Bispebjerg og Frederiksberg Hospital

Clinical Proteomics of Rhabdomyolysis
Molecular Insights Toward Early Detection

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Copenhagen
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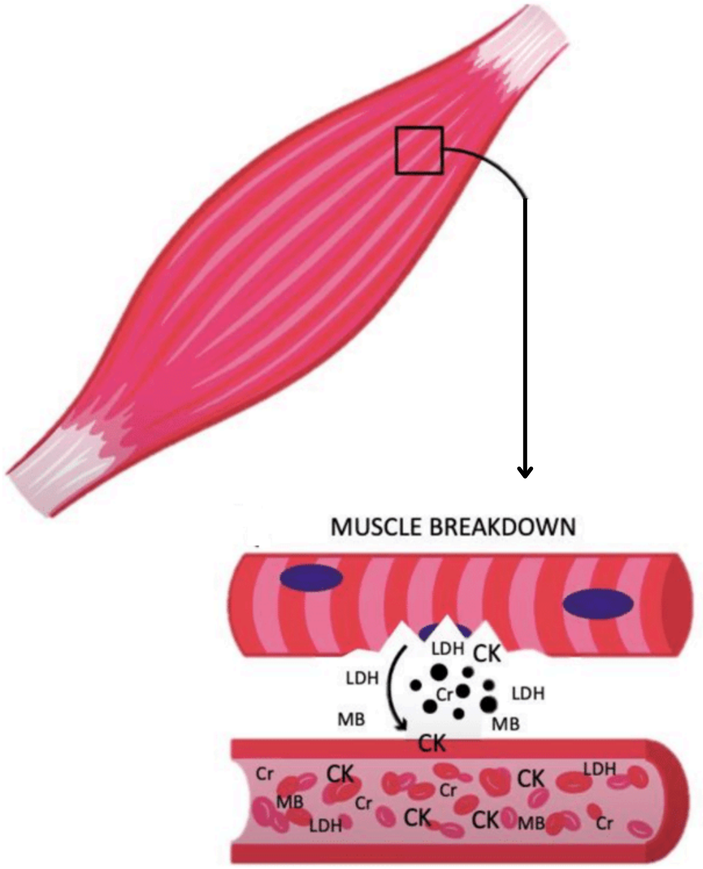
Introduction

Rhabdomyolysis: skeletal muscle breakdown following injury, toxicity, or stress.

Current diagnostic markers: insufficiently sensitive or specific for early detection and risk stratification.

Objective: characterize the proteomic response to acute muscle injury over time, assess the influence of Growth Hormone treatment, and identify recovery biomarkers.



Plan of Action

1

Data Preprocessing:

- Assess missingness
- Impute missing values
- Normalization
- Outlier removal

2

Exploratory Analysis:

- UMAP
- PCA

3

Statistical Modeling:

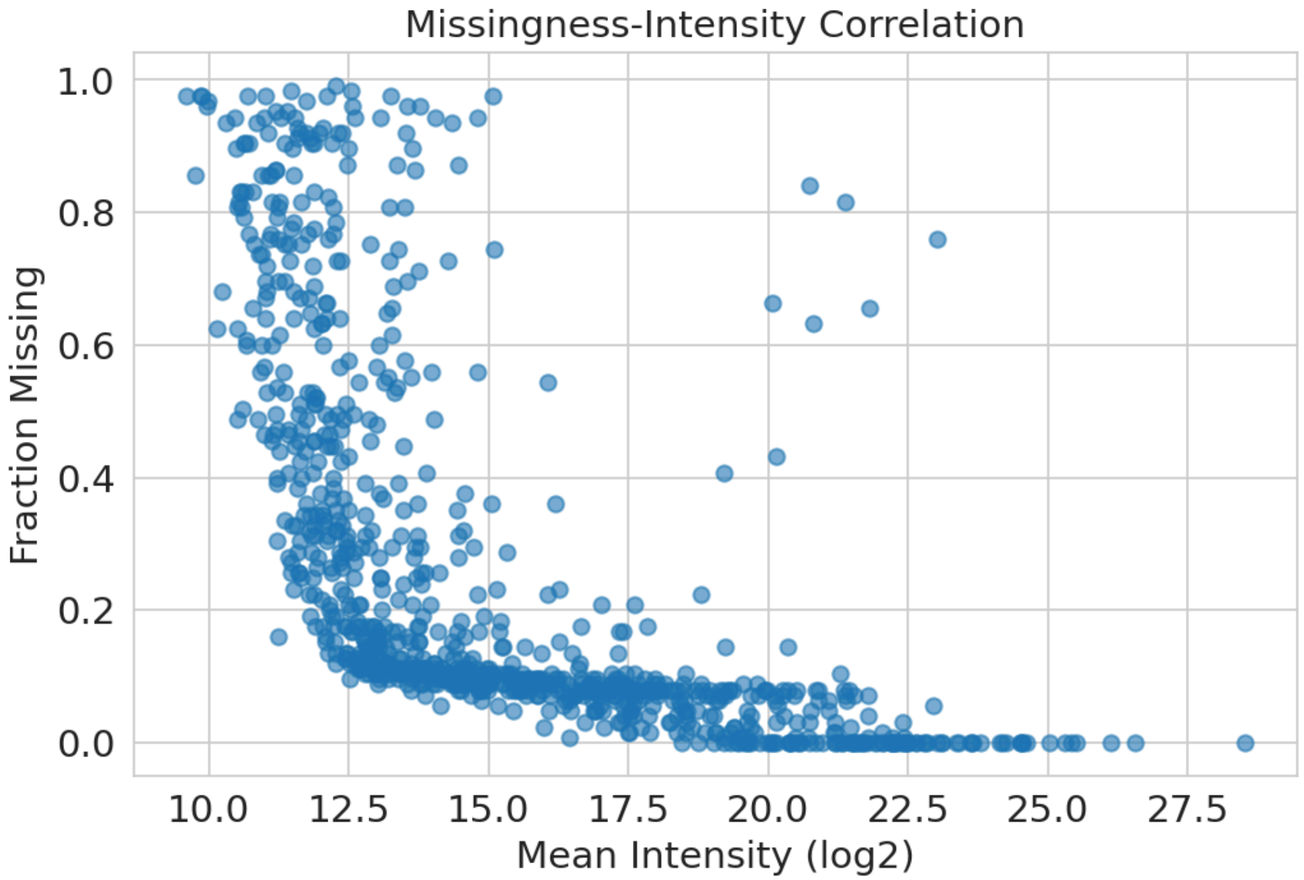
- Mixed linear models for visit and treatment
- Phase-specific proteomic signatures
- Correlations with clinical outcomes

