Use of an exercise challenge system to define a universal proteomic signature of muscle injury in a diverse set of adult individuals with inherited myopathy

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Introduction

Several inherited muscular dystrophies are defined by an enhanced injury response to exercise. However, the molecular definition of muscle injuiry and how injury relates to the underlying genetic lesion remain poorly understood.

To address these questions, we utilized an established exercise intervention system in a diverse set of adult individuals with a range of inherited myopathies. Using the SomaScan 7K high-throughput analysis platfform, we characterized both baseline changes from healthy, as well as the proteomic signatures of injury during the 24 hours following an exercise challenge.

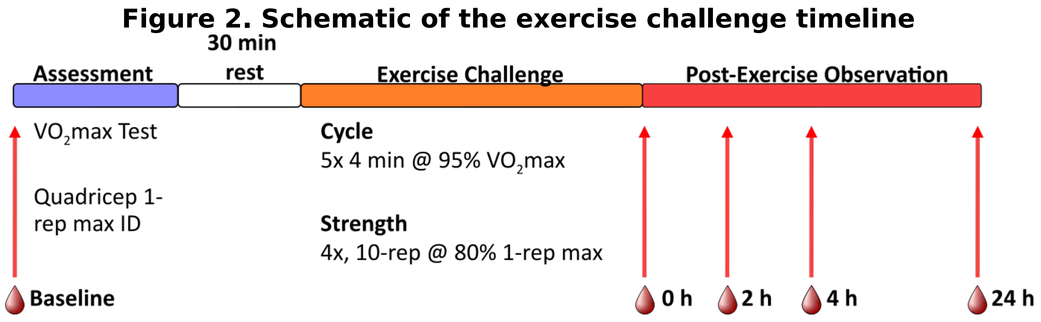
The subjects selected for the study included healthy, Becker muscular dystrophy (BMD), the Limb Girdle muscular dystrphies 2I and 2L (LGMD2I and LGMD2L), as well as an unrelated metabolic myopathy, McArdle's disease. Individuals were chellenged with a high intensity, bimodal exercise regimen consisting of both aerobic and strength components. Blood tests were collected prior to exercise and at controlled intervals up to 24 hours post-exercise, after which the plasma was analyzed by SomaScan for the concentrations of approximately 7,000 proteins.

Study Design

Figure 1. Demographics of study participants.

	N	% Male	Age (yrs)	BMI	$VO2_{Max}$ (mL min^{-1} kg^{-1})	WMax (J sec^{-1})	% HR_{Max}	1-RM (kg)
Control	9	77.8	44 ± 13	24.5 ± 2.4	38.8 ± 3.5	278 ± 53	100 ± 7.2	96 ± 26
BMD	9	100	33 ± 7	23.6 ± 2.9	22.9 ± 8.5	113 ± 107	94.4 ± 8.9	38 ± 41
LGMD2I	8	12.5	30 ± 10	22.6 ± 2.7	26.1 ± 8.5	132 ± 71	95.4 ± 5.3	49 ± 29
LGMD2L	9	66.7	52 ± 9	27.1 ± 4.4	27.6 ± 11.4	176 ± 89	96.6 ± 9.8	70 ± 44
McArdle	2	50	46 ± 28	27.8 ± 5	19.7 ± 5.5	73 ± 25	102.4 ± 13.4	57 ± 13

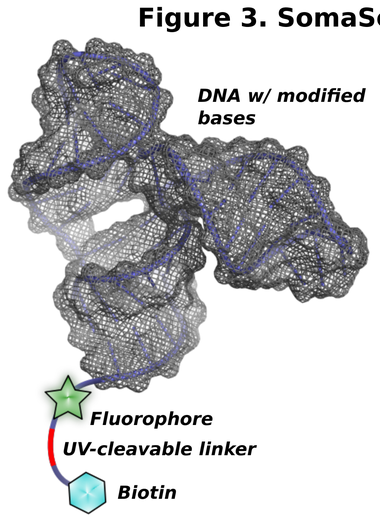
Figure 2. Schematic of the exercise challenge timeline



The exercise challenge consisted of an assessment phase, in which aerobic threshold (VO_{2max}) and strength (quadricep 1-rep max) would be measured, and a strenuous individualized challenge phase, separated by a 30 min recovery period. Blood draws were taken prior to assessment (pre-exercise baseline) and at defined periods of 0, 2, 4, and 24 hours post-challenge.

Analysis of the samples was done using the SomaScan 7K Proteomic Assay.

