Power Amplification Increases With Contraction Velocity During Stretch-Shortening Cycles of Skinned Muscle Fibers

André Tomalka1*, Sven Weidner1, Daniel Hahn2,3, Wolfgang Seiberl4 and Tobias Siebert1,5

1 Department of Motion and Exercise Science, University of Stuttgart, Stuttgart, Germany, 2 Human Movement Science, Faculty of Sports Science, Ruhr University Bochum, Bochum, Germany, 3 School of Human Movement and Nutrition Sciences, University of Queensland, Brisbane, QLD, Australia, 4 Human Movement Science, Bundeswehr University Munich, Neubiberg, Germany, 5 Stuttgart Center for Simulation Science, University of Stuttgart, Stuttgart, Germany

Muscle force, work, and power output during concentric contractions (active muscle shortening) are increased immediately following an eccentric contraction (active muscle lengthening). This increase in performance is known as the stretch-shortening cycle (SSC)-effect. Recent findings demonstrate that the SSC-effect is present in the sarcomere itself. More recently, it has been suggested that cross-bridge (XB) kinetics and non-cross-bridge (non-XB) structures (e.g., titin and nebulin) contribute to the SSC-effect. As XB and non-XB structures are characterized by a velocity dependence, we investigated the impact of stretch-shortening velocity on the SSC-effect. Accordingly, we performed in vitro isovelocity ramp experiments with varying ramp velocities (30, 60, and 85% of maximum contraction velocity for both stretch and shortening) and constant stretch-shortening magnitudes (17% of the optimum sarcomere length) using single skinned fibers of rat soleus muscles. The different contributions of XB and non-XB structures to force production were identified using the XB-inhibitor Blebbistatin. We show that (i) the SSC-effect is velocity-dependent—since the power output increases with increasing SSC-velocity. (ii) The energy recovery (ratio of elastic energy storage and release in the SSC) is higher in the Blebbistatin condition compared with the control condition. The stored and released energy in the Blebbistatin condition can be explained by the viscoelastic properties of the non-XB structure titin. Consequently, our experimental findings suggest that the energy stored in titin during the eccentric phase contributes to the SSC-effect in a velocity-dependent manner.

Keywords: contractile behavior, muscle stretch, muscle shortening, muscle damping, mechanical power, performance enhancement, eccentric contractions

Abbreviations: ATP, adenosine 5′ triphosphate disodium salt hydrate; CK, creatine phosphokinase; Ca2+, calcium; CP, creatine phosphate; E-64, trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid; F/F0, maximum isometric muscle force; F-l, force-length-relation; F-v, force-velocity-relation; rFD, residual force depression; rFE, residual force enhancement; GLH, glutathione; HDTA, 1,6-diaminohexane-N,N,N′,N′-tetraacetic acid; h, height; IMID, imidazole; KOH, potassium hydroxide; KP, potassium propionate; L/L0, optimum muscle fiber length associated with F/F0; L/S0, optimum sarcomere length associated with F/F0; L0, individual sarcomere length; non-XB, non-cross-bridge; P0, maximum power output; PMSF, phenylmethanesulfonyl fluoride; P-v, power-velocity-relation; SSC, stretch-shortening cycle; TES, Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; v/v, volume/volume; w/v, weight/volume; w, width; XB, cross-bridge.
INTRODUCTION

