

CK-2127107 IMPROVES MUSCLE FUNCTION AND EXERCISE CAPACITY IN A RAT HEART FAILURE MODEL

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ABSTRACT

Heart failure (HF) is associated with skeletal muscle dysfunction and exercise intolerance. Small molecule, fast skeletal troponin activators increase sarcomere calcium sensitivity and muscle force at sub-maximal nerve stimulation rates while reducing muscle fatigability in both an *in situ* model of vascular insufficiency and rotarod running. Here we show the fast skeletal troponin activator, CK-2127107, improves exercise capacity in a rat model of heart failure produced by ligation of the left anterior descending artery.

Exercise performance was assessed in HF rats at 11-12 weeks post-surgery using a fatiguing rotarod protocol (5 min forced-run ramped from 14-16 RPM followed by run time assessment during a 10 min ramp from 12 to 25 RPM). HF rats were selected based on a left ventricular fractional shortening <25% and reduced run time compared to sham controls. Rats received CK-2127107 (10 mg/kg PO) or vehicle in a randomized, blinded, 2-way cross-over study design. Vehicle-treated sham rats ran longer than vehicle-treated HF rats (198 ± 26 vs. 111 ± 32 sec, p=0.042, mean ± S.E.). CK-2127107 treated sham rats ran longer than vehicle-treated HF rats (275 ± 31 vs. 198 ± 26 sec, p=0.022, mean ± S.E.). HF rats treated with CK-2127107 increased their rotarod running time approximately 2.5-fold compared to vehicle treatment to 277 ± 32 sec (p=0.0004 by ANCOVA), completely normalizing exercise capacity in HF rats. Hind limb muscles showed no major differences in contractility, fatigability or fiber type composition between groups. The diaphragm was characterized by muscle atrophy and fiber type switching toward slow fibers. Diaphragm tissue exhibited decreased contractility and reduced calcium sensitivity, which was corrected by CK-2127107.

Overall, CK-2127107 increased fatigue resistance in HF rats. These studies suggest that fast skeletal troponin activators, by increasing calcium sensitivity of the fast skeletal muscle sarcomere, may be useful to improve exercise tolerance in patients with HF.

INTRODUCTION

Heart failure affects over 5 million people in the United States alone (Roger *et al.*, 2012). In addition to the increased risk of mortality, heart failure patients exhibit muscle weakness, fatigue, and exercise intolerance, which reduces the quality of life. While such fatigue is common in heart failure patients, there appears to be little correlation between fatigue and the severity of the disease itself, suggesting that the fatigue is due to changes that go beyond poor perfusion due reduced cardiac function (Lunde *et al.*, 2001).

Heart failure is associated with morphological changes in skeletal muscle that include atrophy and changes in fiber type distribution, toward a greater prevalence of fast twitch muscles. These changes lead to decreases in maximum voluntary contractile force and increased fatigability in patients (Sullivan and Hawthorne 1995).

Exercise intolerance and fatigue has been shown to be amenable to modification in both animals and humans (Chung *et al.*, 2011, Bosnak-Gucu *et al.*, 2011). In particular training of respiratory muscles has been shown to be effective in reducing exercise intolerance (Bosnak-Gucu *et al.*, 2011). Whilst exercise has been shown to have no major impact on long term survival in these patients (O'Connor *et al.*, 2009), improvement of fatigue symptoms may improve quality of life.

Small molecule, fast skeletal troponin activators increase sarcomere calcium sensitivity and muscle force at sub-maximal nerve stimulation rates (Russell *et al.*, 2012). I.a model of vascular insufficiency, they reduced the fatigability of the extensor digitorum longus (EDL) muscle *in situ*, increasing the overall tension achieved over the course of the fatigue protocol. Tirasemtiv increased running time on both rotarod and treadmill (Kennedy *et al.*, 2012). CK-2127107 is a novel small molecule activator of the fast skeletal muscle troponin complex that acts in a similar way to those previously published (Russell *et al.*, 2012; Kennedy *et al.*, 2012). As such we have undertaken studies to describe the effects of CK-2127107 on the exercise intolerance induced in a rat model of heart failure.

METHODS

Animals

Female Sprague Dawley rats (175-200g) were obtained from Charles River Laboratories with ligation of the left anterior descending coronary artery performed prior to shipment. Sham operated animals were also obtained from the same source.

