calcium into the sarcoplasmic reticulum ends the cycle and the muscle relaxes.

Muscle force production can be modulated in several ways, specifically by altering how many motor units are activated, or by changing the frequency at which they fire (19). First, recruitment, which is the process of activating additional motor units, a process that follows Henneman's size principle, where small motor units are activated at first, and larger motor units are activated as force requirements increase (15). The reverse occurs for deactivating motor units, where the largest motor units are turned off first as force requirements decrease (15). An additional technique of grading muscular force is through rate coding by increasing the frequency of action potentials in active motor units (15). The use of these strategies seems to depend on muscle size, where smaller muscles rely more on rate coding and larger muscles rely more on recruitment (15). The pattern of muscle activation can also affect muscle force. Sometimes motor units will fire with two discharges very quickly, often referred to as a doublet

(15). These doublets are most prevalent at the onset of muscular contractions, particularly playing an important role when quickly ramping up force levels (20).

Motor unit recruitment processes contribute to the basic functioning of the efferent portion of the neuromuscular system. A prominent characteristic of the system is its adaptability (21). When subjected to a stimulus (acute or chronic), such as training or aging, it can adapt to the altered demands of usage. These adaptations are extensive and can affect most aspects of the system, both morphological and functional. For example, one of the best-known acute adaptations is a phenomenon that is referred to as muscle fatigue.

Muscle Fatigue

Muscle fatigue refers to a decreased force/power generating capacity during and following prolonged or repeated muscle activity (22). Numerous factors influence muscle output during prolonged exercise and generally the study of muscle fatigue in humans focuses on both peripheral and central mechanisms. Peripheral fatigue refers to changes that occur at or distal to the neuromuscular junction (22). Whereas central fatigue refers to changes within the brain and CNS. Both types of fatigue will be explored in the following section.

Peripheral fatigue in individual muscles is studied with percutaneous electrical stimulation of the motor nerve or directly of the muscle, bypassing the brain and spinal cord (23). For example, a single supramaximal stimulus (generally 120-150% of the stimulus that first evokes a contraction) elicits an action potential in the muscle cells and force is generated (23). The resultant compound muscle action potential (M-wave) and the maximal mechanical twitch response (peak twitch force) are often recorded (23). A reduction in M-wave amplitude is interpreted as evidence of impaired neuromuscular transmission or action potential

propagation (24), and if the peak twitch force is similarly reduced, this likely indicates that the impaired force production is due to reduced muscle excitability (25). Additionally, the time to reach peak twitch force and the subsequent half relaxation time are recorded from these evoked twitches. Time to peak twitch force are suggested to represent sequestering of calcium at the sarcoplasmic reticulum, whereas half relaxation time has been suggested to indicate reuptake of calcium at the sarcoplasmic reticulum (26).

There is also evidence for 'central' fatigue, which can be defined as a subset of fatigue associated with alterations in CNS function that cannot reasonably be explained by dysfunction within the muscle itself (27). This definition allows for the possibility that psychological factors like motivation and perception can contribute to fatigue (27). For example, force generation and electromyographic (EMG) activity during repeated maximal voluntary contractions (MVC's) may be enhanced by encouragement (28), and fatigue is more pronounced in participants who are concentrating on their performance and is reduced when they are distracted (29). This is very relevant, because the inability to generate and maintain adequate CNS drive to the working muscle is the most likely explanation of fatigue in most people during activity (27).

The technique routinely used to identify the role of central versus peripheral factors in fatigue is one in which the muscular force that a participant can voluntarily produce is compared to that elicited by supramaximal electrical stimulations (Interpolated Twitch Technique) (27). The rationale for this technique is that if, during an attempted maximal voluntary contraction (MVC), some motor units are either not recruited or firing at a submaximal rate and supramaximal stimulation of the motor nerve (or muscle) should stimulate those units and produce a detectable twitch contraction (30). The force produced by

the electrical stimulation is called the interpolated twitch torque, which decreases as voluntary muscle activation increases (31–33). Data suggests that voluntary activation (VA) of human motor units is suboptimal and typically below true maximal muscle force (22).

Generally, there are two rationales for what causes central fatigue: 1) a reduction in the descending impulses reaching the motor units and/or 2) an inhibition of motor unit excitability by afferent feedback from the muscle (22, 27). A majority of research supports feedback inhibition at the spinal cord level, leading to a proposal by Bigland-Ritchie (34) that inhibition of motor neuron firing rates may result from a reflex involving feedback from mechanoreceptors or free sensory nerve endings, sensitive to metabolite accumulation during fatigue. This has been suggested to be the CNS's attempt to optimize the maximal force that can be produced by fatiguing muscle, so that the most safe and economical pattern of muscle activation can occur. This concept is referred to as the sensory feedback hypothesis.

Reductions in central drive from the brain cannot be discounted as a contributor to central fatigue. A good example of this is data from studies using transcranial magnetic stimulation (TMS). TMS is a technique that can be used to excite areas of the brain such as the motor cortex. Stimulating the motor cortex produces descending volleys in corticospinal neurons, which can be used to evaluate the performance of the corticospinal tract (35). The size of the evoked motor response is influenced not only by the level of cortical excitability but also by the excitability of the motor unit pool (35). Studies using this technique report that stimulations can add progressively more force to that generated voluntarily during sustained MVC's (22), and the magnitude of motor responses elicited by TMS is transiently decreased after exercise (36). This indicates a decrease in the efficiency of motor command generation

within the motor cortex during fatigue (36). Although there are several possible explanations for this effect, researchers (36) suggested that accumulation and depletion of neurotransmitters in the CNS may be the cause.

Heat and Performance

Hyperthermia increases physiological strain on the body (1), leading to systemic changes such as decreases in neuromuscular function and thermal comfort (1, 5) as well as increased cardiovascular (37) and cognitive strain (38). One common effect of hyperthermic fatigue is impairments in the ability to voluntarily activate muscle (1). This was first shown by Nybo et al. (4), who found that voluntary force development during prolonged MVC's (120s) is reduced during hyperthermia, and this reduction is highly associated with decreased central activation. Similar findings were reported in the work of Morrison et al. (5), where there was a progressive impairment in both MVC force and VA in the knee extensors as core temperatures reach levels of ~39.4°C (5), an effect independent of peripheral temperatures (6) and fitness levels (7). This was found using passive heating methods, rather than active (cycling) techniques, minimizing the possible confound of cardiovascular strain on fatigue. Further, Todd et al. (8) reported similar effects, where voluntary force was reduced during hyperthermia in both brief (~2.4%) and sustained (~12%) MVC's. Additionally, superimposed twitch amplitude during sustained MVC contractions was 50% larger, thus the ability to drive the muscle maximally in a sustained fashion was deceased during hyperthermia and additional motor cortical output which could have increased torque, remained untapped by voluntary drive (8).

The Effects of Dopamine on Performance in the Heat

Alterations in neurotransmitters such as dopamine may have a role in this hyperthermia-induced central fatigue. Dopamine is a monoamine catecholamine neurotransmitter, produced in the brain. Dopamine is synthesized through:

- The amino acid tyrosine being converted to L-DOPA by the enzyme tyrosine hydroxylase (39).
- L-DOPA conversion into dopamine by the enzyme aromatic L-amino acid decarboxylase in the cytoplasm.
- Dopamine can be converted into norepinephrine by dopamine-ß-hydroxylase in the synaptic vesicles (40).

Dopamine plays an important role in human thermoregulation, a process for which the pre-optic and anterior hypothalamus (PO/AH) is responsible for (41). The PO/AH region contains neurons that are sensitive to changes in hypothalamic temperature, information that is integrated with that of somatosensory input from the skin and spinal thermoreceptors (42). Hypothetically, this would allow for comparison between central and peripheral thermal information, and the appropriate thermoregulatory responses are carried out to maintain homeostasis (42).

Dopamine is specifically involved in heat loss mechanisms within the PO/AH area. For example, peripheral heating in cats stimulates dopamine release within the PO/AH area, and hypothalamic dopamine injection also elicited dose-dependent decreases in body temperature (43). Additionally, in rat models, dopamine perfusion into PO/AH tissue activates warm-

sensitive neurons and inhibits cold-sensitive neurons, resulting in a stimulation of heat loss responses (44).

Dopamine is also an important neurotransmitter for regulating exercise performance, specifically being involved in processes such as motivation, movement initiation, and goal directed behaviour (10, 45–47). Dopamine synthesis and metabolism increase during exercise (27), and reductions in dopamine are linked to early fatigue (48). In addition, dopamine may play an even more important role in regulating exercise performance in the heat, and it has been reported that dopaminergic pathway activity is a good predictor of exercise tolerance in the heat (49). This is supported by Hasegawa et al. (46), who firstly discovered that during hyperthermia, there is a decrease in dopamine at fatigue, an effect not seen in thermoneutral environments (50). Additionally, they found that when rats were injected with Bupropion; a dual dopamine and norepinephrine reuptake inhibitor, their heat tolerance improved compared to control (46). These findings indicate a role for dopamine in modulating exercise performance and fatigue in the heat.

Furthermore, the individual components of dopamine production have been manipulated to examine any effects they may have on exercise performance in the heat. There appears to be no performance benefit of supplementing with catecholamine precursors such as tyrosine (51) and L-DOPA (52) or norepinephrine re-uptake inhibitors (53) during cycling time trial performance in both thermoneutral or hot conditions. However, Watson et al. (54) found that ingestion of bupropion; a dual dopamine/norepinephrine reuptake inhibitor, improved time trial performance by ~9% but only in 30°C heat and not in 18°C temperatures. Further, Roelands et al. (10) tested the effects of methylphenidate (MPH, common drug name Ritalin)

ingestion (20mg 1hr prior to exercise) on exercise performance, consisting of 30 minutes of cycling at 55% of peak power output, followed by a cycling time trial, in both 18°C and 30°C temperatures. MPH is a dopamine reuptake inhibitor that binds to dopamine transporters, increasing its extracellular concentration (55). While MPH ingestion had no effect on performance during thermoneutral conditions, it significantly improved time-trial performance by ~16%, and power output in 30°C temperatures, with participants having higher heart rates and final T_{core} (compared to placebo) (10). This has led to the conclusion that dopamine content specifically, may play a critical role in exercise performance in hot conditions.

