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Fast Skeletal Muscle Troponin Activator CK-4015089 Improves Muscle Function in a FSHD Mouse Model with Muscle Weakness

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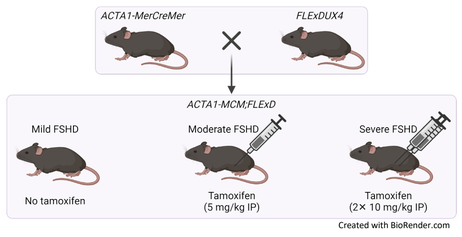
BACKGROUND

- **Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant, sporadic de-repression of DUX4 in skeletal muscle, leading to muscle degeneration, atrophy, and weakness that primarily affects muscles in the face, shoulder girdle, upper arm, and lower leg.**
- **CK-4015089 is a small molecule fast skeletal muscle troponin activator (FSTA) that slows the off-rate of calcium from fast skeletal troponin, leading to increased myosin ATPase activity and muscle force in response to subtetanic nerve stimulation.**
- **The objective of this study was to evaluate the single dose effect of CK-4015089 on muscle function in the tamoxifen-inducible ACTA1-MCM;FLExD mouse model of FSHD.**

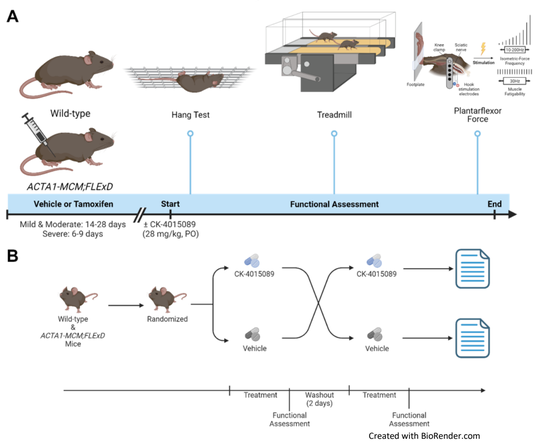
METHODS

Mouse Model Generation



- The *ACTA1-MCM;FLExD* FSHD mouse model has been previously described[1] and were obtained from the Jackson Laboratory and the laboratory of Peter Jones at the University of Nevada, Reno.
- FSHD mice that are not treated with tamoxifen exhibit low levels of DUX4 expression and demonstrate a mild muscle pathology phenotype. Mice dosed with 5 mg/kg or a cumulative 20 mg/kg tamoxifen dose exhibit a moderate and severe phenotype, respectively.

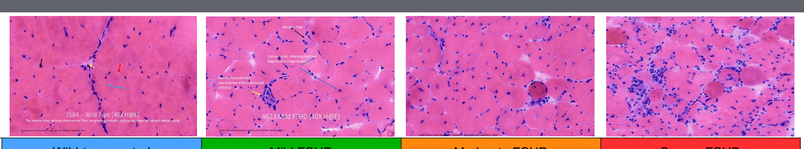
Study Schematic



A. Functional assessment of Wild-type control and *ACTA1-MCM;FLExD FSHD* mice was conducted 14-28 days after vehicle (corn oil) or tamoxifen treatment in Mild and Moderate FSHD mice and 6-9 days after in Severe FSHD mice.
B. Wild-type and *ACTA1-MCM;FLExD* FSHD mice were randomized and treated in a crossover design followed by blinded assessments of hang time and treadmill assessments. Mice were treated with vehicle (0.5% HPMC/0.1% Tween 80, PO) or CK-4015089 (28 mg/kg, PO) prior to each functional assessment, and given at least a two day washout period prior to the next treatment and functional assessment. Following the completion of all in vivo functional assessments, all mice were treated with vehicle or CK-4015089 and underwent terminal plantarflexor force assessment.

RESULTS

FSHD mouse muscle exhibit dystrophic histopathology



	Wild-type control		Mild FSHD		Moderate FSHD		Severe FSHD	
	Mean Group Score	Mean Lesion Score	Mean Group Score	Mean Lesion Score	Mean Group Score	Mean Lesion Score	Mean Group Score	Mean Lesion Score
Necrosis, myocyte	0.2	1	2.2	2.2	2.4	2.4	4	4
Inflammatory cell infiltrate, mononuclear cell	0		1.6	1.6	2.2	2.2	3.2	3.2
Atrophy, myofiber	0		1.6		2		2.8	
Myocyte, degen/regen, (central nuclei)	0		2.4	2.4	2.8	2.8	3.4	3.4
Fibrosis, increased, endomysium/perimysium	0		0.4	1	0.6	1	2.6	2.6
Sum - Scores:	**0.2**	**1**	**8.2**	**7.2**	**10**	**8.4**	**16**	**13.2**

Figure 1. Muscle pathological scoring of Wild-type control, Mild, Moderate, and Severe FSHD mice from Hematoxylin and Eosin-stained cross sections of the gastrocnemius muscle. Markers of degeneration/regeneration, atrophy, mononuclear cell infiltration, necrosis, and fibrosis were present with increasing severity in FSHD mice as indicated by mean Sum Scores. N=5 mice/group.