Echocardiographic assessment of fractional shortening was performed upon arrival and at 2, 4, 7 and 10 weeks after arrival (Figure 1a). Only animals that showed a fractional shortening of less than 25% were used as study animals. Animals that did not show such reductions were excluded from the study.

Post-mortem analysis of heart weight and infarct size was performed on animals used in the study (figure 2).

Measurement of exercise capacity

Rats were assessed according to a protocol developed at Cytokinetics to assess motor coordination under fatiguing conditions (Figure 1b), and that had previously shown CK-2127107 to be effective at increasing running time in young healthy rats (Figure 1c).

On the day of the experiment animals were dosed thirty minutes prior to start of test. The test began with a 5-minute primer session, whereby animals were run at an increasing speed from 14-16 RPM over 5 minutes. Rats were then run at a constantly accelerating rate from 12 RPM to 25 RPM over the course of 10 minutes. Once 25 RPM had been reached, a constant speed of 25 RPM was maintained for an additional 5 minutes. Time to fall was recorded, with the test being terminated at 900 seconds. The following day animals were assessed again in a cross over design, where each animal was assessed with and without treatment.

Assessment of myocardial infarct

Hearts from animals used on study were dissected free, flushed of blood, blotted dry and weighed (Figure 2a). Hearts were fixed and paraffin embedded, sectioned and stained with Masson's trichrome (Figure 2b).

Fiber type analysis

Serial frozen cross sections were cut at 10 μ m and stained for myosin ATPase after preincubation at pH 4.35. Stained fibers were classified Type I, Type IIa, or Type II b/x and measured for individual myofiber cross-sectional area (μ m 2).

Muscle tissue experiments

Muscle tissue for *in vitro* skinned fiber studies were prepared using an adapted protocol based on Lynch and Faulkner (1998). Single skinned fibers were then suspended between a 400A force transducer (Aurora Scientific, Ontario, Canada) and a fixed post. Tension was generated in each fiber by changing fiber buffer over to relax buffer (20 μ M MOPS, 5.5 μ M MgCl₂, 132 μ M potassium acetate, 4.4 μ M ATP, 22 μ M creatine phosphate, 1 mg/ml creatine kinase, 1 mM DTT, 44 ppm antifoam , pH 7) supplemented with 1 mM EGTA and a 15 mM solution of calcium chloride in varying proportions. CK-2127107 was added to these buffers from a DMSO solution (final DMSO concentration =1%).

Diaphragm Force-Frequency relationship

Diaphragm contractile force was measured by electrical field stimulation in an organ bath system (Radnoti) based on a standard operating protocol adapted from the Treat NMD website.

(http://www.treat-nmd.eu/downloads/file/sops/dmd/MDX/MDM_M.1.2.00_2.pdf). Diaphragm strips were set to a length that produced maximum twitch tension (Lo). The force-frequency profile of the muscle was obtained by stimulating the muscle at frequencies between 10-150 Hz (Grass Stimulator, 800 ms train duration, 0.6 ms pulse width). CK-2127107 was dissolved in DMSO and directly added into the bath.

RESULTS

Rotarod running in normal rats

CK-2127107 produced a dose-dependent increase in running time in healthy rats. Following a dose of 10mg/kg an increase of up to 2.5 fold was observed.

Rotarod running in heart failure rats

Heart failure resulted in reduced rotarod running time compared to sham operated controls (198 ± 26 vs. 111 ± 32 sec, p=0.042, mean ± S.E.). CK-2127107 treated sham rats ran longer than vehicle treated sham rats (275 ± 31 vs. 198 ± 26 sec, p=0.022, mean ± S.E.). HF rats treated with CK-2127107 increased their rotarod running time approximately 2.5-fold compared to vehicle treatment to 277 ± 32 sec (p=0.0004 by ANCOVA), completely normalizing exercise capacity in HF rats.

Fiber type distribution and skinned fiber analysis of EDL

Mean EDL cross sectional area and fiber type distribution were not significantly different between SHAM and LAD animals. There was no difference in the force-pCa relationship between SHAM and LAD EDL fibers. CK-2127107 (3 μ M) caused a significant leftward shift in the force-Ca²⁺ relationship in both SHAM and LAD EDL muscle.

