CHRONIC POLYPHENOL SUPPLEMENTATION EFFECTS ON PHYSIOLOGICAL AND COGNITIVE RESPONSES TO COLD WATER IMMERSION

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INTRODUCTION

Polyphenols may mitigate military-relevant cold stress by improving blood flow and priming protective molecular pathways (1, 2).

PURPOSE: We aimed to determine if chronic polyphenol supplementation could attenuate cold water immersion (CWI) stress.

METHODS

In a crossover, double-blind, placebo-controlled study, participants (n=14 [7 male, 7 female], age=26±2y) were randomized to consume a polyphenol-rich (500mg flavonoids+700mg anthocyanins) or placebo supplement bar for 14 days prior to an in-laboratory 25min CWI (~10°C) test (Figure 1).

Measurements included:

1. **Cold Stress Trials:** CWI test had five stages (baseline, pre-CWI, CWI, post-CWI, recovery) with pre-CWI, CWI, and post-CWI in a cold environmental chamber (~1°C)
2. **Salivary Stress Biomarkers:** cortisol, salivary alpha amylase [sAA]; at baseline, CWI [at ~10 and 20min], post-CWI, recovery
3. **Thermal Response**: core (ingestible capsule [Vital Sense, Respironics]) and skin temperature (shoulder, chest, thigh, foot, hand; iButtons)
4. **Cognitive Performance:** attention control tasks (psychomotor vigilance task [PVT], antisaccade task; at baseline, pre-/post-CWI, three times during CWI, recovery)
5. **Dried Blood Spots (DBS)** for proteomics analyses

Proteomics Analyses: Purified peptides were analyzed using LC-MS/MS. Acquired spectral data was processed using bioinformatics software to search protein databases to quantify and identify proteins.

Statistical Analysis: Repeated measures ANOVAs were used to assess intervention x stage main effects or interactions. For proteomics, statistical analysis outcomes included paired t-tests with permutation-based False Discovery Rate (FDR) correction (q<.05). Data are presented as means ± SE, and significance was set at p<.05.

