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

Benjamin Barthel[a,*], Mads G Stemmerik[b,*], Nanna R Andersen[b], Sofie V Skriver[b], Alan J Russell[a], John Vissing[b]

[*]These authors contributed equally to this work, [a]Edgewise Therapeutics, Boulder, CO, USA, [b]Neuromuscular Center, Department of Neurology, University of Copenhagen, Copenhagen, DK

Introduction

Several inherited muscular dystrophies are characterized 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 set of inherited myopathies that differ in their underlying pathophysiology. Using the SomaScan 7K high-throughput analysis platfform, we characterized both baseline differences from healthy, as well as the proteomic signatures of injury during the 24 hours following an exercise challenge.

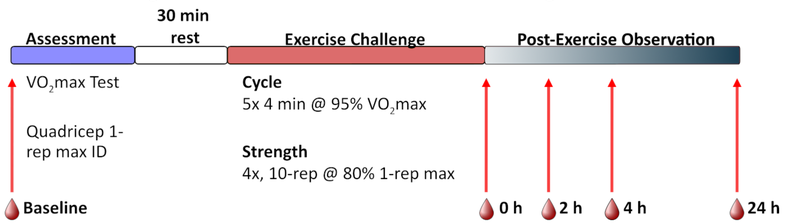
The subjects selected for the study included Becker muscular dystrophy (BMD) and the Limb Girdle muscular dystrophies 2I and 2L (LGMD2I and LGMD2L), as well as a healthy cohort. Individuals were challenged with a high intensity, bimodal exercise regimen consisting of both aerobic and strength components. Blood samples 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.

Exercise Study Design and Analysis

Figure 1. Demographics of Study Participants

	N	% Male	Age (yrs)	BMI	$VO2_{max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	W_{max} ($J \cdot sec^{-1}$)	% 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

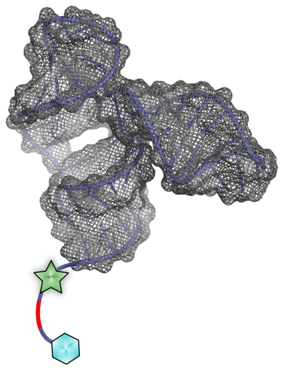
Figure 2. Schematic of the Exercise Challenge Timeline



The exercise challenge consisted of an assessment phase, in which aerobic threshold (VO2max) and strength (quadricep 1-rep max) was measured, and a strenuous individualized challenge phase, separated by a 30 min recovery period. Blood draws were taken prior to 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

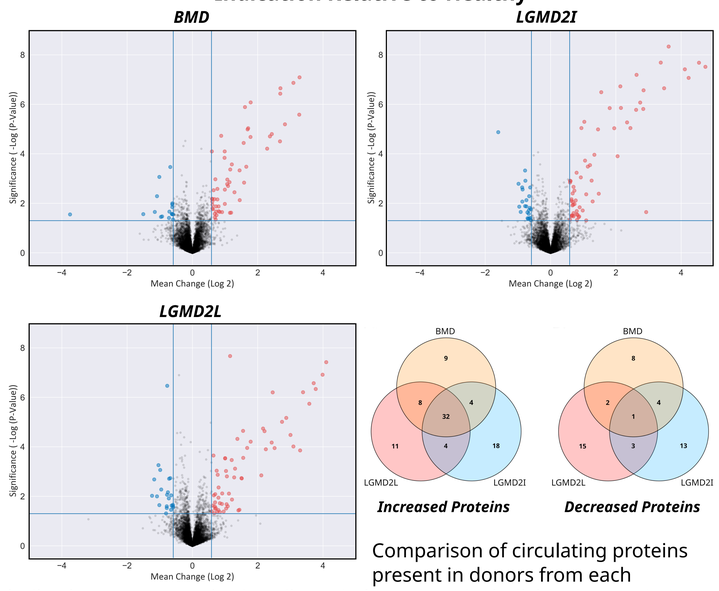


SomaScan is a multistep modified aptamer-based assay for high-throughput, sensitive, and objective biomarker measurement. The current platform screens for approximately 7000 proteins.

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.

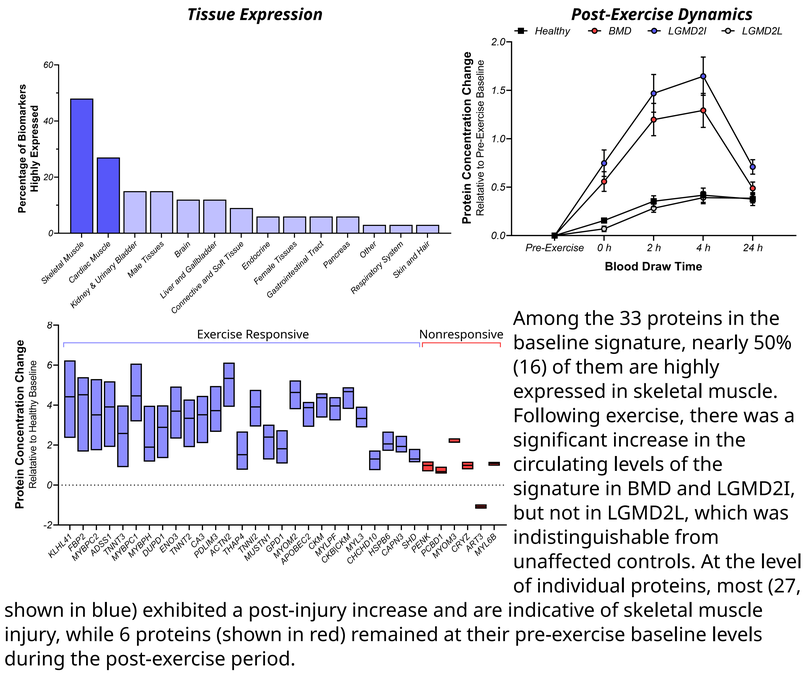
Identification of the Baseline Signature and Post-Exercise Dynamics

