Impact of Concurrent Cold Pressor and Muscle Metaboreflex Responses via the Sympathetic Nervous System

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BIOL 3550 L01: Human Physiology 2

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April 4th, 2025

Abstract

Background: The cold pressor test (CPT) and the muscle metaboreflex (MMR) are both methods for activating the sympathetic nervous system, though they differ in their effects on heart rate due to parasympathetic modulation during the MMR. This study aims to determine whether CPT-induced sympathetic activation can override the parasympathetic influence, hypothesizing that combining both stressors will lead to greater cardiovascular responses.

Methods: Nine healthy participants were subject to three randomized trials: a CPT, static handgrip MMR, and a combined response test. Trials monitored arterial blood pressure and heart rate in response to SNS activation during all three trials. Test procedures were inclusive of baseline data and recovery periods.

Results: Data were analyzed in JASP. Statistical significance existed solely between the heart rate measurements of baseline and cold pressor tests ($p = 0.048$, mean difference = -8.191 ± 2.336 BPM, $t = -3.507$, $df = 8$). All other pairwise tests had p-values greater than 0.05, showing no statistical significance.

Conclusion: Our study did not support our hypothesis that combining the CPT and MMR would amplify cardiovascular responses as only heart rate increased significantly during the CPT alone. These findings suggest that sympathetic activation may have been insufficient to override parasympathetic modulation or that compensatory mechanisms, like baroreflex buffering, limited the observed effects. This highlights the need for future studies with higher exercise intensities, use of sympathetic microneurography, and baseline measurements prior to each task.

Keywords: cold pressor test, muscle metaboreflex, sympathetic nervous system, post-exercise circulatory occlusion, thermoreceptors, baroreflex, parasympathetic nervous system

Introduction

Activation of the sympathetic nervous system can be measured through physiological responses when the body experiences significant stressors (Arza et al., 2019; Goldstein, 1987). The cold pressor test (CPT) is a well-established method used to activate the sympathetic nervous system (SNS) and assess physiological stress responses (Fanninger et al., 2023). During CPT, the immersion of an extremity in ice water stimulates thermoreceptors in the skin which triggers afferent signaling through A-δ and C-fibers to the hypothalamus and brainstem autonomic centers for sympathetic activation, and the somatosensory cortex for cold perception (Silverthorn & Michael, 2013; Fanninger et al., 2023). The resulting SNS activation leads to cardiovascular responses such as increased heart rate, arterial blood pressure, and vascular resistance (Saab et al., 1993; Silverthorn & Michael, 2013). Clinically, CPT can be used to assess autonomic function and baroreflex modulation, aiding in the diagnosis of conditions like hypertension (Lamotte et al., 2021). Previous research suggests that CPT elicits a linear correlation between mean arterial pressure and heart rate, supporting the notion of SNS activation (Cui et al., 2002).

Another related physiological reflex, the muscle metaboreflex (MMR), also increases arterial blood pressure and sympathetic activity (Fischer et al., 2013). This reflex is triggered by the accumulation of muscle metabolites during exercise, activating group III and IV muscle afferents (Kaufman & Rybicki, 1987). It is commonly studied using post-exercise circulatory occlusion (PECO), which traps metabolites in the active muscle, sustaining the afferent stimulation (Fischer et al., 2013). Interestingly, the sympathetic activation from PECO following a static handgrip exercise does not appear to result in a sustained increase in heart rate (Mark et al., 1985). However, some evidence suggests occlusion of larger muscle groups, such as in the leg, results in attenuated cardiac baroreflex sensitivity and a prolonged elevation in heart rate (Hartwich et al., 2011).

Studies suggest the MMR induced by PECO after a static handgrip exercise does not significantly elevate heart rate due to concurrent parasympathetic activation (O'Leary, 1993; Nishiyasu et al., 1994; Iellamo et al., 1999; Fisher et al., 2010). Parasympathetic tone, measured through heart rate variability (HRV), appears to counteract the sympathetic effects of the metaboreflex, limiting heart rate changes (Nishiyasu et al., 1994).