RESULTS

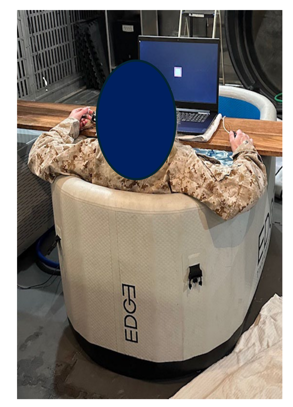

Photo by Dr. Michael Stone courtesy of NHRC

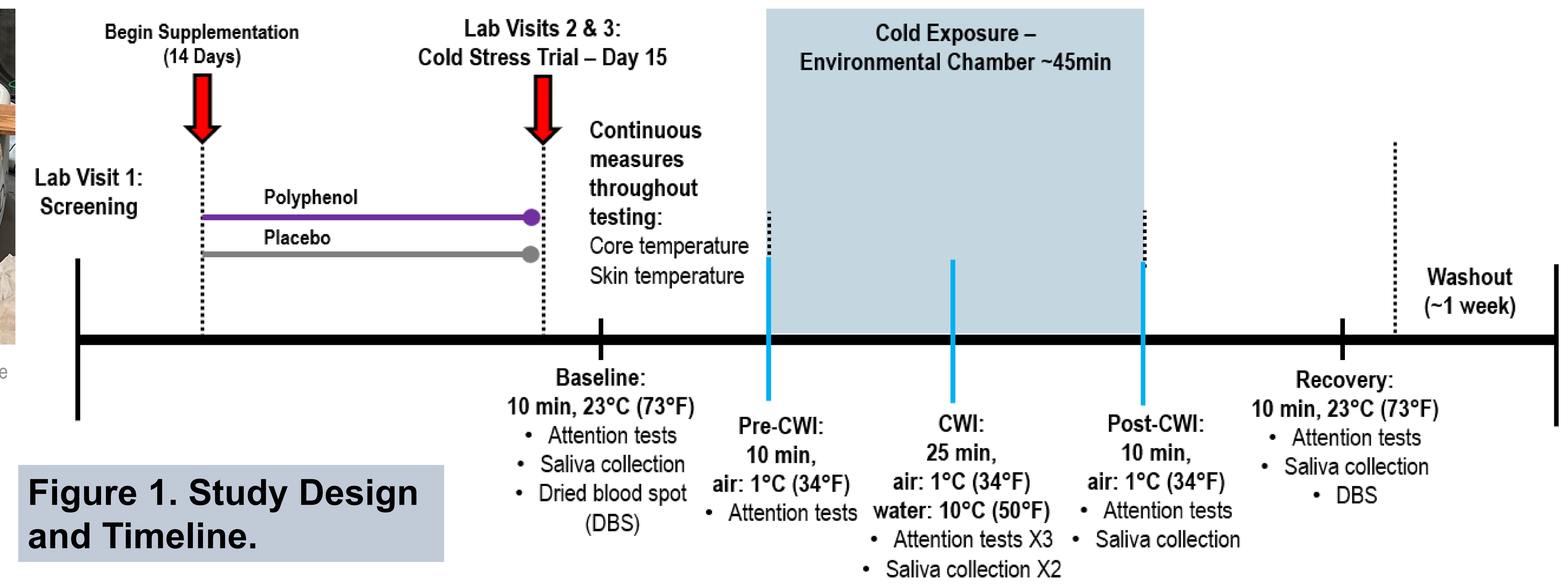

Figure 1. Study Design and Timeline.

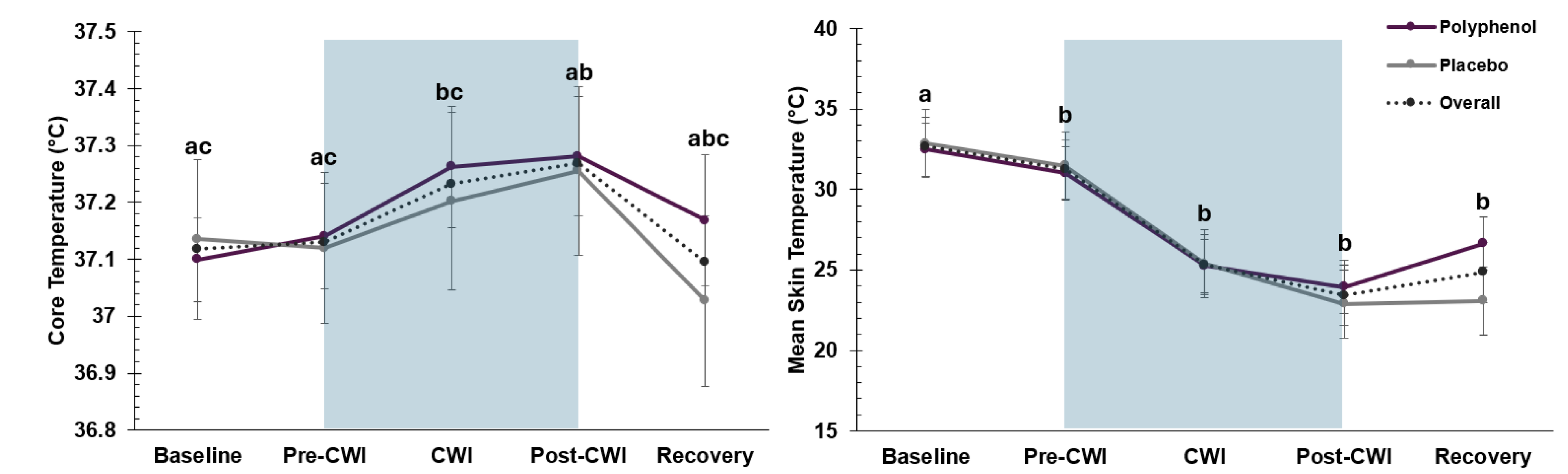

Figure 2. Thermal Response During Cold Stress Trial (different letters denote significance).

No difference in temperature with intervention (p>.05; Fig. 2):
- Overall, core temperature was lower (p<.007) at baseline (37.12 °C) and pre-CWI (37.13 °C) vs. CWI (37.23 °C); recovery (37.09 °C) was lower vs. post-CWI (37.27 °C).
- Overall, mean skin temperature (MSK=[0.5 x chest]+[0.36 x thigh]+[0.14 x shoulder]) was higher (p<.001) at baseline (32.65 °C) vs. pre-CWI (31.26 °C), CWI (24.72 °C), post-CWI (22.99 °C), and recovery (25.43 °C).