4

Enrichment Analysis

- Genes
- Pathway

Missingness Analysis



Spearman correlation: -0.857

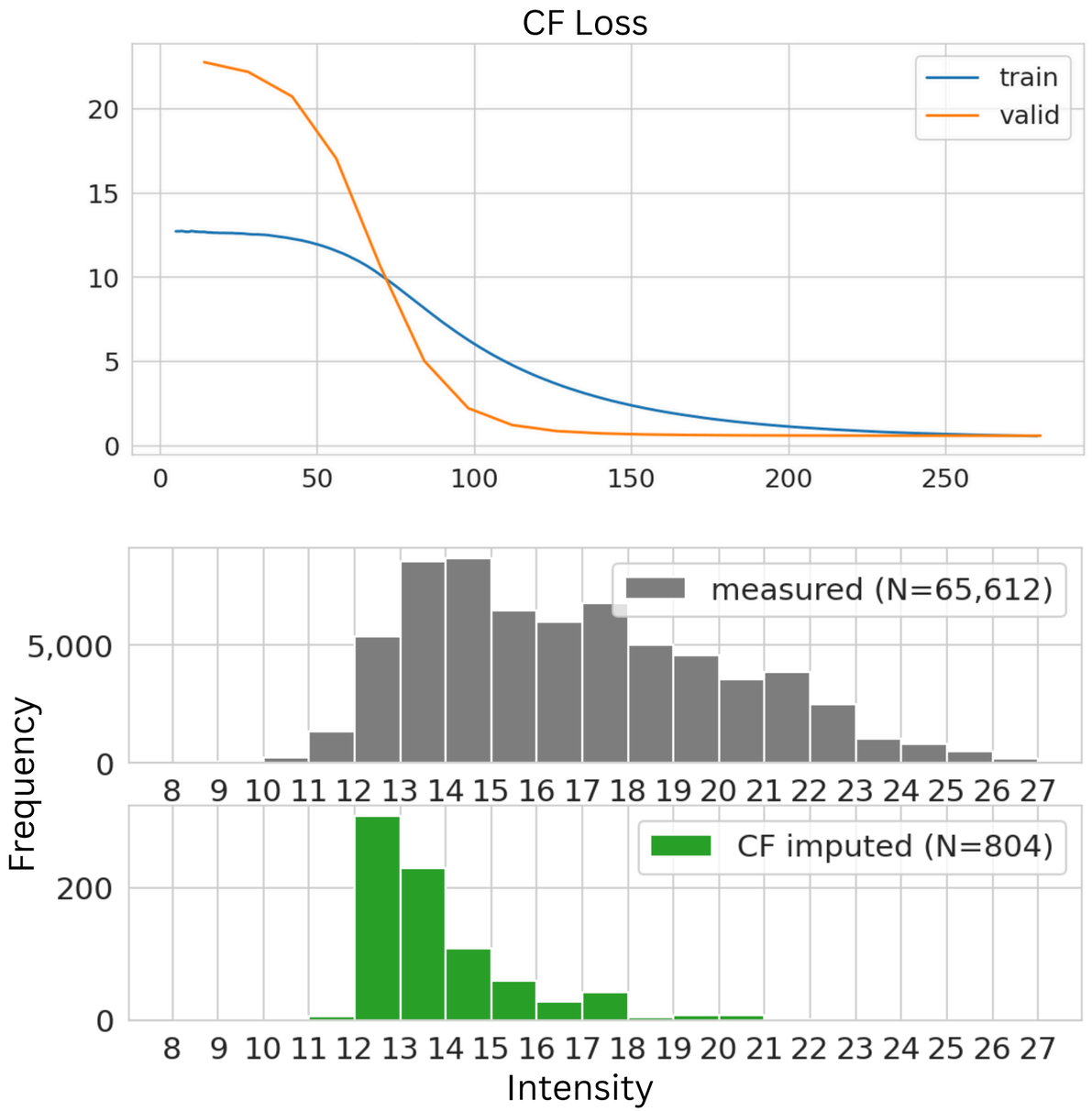
- The data is MNAR
- High correlation between the missingness and the mean intensity of each biomarker
- Likely due to instrument sensitivity