Given the distinct autonomic responses of these two stressors, this study aims to determine whether CPT-induced sympathetic activation can override the parasympathetic modulation observed during isometric handgrip PECO. If CPT enhances the metaboreflex response, we expect to observe greater increases in heart rate, reductions in HRV, shorter pulse transit time, and elevated mean arterial pressure (Kalfon et al., 2015). To test this hypothesis, we will compare cardiovascular responses across three randomized conditions of CPT alone, MMR alone, and a combination of both. HRV will be assessed through R-R interval variability using non-linear poincaré analysis (Malik et al., 1996). The SD1 and SD2 values can be extracted and divided to investigate the unpredictability of the R-R time intervals where a larger SD1/SD2 ratio indicates greater parasympathetic influence (Navarro-Lomas et al., 2020; Shaffer & Ginsberg, 2017). Moreover, pulse transit time will serve as a surrogate for arterial stiffness, reflecting sympathetic activation (Zhang et al., 2011). Prior research has demonstrated that increased sympathetic outflow, as seen in lower body negative pressure tests, directly reduces pulse transit time (Nardone et al., 2018).

Thus, we predict that the combined CPT and MMR condition will elicit more pronounced reductions in pulse transit time and HRV, accompanied by increased mean arterial pressure and heart rate relative to the metaboreflex condition alone. A better understanding of the autonomic regulatory mechanisms when the body is subjected to multiple stressors will not only provide

insight into how these mechanisms operate in tandem but could be useful in identifying autonomic dysfunction which leads to cardiovascular disease progression like hypertension, heart failure and coronary artery disease (Goldberg et al., 2019).

Methods and Materials

Ethical approval

This study was reviewed and approved by the Thompson Rivers University Research Ethics Committee on Human Subjects and by Faculty Supervisor, Dr. Mark Rakobowchuk. Before beginning, participants provided written, informed consent to participate in this study. Each participant understood the risks associated with the physiological tests and received verbal and written descriptions of procedures to be endured in this experiment. Each participant understood fully that they retain their right to withdraw from the study at any point. Consent was received prior to performing each task in the study.

Participants

Nine participants– three men and six women between ages 18 to 30 years –provided written, informed consent to participate in this study. Participants were recruited through word-of-mouth and were not provided with any compensation for their participation. Participants gave between sixty and seventy-five minutes of their time to endure this study. All volunteers had no known significant health concerns and declared themselves healthy and able to endure the experimental physiological tests. Participants were screened verbally for health-related issues such as cardiovascular disease or chronic hypertension before signing the consent form.

Experimental Overview

Procedure

Upon arrival at the lab, participants were assured of confidentiality (each was assigned a random number, with no identifying information recorded apart from the consent form). They were provided with a consent form outlining the study's purpose, procedures (including the estimated time commitment), potential risks and benefits, compensation, confidentiality assurances, the right to withdraw, and contact information.

Participants were then instructed to perform three maximal hand grips using a MLT004/ST Grip Force Transducer connected to a PowerLab 26T (ADInstruments, New Zealand). Participants began the experiment by measuring maximal hand grip three times at 30s intervals over 90s. The minimal and maximal hand grip values (mV) were extracted from the data and set as the lowest (0%) and highest value (100%) of handgrip in AcqKnowledge software via the BIOPAC system. Participants were connected to an ECG via a Philips EPIQ5 ultrasound machine by placing 3M-2560 Red Dot Multi-Purpose Monitoring Electrodes (1000/cs) with Parker Spectra ® 360 electrode gel under their clavicles and on the left hip region, as seen in Figure 1. Arterial blood pressure was continuously monitored by a CNAP monitor (CNSystems, Austria) with an electric sphygmomanometer cuff and finger cuff monitor on the non-dominant hand. Sitting comfortably in a lab chair in a standard position, participants' baseline data was recorded for 5 minutes. Experimental trials were randomized in order but consisted of four conditions: an isometric handgrip PECO MMR, a CPT, and a combined MMR + CPT test (conducted 15s apart).

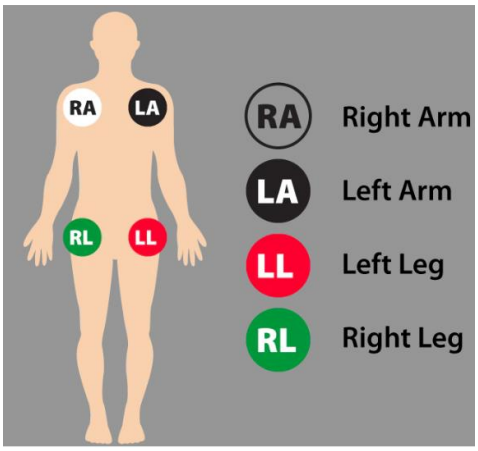

Figure 1. ECG set-up used in the study (excluding right leg electrode).