MPH supplementation and its effects on neuromuscular function in a thermoneutral environment have also been investigated. When supplemented with 20 mg of MPH, there was an increase in isometric grip force by ~5-6% in thermoneutral environments (56). This study also reported an increase in coupling between the left insular cortex and left motor cortex, indicative of an enhanced flow of information, and MPH also induced a negative connectivity between the insular cortex and orbitofrontal cortex (typically there is a positive connectivity with fatigue) indicating that MPH may reduce the perception of fatigue (56). Additional work by Klass et al. (57) reported no changes to central neuromuscular drive with MPH ingestion before and after prolonged cycling in temperature conditions, but this may be due to low thermal strain (10). Currently the effects of MPH ingestion on the progressive impairment in central drive from hyperthermia are not yet known, which is the gap in the literature this project will answer.

Objectives and Hypotheses

The objective of this research project was to determine if methylphenidate ingestion will attenuate the impairment in central drive seen with hyperthermia, compared to placebo. The testable hypotheses for this project were:

1. Voluntary activation will be higher during hyperthermia with methylphenidate ingestion compared to placebo.
2. Isometric maximal force production will be higher during hyperthermia with methylphenidate ingestion compared to placebo.

Methods

Experimental Design

This study was a randomized, double-blinded, placebo-controlled study that consisted of initial participant screening, a familiarization session and two experimental sessions.

Participants

The participants for this study consisted of healthy males between the ages of 18 to 35 years old, recruited from the university and general population. Participants were pre-screened with a Physical Activity Readiness Questionnaire (Appendix A) and screening form and were informed of all details of the experimental procedures and the associated risks and discomforts before they provided their consent. A study physician then individually screened and cleared the participants at his medical discretion before prescribing MPH. Ethical clearance was obtained from Brock University's Bioscience Research Ethics Board (REB 17-123).

Participant Inclusion Criteria

- Males (aged 18-35 years)

Participant Exclusion Criteria

- Diagnosed cardiovascular, respiratory and/or neuromuscular disease
- Prescription of MPH within the past 1 year

Familiarization Session

Upon arrival participants had the experimental procedures explained to them and provided written informed consent. The familiarization session consisted of familiarizing participants with the maximal voluntary wrist flexion contractions (MVC) that were performed

in the trials, while also showing participants what an interpolated muscle twitch feels like. All contractions were performed using the dominant arm in a custom-made device isolating isometric wrist flexion that is connected to a calibrated load cell to measure muscle force. Contractions were performed isometrically to maximize participant force production capabilities and voluntary activation. Participants first performed 3 MVCs, each for 3 seconds, with an interpolated twitch administered in the middle of the contraction, and 2 seconds after the participant has stopped contracting (See Figure 1 for an example), with 2 minutes of rest between contractions. Then, 2 minutes after the final 3 second MVC, a 45 second MVC was performed. This included twitches at the 2, 15, 30, and 45 second marks, with an additional twitch a few seconds after the participant has stopped contracting. The full neuromuscular testing battery is outlined further in Table 1. This concluded the familiarization session.

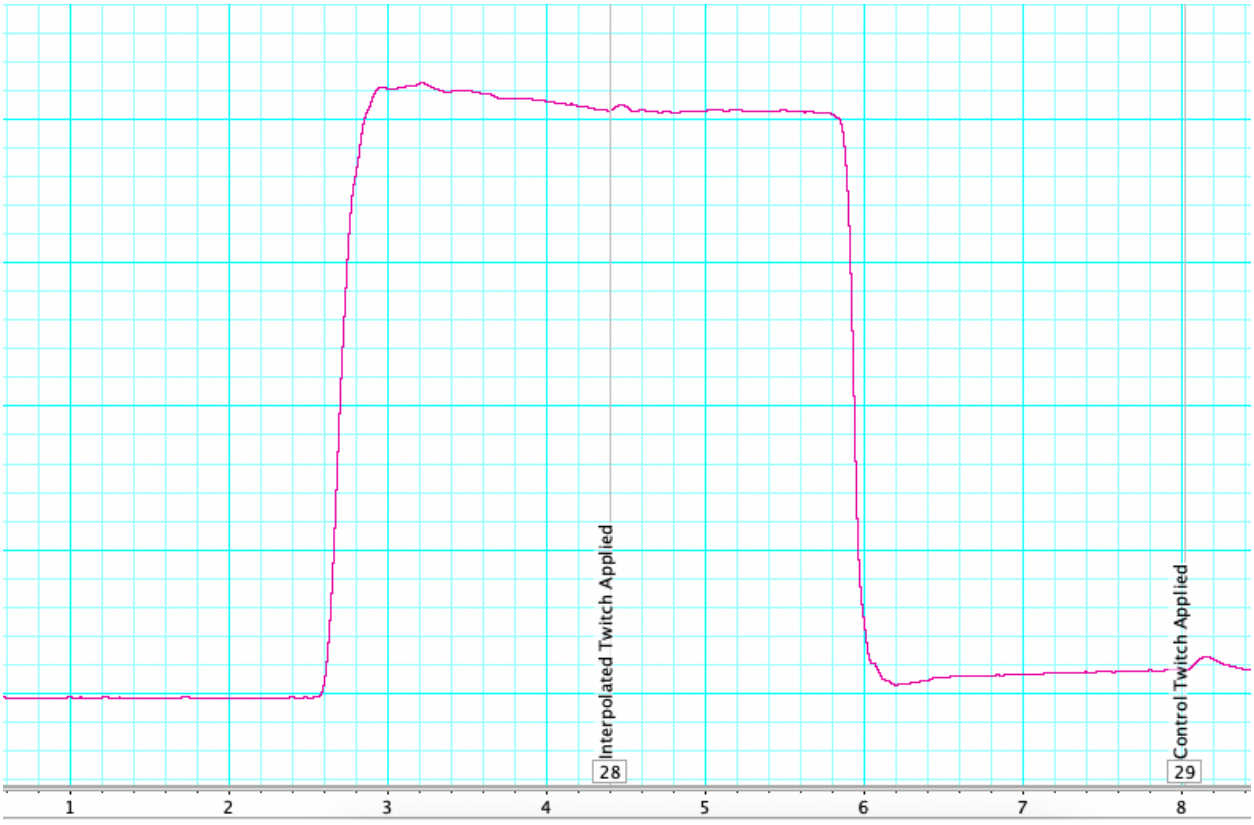

Figure 1. Example 3 second MVC data. As muscle force (Y axis) increases and plateaus, an electrical stimulation is superimposed onto the nerve producing a small increment of additional muscle force (superimposed twitch). This is followed by a control twitch at rest.

Table 1. Full neuromuscular testing battery.

Test	Description	Purpose	Expected Outcome
3-second MVC	-A maximal wrist flexion contraction, performed isometrically (without change in joint angle) for 3 seconds -Interpolated twitch in the middle and at rest	-To determine how much force can be produced when contracting maximally	-MVC force decrements will be attenuated by MPH ingestion compared to placebo
45-second MVC	-A maximal wrist flexion contraction, performed isometrically (without change in joint angle) for 45 seconds -Interpolated twitches at the 2,15,30,45 second marks, and at rest	-To determine how much force can be produced over an extended period of time	-45 second MVC force decrements will be attenuated by MPH ingestion compared to placebo
Interpolated Twitch	-A supramaximal stimulated contraction superimposed on top of an MVC	-To determine the voluntary activation of the muscular system -Determines what % of motor units are not activated during an MVC	-Voluntary activation will be maintained during hyperthermia after ingestion of MPH compared to placebo
Nerve Stimulation	-A supramaximal stimulated contraction, performed while the muscle is rested	To determine peripheral contractile characteristics such as: -Twitch amplitude -Rate of force development -Half-time to muscle relaxation	-MPH ingestion will not significantly affect peripheral contractile characteristics during hyperthermia

Experimental Sessions Drug Manipulation

Participants consumed either 20 mg of methylphenidate (MPH) or 20 mg of a lactose placebo (PLA) that was crushed into a standardized container of apple sauce (113 g, 50 calories, 14 g carbohydrates, 0 g fat, 0.3 g protein) to be indistinguishable for participants. There was a 60-minute wash-in period for the drug. Drug manipulations were double blinded by an independent investigator and trial order was randomized to prevent order bias. Trials were separated by a minimum of seven days to allow for adequate wash-out of MPH and performed at the same time of day to account for circadian fluctuations in core temperature.

Experimental Protocol

The experimental protocol consisted of two randomized trials, with the only difference being in drug manipulation (MPH vs. Placebo). Upon arrival to the laboratory, participants had their hydration status tested using a refractometer (PAL-10S, Atago, WA), with a satisfactory value of <1.020 required for participation. After, participants self-instrumented with a flexible rectal thermistor (Mon-A-Therm Core, Mallinkrodt Medical, St. Louis MO) to measure rectal temperature (T_{re}). Then, we located the participant's FCR and PL motor points. The motor point is the location with a dense number of motor end plates. This area is located in the muscle belly and can be detected with low level electrical stimulation on the skin surface. When stimulating the correct location, a visible muscle twitch will occur. Then the stimulus intensity should be gradually lowered, while ensuring the muscle twitch is still occurring. When the lowest possible electrical stimulation produces a minimal muscle twitch, the motor point has been located (15) and the location was marked on the skin. Surface electromyography (sEMG) electrodes (Delsys Inc, Natick, MA) were placed in a monopolar configuration, with one electrode directly on the motor point for both the FCR and PL. To administer evoked potentials, a cathode was taped (Transpore™, 3M, St. Paul, MN) on top of the median nerve, near the brachial pulse at the elbow crease and the anode was taped directly across from the cathode above the olecranon process, with a ground electrode taped onto the back of the participant's hand for electrical safety and to minimize noise. Also, thermocouples (PVC-T-24-190, Omega Environmental Inc. Laval, QC) were taped to the calf, quadricep, abdominal, chest, upper and lower back regions to give a 6-site mean skin temperature ($\bar{T}_{sk}$) value, and an additional thermocouple was taped on

the testing forearm for a local temperature measure. Lastly, a 3-lead electrocardiogram was setup on the participant, allowing heart rate to be derived from the R-R intervals.

