#729LBP

Protein Biomarkers of Muscle Injury Exhibit Differential Reduction with Subject Age in Adults with Becker Muscular Dystrophy

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Background

Becker (BMD) and Duchenne (DMD) muscular dystrophies are associated with mutations in the dystrophin gene and are characterized by progressive muscle damage and loss, with eventual replacement by fibrotic and fatty tissue. In DMD, where there is no functional dystrophin present, natural history studies have demonstrated that this muscle tissue loss is associated with an age-related decrease in circulating levels of muscle-derived proteins, such as creatine kinase (CK), fast troponin I (TNNI2), and myoglobin (Mb). In contrast to DMD, some dystrophin functionality remains in BMD, wherein individuals present an in-frame deletion within the dystrophin gene, but the onset of progressive decline and muscle loss is variable.[1,2]

Previous natural history studies have indicated that once functional decline begins, the rate of functional loss is predictable at approximately 1.2 NSAA points per year.[3,4,5] As muscle loss and tissue replacement are present in these individuals, markers of muscle damage are also predicted to decrease over time.

Objective

To characterize the age-related decreases in CK, TNNI2, and Mb in individual BMD patients.

Methods & Demographics

Longitudinal BMD serum samples (N = 114 from 36 individuals) were acquired and were submitted for proteomic analysis using the SOMAScan 7K platform (Version 4.1). BMD patients included in the study were ≥18 years old and were diagnosed based on the following criteria: genetic confirmation (in-frame genetic variant) or other genetic variant in the *DMD* gene with a mild clinical phenotype (ambulant >16 years without steroid treatment). Participant demographics are shown in table (below).

Participant Demographics	
Number of Samples	114
Individual Patients	36
Patient Ages	Range: 20 – 71 Median: 44.5 (IQR: 35 – 52)
Average Follow-up Duration	2.3 yrs

For this targeted analysis, values for TNNI2, CKM, and Myoglobin were extracted from the data and converted to concentrations using relationships derived from comparing SOMA values against those from clinically-validated biomarker assays (shown to the right).

$$CK\ \left(\frac{U}{L}\right) = 10^{((0.3274*\log_2 SOMA - 1.045)}$$

$$TNNI2\ \left(\frac{ng}{mL}\right) = 10^{((0.6982*\log_2 SOMA - 9.523)}$$

$$Myoglobin\ \left(\frac{ng}{mL}\right) = 10^{((0.4682*\log_2 SOMA - 2.962)}$$

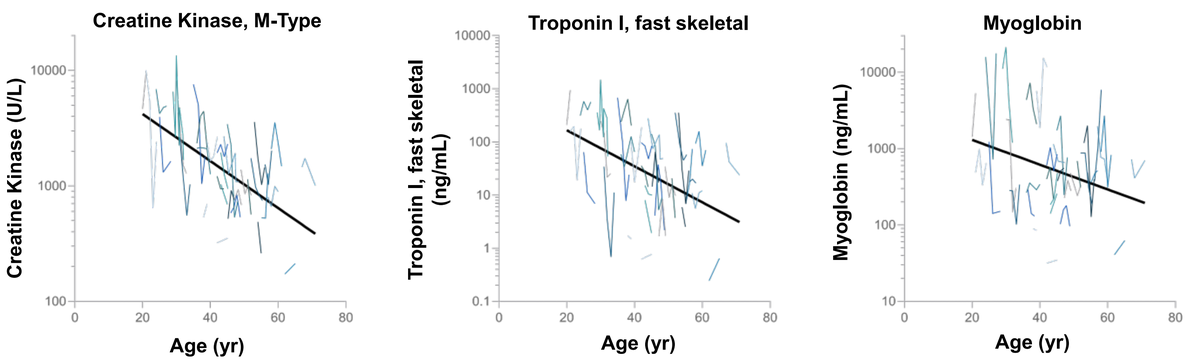
All analysis was performed in Python using custom code written by Edgewise using publicly-available modules (statsmodels, scikit-learn, scipy, pandas, and numpy) or in GraphPad Prism (version 10). Linear mixed modeling of all three biomarkers was performed and allowed for subject-specific random effects on intercepts.