Imputation

with PIMMS

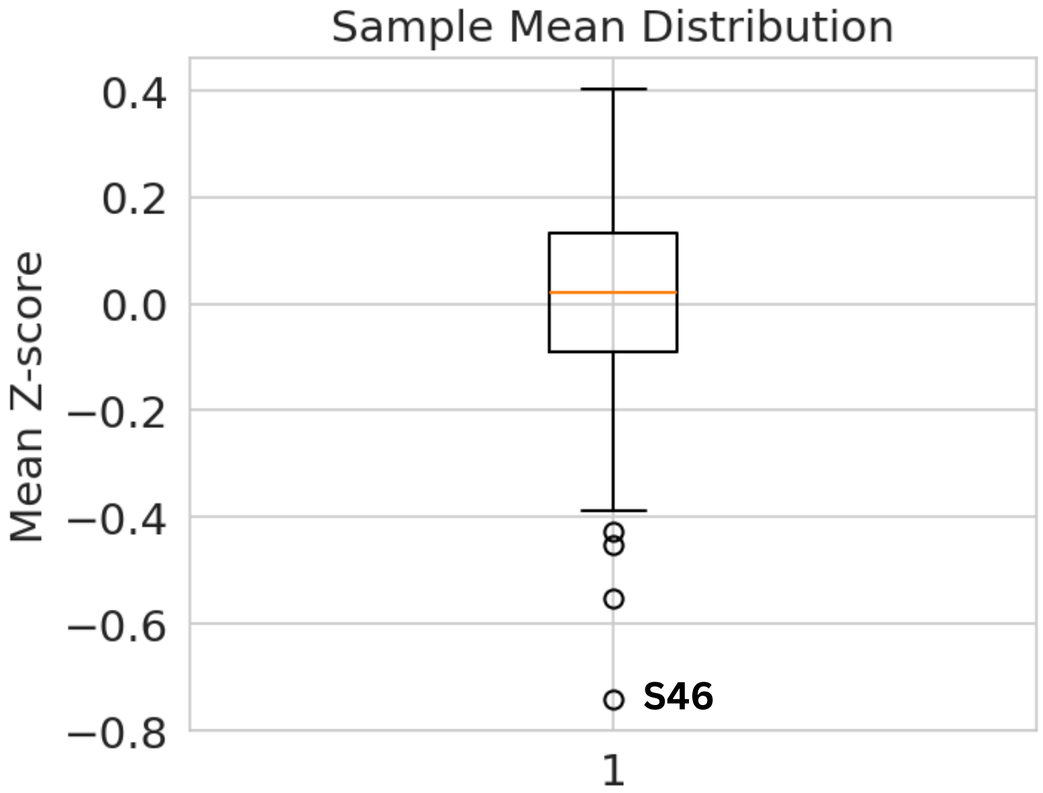
With a final validation loss of 0.600, the CF model greatly outperformed VAE and DAE, as well as all baseline methods (mean, median, zero and random).

The distribution of imputed values indicating that the CF model was successful in predicting low intensities.



Webel, H., Niu, L., Nielsen, A.B. et al., *Imputation of label-free quantitative mass spectrometry-based proteomics data using self-supervised deep learning,* Nat Commun 15, 5405 (2024).