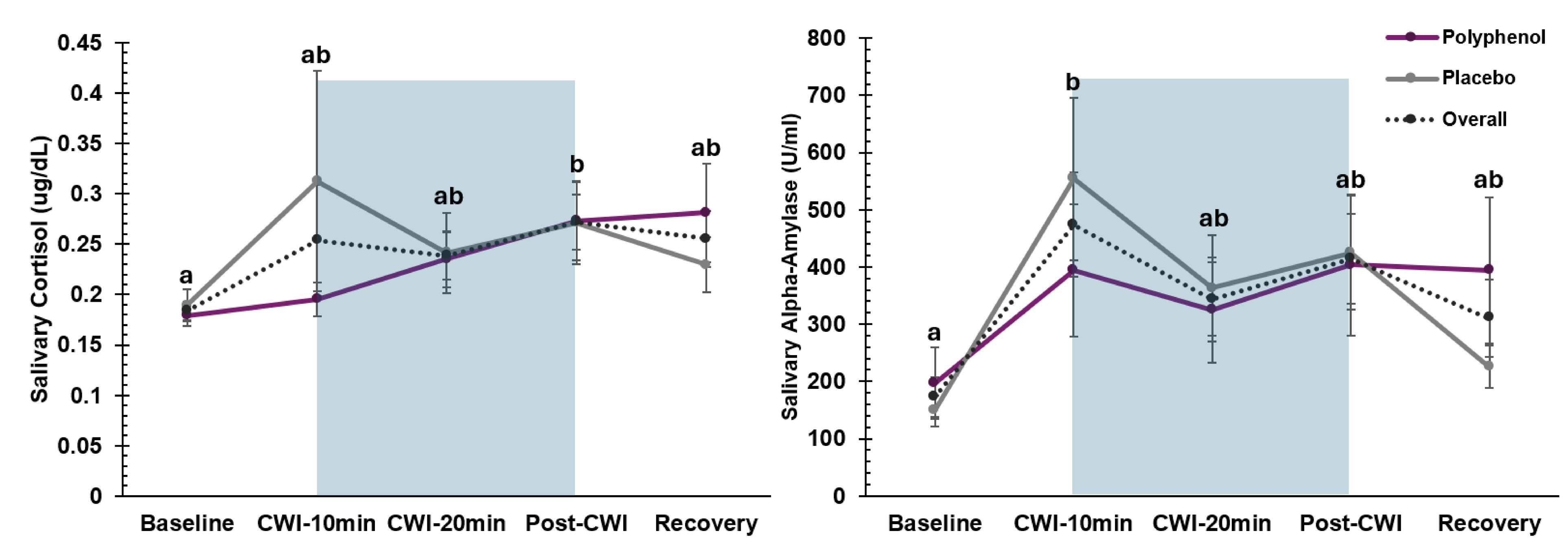

Figure 3. Salivary Hormone Response During Cold Trial (different letters denote significance).

No difference in salivary cortisol or sAA with intervention (p>.05; Fig. 3):
- Salivary cortisol was lower (p=.025) at baseline (0.184 ug/dL) vs. post-CWI (0.272 ug/dL).
- sAA was lower (p=.04) at baseline (173.4 U/ml) vs. CWI at 10 min (473.9 U/ml).