During the MMR, the participants were seated facing a desktop computer displaying the lab chart output. A sphygmomanometer was wrapped around the dominant arm, then participants were instructed to maintain an isometric grip force in their dominant hand at 20 % of maximal voluntary contraction for 3 minutes. When a participant was 15 seconds from ending the exercise, the sphygmomanometer was inflated to 240 mmHg to occlude the brachial artery in the dominant arm. The grip force transducer was then removed from the dominant hand. Participants were previously instructed to remain still while PECO was maintained on the dominant arm for 3 minutes while data was recorded. After the cuff was deflated at 3 minutes post-exercise, data were recorded for 5 minutes.

The CPT was performed on the foot enabling continuous monitoring of variables from the upper body. Baseline data were recorded for 5 minutes before beginning the cold pressor test. A cold-water bath was maintained at a temperature between 4 – 5 ºC using ice. The temperature was monitored with an infrared thermometer. A baseline temperature of the foot was taken for reference also via the infrared thermometer. After baseline data were recorded, the participant was instructed to submerge their foot up to the ankle in the cold-water bath. Data were recorded for 3 minutes

while a researcher kept the water circulating to maintain a consistent temperature, subsequently reducing the ability of the extremity to create a thermal layer while submerged. Immediately after the submersion period, the participant's foot was dried and wrapped in a heating pad to accelerate the rate of the extremity returning to baseline temperature. The participant was instructed to rest for a minimum of 5 minutes.

To perform the concomitant test, the participants maintained an isometric hand grip exercise in their dominant hand at 20 % of maximal voluntary contraction for 3 minutes as per the procedure stated above. When a participant was 15 seconds from ending the exercise, a sphygmomanometer was inflated to 240 mmHg to occlude the brachial artery in the dominant arm. The cold pressor test was delayed by 30 seconds from occlusion onset to cause an additional increase in SNS response to the additional test. Post-exercise occlusion on the arm and simultaneous foot immersion were maintained for 3 min while data were recorded. After 3 min, the sphygmomanometer was released, and following an additional 30s, the foot was removed, dried, and wrapped in a heating pad.

Statistical Analysis

We used AcqKnowledge software to extract heart rate (HR), mean arterial pressure (MAP), and pulse transit times (PTT) for all four conditions. Poor signals from ECG or finger pulse oximeter data were filtered and excluded from mean calculations. Regions of interest of 5 minutes were selected for baseline and all other conditions, then HR, PTT, and MAP means were extracted from the last minute of each condition. Poor signals from ECG and finger pulse data were filtered and excluded from mean calculations. Variables within the regions of interest were transposed into Microsoft Excel software. HRV analysis involved all 5 minutes of the region of interest as per recommendation (Malik et al., 1996). Heart rate data that contained poor signals was manually

filtered in LabChart Reader v7.0.00 (Colorado Springs, USA), so that all QRS complexes were detected, and the R-R intervals were calculated and exported in Microsoft Excel. HRV analysis was conducted using HRVanalysis 1.2 software (Université Jean Monnet, Sainte Étienne, France) to obtain SD1 and SD2 values from Poincaré plots for non-linear measurements of HRV (Pichot et al., 2016).

The experiment utilized a within-subjects design across four conditions, so a repeated measures ANOVA was conducted for statistical analysis. Data were expressed as means ± SD with 95% confidence intervals. All statistical analyses and graphical representations were performed using JASP (v0.19.3, University of Amsterdam, Netherlands). Statistical significance was set at $p < 0.05$. Variables that showed significant effects across conditions were further examined using post-hoc comparisons to identify specific inter-condition differences.

Results

The analysis in Figure 2. shows a significant main effect of condition on HR ($F(3, 24) = 6.424$, $p = 0.002$). Holm-corrected post hoc comparisons showed a significant increase in heart rate from the Baseline to CPT condition (mean difference = -8.191 ± 2.336 BPM, $t = -3.507$, $df = 8$, $p = 0.048$). No other pairwise differences were statistically significant after correction (all $p > 0.05$).

