#### Titin's Role in Power Amplification and Force Re-Development in Stretch-Shortening Cycles

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#### **Summary**

Stretch-shortening cycles (SSCs) enhance muscular performance compared to pure shortening. This SSC effect is influenced by cross-bridge (XB) and non-cross-bridge (non-XB) structures like titin. To examine the mechanisms underlying the SSC effect, *in-vitro* experiments on rat soleus fibres tested different SSC velocities. Blebbistatin was used to distinguish XB and non-XB contributions. SSCs significantly enhanced and accelerated force re-development, compared to pure shortening contractions with power output increasing at higher velocities in both blebbistatin-treated and untreated fibres. Findings highlight titin's velocity-dependent role in SSC-induced force enhancement via viscoelastic and mechanosensory properties, emphasizing its role in elastic energy storage and release.

#### Introduction

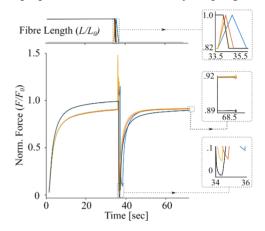
Stretch-shortening cycles (Fig. 1, coloured lines), involving eccentric followed by concentric muscle actions, enhance force, work, and power output, crucial in terrestrial locomotion [1]. The SSC effect has been observed across various scales, from muscle fibres to human movements [2], and is influenced by cross-bridge (XB) and non-XB structures like titin at the myofibrillar level [3, 4]. While recent studies highlight the role of titin in force generation, the detailed mechanisms, especially during force re-development post-SSC, remain unclear. This study hypothesizes that XB and non-XB structures contribute to muscle performance at varying SSC velocities, and that inhibition of XB interactions with blebbistatin will reveal the specific role of non-XB structures in force generation and energy storage.

## Methods

*In-vitro* experiments were conducted on single-skinned rat soleus muscle fibres (n=16) to examine the impact of varying SSC velocities (30%, 60%, and 85% of maximum contraction velocity) and constant stretch-shortening magnitudes (18% of optimum fibre length). The XB inhibitor blebbistatin (20 μmol l<sup>-1</sup>) was used to differentiate the roles of XB and non-XB structures in force generation. Results were analyzed via repeated measures ANOVA and Statistical Parametric Mapping to evaluate contraction conditions, treatment effects, and interactions.

### **Results and Discussion**

SSC-induced force re-development was significantly greater and faster than during pure shortening contractions in untreated and blebbistatin-treated fibres. Power output increased with SSC velocity, and energy return (elastic energy storage-to-release ratio) was higher in blebbistatin-treated fibres than in untreated ones, emphasizing titin's role in elastic energy storage and release. These results suggest that titin contributes to the velocity-dependent SSC effect through its viscoelastic properties and mechanosensory coupling.



**Figure 1**: Length (top) and force-time (bottom) traces of fibres during mechanical experiments (SSCs in colour, pure shortening in black). Fibre activation began at 0 secs, and SSCs started at 33.5 secs.

### **Conclusions**

The SSC effect is velocity-dependent, with titin playing a key role in force and power enhancement through elastic energy storage during eccentric contractions and energy return during shortening. These findings expand our understanding of the SSC effect, emphasizing the contribution of non-XB structures like titin in regulating force production [5]. This modulation likely involves intricate mechanosensory coupling, where stretch influences signal transmission during muscle contraction [6].

### Acknowledgments

This work was supported by the German Research Foundation DFG under project numbers 354863464 and 540349998, with partial funding through the DFG as part of the German Excellence Strategy - EXC 2075 (390740016).

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