The most common form of muscle action during terrestrial locomotion is characterized by eccentric muscle action (active lengthening) immediately followed by concentric muscle action (active shortening). Such stretch-shortening cycles (SSCs) occur in both cyclical and non-cyclical locomotion at the level of the muscle-tendon unit (Komi, 2000; Ishikawa et al., 2005; Aeles and Vanwanseele, 2019; Navarro-Cruz et al., 2019) and the muscle fiber (Gillis and Biewener, 2001; Nikolaïdou et al., 2017). So far, a large number of experiments at the level of the muscle-tendon unit have shown an increase in muscle force, work, and power output during the shortening phase of SSCs compared with pure shortening contractions (Cavagna et al., 1968; Bosco et al., 1981; Gregor et al., 1988; Seiberl et al., 2015). This SSC-effect (i.e., increased muscular performance) is further associated with amplified muscular efficiency accompanied by reduced metabolic energy consumption (Cavagna et al., 1968; Holt et al., 2014). The underlying mechanisms of the SSC-effect that have been discussed in the literature include activation dynamics, contributions of stretch reflexes, storage and release of elastic energy, and history-dependent effects of muscle action associated with residual force enhancement (rFE) (Van Ingen Schenau et al., 1981; Gregor et al., 1988; Seiberl et al., 2015). Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomalka et al. (2020) further showed that both XB-inhibition, Tomala...
six freshly killed female Wistar rats (age: 3 months, weight: 428–520 g, cage-sedentary, 12–12 h light: dark cycle, housing-temperature: 22°C). The skeletal muscle fibers from rats used for this study have been provided by another animal study approved according to the German animal protection law [Tierschutzgesetz, §4 (3); Permit Number: 35-9185.81/0491]. The small bundles (50–100 fibers) were stored in a storage solution (see section “Solutions”) containing 50% glycerol at –20°C for 4–6 weeks. As previously described, single fibers were prepared before the experiment (Tomalka et al., 2020). The fibers were treated with a relaxing solution (see section “Solutions”) containing Triton X-100 (1% v/v) for 1–2 min at 4°C to chemically disrupt the muscle membranes without affecting the contractile apparatus (Fryer et al., 1995). Afterward, a fiber segment 0.8–1.2 mm long was cut from the fiber, and T-shaped aluminum clips were mounted at its extremities for attachment between the lever arms of a high-speed length controller (322 C-I, Aurora Scientific, Canada) and a force transducer (403a, Aurora Scientific, Canada) (Figure 1A). The two ends of the fiber were fixed with glutaraldehyde in rigor solution and glued to the clips with fingernail polish diluted with acetone (Burmeister Getz et al., 1998). The length (L), width (w), and height (h) of the fiber were measured at 0.1 mm intervals in the central segment of the relaxed fiber with a 10× dry-objective (NA 0.30, Nikon) and a 10× eyepiece. The individual sarcomere length ($L_S$) was set to 2.4 ± 0.05 µm, which is within the optimal sarcomere length ($L_{S0}$) range for maximal isometric force ($F_0$) development in the activated state ($p_{Ca} 4.5$) (Stephenson and Williams, 1982). The fiber cross-sectional area was determined assuming an elliptical cross-section of single muscle fibers ($\pi hw/4$) and was 4,844 ± 1,246 µm$^2$ (mean ± standard deviation). The $L_S$ was measured using a high-speed camera system (901B, Aurora Scientific, Canada) in combination with a 20× ELWD dry-objective (NA 0.40, Nikon) and an accessory lens (2.5×, Nikon).

**Experimental Protocol**

Stretch-shortening cycle experiments comprised two conditions of repeated measurements. The control condition (Figures 1B–D) was designed to investigate the dynamic total

---

**FIGURE 1** | Schematic diagram of the permeabilized fiber apparatus (A) and a diagram showing the control experiments’ protocol (B–D). (A) Close-up cut-away view of the eight-well bath plate (top right) showing three chambers filled with solutions and a single fiber mounted onto the hooks with T-clips (bottom right). (B) Representative force-time trace showing the changes in muscle force during an entire stretch-shortening cycle (SSC) with previous pre-activation (Pre-Act) and relaxation period (Rel) (n = 1, raw, unfiltered data). Enlarged views of the force response (C) and the change in sarcomere length (D) for an SSC with 85% maximum contraction velocity at supramaximal activation level (Act; $p_{Ca} = 4.5$). Each of these experiments consists of an isometric phase, then a ramp transient, and then an isometric phase. The force is shown in mN, and the sarcomere length is recorded at maximal activation and is shown in µm. All ramp experiments have been conducted at a constant stretch/shortening magnitude of 0.17 $L_S/L_{S0}$ on permeabilized single fiber segments from rat soleus muscles at 12°C experimental temperature. The vertical lines indicate the time for solution change. The arrow indicates the onset of activation.
force response during varying isovelocity SSCs. The experiments with the XB-inhibitor (Blebbistatin condition) repeated the control condition and suggested the contribution of non-XB elements to force production during isovelocity SSCs. Each SSC experiment consisted of an isometric phase, followed by a ramp phase (lengthening and shortening) and a final isometric phase (Figure 1B).

For the control condition, single skinned fibers (six rats, 14 fibers) were activated at \( \sim 2.0 \, \mu \text{m} \) \( L_S \) stretched to \( L_{S0} \) of \( \sim 2.4 \, \mu \text{m} \) [in the activated state (pCa 4.5)], and then immediately shortened to \( \sim 2.0 \, \mu \text{m} \) \( L_S \) with varying stretch-shortening velocities of 30, 60, and 85\(^\circ\) \( v_0 \) in a randomized order.

An identical protocol was repeated for the same skinned muscle fibers (five rats, 13 fibers), in the presence of 20 \( \mu \text{mol} \, \text{l}^{-1} \) Blebbistatin in all solutions (see section “Solutions”) to identify non-XB contributions to muscle force (Cornachione and Rassier, 2012; Shalabi et al., 2017; Tomalka et al., 2020). Blebbistatin is a photosensitive chemical that blocks the force-generating transition of the bound actomyosin complex from a weakly to a strongly bound state and causes myosin heads to bind to actin without exerting any isometric force (Iwamoto, 2018; Tomalka et al., 2020). Thus (a major population of) XBs remain in the pre-power-stroke state weakly attached to actin (Minozzo and Rassier, 2010; Rahman et al., 2018). Blebbistatin does not affect titin mobility (Shalabi et al., 2017).

