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Serum Creatine Kinase analysis in mouse models of muscular dystrophy.

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Authors	Kanneboyina Nagaraju Heather Gordish Research Center for Genetic Medicine Children's National Medical Center 111 Michigan Avenue, N.W. Washington, DC 20010
Working group members	George Carlson (Dept. Physiology Kirksville College of Osteopathic Medicine Kirksville, MO. 63501-1497, USA) Annamaria De Luca (Sezione di Farmacologia, Dipartimento Farmacobiologico, Facoltà di Farmacia, Università di Bari, Italy)
SOP responsible	Kanneboyina Nagaraju
Official reviewer	George Carlson

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1. OBJECTIVE

Creatine kinase (CK) is a protein found in cardiac and skeletal muscle. Serum CK levels are routinely used as an indicator of muscle damage in dystrophic mice and are even use as a diagnostic tool in human DMD. Levels of this muscle protein are measured in the serum under the assumption that increased serum CK levels in the blood are representative of increased muscle damage, sacrcolemma membrane fragility, and a poor disease phenotype.

2. SCOPE AND APPLICABILITY

Despite the high variability and additional issues, serum CK levels are a useful tool to measure dystropathology and drug efficacy. Collecting blood at sacrifice and analysing the serum CK levels is a useful assay to determine disease phenotype. One important consideration to take into account when measuring serum CK levels is that these levels are based on muscle damage, which is in turn highly affected by activity levels as well as several other factors.

3. CAUTIONS

CK levels are highly variable between mice. In addition because of the high variability, data is limited to comparison of levels within a study and not absolute values between studies (Spurney et al.). CK levels are also highly influential. Serum CK levels can be influenced by multiple factors, including exercise, the method in which the blood is drawn, anaesthesia, as well as age and gender (De Luca et al.; Grounds et al.; Spurney et al.). Finally, apart from mdx, the differences between disease strains and wild type mice are quite small.

4. MATERIALS

- Pointe Scientific Creatine Kinase (CK10) reagent (Fisher Scientific)
- Spectrophotometer

5. METHODS

5.1 Blood Collection

- Collect 250 μL of blood into Eppendorf tube via cardiac puncture under ketamine anaesthesia prior to sacrifice.
- Allow to clot and keep at room temperature. Allow clot contraction prior to centrifugation (10,000 rpm for 10 minutes at 4°C) and serum collection.
- Store collected serum at -80°C until analysis is performed.

5.2 Serum CK Analysis

- Serum creatine kinase activity is measured with Pointe Scientific Creatine Kinase (CK10) reagent (Fisher Scientific)
- Prepare reagent according the instructions
- Add 12.5 µl of serum to 500 µl reagent at 37°C and mix
- At 37°C, the absorbance is measured every min for 3 min at 340nm in a spectrophotometer. Repeat with second sample
- Calculate and average the differences in absorbance for 1 min intervals for both samples. Multiply the average of both samples by a factor 6592. Creatine kinase activity is expressed in units per liter.

6. EVALUATION AND INTERPRETATION OF RESULTS

When compared to wild type BL10 mice, mdx mice can show approximately 7 times higher serum CK levels upon analysis (Figure 1). Of course, this can vary greatly for many reasons discussed above and every effort must be made to minimize this variation by standardizing techniques and using similar animals.

Sample sizes for 4 models were calculated to detect a significant difference from wildtype:

Table 1: comparison of W/T to 5 disease models and sample size calculations

Strain	N	Median (range)	Mean ± SD	Significantly different from W/T (p-value)[1]	N needed per group to detect significant difference with W/T[2]
W/T	44	572 (115 – 1302)	645 ± 323		---
MDX	39	13308 (1339 – 55620)	18532 ± 14577	p<0.001	10
SJL	22	933 (267 – 3748)	1163 ± 857	p=0.021	30
BLAJ	29	998 (341 – 11318)	2517 ± 3094	p<0.001	26
AJ	6	845 (575 – 1368)	924 ± 379	p=NS	30

[1] P-value produced from nonparametric Wilcoxon rank sum test and adjusted for multiple comparisons

[2] Sample size calculations utilize a t-test model. Resulting sample sizes have been adjusted by an Asymptotic Relative Efficiency (ARE) factor 0.864 appropriate for data with no assumption of distribution.

7. REFERENCES

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2. Grounds MD, Torrisi J. Anti-TNFalpha (Remicade) therapy protects dystrophic skeletal muscle from necrosis. FASEB J. 2004 Apr;18(6):676-82.

