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Human Physiology Final Project Proposal

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Title: Impact of Cold Pressor on Muscle Metaboreflex Response

Objective/Research Question: Does the cold pressor test result in an increased muscle metaboreflex response during a handgrip exercise?

Prediction: If the cold pressor test influences the muscle metaboreflex during a handgrip exercise, then we expect to see increased sympathetic activation resulting in increased heart rate, decreased pulse transit time and increased mean arterial pressure when compared to responses from only the cold pressor test or muscle metaboreflex.

Background:

The cold pressor test (CPT) is a procedure that primarily activates the sympathetic nervous system (SNS) and can be used to study physiological and pain stress responses (Fanninger et al., 2023). During the test, participants place an extremity into ice water to stimulate thermoreceptors in the skin (Silverthorn and Michael, 2013, Fanninger et al., 2023). Signals are then transmitted to the somatosensory cortex which stimulates a response throughout effectors of the SNS (Silverthorn & Michael, 2013; Fanninger et al., 2023). Thermoreceptors evoke signals in ascending A-δ and C-fibres in the arm which transmit action potentials to the somatosensory cortex (Fanninger et al., 2023). Efferent nerves deliver stimuli to the effector organs, including the heart and lungs (Fanninger et al., 2023). SNS physiological responses to the CPT can be measured through the cardiovascular system and include increases in heart rate, arterial blood pressure, and vascular resistance (Silverthorn and Michael, 2013). In clinical systems the CPT is used as a measure of baroreflex modulation and is used to diagnose autonomic nervous system dysfunctions such as hypertension (Lamotte et al., 2021). Cui, Wilson, and Crandall (2002) suggested that during the CPT there is a change in heart rate and muscle sympathetic nerve activity to account for baroreflex effects. They also suggest a linear correlation with mean arterial blood pressure and heart rate, further supporting the activation of the SNS during the procedure (Cui, Wilson, and Crandall, 2002).

In relation to the cold pressor test, the muscle metaboreflex is another pressor test that often increases arterial blood pressure and sympathetic activity (Fischer et al., 2013). This reflex occurs when the group III and IV muscle afferents are stimulated in part by the accumulation of muscle metabolites during periods of exercise (Kaufman & Rybicki, 1987). A common method to induce the muscle metaboreflex is by post-exercise circulatory occlusion (PECO) of the muscle that is exercising, thereby trapping the accumulated muscle metabolites within the region of muscle activity and triggering the metabolically sensitive group III and IV afferents (Fischer et al., 2013). A study by Nishiyasu et al. (1994) that had participants undergo an isometric handgrip exercise followed by PECO found that parasympathetic tone, which was measured by observing the magnitude of the heart rate variability, is enhanced. This increase in parasympathetic tone may be able to counteract the sympathetic effects of the metaboreflex on cardiac autonomic tone resulting in only modest increases in heart rate (Fischer et al., 2013). This is why studies have often reported limited effects of the metaboreflex on increasing heart rate (O'Leary, 1993; Nishiyasu et al., 1994; Iellamo et al., 1999; Fisher et al., 2010). Therefore, we want to evaluate if the cold pressor test, which is known to increase cardiac sympathetic activity, is able to override the parasympathetic activity observed during isometric handgrip PECO. This would result in a greater increase in heart rate and elicit a larger metaboreflex response by further decreasing heart rate variability, decreasing pulse transit time, and increasing mean arterial pressure.

To test our hypothesis, we need to compare measurements of heart rate, pulse transit time and mean arterial pressure under three randomized conditions: cold pressor test only, metaboreflex only, and both stressors combined. We can measure heart rate variability by extracting the R-R intervals during 5-minute response periods and then calculating the standard deviation between R-R intervals using time domain statistical analysis (Malik et al., 1996). We can also look at pulse transit time, as a surrogate for pulse wave velocity, to indicate increased sympathetic activation. A study by Nardone et al. (2018) demonstrated that acute increases in sympathetic activity via lower body negative pressure significantly raised central and whole-body pulse wave velocity (PWV) independent of changes in blood pressure, confirming a direct relationship between sympathetic outflow and arterial stiffness. So, we would expect pulse transit time to further decrease in participants undergoing various stress tests with the decreases occurring in the combined CPT and metaboreflex trial.

Procedure/Methods:

1) Recruit participants (e.g., healthy adults aged 18–40) with no known cardiovascular diseases.
2) Obtain informed consent from each participant.
3) Have participant perform maximal handgrip three times with 30s intervals to determine maximum handgrip.
4) Have participant sit comfortably in a chair, feet flat on the ground, with hands resting on the arms of the chair.
5) Attach ECG, CNAP machine, and loosely wrap blood pressure cuff around the working arm.
6) Have participant rest for 5 minutes
7) Measure baseline blood pressure and heart rate for 5 minutes.

A) Muscle Metaboreflex

8) Have participant perform isometric handgrip exercise at 20% of their maximal grip for 3 minutes.
9) Inflate the cuff to 240 mmHg 15s before end of exercise.
10) Maintain occlusion for 3 minutes while participant remains relaxed.
11) Monitor blood pressure and heart rate continuously during the occlusion period.
12) Deflate the cuff and continue to monitor blood pressure and heart rate for 5 minutes.
13) Remove blood pressure cuff/hand grip transducer.
14) Have participant rest in a seated position for 5 more minutes.