After this instrumentation, baseline measures were taken. This firstly consisted of 3 resting M-waves, each separated by 20 seconds. Then participants performed 3 MVCs for 3 seconds each, with an interpolated twitch administered in the middle of the contraction and a few seconds after participants stop contracting, with 2 minutes of rest given between contractions. Then, participants reported their thermal comfort and thermal sensation based on a Likert scale (58) (Appendix A) and blood pressure was taken, both to allow for comparison of values taken during the heating process and helping to confirm the heating protocol was successful. Afterwards, a 45 second MVC was performed. This included twitches at the 2, 15, 30, and 45 second marks, with an additional twitch a few seconds after the participant has stopped contracting. This concluded the baseline testing.

After this baseline test battery, participants ingested applesauce with either a placebo or 20 mg of methylphenidate, with a wash-in time of 1 hour. After the wash-in period, participants completed the exact same testing battery as performed prior to ingestion, to determine the effects MPH ingestion may have in a thermoneutral setting. Upon completion, participants then dressed in a two-piece liquid-conditioning garment (BCS 4 Cooling System, Allen Vanguard, Ottawa, CAN) consisting of 1/8” Tygon tubing sewn into a stretchable suit. The liquid-conditioning garment covered the arms, upper and lower legs, and torso; the face, head, neck, hands, and feet were uncovered. To minimize evaporative heat loss, an impermeable polyvinyl rain suit was worn overtop the liquid-conditioning garment. Passive hyperthermia was performed by circulating 49.5°C water through the liquid conditioning garment, maintained by

a temperature controller (Model 5202, Polyscience, Niles, IL, USA) and pumped (MED-ENG, Pembroke, CAN) at a flow rate of ~1.5 $L \cdot min^{-1}$. Figures 1 and 2 display the whole experimental setup and the participants forearm setup respectively.

Figure 2. Overall experimental setup. The participant rested in a semi-recumbent chair while wearing the liquid cooling garment and impermeable polyvinyl rain suit while having their forearm setup for the isometric wrist flexion contractions.

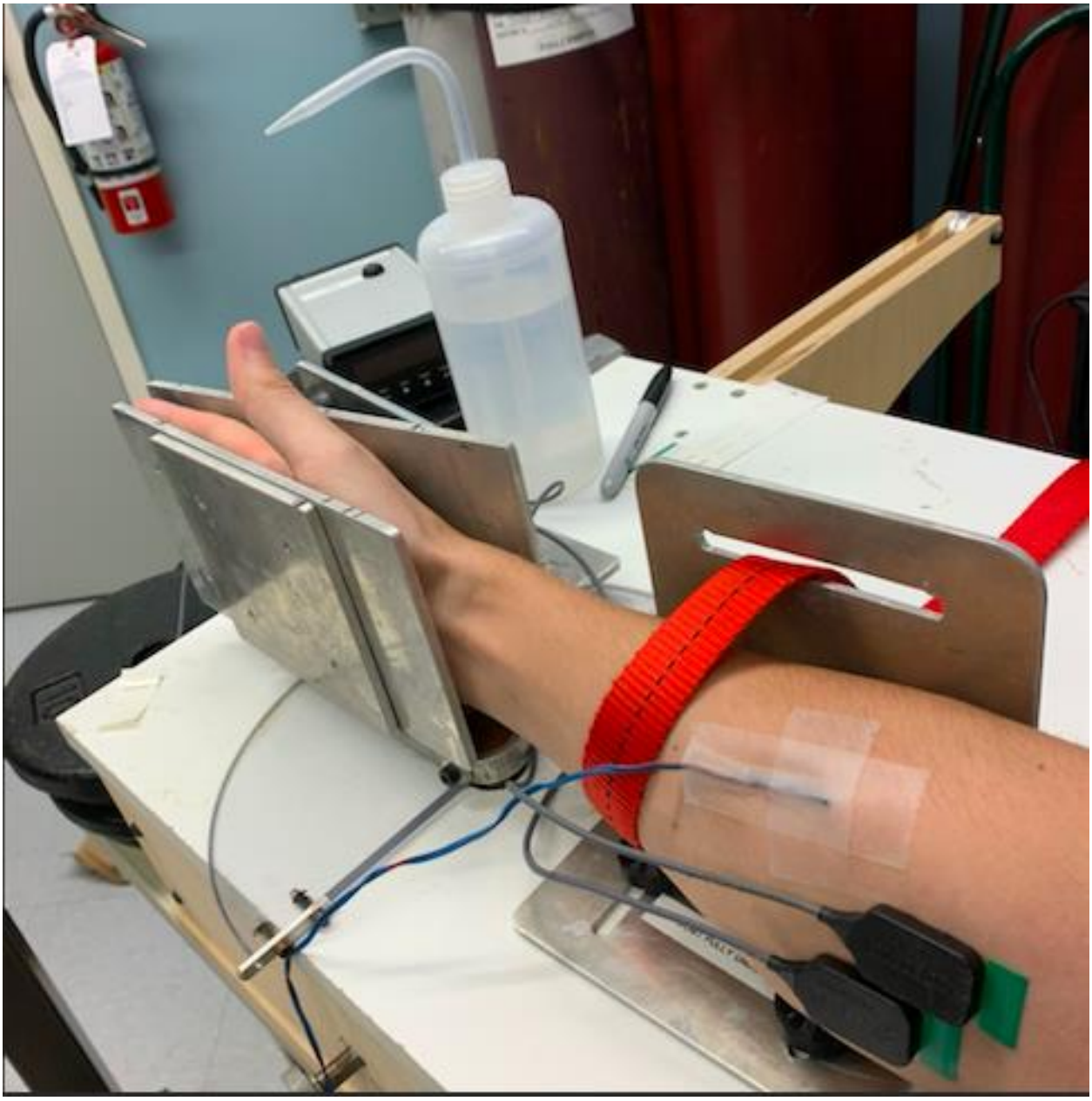

Figure 3. Forearm setup. The wrist is braced by plates to isolate isometric wrist flexion for the MVC's during the study. Also pictured is the skin temperature thermistor and sEMG electrodes.

At this point the passive heating protocol started. Heating lasted until participants felt they had reached their thermal tolerance or until 3 hours of heating had passed. At no point were participants told their T_{re} temperatures, so that voluntary thermal tolerance could be compared between MPH and placebo conditions. A final testing battery was performed when

participants reached their thermal limit (or if 3 hours passed), that consisted of: 3 M-waves, 3 MVC's, blood pressure, thermal comfort, thermal sensation and a final 45 second MVC. The trial ended after this testing battery. Figure 4 outlines the entire experimental protocol.

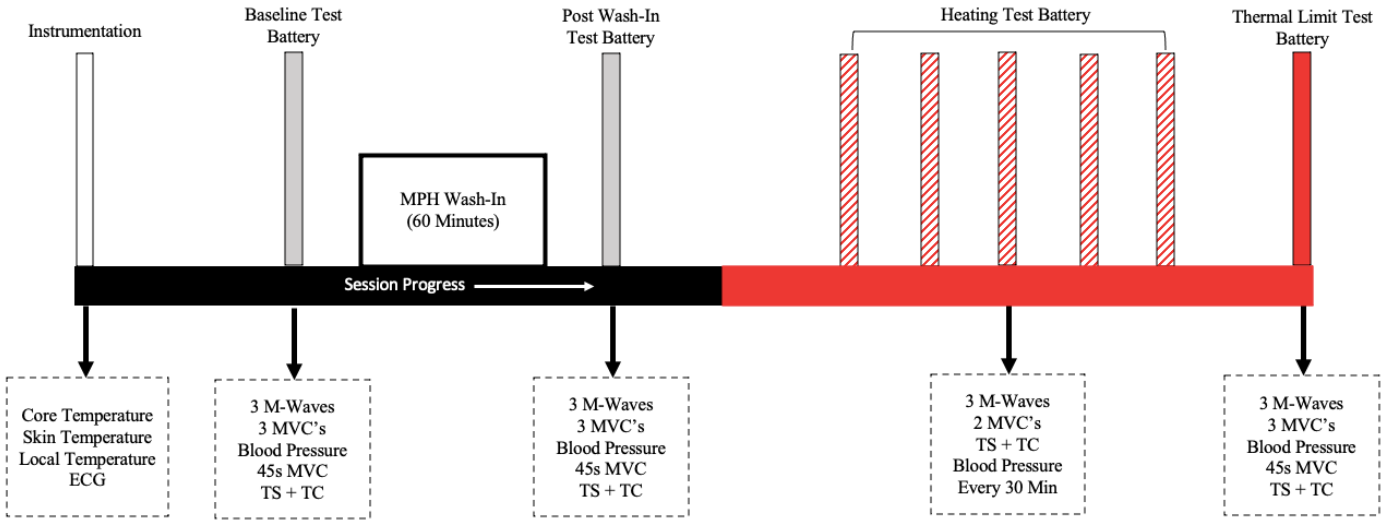

Figure 4. Sample experimental protocol.