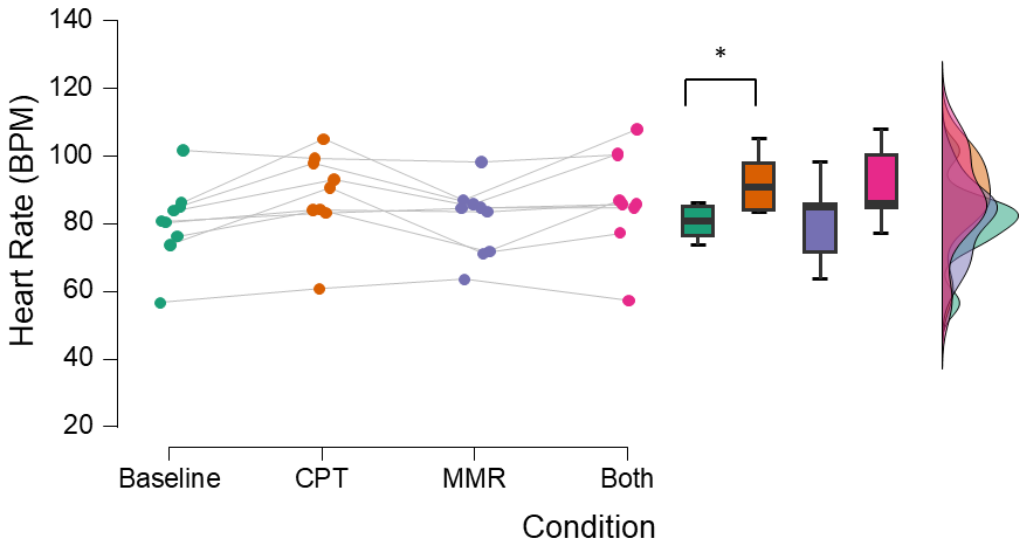

Figure 2. Raincloud plot portraying each participant's mean heart rate (BPM) across all four conditions: Baseline, CPT, MMR, and Both. (n = 9)

The analysis in Figure 3. shows a significant main effect of condition on HRV (SD1/SD2 ratio), with sphericity violated ($F(3, 24) = 3.049$, $p = 0.048$). However, Holm-corrected post hoc comparisons did not reveal any statistically significant differences between conditions (all $p > 0.05$).

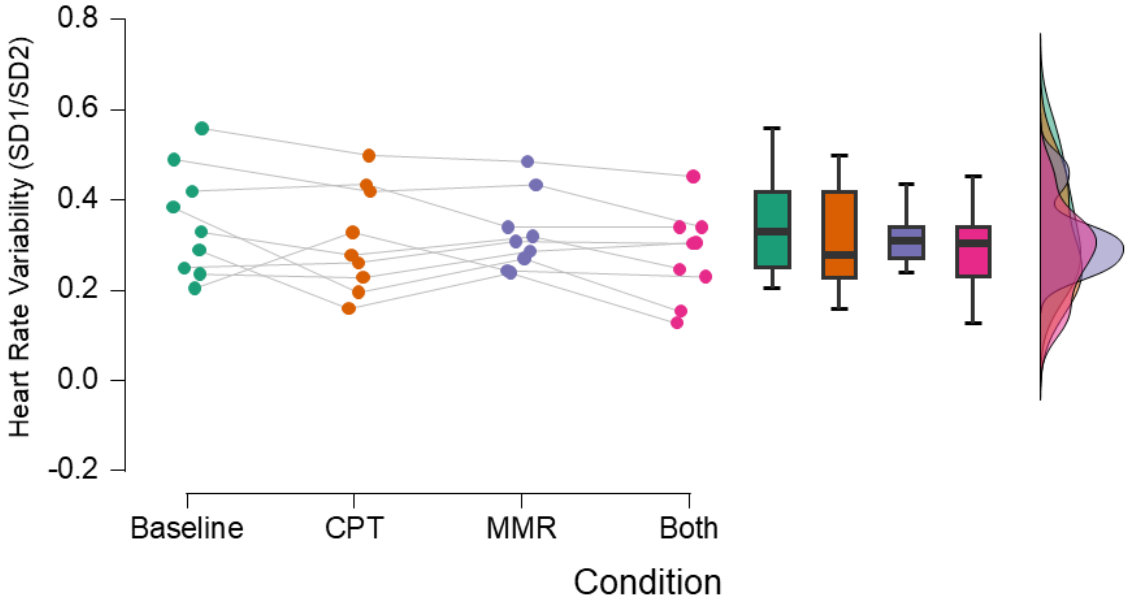

Figure 3. Raincloud plot portraying each participant's Heart Rate Variability (SD1/SD2) across all four conditions: Baseline, CPT, MMR, and Both (n = 9)

The analysis in Figure 4. did not reveal a significant main effect of condition on MAP ($F(3, 24) = 2.459$, $p = 0.087$).

