# Effect of *tirasemtiv* on Submaximal Rodent Diaphragm Strength and Respiratory Function

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DISCLOSURE OF INTERESTS: DTH, AK, FIM, and JRJ are currently employees of **Cytokinetics, Inc.** and were compensated financially for their work.

## Abstract

**Background:** Diaphragm weakness, which is characterized by significant losses in function, is a primary component of the pathophysiological changes that lead to respiratory failure. *Tirasemtiv* is a fast skeletal troponin activator, previously shown to increase submaximal force in rat and human lower leg muscles.

**Objective:** The objective of this study was to characterize the effect of *tirasemtiv* on calcium sensitivity and force production *ex vivo* in rat and mice diaphragm muscle.

**Methods:** For *in vitro* skinned (permeabilized) fiber studies, Sprague Dawley rat diaphragm muscles were rapidly dissected, rinsed in physiological saline, and then incubated in skinning and storage solution. Single muscle fibers were dissected from larger segments of tissue in rigor buffer. The fibers were then suspended between a force transducer and a fixed post. The muscle force-calcium (pCa) relationship in diaphragm muscle was investigated in single rat diaphragm fibers treated with either 1% DMSO (vehicle treatment), or *tirasemtiv* (0.1μM, 1μM, or 10μM) over –log(10) calcium concentrations (pCa) ranging from 8 to 4.

For intact diaphragm muscle, contractile force was measured by electrical field stimulation in an organ bath system. The diaphragm and the last floating rib from B6SJL mice were excised, rinsed in physiological saline, placed in a temperature controlled water-jacketed chamber containing Krebs-Henseleit buffer. Braided silk sutures were tied at the central tendon and floating rib and attached to a force transducer between two platinum electrodes. The force-frequency profile of the muscle was obtained by stimulating the muscle at frequencies between 5-150 Hz. *Tirasemtiv* (1 μM in DMSO) was directly added into the bath.

**Results:** *Tirasemtiv* increased the calcium sensitivity of rat diaphragm muscle, shifting the force-pCa relationship of skinned fibers in a dose-dependent manner. Compared to DMSO-treated skinned diaphragm muscle fibers, 10μM *tirasemtiv* increased the pCa at 50% maximum tension (pCa50) 10-fold (vehicle: 5.43 ± 0.05, 10μM *tirasemtiv*: 6.74 ± 0.02, n=5/group). In intact muscle, at submaximal stimulation frequencies less than 20Hz, *tirasemtiv* (1μM) increased mouse diaphragm tension *ex vivo* compared to vehicle-treated diaphragm strips (vehicle n=11, *tirasemtiv* n=5, p<0.05 at 5, 10, and 20 Hz).

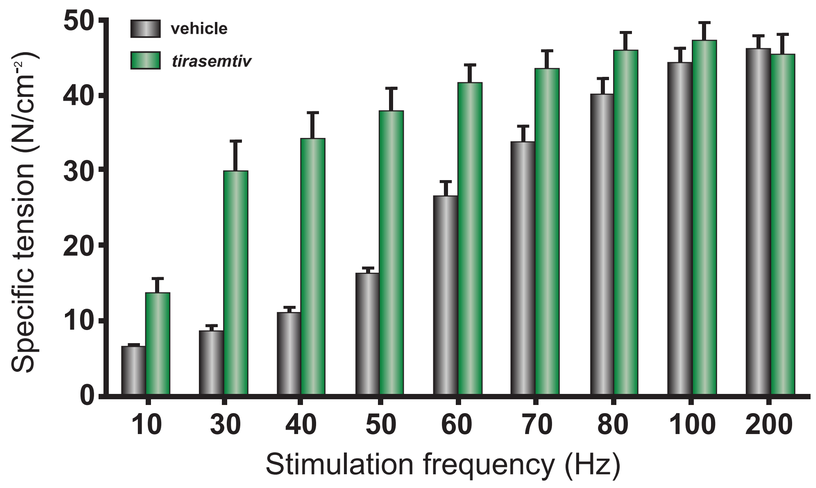
**Discussion and conclusions:** Pathological conditions that lead to diaphragm weakness can have severe consequences, ranging from dyspnea and reduced quality of life to respiratory failure and death. The fast skeletal troponin activator, *tirasemtiv*, increased calcium ($Ca^{2+}$) sensitivity in a dose-dependent manner and increased submaximal force production *ex vivo*. These results suggest that *tirasemtiv* and other fast skeletal muscle troponin activators may be viable therapeutics for improving respiratory muscle function.