More Preprocessing



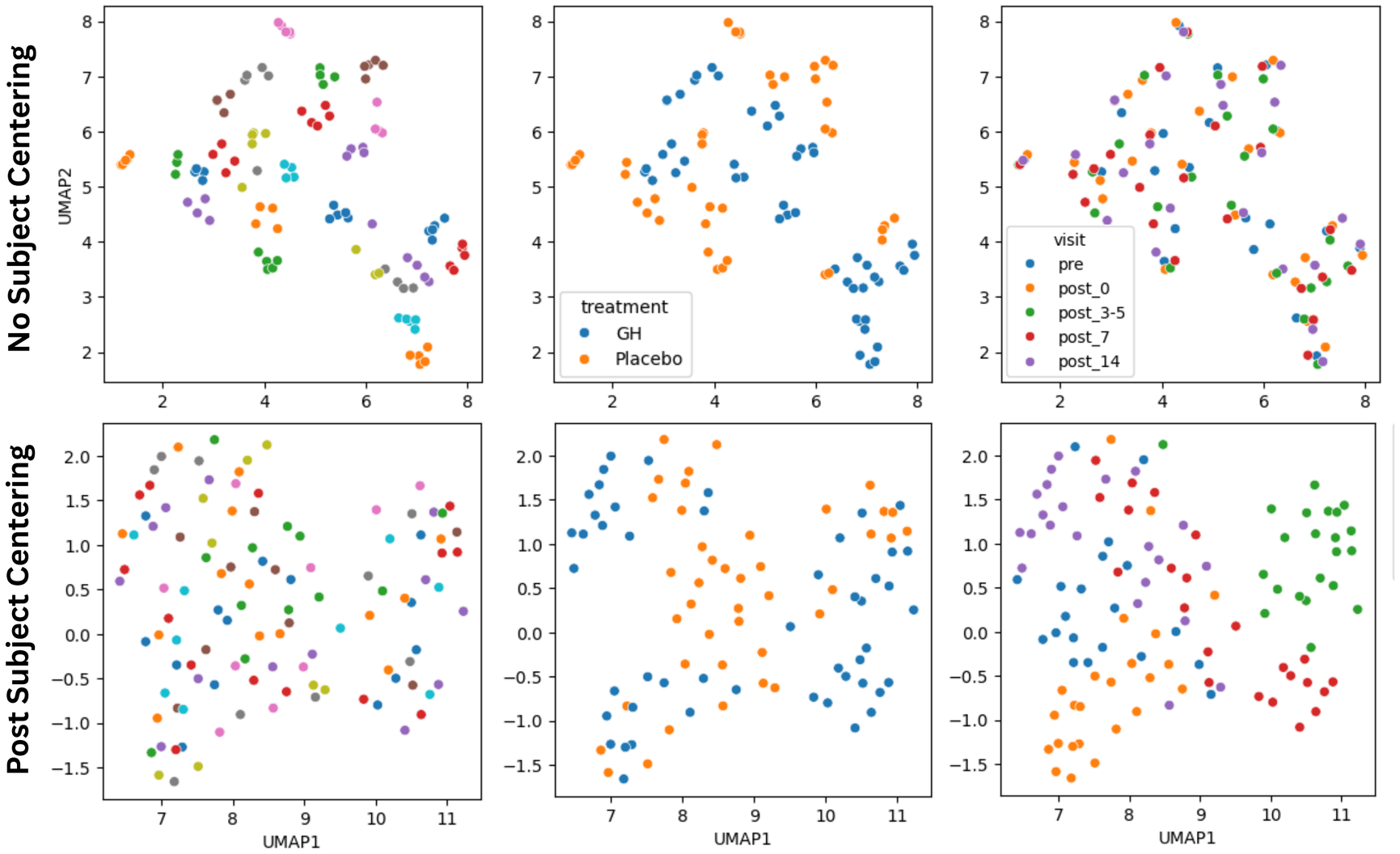
- Z-score normalization for all protein distributions
- Outlier removal
  - Z-score thresholding (|z|>3) identified one outlier (S46)
- Subject centering (only for exploratory purposes)

UMAP

Before subject centering:
Data mainly clusters by subject

After subject centering:
Subject clusters disappear and new patterns appear for visit

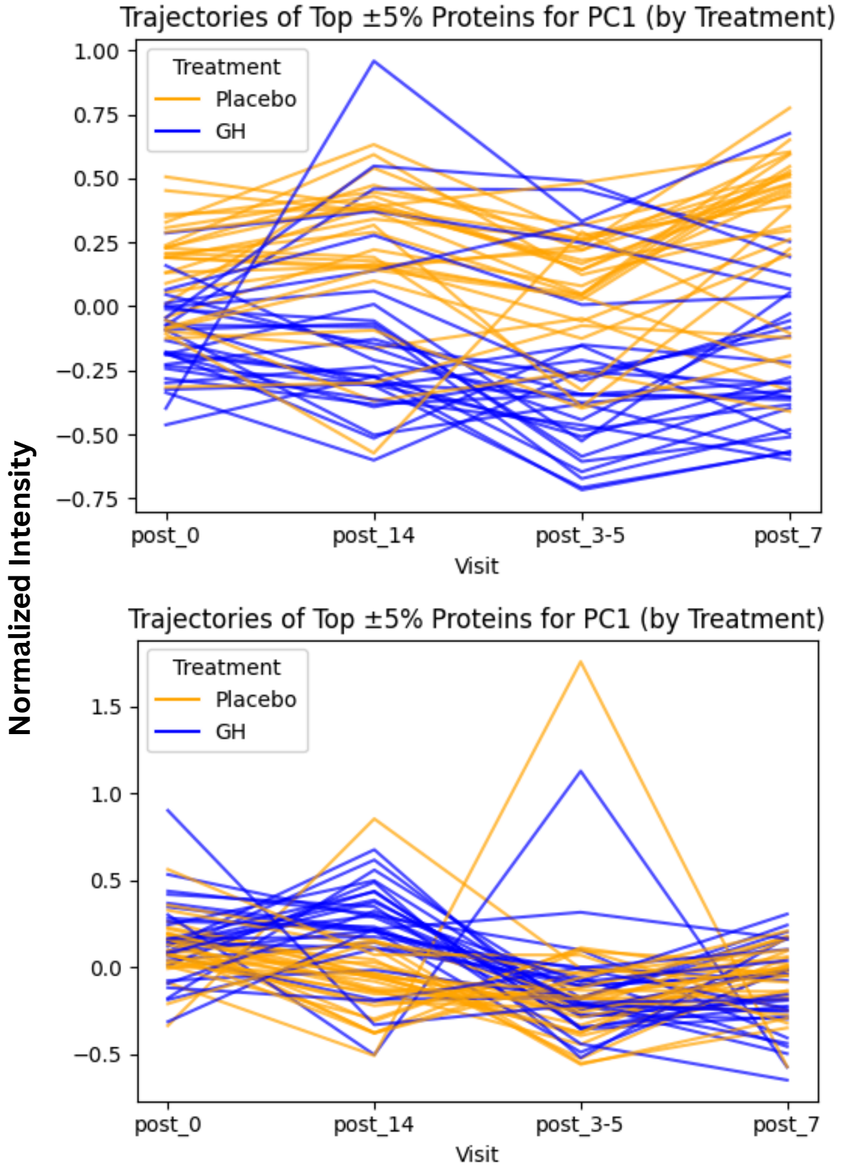
NOTE: removing subject variance affect treatment