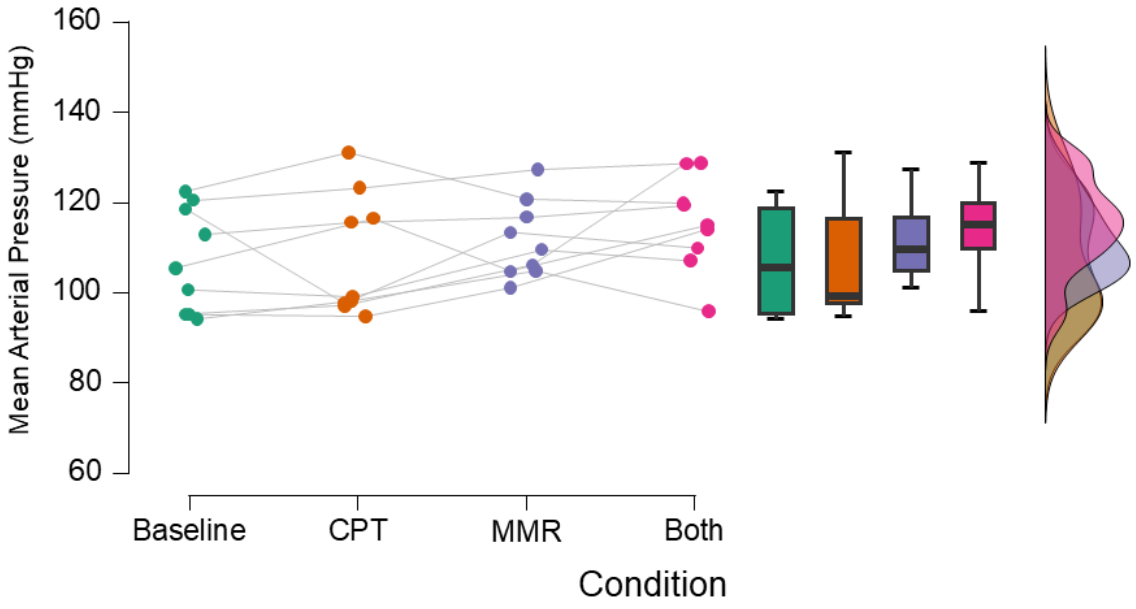

Figure 4. Raincloud plot portraying each participant's Mean Arterial Pressure (mmHg) across all four conditions: Baseline, CPT, MMR, and Both (n = 9).

The analysis in Figure 5. showed no significant effect of condition on PTT ($F(3, 24) = 1.021$, $p = 0.401$).