FSHD mice exhibit significant muscle atrophy and reductions in ankle plantarflexor muscle force

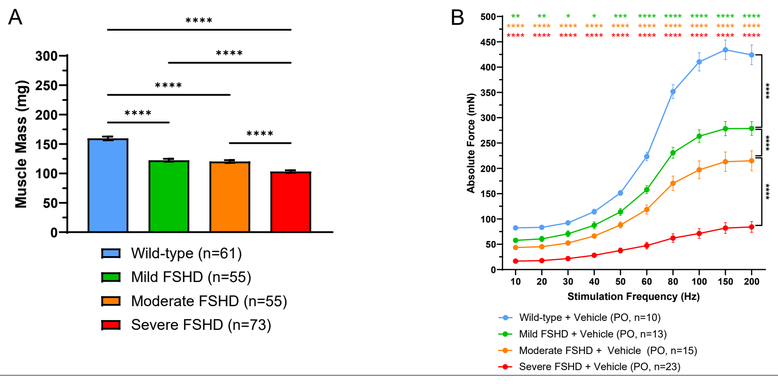


Figure 2A. Gastrocnemius muscle mass in male and female Wild-type control, Mild, Moderate, Severe FSHD mice.
2B. Absolute isometric force in situ in response to electrical stimulation frequencies ranging from 10-200 Hz (1 ms pulse width, 350 ms train duration) in male and female Wild-type control, Mild, Moderate, Severe FSHD mice. Data are expressed as mean ± SEM and analyzed by two-way ANOVA. Asterisks (*) of group colors indicate significant differences compared with Wild-type (Vehicle) or via selected pairs (black): *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

CK-4015089 increases ankle plantarflexor muscle force production in FSHD mice

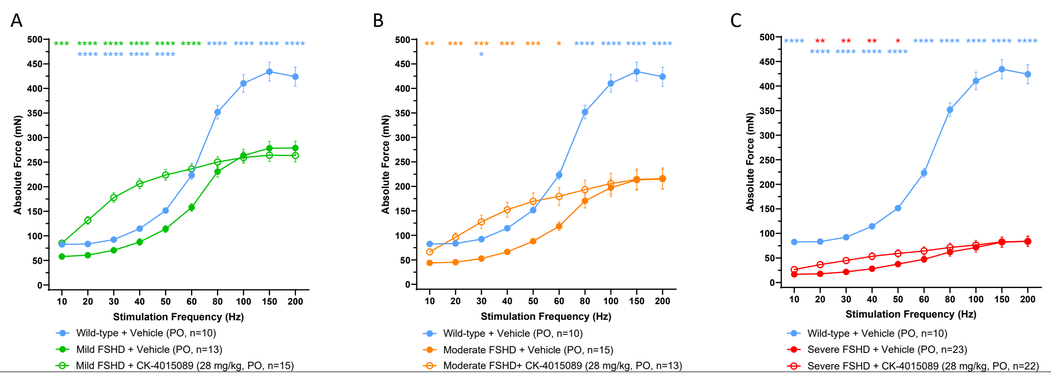


Figure 3. Absolute isometric force in situ in response to electrical stimulation frequencies ranging from 10-200 Hz (1 ms pulse width, 350 ms train duration) in male and female **A.** Mild, **B.** Moderate, and **C.** Severe FSHD mice treated with Vehicle (filled circles) or CK-4015089 (28 mg/kg, PO, open circles). As a comparator, vehicle-treated Wild-type control force data is plotted with each FSHD group. Data are expressed as mean ± SEM and analyzed by two-way ANOVA. Asterisks (*) indicate significant differences compared with each respective FSHD severity treated with CK-4015089: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

CK-4015089 attenuates repetitive nerve stimulation-induced muscle fatigue in FSHD mice