To characterize data variance on short timeframes, longitudinal measurements within each subject were normalized by years after first measurement, then averaged to derive subject-specific biomarker change values. Biomarker correlations were assessed by Pearson correlation analysis.

Results

CKM, TNNI2, and Myoglobin all exhibit significant decrease with age.

CK, TNNI2, and Mb all showed significant decrease with age in a linear mixed model analysis. The grouping of datapoints by subject was important to explaining the observed variance, most particularly for TNNI2, where a high proportion of the variance in the circulating protein level was due to differences between individuals, rather than within individual subjects.



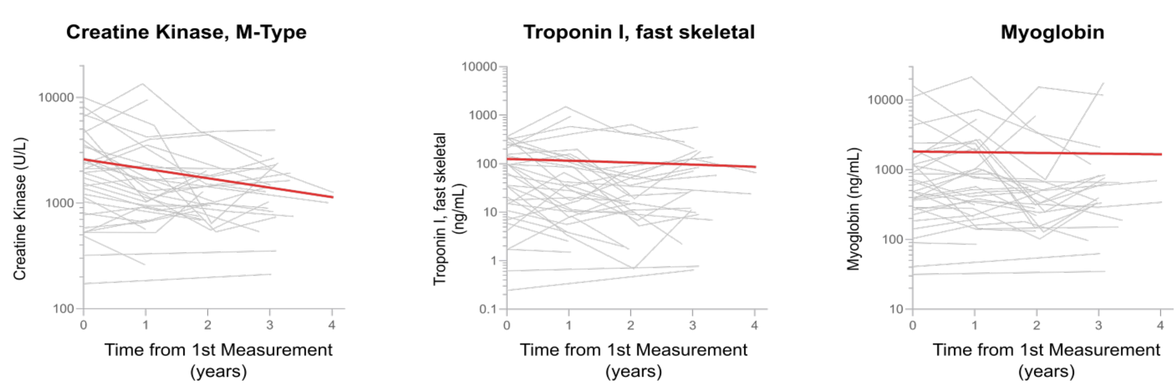
	Correlation vs age Significance	Intraclass Correlation Coefficient
Creatine Kinase, M-type	P < 0.0001	0.7185
Troponin I, fast skeletal	P < 0.001	0.8597
Myoglobin	P = 0.02	0.6583

Results

Muscle injury biomarkers show small overall change within 4 years of first measurement.

Age-related decrease in circulating protein concentrations for muscle injury were minimal when considered on a time frame relevant to the longitudinal sampling of each subject. TNNI2 decreased the most, losing approximately 7.5% per year, while myoglobin decreased at approximately half that rate.

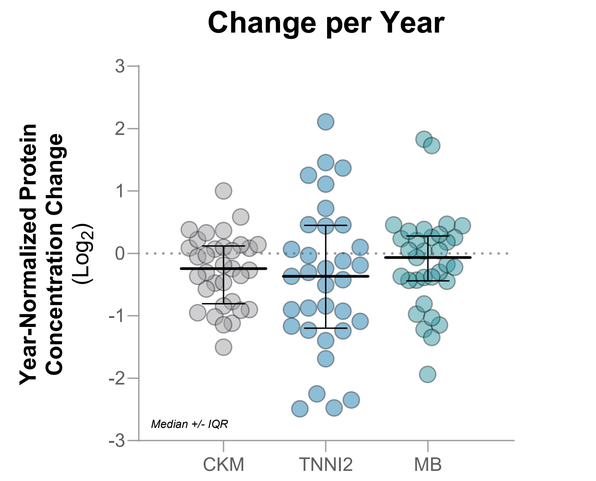
Individual subjects are shown in grey, while the overall correlation is shown in red.