Data Processing

The force signal was collected at 2 k/s (PowerLab, ADInstruments, Colorado Springs, USA) and temperature (T_{re}, $\bar{T}_{sk}$, $T_{forearm}$) data were collected at 1000 Hz (PowerLab, ADInstruments, Colorado Springs, USA). Both were stored on a personal computer to be analyzed and processed offline using LabChart (Version 8, ADInstruments, Colorado Springs, USA). During analysis, force data was filtered using a low pass filter with a 15 Hz cut-off.

For the 3s MVC's, force was obtained from the 1s of data pre and post interpolated twitch and averaged, giving a force value for each contraction. Then an overall force average for each test battery was calculated from the three contractions that were performed. 3s MVC VA was calculated as $\%VA = \left(1 - \frac{superimposed\ twitch}{potentiated\ twitch}\right) x\ 100$, and was averaged over the 3 MVC's that were performed in each test battery. For the 45s MVC's, force was obtained from the 1s of data

pre and post each interpolated twitch (twitches at 2s, 15s, 30s, 45s). It was then averaged between the 4 timepoints to create a value for the entire contraction. 45s MVC VA was calculated identically to the 3s MVC VA, and was taken at the 2s, 15s, 30s and 45s marks of the contraction. Then, those 4 values were averaged to create a value for the entire contraction. The force output from the resting nerve stimulations was used to calculate peak twitch force, time to peak force and half relaxation time were calculated from the force of the m-waves, and peak-to-peak amplitude was obtained from the sEMG data, which was filtered to a bandwidth between 20 Hz and 450 Hz by a Delsys Bagnoli Pre-Amplifier (Delsys Inc, Natick, MA).

Statistical Analysis

Data were normally distributed as assessed by skewness and kurtosis measures, and by visual inspection of histograms. Results are presented in means ± standard deviation (SD). To evaluate differences in variables from baseline and post-wash-in (PW) to participants thermal limits (T_{Lim}), a two factor repeated measures ANOVA (drug x time) was used. Bonferroni post hoc comparisons were used to determine where differences occurred if a significant interaction was found. Paired samples T-tests were run to compare delta T_{core} change and heating time. Statistical analyses were performed using SPSS 27 (SPSS Inc., Chicago, USA) and statistical significance was accepted at $P < 0.05$.

Results

6 men aged 23 ± 1.5 years old participated in the current study, with all participants completing both experimental trials. On average, the participants weighed 72.2 ± 6.5 kg and were 176.8 ± 8.5 cm tall. Data collection was halted March 11, 2020 due to restrictions from the COVID-19 pandemic. This thesis will restrict itself to only the analyses and interpretations possible from the available data, though the low participant numbers are a clear limitation.

Thermal Strain

Participants heated for 152 ± 25 minutes in the placebo (PLA) trials and 160 ± 25 minutes in the MPH trials, with MPH having no influence on heating time ($p = 0.419$). 3 of the 6 participants tolerated the entire 3 hours of heating and they did so in both of their experimental trials. During the heating protocol T_{re} levels rose ($p < 0.001$, $F = 244.003$) (Figure 4), from 36.6 $\pm$ 0.1°C to 38.4 ± 0.5°C (PLA) and 36.6 ± 0.1°C to 38.7 ± 0.2°C (MPH). Further, T_{re} levels were ~0.3°C higher at participants thermal limits in the MPH trial, but this did not reach statistical significance ($p = 0.065$, $F = 3.637$). Additionally, 5/6 participants had a higher ΔT_{re} with MPH, and the effect size for this was 1.05. $\bar{T}_{sk}$ rapidly increased ($p < 0.001$, $F = 312.953$) during heating, from 33.4 ± 0.7°C to 38.7 ± 0.4°C (PLA) and 32.9 ± 1°C to and 38.7 ± 0.2°C (MPH). $T_{forearm}$ levels mirrored this change ($p = 0.001$, $F = 12.109$), rising from 30.9 ± 1.1°C to 34.3 ± 1.1°C (PLA) and 30.9 ± 0.9°C to 34.6 ± 0.9°C (MPH). Drug had no significant effect on any temperature measures. In addition to temperature, participants' thermal comfort and thermal sensation ratings were also significantly influenced by passive heating ($P < 0.001$, $F = 259.286$). Specifically, thermal comfort ratings worsened, and thermal sensation scores increased during

heating. However, there were no drug or drug x time effects on thermal comfort and sensation. Changes in $\bar{T}_{sk}$, $T_{forearm}$, thermal comfort and thermal sensation are further outlined in Table 2.

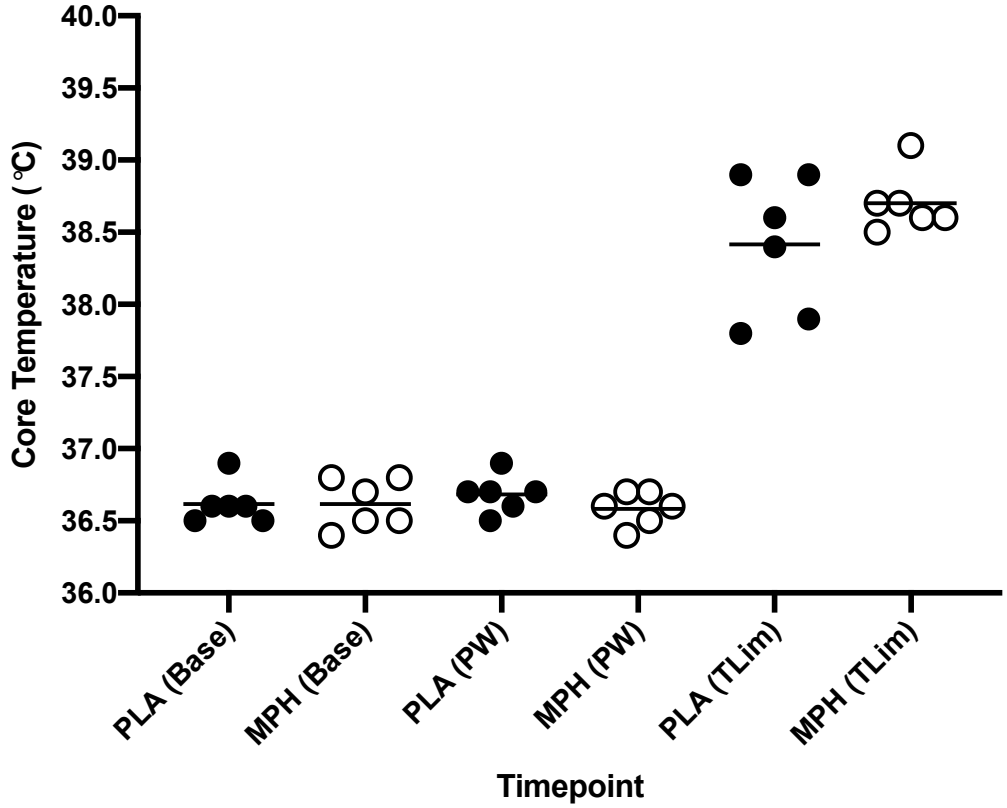

Figure 5. Core temperature at baseline, post-wash-in and thermal limit time points in the PLA (Black) and MPH (White) conditions (n=6). Horizontal bars represent group means.

Table 2. Changes in additional thermal strain variables at the baseline, post-wash-in and thermal limit time points, in both PLA and MPH conditions.

Variable	PLA (Base)	PLA (PW)	PLA (TLim)	MPH (Base)	MPH (PW)	MPH (TLim)
$\bar{T}_{sk}$	33.3±0.5°C	33.4±0.7°C	38.7±0.4°C*	32.6±0.7°C	32.9±1°C	38.7±0.2°C*
$T_{forearm}$	31±0.7°C	30.9±1.1°C	34.3±1.1°C*	31.2±1°C	30.9±0.9°C	34.6±0.9°C*
TC	1.2±0.4	1.3±0.5	4.0±0.4*	1±0	1.2±0.4	4±0*
TS	3.2±0.4	3.2±0.4	6.8±0.4*	2.8±0.4	3.2±0.8	6.8±0.4*

*Significantly different from post-wash in ($P < 0.05$)
Due to technical issues skin temperature was only available for 5 participants
$\bar{T}_{sk}$skin temperature, $T_{Forearm}$ forearm temperature, *TC* thermal comfort, *TS* thermal sensation

Neuromuscular Function

3 s MVC force decreased from the start (PW) to the end of heating (T_{Lim}) ($p = 0.002$, $F = 12.440$) in both trials, from 161 ± 68 N to 120 ± 64 N (PLA) and 157 ± 55 N to 124 ± 51 N (MPH). Drug ingestion had non-significant interactions on 3s MVC force ($p = 0.746$, $F = 0.117$). The drug x time interaction on 3s MVC force was also non-significant ($p = 0.431$, $F = 0.917$). Passive heating also significantly reduced ($p = 0.006$, $F = 7.636$) VA in the 3 s MVCs ($n = 4$) from 81 ± 5% to 70 ± 12% (PLA) and 82 ± 17% to 68 ± 19% (MPH). Both drug ($p = 0.565$, $F = 0.416$) and drug x time ($p = 0.235$, $F = 0.973$) interactions on VA were non-significant. No interactions were found between drug and force, or drug and VA. VA was limited to 4 participants due to inaccurate control twitch force values in two trials, which resulted in abnormally low VA values. This was likely a result of the subject's hands not pressing onto the plate connected to the force transducer during the control twitch in many of the contractions during 2 trials. This led to very small control twitch force values and inaccurate VA calculations. These interactions can be seen in Figure 5.