Diaphragm contractility

Mean diaphragm cross sectional area was significantly lower in LAD diaphragm muscle. Within individual fiber types, significant atrophy in type IIa and type IIb/x fibers was observed in LAD diaphragms. Fiber type distribution characterized by myosin ATPase activity was not significantly different between SHAM and LAD animals.

Compared to SHAM diaphragms, HF diaphragm fibers had significantly lower Ca²⁺ sensitivity. CK-2127107 (30 μ M) significantly increased Ca²⁺ sensitivity in both SHAM and HF diaphragm fibers.

The force-frequency relationship of SHAM and LAD diaphragms were measured ex vivo by electrical field stimulation.

LAD diaphragm muscle produced significantly lower force compared to Sham diaphragm muscle. CK-2127107 (30 μ M) significantly increased force in both SHAM and LAD diaphragms at submaximal frequencies of electrical stimulation.

Overall, CK-2127107 improved the impaired force production in LAD diaphragms.

Model Development

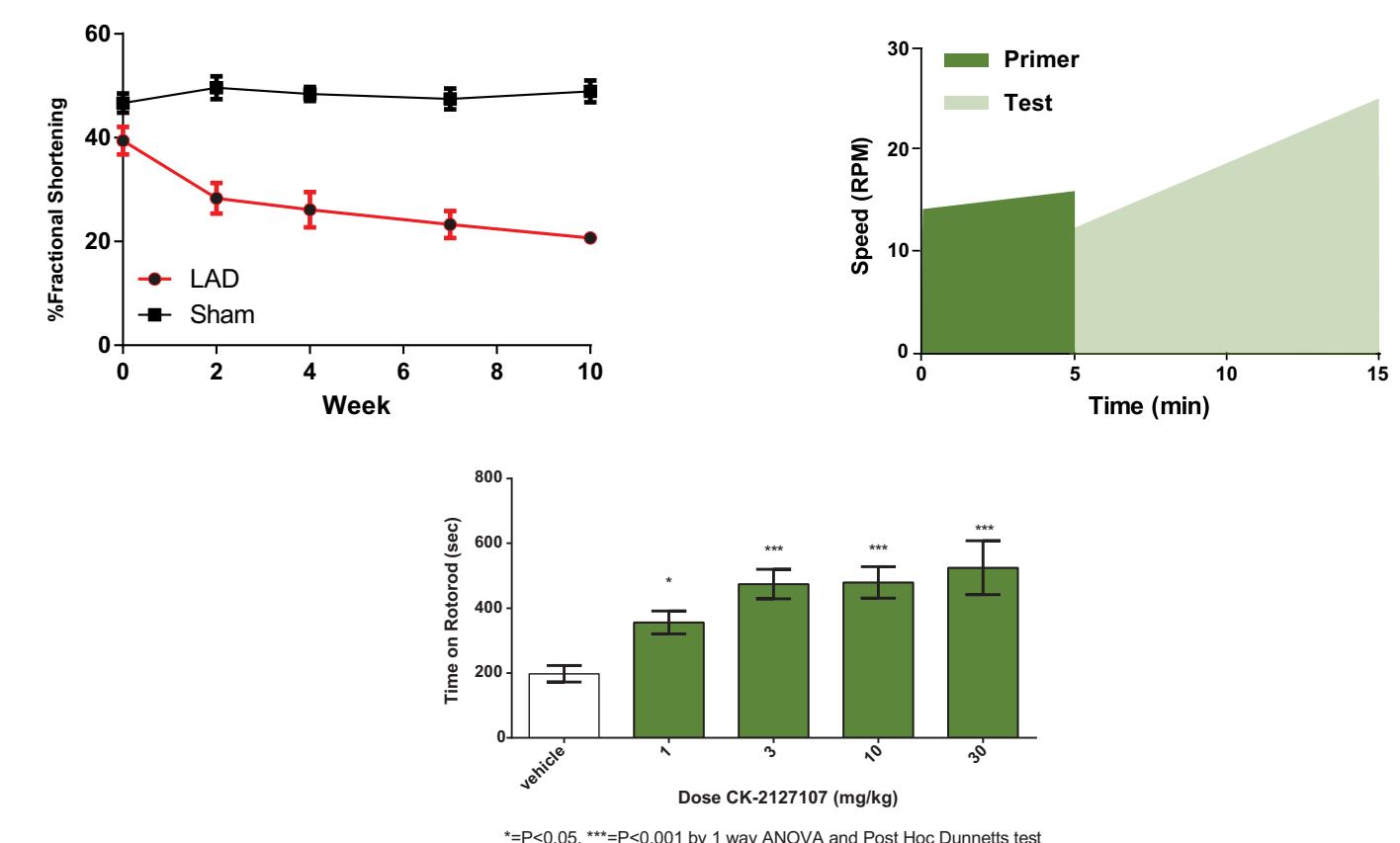