3. Spurney CF, Gordish-Dressman H, Guerron AD, Sali A, Pandey GS, Rawat R, Van Der Meulen JH, Cha HJ, Pistilli EE, Partridge TA, Hoffman EP, Nagaraju K. Preclinical drug trials in the mdx mouse: assessment of reliable and sensitive outcome measures. Muscle Nerve. 2009 May;39(5):591-602.

8. APPENDIX

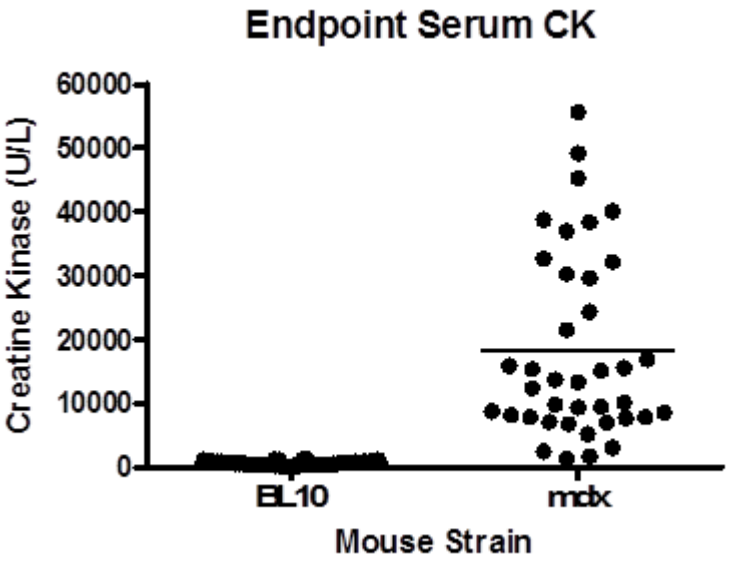

Figure 1: Serum CK levels in C57BL10 (n=44) and mdx (n=39).

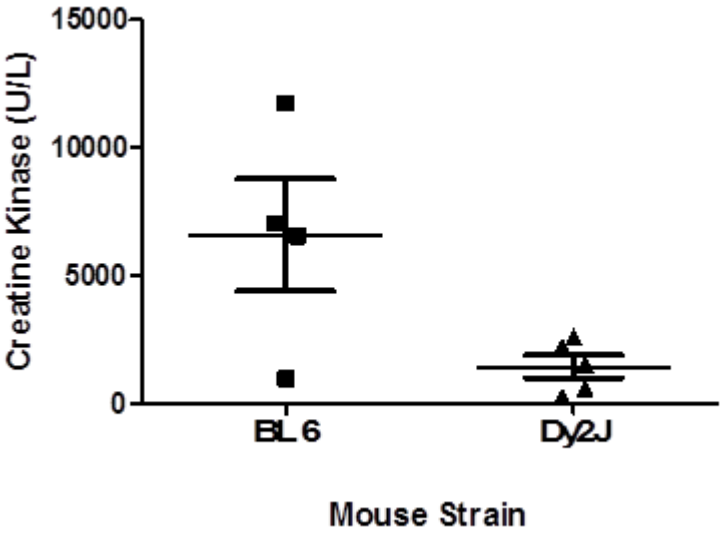

Figure 2: Serum CK levels in Dy2J mice. Dy2J homozygous mice had significantly lower CK when compared to the BL6 group. Note that the CK variations in BL6 controls were bigger than normally seen.

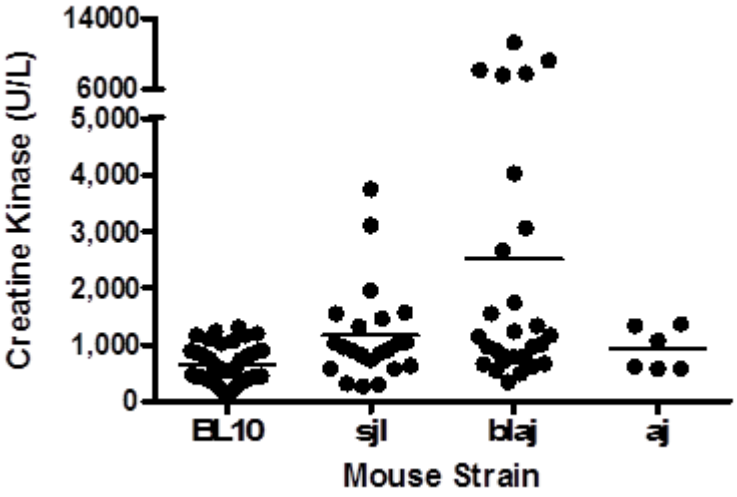

Figure 3: Serum CK levels in sjl (n=22), blaj (n=29), and aj (n=6) compared to wild type C57BL10.