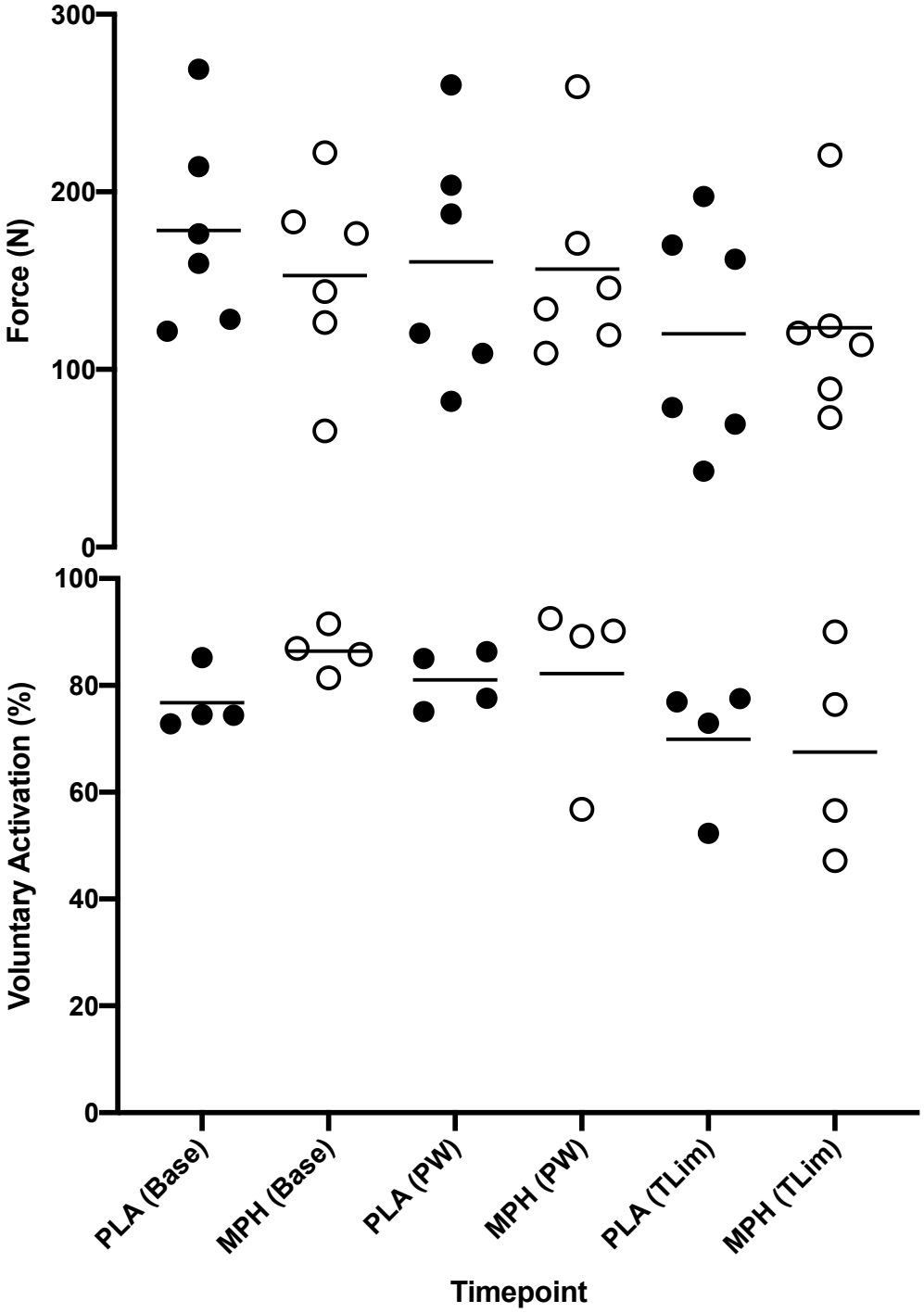

Figure 6. Changes in 3s MVC Force (Top, n=6) and VA (Bottom, n=4) during passive heating, in the PLA (black) and MPH (white) condition. Horizontal bars represent group means.

45 s MVC force decreased significantly ($p = 0.018$, $F = 6.175$) after passive heating, from 148 ± 70 N to 115 ± 61 N (PLA) and 148 ± 63 N to 124 ± 56 N (MPH). Similarly, 45 s MVC VA

declined (n=4) during passive heating ($p = 0.02$) in both conditions, decreasing from 78 ± 14% to 60 ± 15% (PLA) and 83 ± 12% to 66 ± 15% (MPH). VA was limited to 4 participants due to inaccurate twitch force values in two trials, which resulted in abnormally low VA values. There were no drug x time interactions for both 45 s MVC force ($p = 0.059$, $F = 3.235$) and VA ($p = 0.165$, $F = 2.052$). Drug also had non-significant interactions on force ($p = 0.811$) and VA ($p = 0.456$) during 45s MVCs. The aforementioned interactions can be seen in Figure 6.

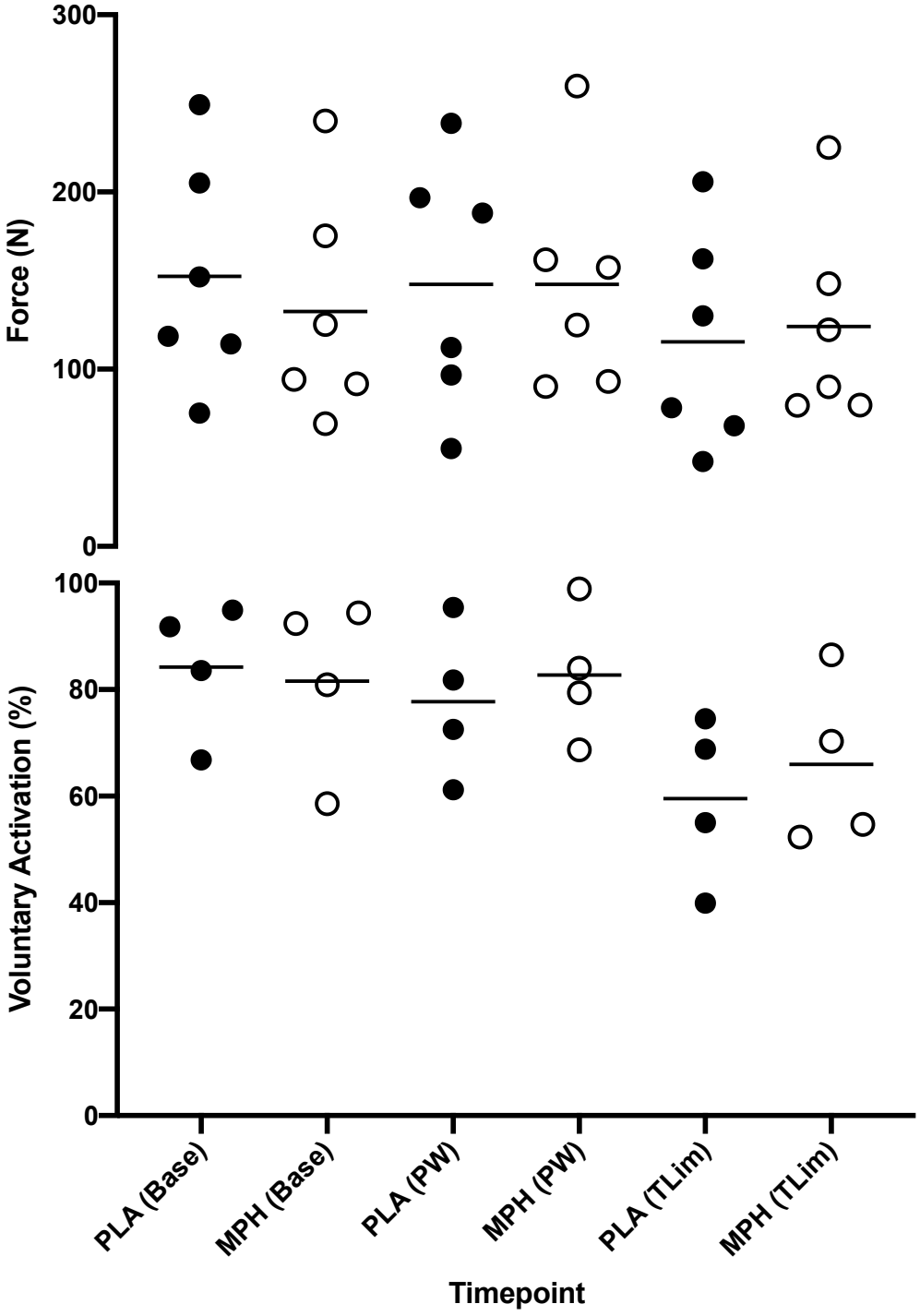

Figure 7. Changes in 45s MVC force (Top, n=6) and VA (Bottom, n=4) during passive heating in both PLA (black) and MPH (white) conditions. Horizontal bars represent group means.

Table 3. Changes in M-Wave and Twitch Characteristics at the baseline, post-wash-in and thermal limit time points, in both PLA and MPH conditions.