Figure 1: a) Fractional shortening LAD animals is decreased over time, when compared to baseline or sham controls. b) Example of rotarod protocol. c) Dose dependent improvement in running time for young female rats in the fatiguing rotarod assay.

Rotarod Running in HF Rats

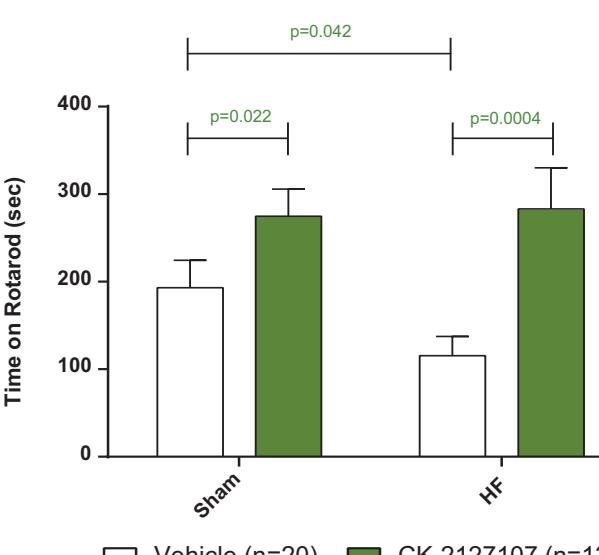


Figure 2: CK-2127107 (10mg/kg, PO) increases rotarod running time in rats with heart failure-induced exercise intolerance and control rats. Running time was significantly decreased in heart failure animals, with CK-2127107 increasing run time in both heart failure and sham animals to a level, not significantly different from one another.

Changes in EDL muscle in HF rats

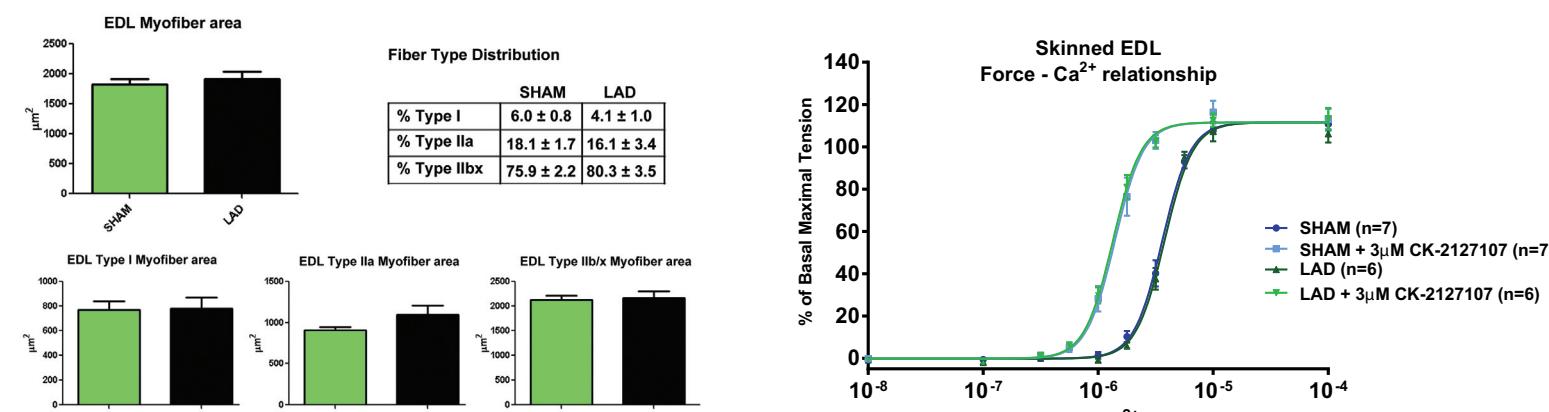


Figure 3: a) Heart failure is not associated with changes in fiber type distribution or cross sectional area. b) Skinned fibers from EDL muscles respond to CK-2127107 by a leftward shift in the pCa curve. No difference is seen between LAD and Sham treated animals.