Figure 3. SomaScan Analysis of Protein Concentration in Plasma

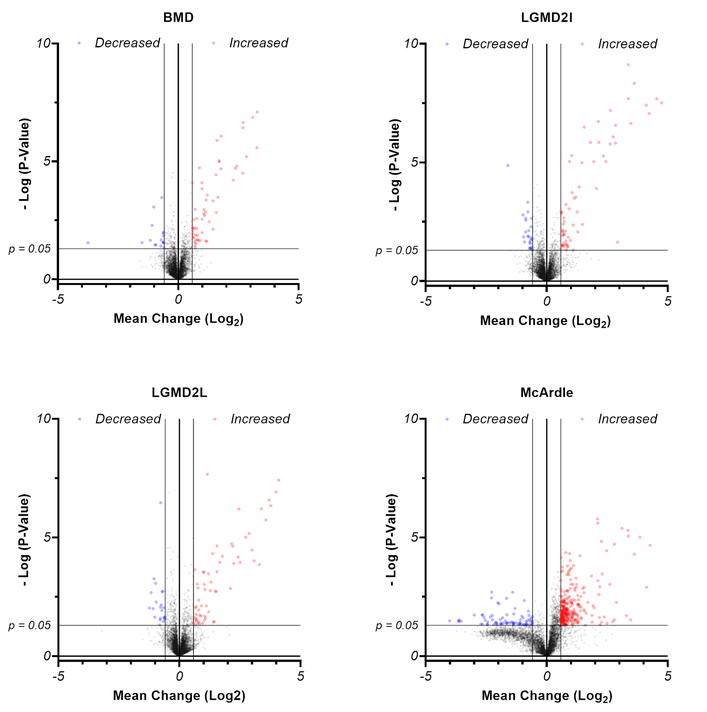


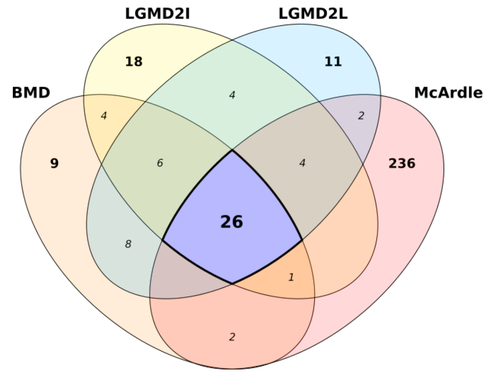
SomaScan is a multistep modified aptamer-based assay for high-throughput, sensitive, and objective biomarker measurement. The current platform screens for approximately 7000 proteins.

Proteins in the samples are selectively bound with fluorescent aptamers, captured with beads, eluted, and quantified on a chip array to yield relativistic measures of protein concentrations in the sample.

Identification of a Common Set of 26 Baseline-Elevated Proteins

Figure 4. Proteomic changes in each indication at pre-exercise baseline.

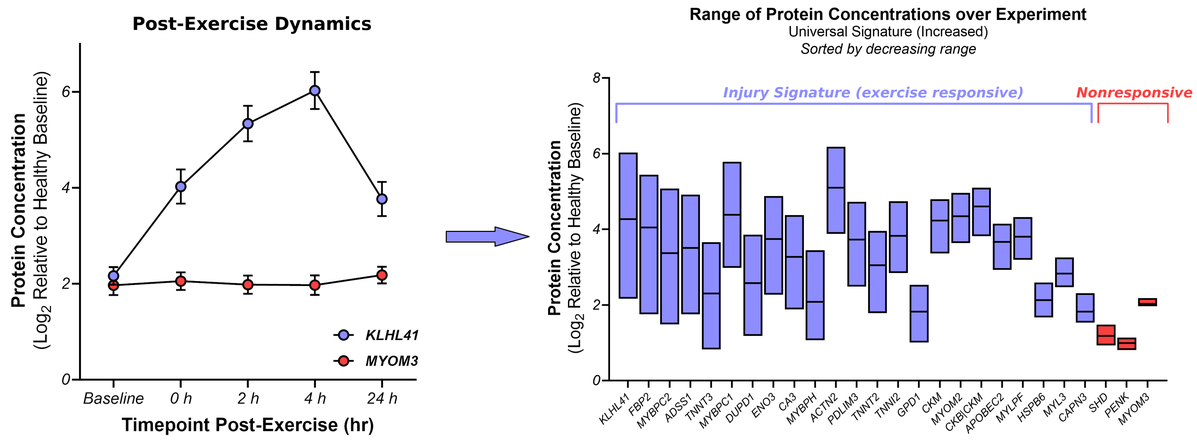




Comparison of circulating proteins present in donors from each indication at pre-exercise baseline relative to healthy individuals revealed several dozen elevated proteins (red points) and decreased proteins (blue points) Despite the distinct genetic lesions associated with each myopathy, a core set of 26 elevated proteins common to all indications was identified.

