# Cross Compartment Proteomic Profiling of Ischemia Reperfusion Injury

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**INTRODUCTION:** Skeletal muscle ischemia reperfusion injury (IRI) is a serious complication of crush injury, compartment syndrome, and vascular compromise, leading to persistent weakness, atrophy, and disability. Despite its clinical importance, the molecular programs underlying acute damage and subsequent regeneration in skeletal muscle remain poorly characterized. Prior work has shown that IRI induces extracellular matrix (ECM) remodeling, metabolic dysfunction, and mitochondrial disruption in muscle tissue. To address this, we performed time resolved proteomic profiling of skeletal muscle and serum after hindlimb IRI to map compartment specific protein dynamics and convergent biological pathways. Our goal was to define both local mechanisms of damage/regeneration and systemic signaling programs. We focused on metabolic/bioenergetic, mitochondrial, and ECM/cytoskeletal responses as central coordinators of muscle injury and repair. Finally, we asked whether subsets of proteins whether shared or unique between muscle and serum organize into high confidence protein-protein interaction modules, providing insight into both tissue intrinsic and circulating signatures of musculoskeletal recovery.
**METHODS:** Unilateral right hindlimb IRI was performed wild type C57BL/6 mice of both sexes (3 months old, n = 12; 3 per group) as previously described [1]. The right tibialis anterior (RTA) muscle was collected at 1, 3, and 14 days after IRI, along with sham-controls, then homogenized for proteomic analysis. Proteins were profiled by LC-MS (Orbitrap Eclipse or timsTOF/Evosep) using DIA in label-free or TMT modes and searched against UniProt (Spectronaut/Proteome Discoverer/Scaffold). Data were log-transformed, filtered (≥2 values/group), and differential proteins defined ($|\log_2 FC| \geq 1.5$, FDR < 0.05). Signals were z-scored and clustered by temporal trend (k-means, k=4); GO/KEGG enrichment was performed, and pathways shared by muscle and serum were mapped to STRING v12 (mouse; combined score ≥ 0.7) to visualize the largest PPI component. The largest connected component was visualized in R. All analyses used fixed random seeds. This study was approved by IACUC.
**RESULTS SECTION:** KEGG analysis revealed 30 pathways common to muscle and serum. Shared metabolic programs were prominent, including Glycolysis/Gluconeogenesis (6 proteins in the overlap: Aldoa, Bpgm, Gapdh, Ldhb, Pgam2, Pgm1), Pyruvate metabolism (6: Glo1, Hagh, Ldha, Ldhb, Me1, Pkm), and Carbon metabolism (4: Aldoa, Gapdh, Me1, Pgam2), consistent with early metabolic reprogramming. A structural/mechano-transductive signal was also present, with motor/contractile proteins observed in the overlap (3: Actb, Myl6, Tpm1). Mapping the overlap to STRING (mouse, v12) and filtering for high confidence (combined score ≥700) produced a single connected PPI subnetwork containing 24 proteins linked by 44 undirected interactions, average degree 3.7 and density 0.16. Community detection resolved four coherent modules: (i) a glycolytic/pyruvate cluster (n=7; Aldoa, Bpgm, Ldha, Ldhb, Me1, Pgam2, Pkm), (ii) a calcium/contractile module (n=5; Calm1-3, Myl6, Tpm1), (iii) a glycogen-handling module (n=4; Agl, Pgm1, Pygm, Ugp2), and (iv) a mixed cytoskeleton/oxidative-stress group (n=8; Actb, Cfl1, Fn1, Gapdh, Glo1, Hagh, Park7, Sod1). Within this network, the most connected nodes were Pgm1 (k=10), Calm2 (k=9), Pygm (k=8), Gapdh (k=7), and Pkm (k=7), while betweenness centrality highlighted Calm2 (0.130), Pgm1 (0.116), Pygm (0.095), Calm3 (0.089), and Calm1 (0.081) as potential bottlenecks. Several candidates point toward mitochondria-related stress and redox control in injured muscle (Me1, Park7, Sod1), and the ECM/cytoskeletal components (Fn1, Actb, Tpm1, Myl6) suggest a shared mechano responsive axis with potential relevance to muscle-bone crosstalk. The data define a compact module centered on energy metabolism and CaM-linked contractile signaling, suggesting a focused protein panel to monitor muscle injury and recovery after IRI.
**DISCUSSION:** Our cross-compartment profiling revealed a coordinated response to limb IRI centered on energy metabolism and ECM/cytoskeletal programs, with overlapping signatures detectable in both muscle and serum. We focused on proteins that changed in both muscle and serum to highlight potential factors with systemic relevance that may reflect muscle states while being measurable in circulation. This approach was intended to nominate candidate pathways. STRING network analysis connected glycolytic/glycogen enzymes with calmodulin associated contractile and redox proteins, outlining an integrated stress response module. These findings suggest that serum profiling may provide pathway aligned indicators of muscle injury and recovery. Limitations include reliance on an animal model, bulk proteomic analysis without cell type resolution, and the absence of functional or clinical validation. Future studies should prioritize targeted assays to verify candidate proteins, integration with cell type specific and metabolomic data, and evaluation in human cohorts to test whether serum signatures can serve as practical biomarkers of musculoskeletal recovery.
**SIGNIFICANCE/CLINICAL RELEVANCE:** Shared muscle-serum pathways provide a pathway level framework for studying how metabolic and structural responses in injured muscle coordinate with circulating signals after IRI injury.
**REFERENCES:** [1] Zhang et al.