Changes in Diaphragm Muscle in HF Rats

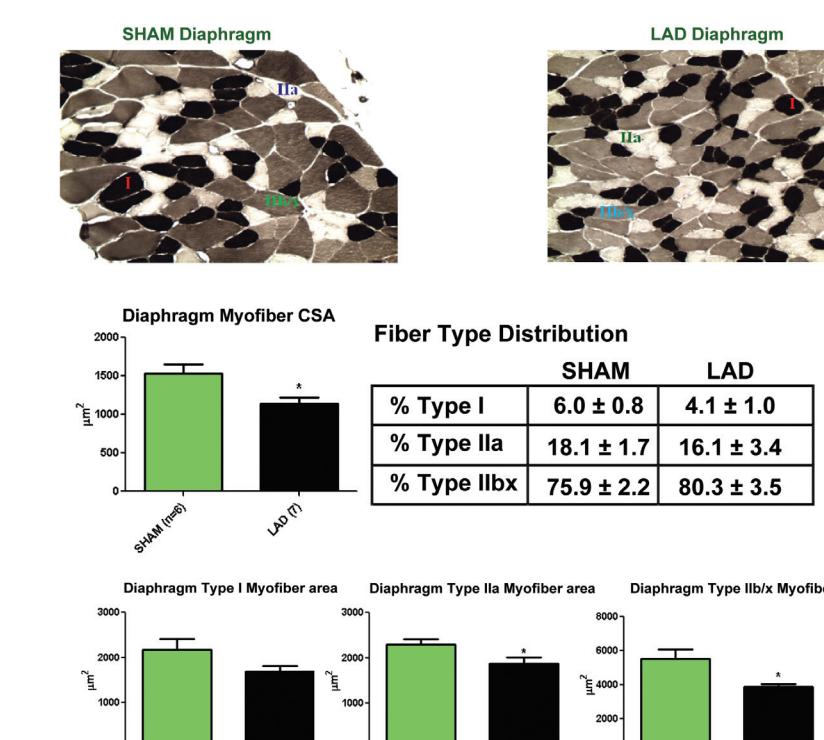


Figure 4: Heart Failure induces significant atrophy in the Diaphragm of LAD animals. a) Photomicrographs of ATPase stained diaphragm from LAD and sham animals. b) Changes in fiber-type distribution and cross-sectional area in diaphragm from LAD animals compared to Sham.