**Acknowledgements:** All authors are currently employees of Cytokinetics, Inc. and were compensated financially for their work.

## Introduction

- Diaphragm weakness is a primary component of the pathophysiological changes that lead to respiratory failure
- About half of the diaphragm is composed of Type II fast skeletal muscle fibers (Humans: 50% Type II; Rodents: 60% Type II)
- *Tirasemtiv* is a fast skeletal troponin activator that increases sarcomere $Ca^{2+}$ sensitivity
- *Tirasemtiv* has previously been shown to increase force at submaximal rates of nerve stimulation rat and human lower leg muscles[1,2]
- The objective of this study was to characterize the effect of *tirasemtiv* on calcium sensitivity, force production, and respiratory function in rat and mice diaphragm muscle

1 Russell, *et al.* Nat Med. 2012 Feb 19;18(3):452-5
2 Hansen, *et al*. Muscle Nerve. 2014 Mar 14. doi: 10.1002/mus.24239




**Background. *Tirasemtiv* increases hindlimb muscle force in response to submaximal rates of nerve stimulation.** *Tirasemtiv* causes a leftward shift in the isometric force frequency relationship in the rat hindlimb extensor digitorum longus muscle. Figure reproduced from Russell, *et al. Nat Med.* 2012 Feb 19;18(3):452-5.

## Methods

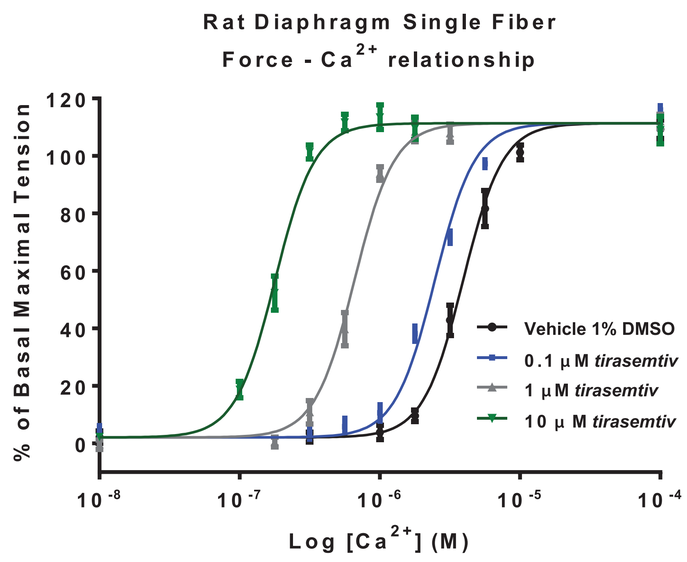
***In vitro* muscle force-$Ca^{2+}$ relationship:** Sprague Dawley rat diaphragm muscles were rapidly dissected, rinsed in physiological saline, and then incubated in skinning and storage membrane permeabilization solution. Single muscle fibers were dissected from larger segments of tissue in rigor buffer. The fibers were then suspended between a force transducer and a fixed post. The muscle force-calcium (pCa) relationship in diaphragm muscle was investigated in single rat diaphragm fibers treated with either 1% DMSO (vehicle treatment), or *tirasemtiv* (0.1μM, 1μM, or 10μM) over –log(10) calcium concentrations (pCa) ranging from 8 to 4.