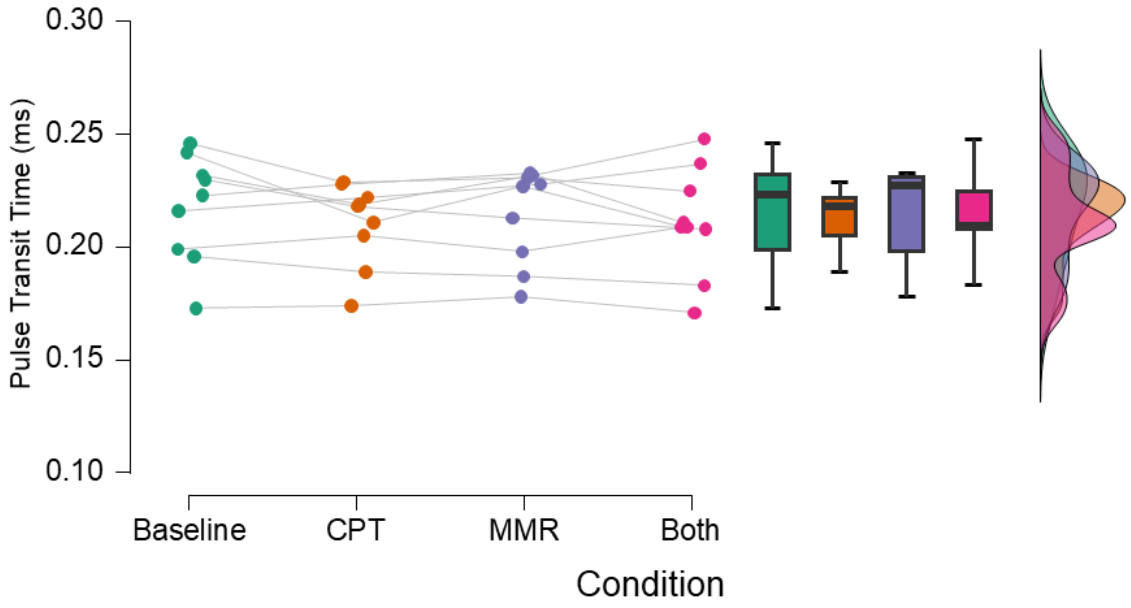

Figure 5. Raincloud plot portraying each participant's Mean Pulse Transit Time (ms) across all four conditions: Baseline, CPT, MMR, and Both (n = 9).

Discussion

Main Findings

We predicted the combined CPT and MMR condition would give rise to more profound reductions in PTT and HRV, accompanied by increased MAP and HR relative to the MMR and CPT conditions separately (Kalfon et al., 2015). Surprisingly, the only significant finding was that the cold pressor test resulted in a significant increase in HR relative to only the baseline condition. This is expected given that sympathetic activation results in the release of norepinephrine on the sinoatrial node leading to elevations in heart rate (Elias & Ayji, 2019; Victor et al., 1987). However, this effect was not accompanied by any increases in MAP, or decreases in HRV and PTT, unlike what is expected from previous studies investigating these responses to sympathetic activation (Lu et al., 2022; Sràmek et al., 2000; Victor et al., 1987). Changes in HRV were not observed during the CPT. This is possibly explained by large inter-subject variability in HRV responses including increases or decreases in parasympathetic tone during CPT, deeming the measure unreliable for cardiovascular reflex tests (Jàuregui-Renaud et al., 2001).

It is not surprising that the MMR during PECO resulted in no changes to heart rate relative to baseline because previous research indicates that baroreflex-mediated cardiovagal reactivation restores heart rate to resting levels regardless of metaboreflex sympathetic outflow (Iellamo et al., 1999). In contrast to previous studies, PECO following 3 minutes of isometric handgrip exercise did not result in any significant changes in MAP (Fisher et al., 2013). It is possible that the arterial baroreflex could be activated during exercise in response to the muscle metaboreflex, and then during PECO continue to buffer any blood pressure increases by restricting sympathetic activation and increases in peripheral vascular resistance (Kaur et al., 2018). Without significant differences in MAP between conditions we can postulate that arterial vasoconstriction was not sufficient under our testing conditions since vasoconstriction is shown to increase arterial stiffness which is reflected in the time it takes for the blood pressure waveform to travel along the arterial path (Fok

et al., 2012). Like the CPT, HRV analysis showed no significant changes during the MMR only condition.

When considering the combined condition of the CPT and MMR, it is important to note the intensity of the stimuli as this can alter the degree of muscle sympathetic nerve activity (MSNA) (Kregel et al., 1992). Since MSNA is typically directly monitored through microneurography—which involves placing a needle in the efferent axon of a peripheral nerve — our study lacks a direct measurement of sympathetic activity (White et al., 2015; Yucha, 2000). Therefore, it is not possible to absolutely conclude sufficient sympathetic outflow was achieved. Furthermore, it has been shown that arterial baroreflex sensitivity can adjust accordingly to increased MSNA induced by the CPT and MMR, and because we evaluated our cardiovascular response variables during the final minute of the condition, it is possible the body was able to adjust to the sympathetic activation resulting in no changes to MAP, HR, PTT and HRV (Cui et al., 2001). Moreover, there is some evidence that baroreflex sensitivity is attenuated by the muscle metaboreflex during post-exercise circulatory occlusion after leg cycling, but not isometric handgrip exercise (Hartwich et al., 2011). This indicates that the activation of larger muscle groups or increases in exercise intensity may decrease potential baroreflex autonomic regulation and limit interference with sympathetic activity. Therefore, isometric handgrip exercise at 20% of maximum voluntary contraction may be insufficient to reduce the baroreflex response which is why our results were inconclusive (Fisher et al., 2008). Future studies could analyze continuous responses across pre-determined time intervals to better understand physiological changes over the whole response period. This would illustrate if initial changes were subsequently buffered.