Variable	PLA (Base)	PLA (PW)	PLA (TLim)	MPH (Base)	MPH (PW)	MPH (TLim)
P-P Amplitude FCR (mV)	8.5±3.4	7.5±1.9	10.7±4.5	7.8±4.7	5.7±1.4	7±2.2
P-P Amplitude PL (mV)	6.4±1.3	7.8±0.7	9.5±3.6	6.3±0.2	7.8±0.2	7.6±0.1
Twitch Force (N)	7±4.1	6.6±3.8	7.3±2.7	3.8±0.7	5±2.7	6.8±3.2
Time to Peak (ms)	89.8±10.6	85.2±10.8	80.8±11.1	87.2±12	92.7±11.3	89.7±29.5
Half Relaxation Time (ms)	80.2±19.1	72.2±36.3	58.3±7.9	83.3±29.3	110.8±46.9	73.3±15.1

Peak-to-peak amplitude of the FCR M-wave (N = 5) increased after heating (PW to TLim), by 43% (PLA) and 22% (MPH), but the overall time interaction did not reach statistical significance ($p = 0.359$, $F = 1.167$, $d = 0.190$). Additionally, there were no drug ($p = 0.225$, $F = 2.058$, $d = 0.199$) or drug x time interactions ($p = 0.275$, $F = 1.522$, $d = 0.236$) on peak-to-peak amplitude of the FCR m-wave. Peak-to-peak amplitude of the PLA m-wave (N = 5) increased after heating by 18% in the PLA trial but decreased by 1% in MPH trial and there was a significant time effect ($p = 0.016$, $F = 7.166$). But similarly, to the FCR m-wave, there were no drug ($p = 0.361$, $F = 1.060$) or drug x time ($p = 0.599$, $F = 0.546$) interactions. There were only slight changes in twitch characteristics in the study. There was a significant drug effect on twitch force (n = 5), where it was significantly smaller ($p = 0.042$, $F = 8.746$) in the MPH trials. However, the time interaction was not significant ($p = 0.355$, $F = 1.183$) and there was no drug x time interaction ($p = 0.542$, $F = 0.661$). There were no significant interactions of drug ($p = 0.349$, $F = 1.067$), time ($p = 0.850$, $F = 0.165$) or drug x time ($p = 0.650$, $F = 0.450$) on time to peak twitch force. Similarly, half relaxation time had non-significant interactions with time ($p = 0.123$, $F = 3.447$), drug ($p = 0.172$, $F = 2.106$) or drug x time ($p = 0.085$, $F = 3.182$).

Discussion

The primary finding of this study was that MPH ingestion allowed participants to tolerate higher volitional T_{re} levels by ~0.3°C, with 5/6 participants exhibiting higher ΔT_{re} with MPH. This occurred despite there being no difference in thermal comfort ratings between trials, further suggestive of an attenuation of thermal perception from elevated dopamine activity. However, despite this altered thermal perception, the second major finding of this study was that MPH ingestion did not attenuate the declines in force and VA that occurred at the point of volitional cessation of passive heating.

Neuromuscular fatigue can be caused by either central failure to maximally activate muscles, alterations in local muscle characteristics, or a combination of both (59, 60). Previous studies have demonstrated that multiple maximal contractions can cause peripheral fatigue in addition to central fatigue (59, 61). We thus chose to limit the number of contractions in our study, so that any decreases in force and VA throughout our heating protocol would be caused by central fatigue induced through hyperthermia rather than through local fatigue from multiple contractions. Further, twitch force was also unaffected by heating in our study, which is in line with previous findings (5, 6). Together, this helps us confirm that central fatigue was limiting the ability to maximally recruit muscle, and that local muscle capacity was not affected by heating. Interestingly, we did not observe changes in the speed of muscle twitch after heating. This would contradict much of the existing literature, which shows that twitch speed increases with heating (5, 6, 62). However, this was likely an effect of our small sample size, as we observed that half relaxation time significantly decreased during passive heating when we doubled our sample size. Additionally, our results indicate that dopamine does not have any

effect on peripheral muscle characteristics. This supports findings from Klass et al. (11) who reported that twitch torque was similar in all three conditions of their study, where they compared the effects of MPH, placebo and reboxetine (a noradrenaline reuptake inhibitor) on fatigue after prolonged exercise in a thermoneutral environment. Further, the use of drugs such as indomethacin to selectively reduce cerebral blood flow has been shown to have no effect on peripheral contractile characteristics (63). Findings from the aforementioned studies indicate that drugs that act primarily on the cerebral level do not seem to have downstream effects on peripheral muscle contractile properties. Overall, we are confident that peripheral muscle capacity was not altered in our study, and that deficits in muscle force and VA were a result of central fatigue induced from passive heating.

MPH has ergogenic benefits during exercise in hot conditions that are not apparent in thermoneutral environments, specifically improving cycling time trial performance and wattage output, while also allowing for tolerance of higher core temperatures (10). One possible explanation for this effect is that the brain is allowing for higher amounts of voluntary muscle activation and this study was designed to test if this was the case. Previous work by Klass et al. (11) has indicated that MPH did not attenuate central fatigue. However, that study was performed in thermoneutral conditions (18°C) and dopamine appears to have a more important role in modulating exercise in high ambient temperatures (10), hence our use of a passive heating model. Force and VA were unaffected by MPH ingestion in our study at both thermoneutral baseline and also with a T_{re} rise of > +1.5°C, which firstly indicates that temperature was not the reason for the lack of effect of MPH on muscle activation in previous work (11). Our results also suggest that dopamine may not be as significantly involved in

neuromuscular activation as we initially thought. Despite there being non-significant effects of MPH ingestion on neuromuscular activation in both our study and work by Klass et al. (11) there is one study that implicates that dopamine levels do indeed modulate neuromuscular activation. Thorstensen et al. (64), blocked one of dopamine's pathways of action, specifically blocking the D_2 dopamine receptors, which have an important role in modulating movement. They found that this significantly decreased force and voluntary activation during elbow flexion in unfatigued muscle, and also exacerbated the declines in force and voluntary activation in fatigued muscle, suggesting that CNS dopamine pathways do affect VA during maximal isometric contractions (64). This indicates that the blockage or total removal of dopamine does directly impair neuromuscular activation. However, combined with our findings and those of Klass et al. (11), it may be that there no dose response exists beyond a minimum threshold of dopamine activity.

While many of the previously mentioned studies have examined dopamine reuptake inhibition, it is not the only neurotransmitter that has a role in neuromuscular activation. Norepinephrine (NE) is a catecholamine neurotransmitter that has been implicated in the control of arousal levels, consciousness and reward mechanisms in the brain (47). Based on this, one would expect that NE reuptake inhibition would also lead to a delay in exercise fatigue; however, much of the existing literature has shown that increased NE activity significantly impairs exercise performance in both thermoneutral and hot conditions (11, 65). Further, NE also appears to have a critical role in modulating neuromuscular activation. Klass et al. (11), used reboxetine; a NE reuptake inhibitor and found that it not only worsened cycling time trial performance, but also caused greater amounts of central fatigue than placebo in a

thermoneutral environment (11). This occurred despite no change in corticospinal excitability measured using transcranial magnetic stimulation, indicating that reboxetine likely affected supraspinal circuits located before the motor cortex (11). More recent work by Klass and colleagues (66) demonstrated that NE reuptake inhibition caused a greater rate of decline in MVC force and VA, helping confirm previous statements suggesting that NE has a role in modulating central/supraspinal fatigue (66). In addition to NE, serotonin (5-HT) may also have a role in central fatigue. 5-HT has been well researched as a possible modulator for exercise fatigue, given that it has well known effects on sleep, lethargy and loss of motivation (67). It was recently discovered that ingestion of paroxetine, a 5-HT reuptake inhibitor, increased VA and torque generation in brief unfatigued contractions (68). However in contrast to this, the ability to generate maximal torque with 5-HT reuptake inhibition was compromised under fatigued conditions, suggesting that 5-HT also modulates central fatigue (68). Overall, it is seems unlikely that one neurotransmitter system is responsible for central fatigue, and central fatigue is most likely caused by a complex interplay between these different neurotransmitter systems (47).

Care must be taken in extrapolating results from isolated muscle movements to whole body exercise, because discrepancies exist in neuromuscular responses to hyperthermia depending on the type of contraction (69). For example, force production and VA are clearly impaired at high core temperatures when performing isometric contractions (5, 6), but isokinetic contractions at three different speeds are not affected by elevations in core temperature with the same passive heating model, and T_{sk} appeared to be more important with dynamic movements (70). This potential difference in thermal influence between isometric and

other movements was confirmed by Coletta et al. (71) who examined differences between surface electromyography in isometric and iso-inertial tasks during passive heating and cooling. They demonstrated that core temperature seems to be the primary thermal afferent for isometric tasks, and skin temperature is the primary thermal afferent for iso-inertial tasks, indicating that temperature has a task-dependent impact on neuromuscular responses. Additionally, maximal isometric contractions are poorly related to dynamic movements such as sprinting and vertical jump test performance when comparing muscle activation patterns (72), and muscle activation during even high-intensity cycling is significantly lower than that of isometric MVC's (73), indicating that MVC's are not representative of whole-body exercise. Our study was designed to specifically examine if the aforementioned ergogenic benefits MPH appears to have when exercising in the heat (10), are due to enhanced neuromuscular activation. Thus, we chose an isometric task, which allowed us to assess VA using interpolated twitch technique, as a measure of central drive.

We observed that dopamine reuptake inhibition apparently improved thermal tolerance in our study. While we did not observe statistical significance ($p = 0.07$), we did find that the large majority of participants (5/6) tolerated higher core temperatures at voluntary cessation of heating in the MPH trial. We did this with a passive heating model, allowing us to limit both the amount of cardiovascular strain and metabolic heat production that would have occurred from exercise, thus allowing us to better isolate how dopamine affects thermal tolerance. One possible mechanism that could explain how dopamine improves thermal tolerance is that it may skew thermal perception. Previous work has shown that participants report similar thermal discomfort ratings despite reaching higher T_{core} levels (10, 54), and our results also

support this, as thermal comfort and sensation ratings were identical when comparing between trials. Overall, thermal perception seems to be strongly linked to thermal tolerance, and if thermal perception is skewed it may be possible to tolerate higher core temperatures.

Limitations

A primary limitation to this study was the restricted sample size of 6 participants due to COVID-19. This is a low number compared to the desired n=12 based on *a priori* power analyses, making it challenging to generalize the results coupled with the large variability in many of the measures. An additional limitation of this study design is that we chose isometric contractions, which do not replicate whole-body exercises such as cycling. The rationale for choosing an isometric task was that it allowed us to isolate whether dopamine is a contributor to neuromuscular activation, by using the interpolated twitch technique to measure VA. Thus, this study is not meant to be a direct replication of previous work done by Roelands et al. (10) and only set out to examine if dopamine has a role in central neuromuscular activation. Another potential limitation may have been the 20 mg dose of methylphenidate being insufficient to sufficiently raise dopamine activity to see a significant effect, especially as the dosage was absolute rather than based on participant body mass. The absolute dosage of 20 mg was based on the same protocol as that of the original study finding improved exercise capacity in the heat (65). Furthermore, typical clinical prescription of methylphenidate is typically 2x20 mg doses per day rather than based on body mass.