***Ex vivo* diaphragm force:** Diaphragm isometric force was measured by electrical field stimulation in an organ bath system. The diaphragm and the last floating rib from B6SJL mice were excised, rinsed in physiological saline, placed in a temperature controlled water-jacketed chamber containing Krebs-Henseleit buffer. Braided silk sutures were tied at the central tendon and floating rib and attached to a force transducer between two platinum electrodes. The force-frequency profile of the muscle was obtained by stimulating the muscle at frequencies between 5-150 Hz. *Tirasemtiv* (1 μM in DMSO) was directly added into the bath.

***In vivo* respiratory function:** B6SJL-$SOD1^{G93A}$ mice were orally dosed with vehicle or 10 mg/kg *tirasemtiv* in a blinded, cross-over design. After oral treatment, mice were placed in unrestrained whole body plethysmography chambers for 30 minutes of acclimation. After acclimation, respiratory parameters, including tidal volume, respiratory rate, and minute ventilation, were monitored for 10 minutes at room air.

## Results

### Rat Diaphragm Single Fiber Force – $CA^{2+}$ Relationship




| | $pCa_{50}$ Log $[Ca^{2+}]$ (M) |
|---|---|
| Vehicle 1% DMSO | 5.43 ± 0.054 |
| 0.1 μM *tirasemtiv* | 5.59 ± 0.007 |
| 1 μM *tirasemtiv* | 6.19 ± 0.018 |
| 10 μM *tirasemtiv* | 6.74 ± 0.028 |

**Figure 1. *Tirasemtiv* increases diaphragm fiber $Ca^{2+}$ sensitivity in a concentration-dependent manner.** Permeabilized rat diaphragm fibers demonstrate a concentration-dependent leftward shift in the force-$Ca^{2+}$ relationship. The table describes the $pCa_{50}$ at each concentration of *tirasemtiv*. (n=5/group).