B) Cold Pressor Test

15) Measure baseline temperature of the foot with the thermometer.
16) Instruct the participant to immerse one foot (to the ankle) in the cold pressor (~ 4°C water) for 3 minutes.
17) Measure BP and HR continuously throughout.
18) Remove foot and measure BP & HR for additional 5 minutes.
19) Have participant rest for 10 minutes or until foot is at baseline temperature
20) Ensure baseline temperature has returned to participant's foot before moving on.

C) Perform both the cold pressor test and muscle metaboreflex at the same time while continuously measuring BP & HR.

21) Perform 20% isometric handgrip for 3 minutes.
22) Start recording HR and blood pressure while inflating the cuff to 240mmHg 15s before end of isometric exercise, then 30s into occlusion immerse the foot in cold water.
23) Maintain cuff inflation and foot immersion for 3 minutes while recording HR and BP.
24) Deflate the cuff and remove the foot while still recording HR and BP for another 5 mins.

Note: procedures **A)**, **B)**, and **C)** will be randomized to account for confounding factors from each previous test. Participant will also have non-exercising arm in an arm sling to be level with the heart.

Supplies/Equipment:

- CNAP machine for continuous blood pressure measurements
- ECG device
- Grip Force Transducer
- Sphygmomanometer

- Power lab
- Desktop computer
- Lab Chart
- Cold pressor (bucket with ice water, temperature ~0–4°C)
- Timer or stopwatch
- Infrared thermometer

References

Cui, Jian, Thad E. Wilson, and Craig G. Crandall. "Baroreflex Modulation of Muscle Sympathetic Nerve Activity during Cold Pressor Test in Humans." *American Journal of Physiology-Heart and Circulatory Physiology* 282, no. 5 (May 1, 2002): H1717–23. https://doi.org/10.1152/ajpheart.00899.2001.

Fanninger, Selina, Paul L. Plener, Michael J. M. Fischer, Oswald D. Kothgassner, and Andreas Goreis. "Water Temperature during the Cold Pressor Test: A Scoping Review." *Physiology & Behavior* 271 (November 2023): 114354. https://doi.org/10.1016/j.physbeh.2023.114354.

Fisher, J. P., Seifert, T., Hartwich, D., Young, C. N., Secher, N. H., & Fadel, P. J. (2010). Autonomic control of heart rate by metabolically sensitive skeletal muscle afferents in humans. *The Journal of Physiology*, *588*(Pt 7), 1117–1127. https://doi.org/10.1113/jphysiol.2009.185470

Fisher, J. P., Adlan, A. M., Shantsila, A., Secher, J. F., Sørensen, H., & Secher, N. H. (2013). Muscle metaboreflex and autonomic regulation of heart rate in humans. *The Journal of Physiology*, *591*(15), 3777–3788. https://doi.org/10.1113/jphysiol.2013.254722

Iellamo, F., Pizzinelli, P., Massaro, M., Raimondi, G., Peruzzi, G., & Legramante, J. M. (1999). Muscle metaboreflex contribution to sinus node regulation during static exercise: Insights from spectral analysis of heart rate variability. *Circulation*, *100*(1), 27–32. https://doi.org/10.1161/01.CIR.100.1.27

Kaufman, M. P., & Rybicki, K. J. (1987). Discharge properties of group III and IV muscle afferents: Their responses to mechanical and metabolic stimuli. *Circulation Research*, *61*(4 Pt 2), I60-65.

Lamotte, Guillaume, Christopher J. Boes, Phillip A. Low, and Elizabeth A. Coon. "The Expanding Role of the Cold Pressor Test: A Brief History." *Clinical Autonomic Research* 31, no. 2 (April 2021): 153–55. https://doi.org/10.1007/s10286-021-00796-4.

Malik, M., Bigger, J. T., Camm, A. J., Kleiger, R. E., Malliani, A., Moss, A. J., & Schwartz, P. J. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. European Heart Journal, 17(3), 354–381. https://doi.org/10.1093/oxfordjournals.eurheartj.a014868

Nardone, M., Incognito, A. V., & Millar, P. J. (2018). Evidence for pressure-independent sympathetic modulation of central pulse wave velocity. Journal of the American Heart Association, 7(3), e007971. https://doi.org/10.1161/JAHA.117.007971

Nishiyasu, T., Tan, N., Morimoto, K., Nishiyasu, M., Yamaguchi, Y., & Murakami, N. (1994). Enhancement of parasympathetic cardiac activity during activation of muscle metaboreflex in humans. *Journal of Applied Physiology*, *77*(6), 2778–2783. https://doi.org/10.1152/jappl.1994.77.6.2778

O'Leary, D. S. (1993). Autonomic mechanisms of muscle metaboreflex control of heart rate. *Journal of Applied Physiology*, *74*(4), 1748–1754. https://doi.org/10.1152/jappl.1993.74.4.1748

Silverthorn, Dee U., and Joel Michael. “Cold Stress and the Cold Pressor Test.” *Advances in Physiology Education* 37, no. 1 (March 2013): 93–96. https://doi.org/10.1152/advan.00002.2013.