In conclusion, MPH ingestion allowed participants to tolerate higher volitional T_{re} levels. This occurred in the absence of any differences in thermal strain ratings between trials. However, despite thermal perception being altered, MPH ingestion was not able to attenuate

the declines in force and VA that occurred at the point of volitional cessation of passive heating. Therefore, the higher upregulation of power output in the study by Roelands et al. (10) was not a direct result of dopamine action on either local muscle capacity or central capacity for maintained neuromuscular activation in the heat.

Bibliography

1. **Cheung SS**, **Sleivert GG**. Multiple triggers for hyperthermic fatigue and exhaustion. *Exercise and sport sciences reviews* 32: 100–106, 2004.

2. **Kluger BM**, **Krupp LB**, **Enoka RM**. Fatigue and fatigability in neurologic illnesses: Proposal for a unified taxonomy. *Neurology* 80: 409–416, 2013. doi: 10.1212/WNL.0b013e31827f07be.

3. **Enoka RM**, **Duchateau J**. Translating Fatigue to Human Performance: *Medicine & Science in Sports & Exercise* 48: 2228–2238, 2016. doi: 10.1249/MSS.0000000000000929.

4. **Nybo L**, **Nielsen B**. Hyperthermia and central fatigue during prolonged exercise in humans. *Journal of applied physiology* 91: 1055–1060, 2001.

5. **Morrison S**, **Sleivert GG**, **Cheung SS**. Passive hyperthermia reduces voluntary activation and isometric force production. *European Journal of Applied Physiology* 91: 729–736, 2004. doi: 10.1007/s00421-004-1063-z.

6. **Thomas MM**, **Cheung SS**, **Elder GC**, **Sleivert GG**. Voluntary muscle activation is impaired by core temperature rather than local muscle temperature. *Journal of Applied Physiology* 100: 1361–1369, 2006. doi: 10.1152/japplphysiol.00945.2005.

7. **Morrison SA**, **Sleivert GG**, **Cheung S**. Aerobic influence on neuromuscular function and tolerance during passive hyperthermia. *Medicine & Science in Sports & Exercise* 38: 1754–1761, 2006. doi: 10.1249/01.mss.0000230120.83641.98.

8. **Todd G**, **Butler JE**, **Taylor JL**, **Gandevia S**. Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* 563: 621–631, 2005. doi: 10.1113/jphysiol.2004.077115.

9. **Nybo L**. Hyperthermia and fatigue. *Journal of Applied Physiology* 104: 871–878, 2008. doi: 10.1152/japplphysiol.00910.2007.

10. **Roelands B**, **Hasegawa H**, **Watson P**, **Piacentini MF**, **Buyse L**, **De Schutter G**, **Meeusen RR**. The Effects of Acute Dopamine Reuptake Inhibition on Performance: *Medicine & Science in Sports & Exercise* 40: 879–885, 2008. doi: 10.1249/MSS.0b013e3181659c4d.

11. **Klass M**, **Roelands B**, **LéVéNez M**, **Fontenelle V**, **Pattyn N**, **Meeusen R**, **Duchateau J**. Effects of Noradrenaline and Dopamine on Supraspinal Fatigue in Well-Trained Men: *Medicine & Science in Sports & Exercise* 44: 2299–2308, 2012. doi: 10.1249/MSS.0b013e318265f356.

12. **Donoghue JP**, **Sanes JN**. Motor areas of the cerebral cortex. *J Clin Neurophysiol* 11: 382–396, 1994.

13. **Crossman AR**, **Neary D**. *Neuroanatomy*. 5th ed. Churchill Livingstone, 2015.

14. **Welniarz Q**, **Dusart I**, **Roze E**. The corticospinal tract: Evolution, development, and human disorders. *Developmental Neurobiology* 77: 810–829, 2017. doi: 10.1002/dneu.22455.

15. **Gabriel DA**, **Kamen G**. *Essentials of Electromyography*. Human Kinetics, 2010.

16. **Powers JD**, **Malingen SA**, **Regnier M**, **Daniel TL**. The Sliding Filament Theory Since Andrew Huxley: Multiscale and Multidisciplinary Muscle Research. *Annual Review of Biophysics* 50: 373–400, 2021. doi: 10.1146/annurev-biophys-110320-062613.

17. **Huxley AF**, **Niedergerke R**. Structural Changes in Muscle During Contraction: Interference Microscopy of Living Muscle Fibres. *Nature* 173: 971–973, 1954. doi: 10.1038/173971a0.

18. **Huxley H**, **Hanson J**. Changes in the Cross-Striations of Muscle during Contraction and Stretch and their Structural Interpretation. *Nature* 173: 973–976, 1954. doi: 10.1038/173973a0.

19. **Fuglevand AJ**, **Winter DA**, **Patla AE**. Models of recruitment and rate coding organization in motor-unit pools. *Journal of Neurophysiology* 70: 2470–2488, 1993. doi: 10.1152/jn.1993.70.6.2470.

20. **Garland SJ**, **Griffin L**. Motor Unit Double Discharges: Statistical Anomaly or Functional Entity? *Can J Appl Physiol* 24: 113–130, 1999. doi: 10.1139/h99-010.

21. **Enoka RM**, **Stuart DG**. Neurobiology of muscle fatigue. .

22. **Gandevia SC**. Spinal and supraspinal factors in human muscle fatigue. *Physiological reviews* 81: 1725–1789, 2001.

23. **Place N**, **Yamada T**, **Bruton JD**, **Westerblad H**. Muscle fatigue: from observations in humans to underlying mechanisms studied in intact single muscle fibres. *Eur J Appl Physiol* 110: 1–15, 2010. doi: 10.1007/s00421-010-1480-0.

24. **Bigland-Ritchie B**, **Johansson R**, **Lippold OC**, **Smith S**, **Woods JJ**. Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J Physiol* 340: 335–346, 1983.

25. **Zory R**, **Boërio D**, **Jubeau M**, **Maffiuletti NA**. Central and Peripheral Fatigue of the Knee Extensor Muscles Induced by Electromyostimulation. *Int J Sports Med* 26: 847–853, 2005. doi: 10.1055/s-2005-837459.

26. **Ortenblad N**, **Sjøgaard G**, **Madsen K**. Impaired sarcoplasmic reticulum Ca(2+) release rate after fatiguing stimulation in rat skeletal muscle. *J Appl Physiol (1985)* 89: 210–217, 2000. doi: 10.1152/jappl.2000.89.1.210.

27. **Davis JM**, **Bailey SP**. Possible mechanisms of central nervous system fatigue during exercise. *Med Sci Sports Exerc* 29: 45–57, 1997.

28. **Rube N**, **Secher NH**. Paradoxical influence of encouragement on muscle fatigue. *Europ J Appl Physiol* 46: 1–7, 1981. doi: 10.1007/BF00422169.

29. **Asmussen E**, **Mazin B**. Recuperation after muscular fatigue by "diverting activities." *Europ J Appl Physiol* 38: 1–7, 1978. doi: 10.1007/BF00436747.

30. **Hales JP**, **Gandevia SC**. Assessment of maximal voluntary contraction with twitch interpolation: an instrument to measure twitch responses. *Journal of Neuroscience Methods* 25: 97–102, 1988. doi: 10.1016/0165-0270(88)90145-8.

31. **Belanger AY**, **McComas AJ**. Extent of motor unit activation during effort. *Journal of Applied Physiology* 51: 1131–1135, 1981. doi: 10.1152/jappl.1981.51.5.1131.

32. **Suter E**, **Herzog W**. Effect of number of stimuli and timing of twitch application on variability in interpolated twitch torque. *Journal of Applied Physiology* 90: 1036–1040, 2001. doi: 10.1152/jappl.2001.90.3.1036.

33. **Suter E**, **Herzog W**, **Huber A**. Extent of motor unit activation in the quadriceps muscles of healthy subjects. *Muscle & Nerve* 19: 1046–1048, 1996. doi: 10.1002/mus.880190804.

34. **Bigland-Ritchie B**, **Thomas CK**, **Rice CL**, **Howarth JV**, **Woods JJ**. Muscle temperature, contractile speed, and motoneuron firing rates during human voluntary contractions. *Journal of Applied Physiology* 73: 2457–2461, 1992. doi: 10.1152/jappl.1992.73.6.2457.

35. **Taylor JL**, **Gandevia SC**. Noninvasive stimulation of the human corticospinal tract. *Journal of Applied Physiology* 96: 1496–1503, 2004. doi: 10.1152/japplphysiol.01116.2003.

36. **Brasil-Neto JoaquimP**, **Pascual-Leone A**, **Valls-Sol**  **J**, **Cammarota A**, **Cohen LeonardoG**, **Hallett M**. Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. *Exp Brain Res* 93, 1993. doi: 10.1007/BF00227794.

37. **González-Alonso J**, **Crandall CG**, **Johnson JM**. The cardiovascular challenge of exercising in the heat. *The Journal of Physiology* 586: 45–53, 2008. doi: 10.1113/jphysiol.2007.142158.

38. **Hancock PA**, **Ross JM**, **Szalma JL**. A Meta-Analysis of Performance Response Under Thermal Stressors. *Human Factors: The Journal of the Human Factors and Ergonomics Society* 49: 851–877, 2007. doi: 10.1518/001872007X230226.

39. **Daubner SC**, **Le T**, **Wang S**. Tyrosine hydroxylase and regulation of dopamine synthesis. *Archives of Biochemistry and Biophysics* 508: 1–12, 2011. doi: 10.1016/j.abb.2010.12.017.