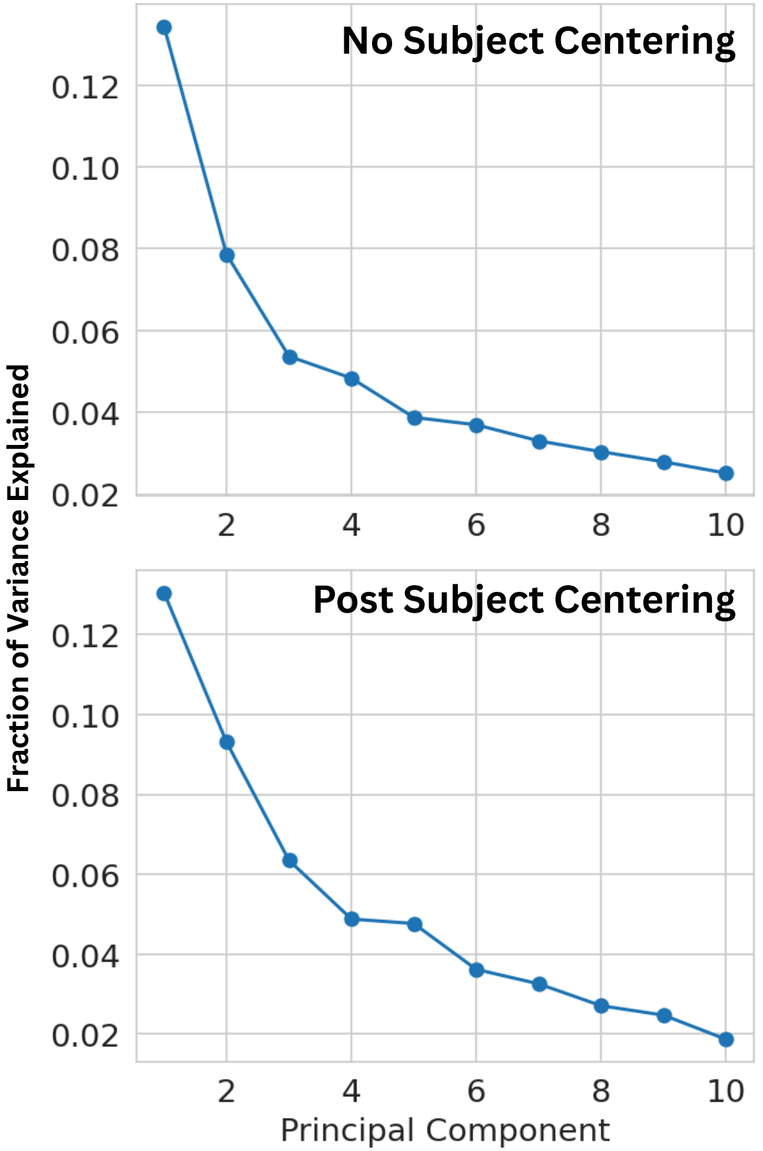


PCA

Subject centering reduces the variance explained by PC1 → the main contributor to PC1 is subject

Treatment trajectories show a clear separation before subject centering → uncentered data must be used to study treatment effects





Model strategy

Models:

- mixed linear models
- model subject as random effect

Single time point analysis:

- Spearman correlation

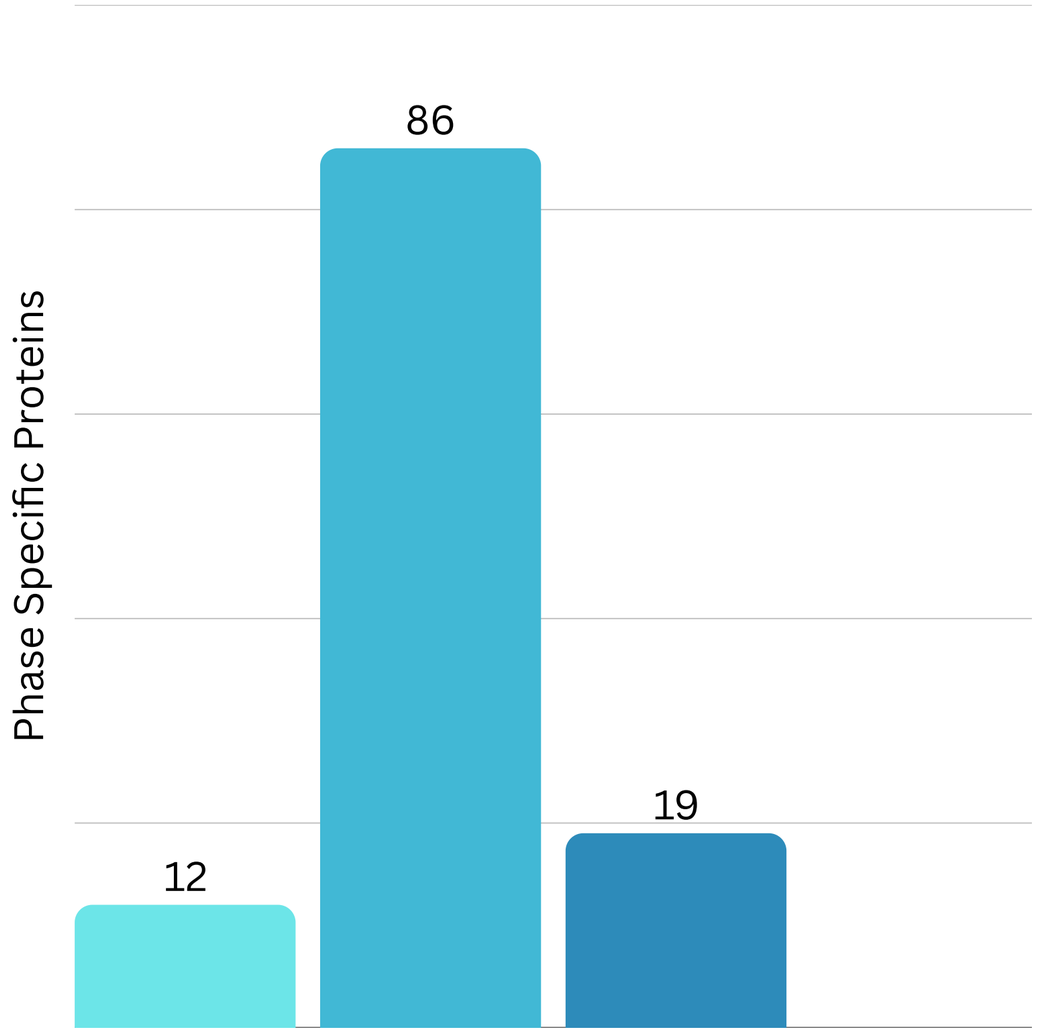
Dataset:

- use non subject-centered dataset

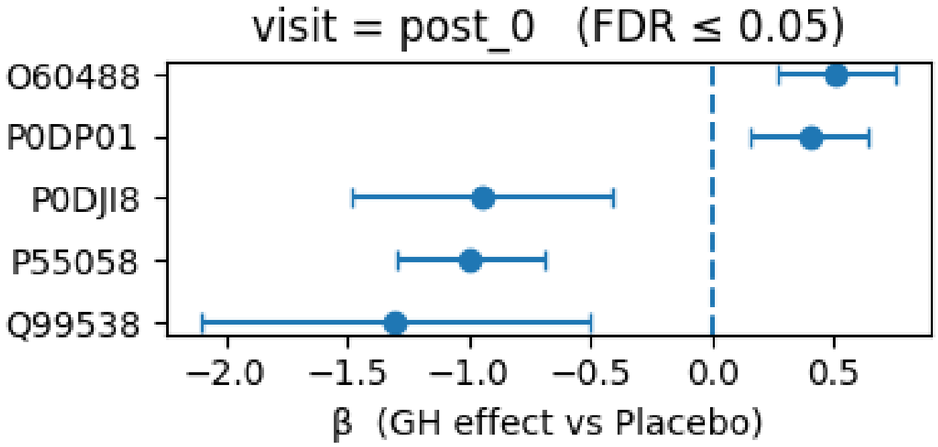
Mixed LM by visit

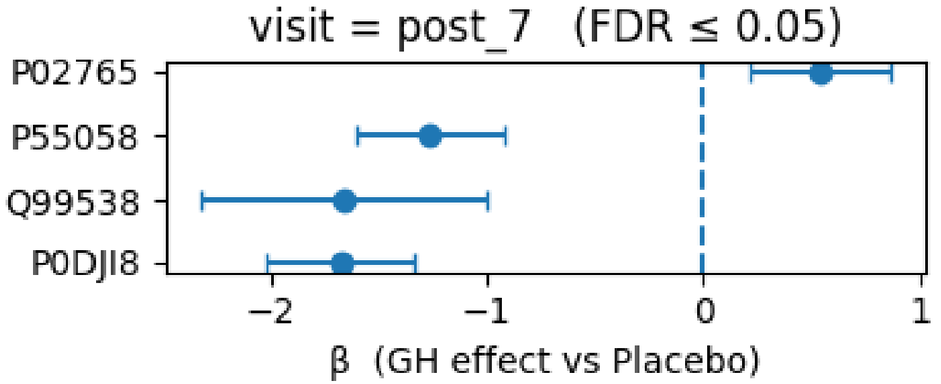
211 proteins show a significant change over different visits (adj. P value < 0.05)

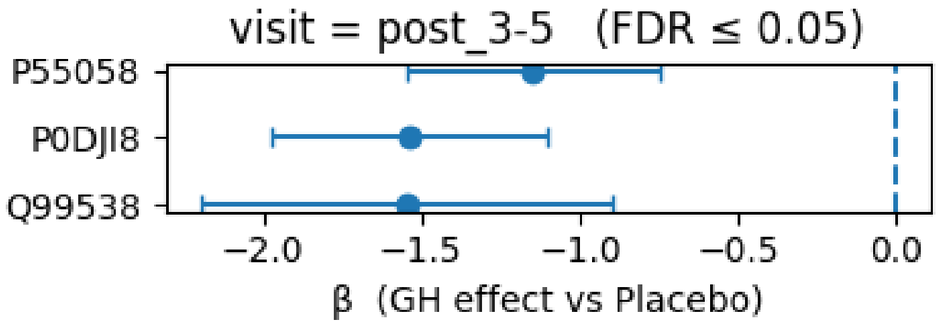
By filtering the proteins that have P value < 0.05 for that phase and a log2 fold change > 0.5 for the first time at that phase, we can assign proteins to the phase where they first arise or drop.

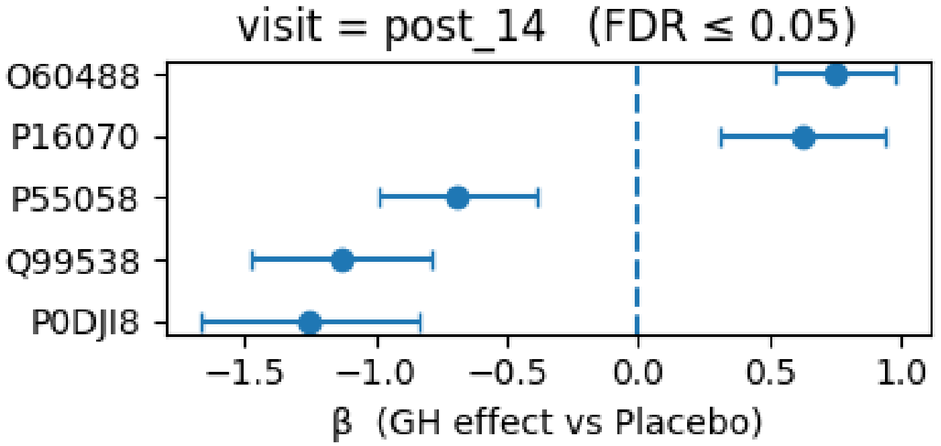


Mixed LM by treatment * visit









Mixed LM by treatment * visit

Upregulated by GH

Acute phase:
↑ Fatty acid utilisation
↑ Debris clearance

Post 7 days:
↑ Platelet activation

Post 14 days:
↑ Membrane biosynthesis and architectural remodeling

Downregulated by GH

At all phases
↓ Cytokine signalling
↓ ECM digestion
↓ HDL remodelling

Mixed LM by CK

164 proteins have a significant correlation with CK

Positive Corr.

- ↑ Complement activation
- ↑ Platelet degranulation
- ↑ Acute phase response
- ↑ Extracellular vescicles,
- ↑ Leaked contractile structures