### *Tirasemtiv* Increases Diaphragm Force in Response to Submaximal Rates of Nerve Stimulation

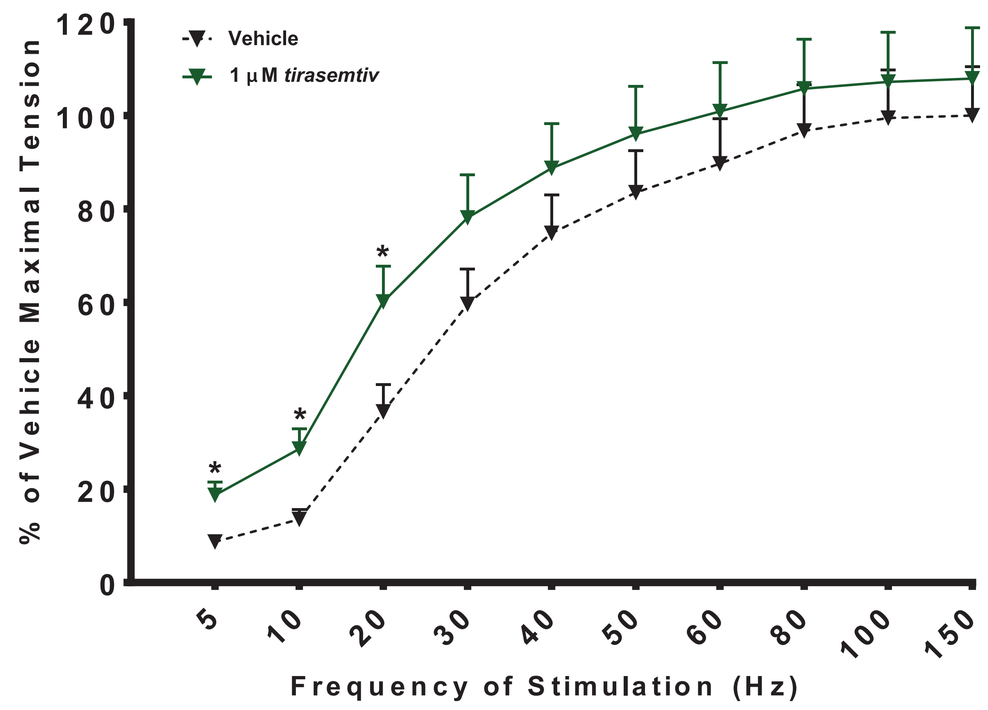



**Figure 2. *Tirasemtiv* increases diaphragm force in response to submaximal rates of nerve stimulation.** Treatment with 1μM *tirasemtiv* increased force in response to 5, 10, and 20Hz electrical stimulation in B6SJL mouse diaphragm muscle. (vehicle N=11, *tirasemtiv* N=5). *p< 0.05 veh vs. *tirasemtiv* at stimulation frequency.

### Tidal Volume

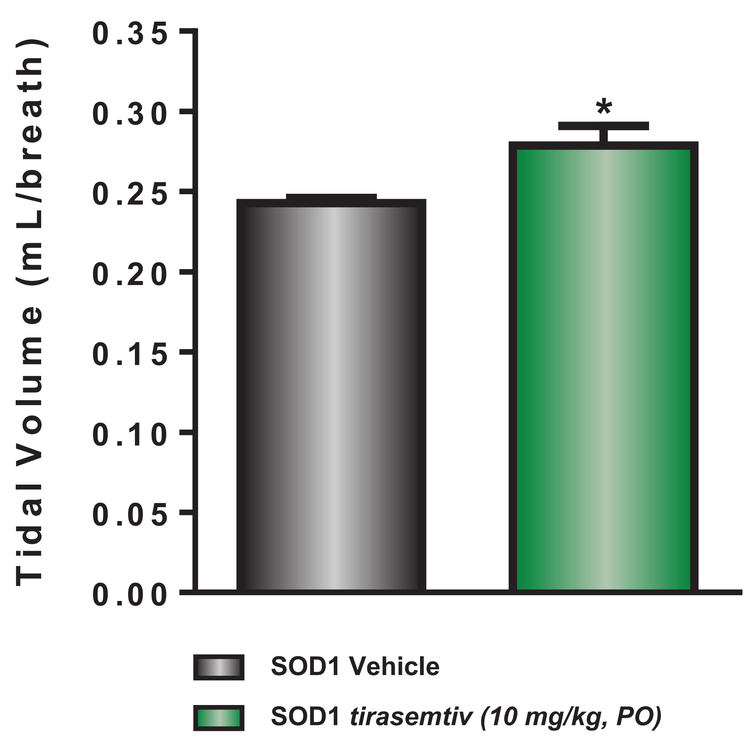
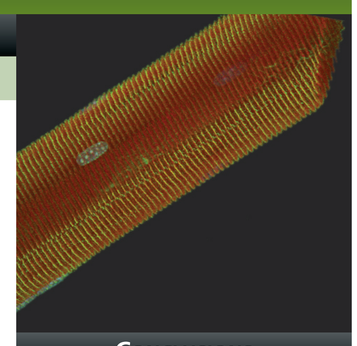



**Figure 3. *Tirasemtiv* increases tidal volume in a mouse model of ALS.** B6SJL-$SOD1^{G93A}$ mice were dosed with vehicle or *tirasemtiv* (10 mg/kg, PO) in a cross-over fashion. Tidal volume, which is the amount of air (mL) inhaled per breath, increased when B6SJL-$SOD1^{G93A}$ mice were treated with *tirasemtiv*. (n=5/group). *p < 0.05.



## Conclusions

- Pathological conditions that lead to diaphragm weakness can have severe consequences, ranging from dyspnea and reduced quality of life to respiratory failure and death
- The fast skeletal tro-ponin activator *tirasemtiv* increased:
  - Rat diaphragm fiber $Ca^{2+}$ sensitivity in a concentration-dependent manner
  - Mouse diaphragm submaximal force production *ex vivo*
  - Tidal volume *in vivo* in a mouse model of ALS
- These results suggest that *tirasemtiv* and other fast skeletal muscle troponin activators may be viable therapeutics for improving respiratory muscle function

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