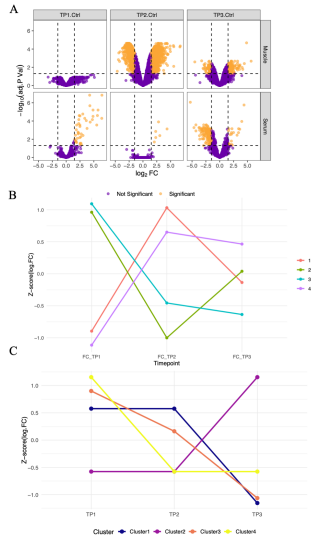


Figure 1. Global proteomic responses to limb IRI.
(A) Volcano plots of differential protein abundance in muscle (top) and matched serum (bottom) at 1, 3, and 14 days post-IRI versus sham (n=3/group). x-axis: $\log_2$ fold-change (IRI vs. sham); y-axis: $-\log_{10}$ FDR-adjusted P value. Vertical dashed lines mark $|\log_2 FC| \geq 1.5$; the horizontal dashed line marks FDR < 0.05. (B–C) Temporal trend clustering of z-scored $\log_2$ fold-change relative to sham for (B) muscle and (C) serum at TP1 (day 1), TP2 (day 3), and TP3 (day 14). Proteins were grouped by k-means (k=4); colored lines show cluster mean trajectories used for downstream KEGG/GO enrichment.

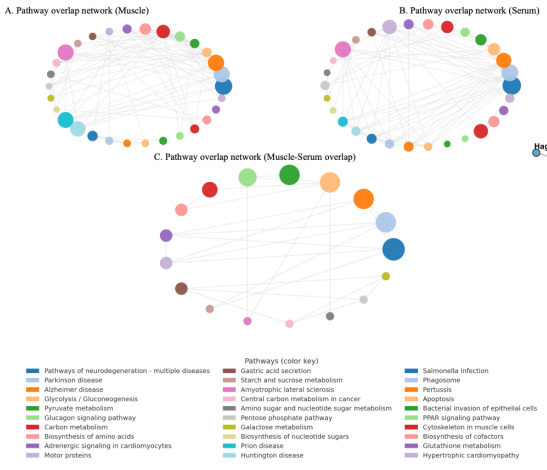


Figure 2. KEGG pathway overlap networks in muscle, serum, and their intersection.
(A) Muscle and (B) serum networks built from enriched KEGG. Each node is a pathway; an edge connects two pathways when they share ≥2 proteins in that compartment. Node size scales with the number of proteins mapped to that pathway. (C) Intersection network showing pathways detected in both muscle and serum (n = 30). Circular layout used in all panels.

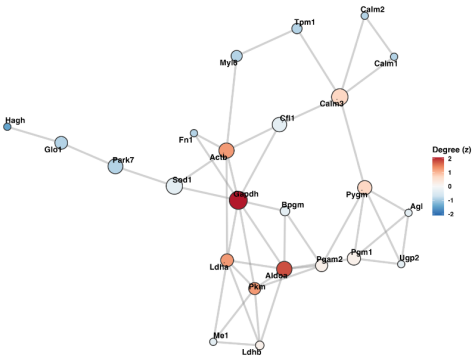


Figure 3. Shared high-confidence PPI module.
KEGG overlap proteins were mapped to STRING (mouse, v12) and filtered at combined score ≥0.7; the largest component is shown (24 nodes, 44 edges). Node size = betweenness, color = degree z-score (red higher), labels beside nodes. The module highlights glycolysis/pyruvate enzymes, a glycogen-handling arm (PGM1/PYGM/UGP2/AGL), and a CaM-contractile cluster (CALM1-3/MYL6/TPM1).