Functional Changes in the Diaphragm of HF Rats

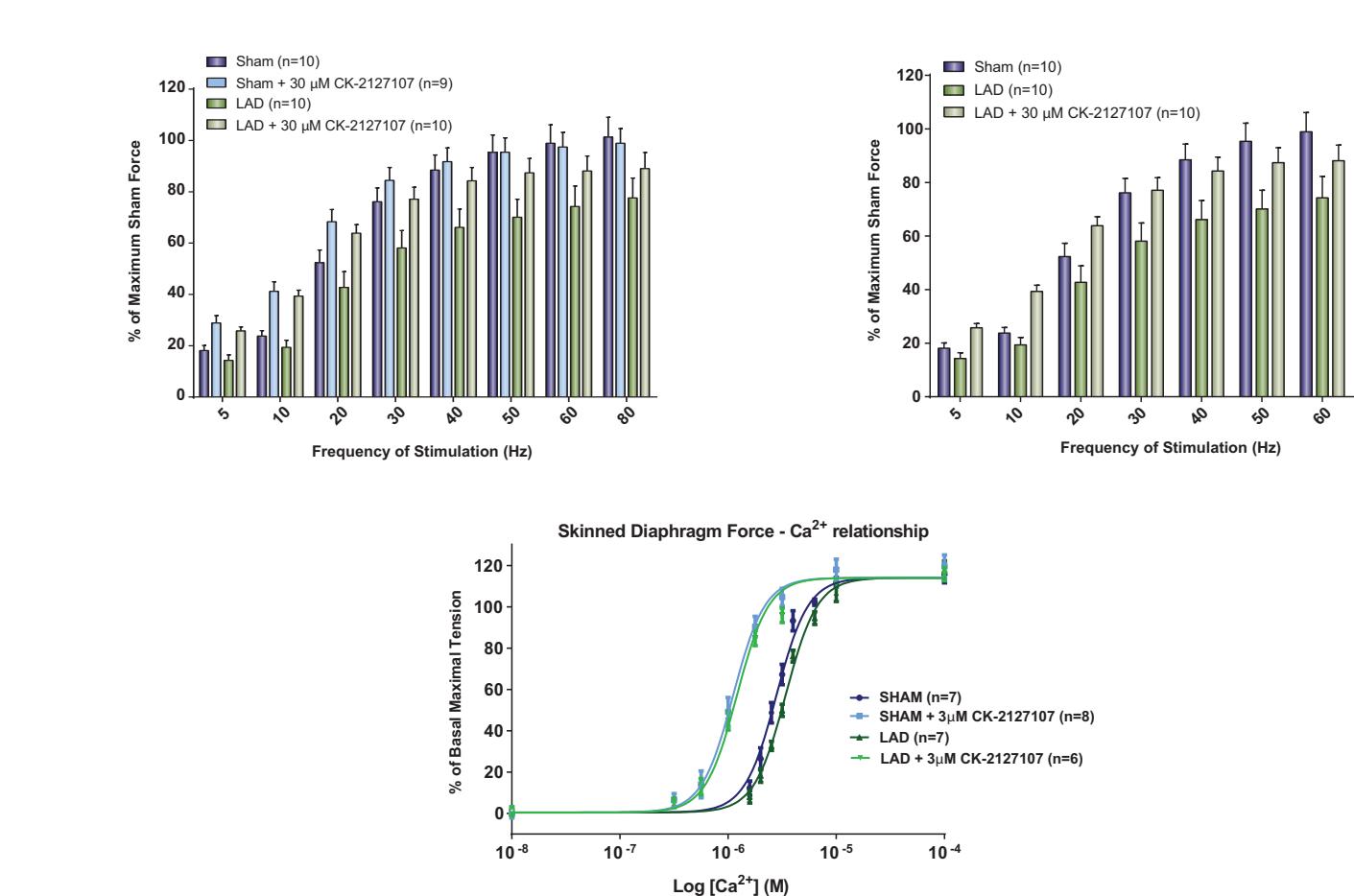
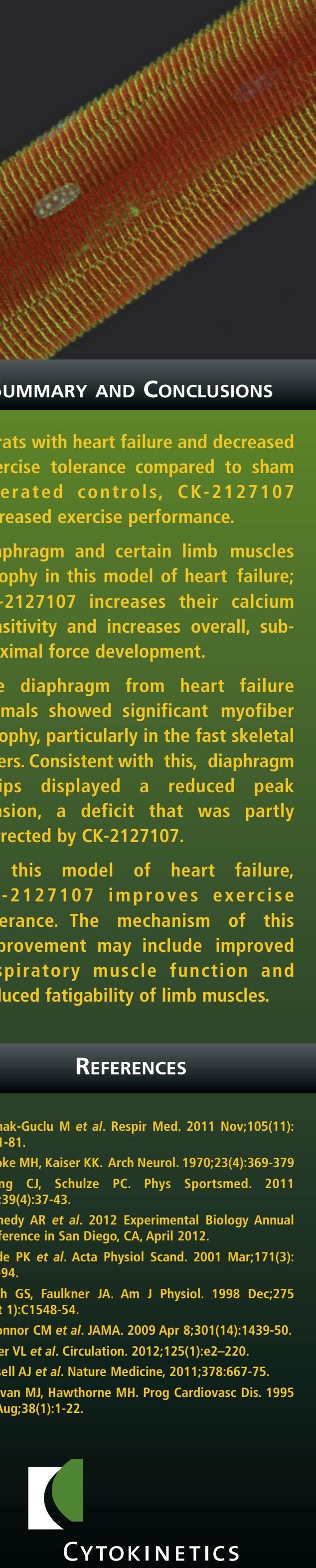


Figure 5: Heart failure leads to atrophy of the diaphragm. a) CK-2127107 increases sub maximal tension in diaphragm strips from both LAD and sham animals. (Data is expressed as a % of maximum sham force). b) Comparison of vehicle and CK-2127107 effects on Diaphragm strips from LAD animals to untreated strips from sham animals, showing a partial return to sham levels. c) Skinned Diaphragm Force - Ca²⁺ relationship.



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