	Decay rate constant (k; 1/yr)	1-Year Decline	2-Year Decline
Creatine Kinase, M-type	0.0469 +/- 0.0025	4.6 +/- 0.2 %	8.95 +/- 0.5 %
Troponin I, fast skeletal	0.0781 +/- 0.0053	7.5 +/- 0.4 %	14.46 +/- 0.8 %
Myoglobin	0.03734 +/- 0.0048	3.7 +/- 0.5 %	7.20 +/- 1.0 %

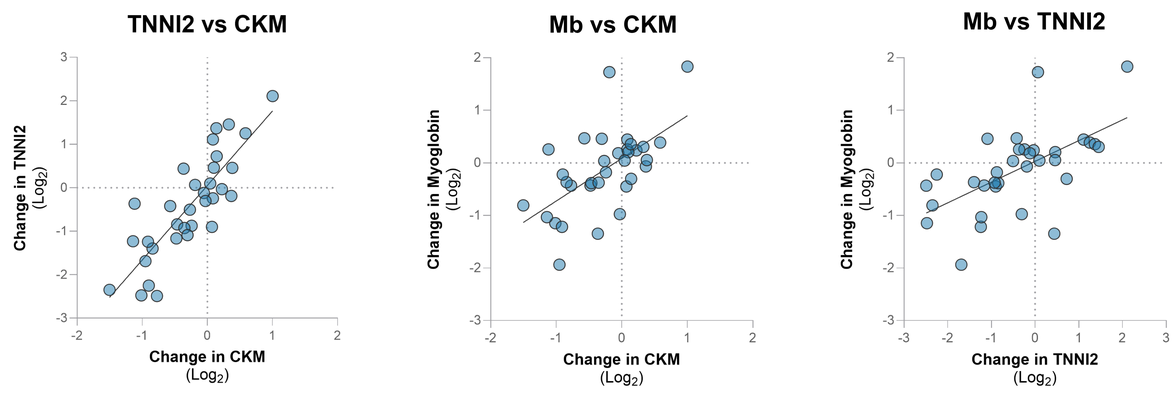
Individual subjects show high individual variability in change per year.

When converted to change per year, individual subjects exhibited a wide range of average yearly biomarker changes for the three studied proteins. TNNI2 exhibited the most marked spread, where subjects' average yearly change varied between approximately 5.6-fold increased and decreased. Mean yearly change for all proteins was negative and the majority of subjects exhibited negative average yearly decreases (CKM: 61%; TNNI2: 67%; MB: 51%) with TNNI2 showing the greatest observed mean yearly decrease of individual subjects (median -23%, IQR = -55%, +35%)



Individual subject changes in muscle injury biomarkers are correlated.

To demonstrate that the large differences in biomarker responses were likely real and not due to noise, each subject's average yearly changes in CKM, TNNI2, and Myoglobin were correlated to each other. All three pairwise correlations exhibited strong significance by a Pearson test (TNNI2 vs CKM: p < 0.0001; Mb vs CKM: p = 0.0002; Mb vs TNNI2: p = 0.0002)



Conclusions

In a multiyear natural history study of muscle injury biomarker changes in BMD subjects, circulating levels of CKM, TNNI2, and Myoglobin all significantly declined with increasing subject age and exhibited unique rates of age-related decrease.

By regression, single-year changes in muscle injury proteins were minimal, with all three proteins exhibiting less than 10% decrease per year. Over short timeframes, individual subject variability in biomarkers was large relative to the expected change from populational, age-related decrease. Despite highly variable individual subject changes in all three biomarkers, the correlative relationships between the changes in the proteins with respect to each other was significant, suggesting that the observed variability represented real differences in ongoing muscle injury in different subjects, likely due to difference in daily activity, which average out in a group setting.

These data contribute to the understanding of the natural history of BMD, both at the individual subject level and at the population level. They also provide a reference against which therapeutic interventions may be compared.

References

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