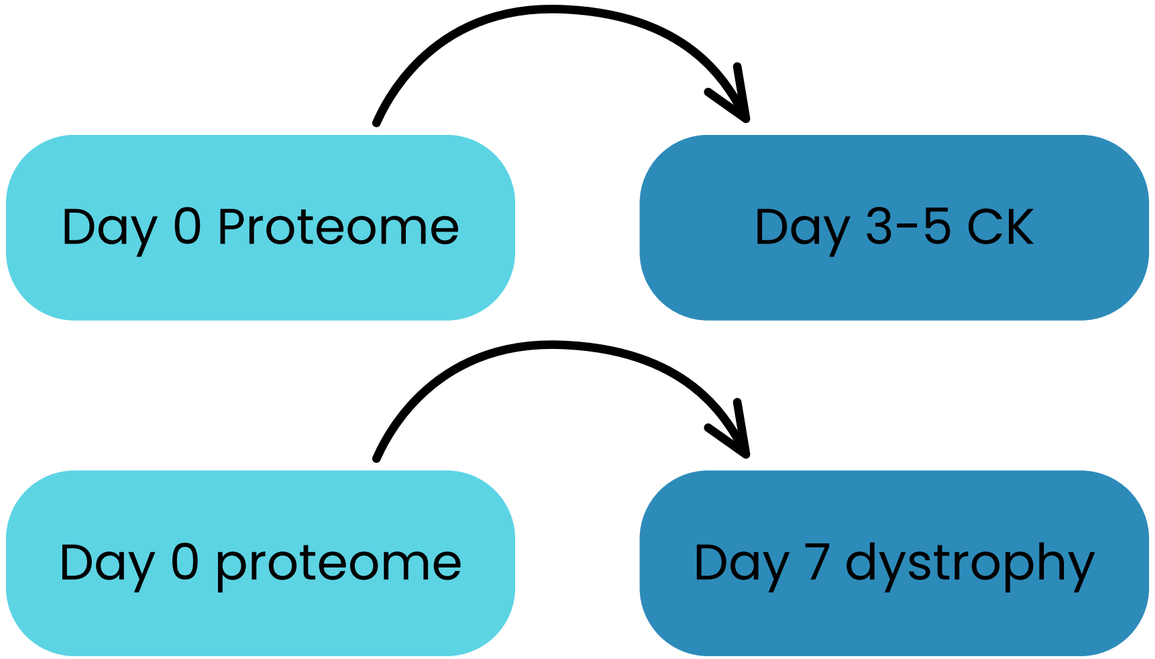
Negative Corr.

- ↓ Immunoglobulin complex
- ↓ Lipoprotein particles, lipid transport
- ↓ Protease inhibitors

Long Term Prediction

Objective:
Can day 0 intensities predict day 3-5 CK and day 7 dystrophy?

Obstacle:
Using only one observation per subject, the dataset is reduced to <25 observations.

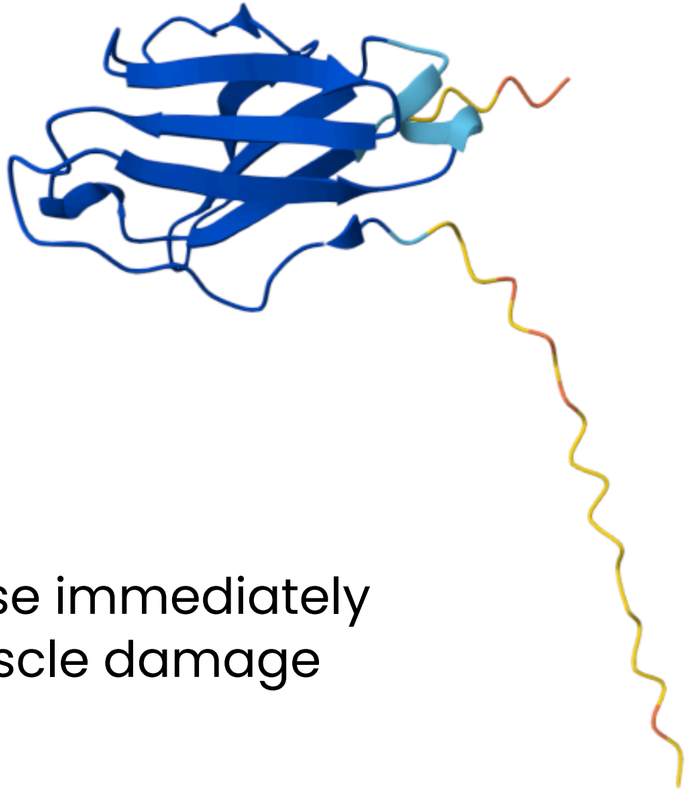


Long Term Prediction

CK prediction

Immunoglobulin-λ light-chain variable region (IGLV2-18)

When measured at day 0 it has a strong, positive association with the rise in CK measured 3-5 days later.



ρ≈0.85 **FDR≈0.001**

Plausible interpretation:
Participants who already had a brisk systemic immune response immediately after injury went on to mount a larger inflammatory-driven muscle damage response, producing higher CK.

Objectives Achieved

- Characterize the proteomic response to acute muscle injury and evaluate how this response evolves over time and under the influence of Growth Hormone treatment
- Identify protein signatures associated with distinct phases of muscle damage and recovery
- Assess correlations with clinical markers such as creatine kinase
- Explore whether proteomic profiles can predict longer-term outcomes such as the percentage of dystrophic fibers or the creatine kinase concentration

Future Perspectives

- Expand the dataset to obtain more significant results and possibly expand the list of differentially expressed proteins under various conditions.
- Test the key proteins in a larger mix of rhabdomyolysis patients to study different causes, ages, and severities.
- The data keep pointing to extracellular vesicles, clotting/complement proteins, and collagen/ECM remodelling. Isolate vesicles, look at their cargo, and use cell or animal injury models to see how these pathways drive muscle damage or repair.

THANK YOU!