Sources of Error and Limitations

There are some significant limitations in our study. First, we did not collect baseline data prior to the start of each condition trial, subsequently limiting our ability to verify that the cardiovascular dependent measures returned to baseline. After completion of each condition, baseline measurements were assumed to have returned to homeostasis after a minimum of 5 minutes of rest considering MAP and HR return to baseline after 3 minutes post-recovery from CPT immersion and 2 minutes after isometric handgrip PECO (Mizushima et al., 1998; Ubolsakka-Jones et al., 2017). We also ensured participant skin temperature returned to baseline prior to continuing with testing protocols. A lack of continuous measurement also made it more difficult to quantify the physiological changes that were occurring over the whole condition period which would have helped us see if the body is rapidly adapting to the two stressors.

During CPT analysis, we extracted mean data from the third minute of the test to be consistent with wanting to observe the combined effects of sustained CPT and PECO pressor responses without the influence of the exercise period. However, it is suggested that due to the increase in cardiac output and blood pressure at the start of the test, the first 30 seconds of the CPT is the most effective period for measuring these effects (Moriyama & Ifuku, 2007).

Another limitation of this study was the elevated baseline MAP observed in our participants (107 ± 11 mmHg), which is higher than typically reported in healthy, young adults (~85 - 90 mmHg) (Fabian et al., 2016; Diaz et al., 2018). This difference may be attributed to the majority of our participants being university students (eight of nine), a population which has reportedly higher psychological distress when compared to non-university peers of their age (Dyrbye et al., 2006). Increased stress and anxiety are correlated with increased resting MSNA which could indicate increased sympathetic outflow prior to testing resulting increased blood pressure (Bigalke et al.,

2023). This could be a confounding factor explaining why we didn't see any significant alterations in blood pressure and other cardiovascular responses during testing.

An *A Priori* power analysis was performed using JASP software which suggested a sample size of at least 17 participants would be necessary to determine an effect size of 0.741 for MAP, 77 participants would be necessary to determine an effect size of 0.324 for PTT, 18 participants for effect size of 0.712 for HRV, and 21 participants for effect size of 0.657 for HR, whereas our study had a sample size of 9 participants. Our study had a higher chance of type 2 error, meaning our failure to reject the null hypothesis might be false because of a small sample size.

Future Research

Because the MMR did not increase the heart rate response when combined with the CPT, conditions can be altered to induce a response between the combined tests, such as increasing test duration, or increasing the intensity of the isometric exercise to add motor unit recruitment. Future studies may include muscle nerve innervation to assess autonomic nerve activity functions as well as provide more accurate results through the confirmation of muscle sympathetic nervous recruitment during these tests.

Future research involving the combined muscle metaboreflex and cold pressor test could be refined. This could include testing chronically hypertensive subjects and comparing their results to normotensive individuals to see if there are exaggerated responses to sympathetic activation

(Delaney et al., 2010). We could also incorporate blood measurements to detect metabolite accumulation after varying levels of exercise intensity in post-exercise circulatory occlusion, to see if higher intensity exercise has greater effects on heart contractility and cardiac output compared to the increases in systemic vascular resistance observed at lower intensities (Crisafulli et al., 2006). This could help to understand the various mechanisms of sympathetic stimulation during the isolated metaboreflex, which may or may not be impacted by the cold pressor test. One could also incorporate pharmacological antagonists to observe how much both reflexes are modulated by say sympathetic activation compared to parasympathetic withdrawal. Fisher et al. (2013) examined the muscle metaboreflex under parasympathetic and then sympathetic blockade to better understand how cardiac responses are modulated by different arms of the autonomic nervous system (Fisher et al., 2013). This figure shows how norepinephrine binds to beta-adrenergic receptors to increase heart rate and contractility, but a beta-blocker would block these receptors, so we can see the effects of parasympathetic activity by itself. Finally, having participants perform Valsalva maneuvers prior to testing could help evaluate the baroreceptor reflex and could give a better idea of basal autonomic regulation (Zygmunt & Stanczyk, 2010).

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