Metabolomic Profiling Reveals Sex Differences and a Reduction in Metabolism after Remote Ischemic Preconditioning

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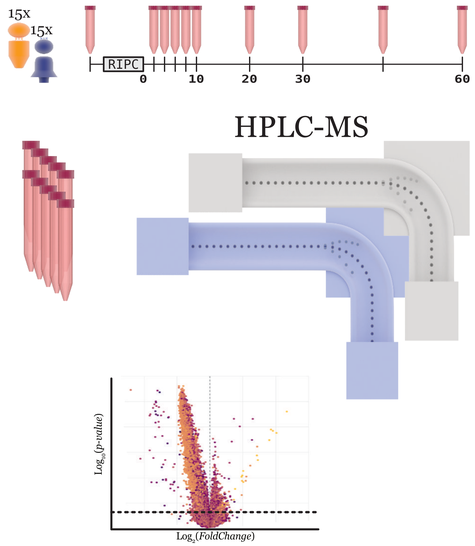
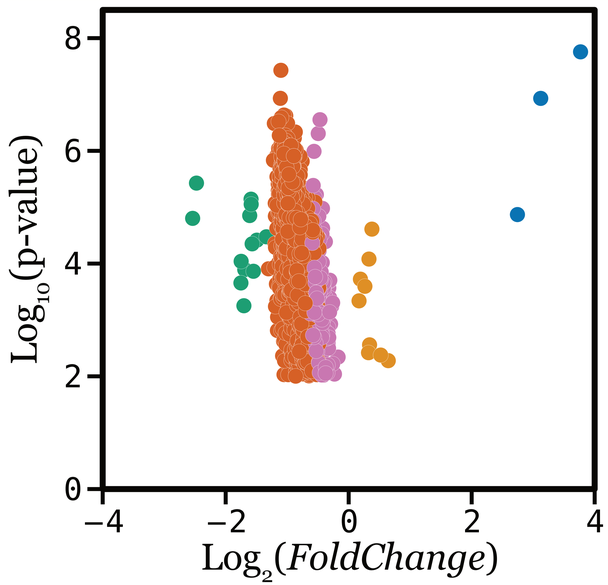
Graphical Abstract



Summary

- Remote ischemic preconditioning (RIPC) is a low-cost low-risk intervention in which repeated cycles of upper extremity ischemia induce organ protection by an unknown mechanism.
- We used plasma samples collected from healthy volunteers to probe metabolism dynamics in response to RIPC.
- Absolute (n=33) and non-targeted metabolomic feature fold-change profiles (n=73,815) were extracted from plasma samples using HPLC-MS.
- Isomap was used to cluster non-targeted features according to their fold-change dynamics.
- Following RIPC, we found tightly regulated changes in many metabolites that persist as long as we measured (60min)
- Sex differences appear to influence these

Results



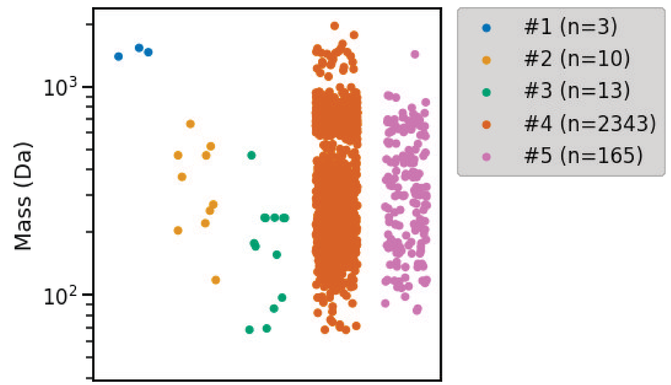


Figure 1) Fold-change from baseline at 10 min after RIPC for untargeted metabolomic features (n=73815) Welch's t-test was performed to estimate statistical significance. 2250 features with statistically significant (p>=0.01, dashed line) concentration changes were selected for clustering.