Figure 4. Baseline Proteomic Differences in Each Indication Relative to Healthy



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). The baseline signature is the 33 proteins (32 increased, 1 decreased) common to all myopathies.

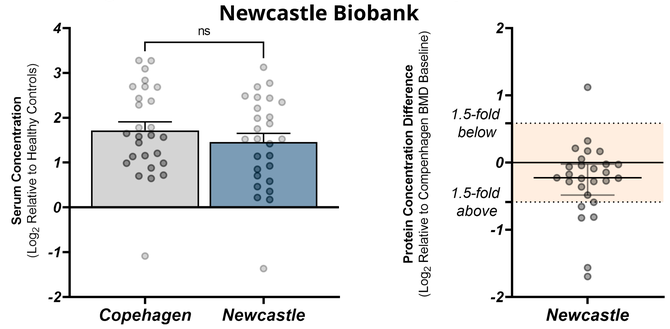
Figure 5. Expression and Post-Exercise Dynamics of the Baseline Signature



Among the 33 proteins in the baseline signature, nearly 50% (16) of them are highly expressed in skeletal muscle. Following exercise, there was a significant increase in the circulating levels of the signature in BMD and LGMD2I, but not in LGMD2L, which was indistinguishable from unaffected controls. At the level of individual proteins, most (27, shown in blue) exhibited a post-injury increase and are indicative of skeletal muscle injury, while 6 proteins (shown in red) remained at their pre-exercise baseline levels during the post-exercise period.

Validation of the Signature in a Large Cross-Sectional Dataset

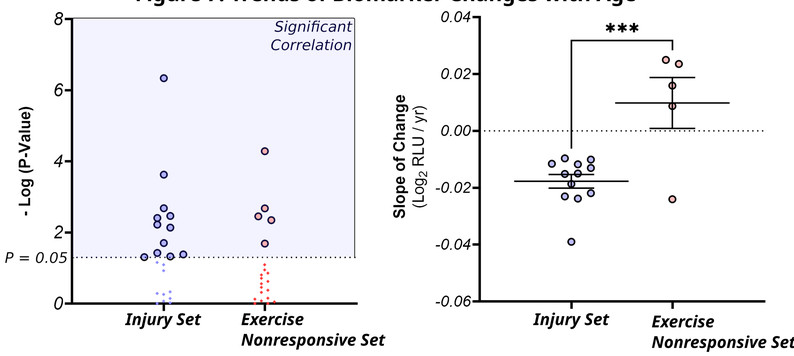
Figure 6. Baseline Signature in Samples from Newcastle Biobank



The baseline signature was applied to a cross-sectional set of 55 BMD serum samples from the Newcastle Biobank. The signature showed similar average change 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.

Age Dynamics of Injury Signature and Exercise Nonresponsive Proteins

Figure 7. Trends of Biomarker Changes 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 3 neuromuscular disorders studied, despite unique genetic etiology. Within that signature, we described a proteomic signature of muscle injury that further elevates upon exercise in BMD and LGMD2I and that this injury signature tends to be negatively associated with age. Conversely, the subpopulation or proteins that are baseline-elevated but are not responsive to exercise or derived from muscle tend to increase with age, raising the possibility that these proteins may serve as direct markers of disease progression.

References

Lippi, G., Schena, F., & Ceriotti, F. (**2019**). Diagnostic biomarkers of muscle injury and exertional rhabdomyolysis. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 57(2), 175–182.

Mercuri, E., Bönnemann, C. G., & Muntoni, F. (**2019**). Muscular dystrophies. *The Lancet*, 394(10213), 2025–2038.

Robbins, J. M., et al. (**2021**). Human plasma proteomic profiles indicative of cardiorespiratory fitness. *Nature Metabolism*, 3(6), 786–797.

Sheikh, A. M., & Vissing, J. (**2019**). Exercise therapy for muscle and lower motor neuron diseases. *Acta Myologica : Myopathies and Cardiomyopathies : Official Journal of the Mediterranean Society of Myology*, 38(4), 215–232.

Somalogic, Inc. (**2021**). SOMAscan Assay v4.1 Technical Note. SomaLogic, Inc. https://somalogic.com/technology/