40. **Jongkees BJ**, **Hommel B**, **Kühn S**, **Colzato LS**. Effect of tyrosine supplementation on clinical and healthy populations under stress or cognitive demands—A review. *Journal of Psychiatric Research* 70: 50–57, 2015. doi: 10.1016/j.jpsychires.2015.08.014.

41. **Boulant JA**, **Dean JB**. Temperature Receptors in the Central Nervous System. *Annual Review of Physiology* 48: 639–654, 1986. doi: 10.1146/annurev.ph.48.030186.003231.

42. **Boulant JA**. Role of the Preoptic-Anterior Hypothalamus in Thermoregulation and Fever. *Clinical Infectious Diseases* 31: S157–S161, 2000. doi: 10.1086/317521.

43. **Ruwe WD**, **Myers RD**. Dopamine in the hypothalamus of the cat: pharmacological characterization and push-pull perfusion analysis of sites mediating hypothermia. *Pharmacol Biochem Behav* 9: 65–80, 1978.

44. **Scott IM**, **Boulant JA**. Dopamine effects on thermosensitive neurons in hypothalamic tissue slices. *Brain Res* 306: 157–163, 1984.

45. **Balthazar CH**, **Leite LHR**, **Rodrigues AG**, **Coimbra CC**. Performance-enhancing and thermoregulatory effects of intracerebroventricular dopamine in running rats. *Pharmacology Biochemistry and Behavior* 93: 465–469, 2009. doi: 10.1016/j.pbb.2009.06.009.

46. **Hasegawa H**, **Piacentini MF**, **Sarre S**, **Michotte Y**, **Ishiwata T**, **Meeusen R**. Influence of brain catecholamines on the development of fatigue in exercising rats in the heat: Effect of bupropion on thermoregulation and exercise performance. *The Journal of Physiology* 586: 141–149, 2008. doi: 10.1113/jphysiol.2007.142190.

47. **Roelands B**, **Meeusen R**. Alterations in Central Fatigue by Pharmacological Manipulations of Neurotransmitters in Normal and High Ambient Temperature: *Sports Medicine* 40: 229–246, 2010. doi: 10.2165/11533670-000000000-00000.

48. **Bailey SP**, **Davis JM**, **Ahlborn EN**. Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. *Journal of Applied Physiology* 74: 3006–3012, 1993.

49. **Bridge MW**, **Weller AS**, **Rayson M**, **Jones DA**. Responses to exercise in the heat related to measures of hypothalamic serotonergic and dopaminergic function. *European Journal of Applied Physiology* 89: 451–459, 2003. doi: 10.1007/s00421-003-0800-z.

50. **Hasegawa H**, **Yazawa T**, **Yasumatsu M**, **Otokawa M**, **Aihara Y**. Alteration in dopamine metabolism in the thermoregulatory center of exercising rats. *Neuroscience Letters* 289: 161–164, 2000. doi: 10.1016/S0304-3940(00)01276-3.

51. **Tumilty L**, **Davison G**, **Beckmann M**, **Thatcher R**. Failure of Oral Tyrosine Supplementation to Improve Exercise Performance in the Heat: *Medicine & Science in Sports & Exercise* 46: 1417–1425, 2014. doi: 10.1249/MSS.0000000000000243.

52. **Cordery P**, **James LJ**, **Peirce N**, **Maughan RJ**, **Watson P**. A Catecholamine Precursor Does Not Influence Exercise Performance in Warm Conditions: *Medicine & Science in Sports & Exercise* 48: 536–542, 2016. doi: 10.1249/MSS.0000000000000791.

53. **Francesca Piacentini M**, **Meeusen R**, **Buyse L**, **De Schutter G**, **Kempenaers F**, **Van Nijvel J**, **De Meirleir K**. No effect of a noradrenergic reuptake inhibitor on performance in trained cyclists: *Medicine & Science in Sports & Exercise* 34: 1189–1193, 2002. doi: 10.1097/00005768-200207000-00021.

54. **Watson P**, **Hasegawa H**, **Roelands B**, **Piacentini MF**, **Looverie R**, **Meeusen R**. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *The Journal of Physiology* 565: 873–883, 2005. doi: 10.1113/jphysiol.2004.079202.

55. **Leonard BE**, **McCartan D**, **White J**, **King DJ**. Methylphenidate: a review of its neuropharmacological, neuropsychological and adverse clinical effects. *Hum Psychopharmacol* 19: 151–180, 2004. doi: 10.1002/hup.579.

56. **King M**, **Rauch LHG**, **Brooks SJ**, **Stein DJ**, **Lutz K**. Methylphenidate Enhances Grip Force and Alters Brain Connectivity: *Medicine & Science in Sports & Exercise* 49: 1443–1451, 2017. doi: 10.1249/MSS.0000000000001252.

57. **Klass M**, **Roelands B**, **Levenez M**, **Fontenelle V**, **Pattyn N**, **Meeusen R**, **Duchateau J**. Effects of Noradrenaline and Dopamine on Supraspinal Fatigue in Well-Trained Men. *Medicine & Science in Sports & Exercise* 44: 2299–2308, 2012.

58. **Gagge AP**, **Stolwijk JAJ**, **Hardy JD**. Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Environmental Research* 1: 1–20, 1967. doi: 10.1016/0013-9351(67)90002-3.

59. **Nordlund MM**, **Thorstensson A**, **Cresswell AG**. Central and peripheral contributions to fatigue in relation to level of activation during repeated maximal voluntary isometric plantar flexions. *Journal of Applied Physiology* 96: 218–225, 2004. doi: 10.1152/japplphysiol.00650.2003.

60. **Kent-Braun JA**. Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *Eur J Appl Physiol Occup Physiol* 80: 57–63, 1999. doi: 10.1007/s004210050558.

61. **Kawakami Y**, **Amemiya K**, **Kanehisa H**, **Ikegawa S**, **Fukunaga T**. Fatigue responses of human triceps surae muscles during repetitive maximal isometric contractions. *Journal of Applied Physiology* 88: 1969–1975, 2000. doi: 10.1152/jappl.2000.88.6.1969.

62. **Davies CT**, **Young K**. Effect of temperature on the contractile properties and muscle power of triceps surae in humans. *Journal of Applied Physiology* : 191–195, 1983.

63. **Hartley GL**. The Influence of Cerebral Blood Flow and Carbon Dioxide on Neuromuscular Responses During Environmental Stress. .

64. **Thorstensen JR**, **Tucker MG**, **Kavanagh JJ**. Antagonism of the D2 dopamine receptor enhances tremor but reduces voluntary muscle activation in humans. *Neuropharmacology* 141: 343–352, 2018. doi: 10.1016/j.neuropharm.2018.08.029.

65. **Roelands B**, **Goekint M**, **Heyman E**, **Piacentini MF**, **Watson P**, **Hasegawa H**, **Buyse L**, **Pauwels F**, **De Schutter G**, **Meeusen R**. Acute norepinephrine reuptake inhibition decreases performance in normal and high ambient temperature. *Journal of Applied Physiology* 105: 206–212, 2008. doi: 10.1152/japplphysiol.90509.2008.

66. **Klass M**, **Duchateau J**, **Rabec S**, **Meeusen R**, **Roelands B**. Noradrenaline Reuptake Inhibition Impairs Cortical Output and Limits Endurance Time. *Medicine & Science in Sports & Exercise* 48: 1014–1023, 2016.

67. **Meeusen R**, **Watson P**, **Hasegawa H**, **Roelands B**, **Piacentini MF**. Central fatigue: the serotonin hypothesis and beyond. *Sports medicine (Auckland, NZ)* 36: 881–909, 2006.

68. **Kavanagh JJ**, **McFarland AJ**, **Taylor JL**. Enhanced availability of serotonin increases activation of unfatigued muscle but exacerbates central fatigue during prolonged sustained contractions. *The Journal of Physiology* 597: 319–332, 2019. doi: https://doi.org/10.1113/JP277148.

69. **Cheung SS**. Hyperthermia and voluntary exhaustion: integrating models and future challenges. *Appl Physiol Nutr Metab* 32: 808–817, 2007. doi: 10.1139/H07-043.

70. **Cheung SS**, **Sleivert GG**. Lowering of skin temperature decreases isokinetic maximal force production independent of core temperature. *European Journal of Applied Physiology* 91: 723–728, 2004. doi: 10.1007/s00421-004-1062-0.

71. **Coletta NA**, **Mallette MM**, **Gabriel DA**, **Tyler CJ**, **Cheung SS**. Core and skin temperature influences on the surface electromyographic responses to an isometric force and position task. *PLOS ONE* 13: e0195219, 2018. doi: 10.1371/journal.pone.0195219.

72. **Mero A**, **Luhtanen P**, **Viitasalo J**, **Komi P**. Relationships between the maximal running velocity, muscle fiber characteristics, force production and force relxation of sprinters. *Scandinavian Journal of Sports Science* 3: 16–22, 1981.

73. **Bieuzen F**, **Lepers R**, **Vercruyssen F**, **Hausswirth C**, **Brisswalter J**. Muscle activation during cycling at different cadences: Effect of maximal strength capacity. *Journal of Electromyography and Kinesiology* 17: 731–738, 2007. doi: 10.1016/j.jelekin.2006.07.007.

Appendices

Appendix A

Table 4. Gagge et al. (1967) Thermal Comfort Scale

Numerical Value	Descriptive Information
1	Comfortable
2	Slightly Uncomfortable
3	Uncomfortable
4	Very Uncomfortable

Table 5. Gagge et al. (1967) Thermal Sensation Scale

Numerical Value	Descriptive Information
1	Cold
2	Cool
3	Slightly Cool
4	Neutral
5	Slightly Warm
6	Warm
7	Hot

2021 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://www.who.int/publications/i/item/9789240015128).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.epafmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

01-11-2020

Figure 8. Sample PAR-Q