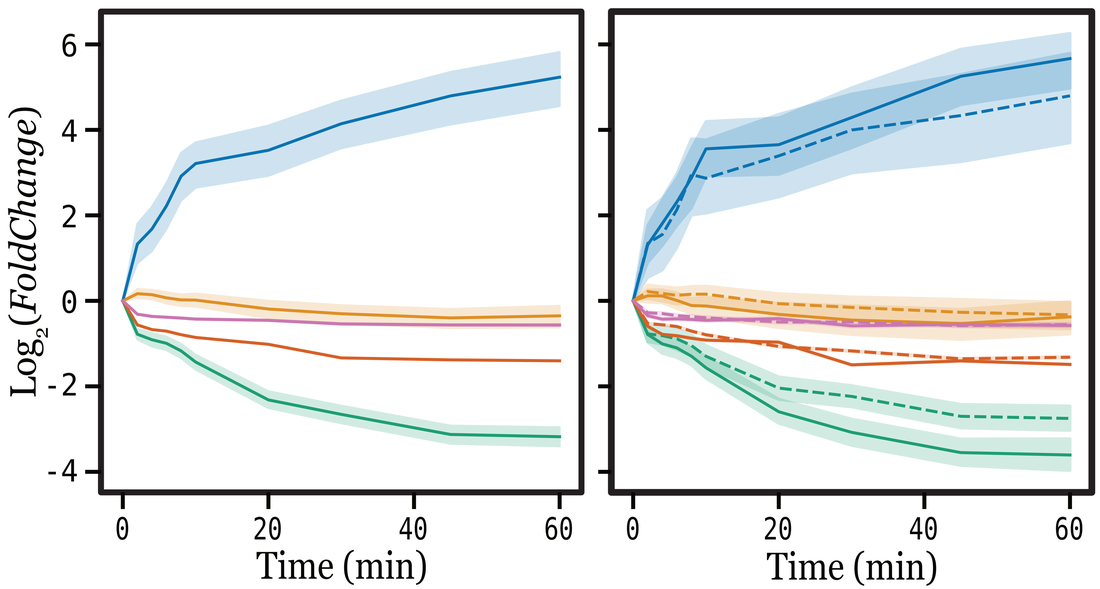


Figure 2) Isomap, a non-linear dimensionality reduction method, was used to cluster the untargeted features (n=2250) into 5 groups according to their temporal fold-change profiles. The mean fold-change over time of each group is shown with their confidence interval (95%) shaded. When the temporal profiles of each cluster group are separated by gender some groups demonstrate sex-specific differences.

Methods

Sample collection and processing
30 healthy volunteers (15 male, 15 female) underwent one cycle of RIPC with plasma samples collected both before and for 60 mins after.

We performed ultra-high performance liquid chromatography mass spectrometry (HPLC-MS) to profile metabolomic response. 33 metabolites were measured with absolute quantification by referencing to stable isotope labelled standards spiked into the samples at known quantification. We also performed an untargeted metabolomic analysis using automated feature detection software (Compound Discoverer) to detect 73,815 features.

Normalization and feature scaling
Untargeted features and absolute quantifications from each patient's plasma samples were normalized by the pre-trial sample (baseline) and scaled by base-2 logarithm to get $\log_2$(fold-change). Log_2(fold-change) mean and variance across all 30 patients were calculated at each timepoint and Welch's t-test was used to calculate significance of each metabolite and feature fold-change. Mean $\log_2$(fold-change) and statistical p-values were used to produce volcano plots for each sampling timepoint.

Untargeted feature clustering: Statistically significant ($p < 0.01$) fold change profiles over time were clustered using Isomap dimensionality reduction[4]. Th five distinct clusters or groups of features were observed after Isomap embedding. Features belonging to each of the five groups were combined and fold-change profiles were plotted over time by sex. Molecular weight scatter plots of each feature group were also produced. Feature group profiles were compared to several profiles from absolute metabolomic quantifications.

Conclusion

RIPC induces a significant reduction in metabolism as measured by a decrease in the majority of metabolites that persists for at least 60 minutes. We noted significantly lower glutamate levels in females after RIPC. RIPC may induce organ protection by reducing oxygen requirements through a systemic reduction in metabolism.

Acknowledgements