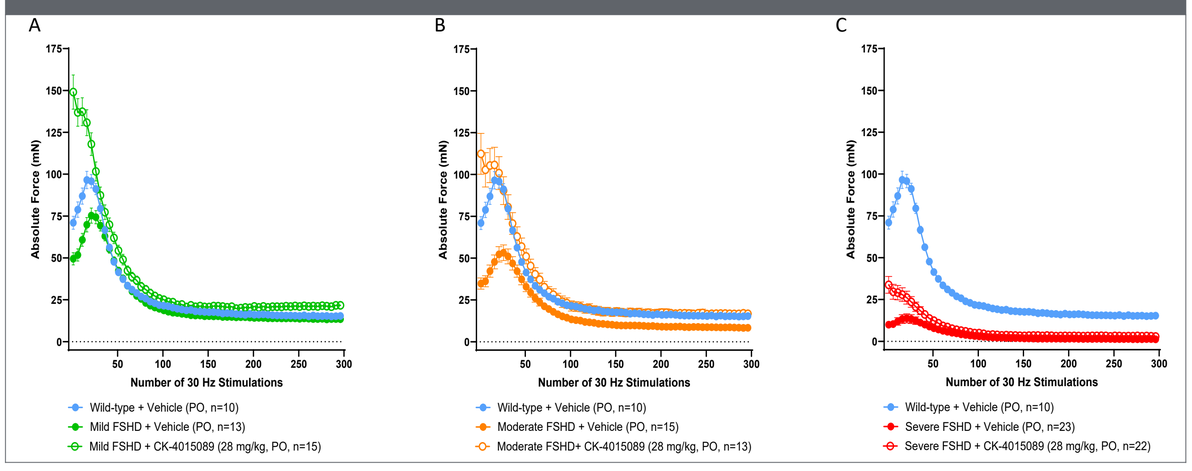


Figure 4. Absolute isokinetic force in response to 300 repeated 30Hz stimulations (3.1 rad/s, one stimulation per 1.5 sec) over an approximate 10 minute period in male and female **A.** Mild, **B.** Moderate, and **C.** Severe FSHD mice treated with Vehicle (filled circles) or CK-4015089 (28 mg/kg, PO, open circles). As a comparator, vehicle-treated Wild-type control force data is plotted with each FSHD group. Data are expressed as mean ± SEM.

CK-4015089 increased total muscle work in FSHD mice under fatigue-induced conditions

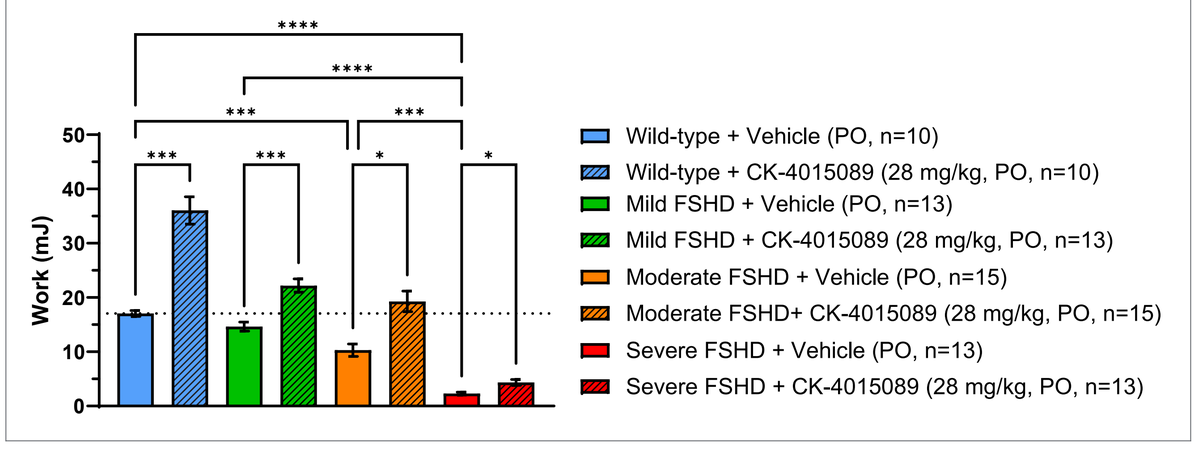


Figure 5. Total muscle work produced in male and female Wild-type control, Mild, Moderate, and Severe FSHD mice treated with Vehicle or CK-4015089 (28 mg/kg, PO, fill pattern). Dashed line represents the vehicle-treated Wild-type control total work. Data are expressed as mean ± SEM and analyzed by one-way ANOVA. Asterisks (*) indicate significant differences between bracketed groups: *p<0.05; ***p<0.001;****p<0.0001.