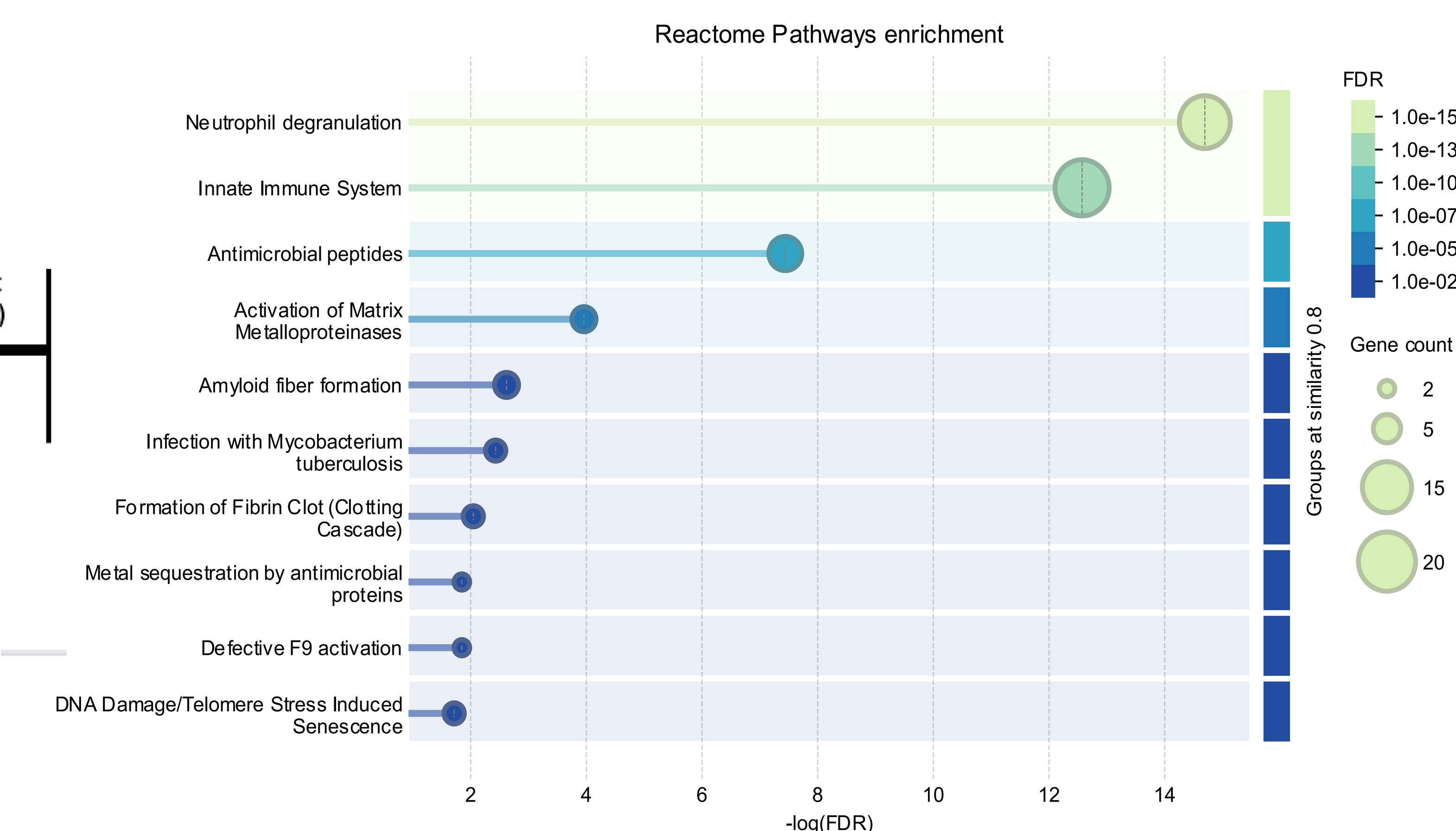

Figure 4. Pathway Enrichment in Differentially Expressed Proteins for Cold Stress.

Reactome pathway enrichment (Fig. 4), cold stress induced significant changes in 29 upregulated, differentially expressed proteins (q<0.05; |log2 fold change| > 0.35; Fig. 4). No intervention effect.

Cognitive Outcomes:
- PVT response time was slower during CWI (334, 334, 346 ms), post-CWI (355 ms), and recovery (350 ms) compared with baseline (315 ms; p<.03).
- The number of correct responses during the antisaccade task did not differ among stages (p>.05).

CONCLUSION

Chronic polyphenol supplementation did not affect thermal, cognitive, stress, or protein response to CWI.

- Proteins upregulated due to cold stress were enriched in hemostasis, response to stress, regulation of body fluid level, and innate immune responses.
- To our knowledge, this is the first proteomics signature assessed for military-relevant cold stress

References
1. Grosso G, Godos J, Currenti W, Micek A, Falzone L, Libra M, Giampieri F, Forbes-Hernández TY, Quiles JL, Battino M, et al. The Effect of Dietary Polyphenols on Vascular Health and Hypertension: Current Evidence and Mechanisms of Action. Nutrients. 2022; 14(3):545. https://doi.org/10.3390/nu14030545
2. Leri M, Scuto M, Ontario ML, Calabrese V, Calabrese EJ, Bucciantini M, Stefani M. Healthy Effects of Plant Polyphenols: Molecular Mechanisms. International Journal of Molecular Sciences. 2020; 21(4):1250. https://doi.org/10.3390/ijms21041250

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