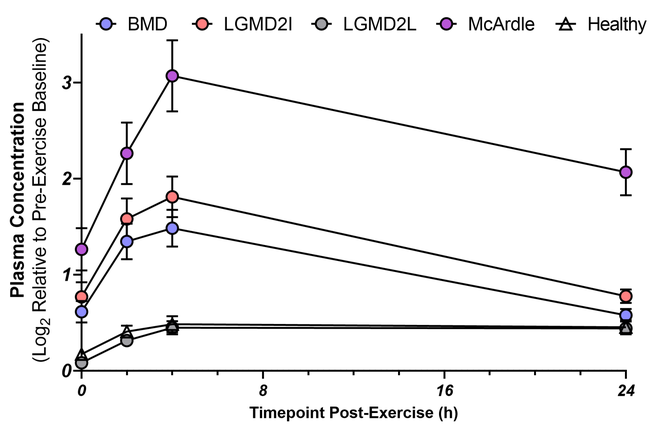
Proteomic Signature of Exercise-Induced Injury

Figure 5. Post-Exercise dynamics of universally-elevated baseline proteins



Among the elevated proteins universal baseline signature, 23 proteins were further elevated by an exercise challenge (Injury Signature), while 3 proteins were unaffected by exercise and maintained baseline levels of elevation over healthy volunteers.

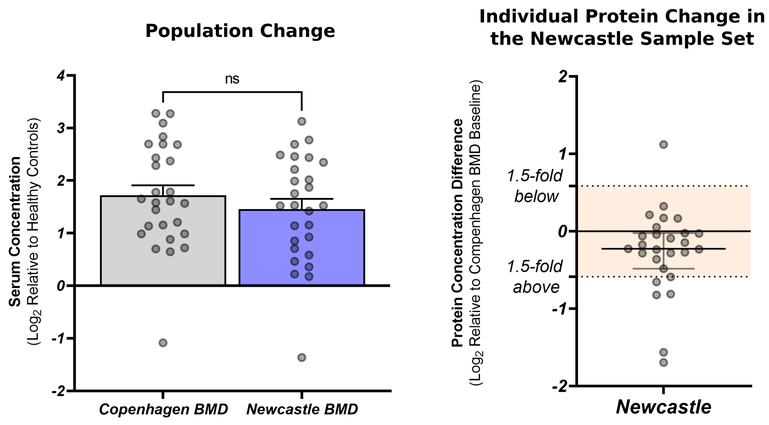
Figure 6. Post-exercise dynamics of the injury signature in each myopathy



Myopathy-focused changes indicate that while BMD, LGMD2I, and McArdle all responded to the exercise challenge with an exaggerated injury response, LGMD2L exhibited no differentiation from the normal cohort.

Validation of Profile in a Large Cross-Sectional Dataset

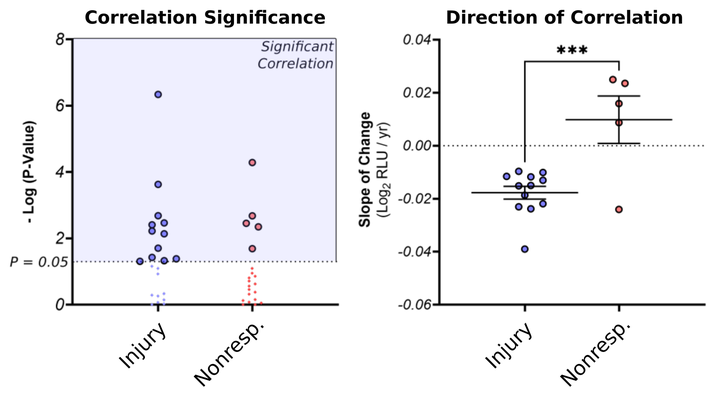
Figure 7. Baseline Profile in Samples from Newcastle Biobank



The universal baseline signature is applicable to a cross-sectional set of 55 BMD serum samples from the Newcastle Biobank. The signature shows equal elevation over healthy in both datasets and, individually, most proteins in the signature are within 1.5-fold of the levels seen in the exercise dataset.

Opposing Age Dynamics of Injury Signature and Exercise Nonresponsive Protieins

Figure 8. Trends of Exercise Responsive and Nonresponsive Subsets with Age



Proteins in the injury signature and exercise nonresponsive proteins in BMD were analyzed for correlation with age (left). Among those significantly associated, all injury signature proteins decreased with age (right). However, with the exception of muscle-derived myomesin 3, all of the exercise nonresponsive proteins increased with age, suggesting one or more non-muscle sources for baseline increases in this subset.

Conclusions

We identified a shared circulating baseline biomarker signature amongst the 4 neuromuscular disorders studied, despite unique genetic lesions and symptom profiles accompanying each. Within that signature, we described a proteomic signature of muscle injury that further elevates upon exercise in BMD, LGMD2I, and McArdle and that this injury signature tends to be negatively associated with age. Conversely, the subpopulation that are baseline-elevated but are not responsive to exercise or derived from muscle increase with age, raising the possibility that these proteins may serve as less variable markers of disease progression.

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