CK-4015089 increased treadmill performance in moderately diseased FSHD mice

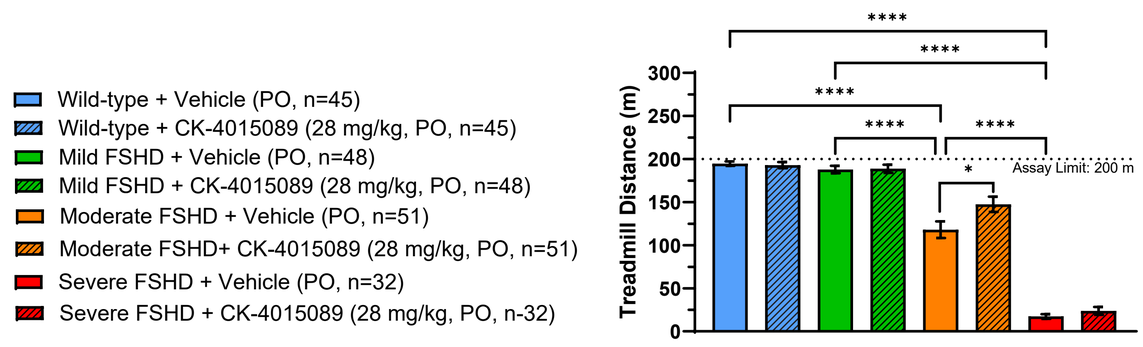


Figure 6. Treadmill performance in male and female Wild-type control, Mild, Moderate, and Severe FSHD mice treated with Vehicle or CK-4015089 (28 mg/kg, PO, fill pattern). The treadmill distance assay limit was 200 meters. Data are expressed as mean ± SEM and analyzed by one-way ANOVA. Asterisks (*) indicate significant differences between bracketed groups : *p<0.05; ***p<0.001; ****p<0.0001.

CK-4015089 increased hang time duration in all FSHD mice

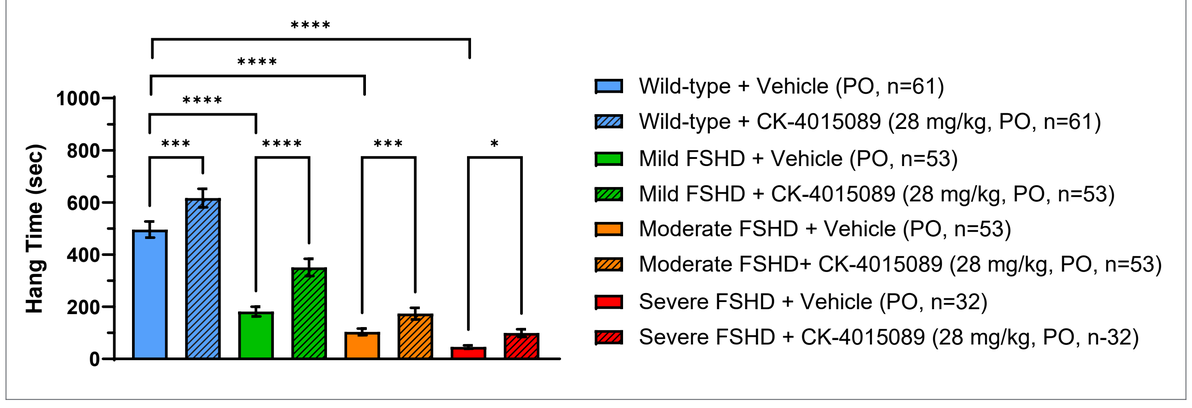


Figure 7. Inverted grid hang time performance in male and female Wild-type control, Mild, Moderate, and Severe FSHD mice treated with Vehicle or CK-4015089 (28 mg/kg, PO, fill pattern). Data are expressed as mean ± SEM and analyzed by one-way ANOVA. Asterisks (*) indicate significant differences between bracketed groups : *p<0.05; ***p<0.001; ****p<0.0001.

CONCLUSIONS

- Mild, Moderate, and Severe ACTA1-MCM;FLExD FSHD mice exhibit markers of muscle disease pathology, significant muscle atrophy, and progressively lower in situ muscle force and in vivo hang time performance that corresponded with increasing disease severity
- In Wild-type and FSHD mice, single doses of FSTA CK-4015089:
  - significantly increased absolute muscle force in response to subtetantic nerve stimulation
  - improved resistance to fatigue, with increases in total work by 111%, 51%, 87%, and 91% in Wild-type, Mild, Moderate, and Severe FSHD mice, respectively
  - increased hang time performance by 24%, 93%, 67%, and 116% in Wild-type, Mild, Moderate, and Severe FSHD mice, respectively
- FSTA CK-4015089 may be a therapeutic approach to mitigate muscle weakness in FSHD

References

1. Jones, T. I., Chew, G. L., Barraza-Flores, P., Schreier, S., Ramirez, M., Wuebbles, R. D., Burkin, D. J., Bradley, R. K., & Jones, P. L. Transgenic mice expressing tunable levels of DUX4 develop characteristic facioscapulohumeral muscular dystrophy-like pathophysiology ranging in severity. Skelet Muscle. 2020 Apr 11;10(1):8

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Disclosures

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