Only the data of one muscle fiber were discarded due to insufficient generation capability by Blebbistatin (remaining active isometric force at \( L_{S0} > 20\% \, F_0 \)).

The average active isometric force at optimum sarcomere length \( L_{S0} \) was 0.29 ± 0.08 mN, while the mean optimum muscle fiber length was 0.77 ± 0.09 mm. The isometric force corresponds to relative average stress of 60.04 ± 9.49 kPa. The maximum shortening velocity of the skinned soleus muscle fibers from adult male Wistar rats was 0.46 ± 0.13 \( L_0 \, \text{s}^{-1} \) (n = 6), and the curvature factor of the force-velocity-relation was \( \text{curv} = 0.07 \pm 0.02 \). In separate experiments, the fiber specific \( v_0 \) was calculated based on our experimental data from six to eight isotonic contractions against forces in the range of 0.1 \( F_0 \) to 0.9 \( F_0 \) (two fibers each from two rats and one fiber each from two other rats).

To preserve the structural and mechanical properties in maximally activated fibers over a longer period and to reduce sarcomere inhomogeneities, the “cycling protocol” by Brenner (1983) was used. To ensure the structural and mechanical integrity of fibers in the experiments, the following criteria were applied to discard fibers: (1) isometric force in reference contractions was decreased by more than 10%; (2) aberrant behavior of force-traces, evidenced by artifacts, oscillations, or abrupt flattening was noted; and (3) lesions, ruptures, or fiber contortion were identified visually. For the determination of force degradation, isometric reference contractions at \( L_{S0} \) were performed before and after each ramp contraction. In the ramp experiments (control condition), the isometric force in successive activations decreased at an average rate of approximately 3.2% per activation. All experiments were conducted at a constant temperature of 12 ± 0.1°C. At this temperature, the fibers proved very stable and were able to withstand rapid ramp perturbations over an extended period as well as prolonged activations (Ranatunga, 1982, 1984; Bottinelli et al., 1996; Tomalka et al., 2017).

## Calculations of XB- and Non-XB Forces

To separate XB and non-XB forces during SSCs, the forces obtained during the Blebbistatin condition were subtracted from the forces obtained during the control condition. This rather simple method was used previously (Tomalka et al., 2017) and is based on the following assumptions: First, XBs produce a constant average force during isokinetic stretch after an initial equilibrium of XB-distributions (Huxley, 1957; Huxley and Simmons, 1971). Second, Blebbistatin suppresses the active XB-based forces to a negligible level. This was assessed by comparing the initial isometric force at \( 2.0 \, \mu \text{m} \) \( L_S \) with and without XB-inhibitors. Administering Blebbistatin suppressed active XB forces by 98%, so it can be expected that Blebbistatin suppresses active forces during SSCs to a similar extent. Third, is assumed that Blebbistatin does only affect the active XB-based force production during SSCs (see section “Isolated XB Forces During the SSC”).

## Solutions

The relaxing solution contained (in mM) 100 TES, 7.7 MgCl\(_2\), 5.44 Na\(_2\)ATP, 25 EGTA, 19.11 Na\(_2\)CP, and 10 GLH (pCa 9.0). The preactivating solution contained (in mM) 100 TES, 6.93 MgCl\(_2\), 5.45 Na\(_2\)ATP, 0.1 EGTA, 19.49 Na\(_2\)CP, 10 GLH, and 24.9 HDTA. The activating solution contained (in mM) 100 TES, 6.76 MgCl\(_2\), 5.46 Na\(_2\)ATP, 19.49 Na\(_2\)CP, 10 GLH, and 25 CaEGTA (pCa 4.5). The skimming solution contained (in mM) 170 potassium propionate, 2.5 MgCl\(_2\), 2.5 Na\(_2\)ATP, 5 EGTA, 10 IMID, and 0.2 PMSF. The storage solution is the same as the skimming solution, except for the presence of 10 mM GLH and 50% glycerol (v/v). Cysteine and cysteine/serine protease inhibitors [trans-epoxysuccinil-l-leucylamido-(4-guanidino) butane, E-64, 10 mM; leupeptin, 20 \( \mu \text{g} \, \text{ml}^{-1} \)] were added to all solutions to preserve lattice proteins and thus sarcomere homogeneity (Linari et al., 2007; Tomalka et al., 2017). pH (adjusted with KOH) was 7.1 at 12°C. 450 U ml\(^{-1}\) of CK was added to all solutions, except for skimming and storage solutions. CK was obtained from Roche (Mannheim, Germany), and Blebbistatin was obtained from Enzo Life Sciences Inc. (NY, United States). All other chemicals were obtained from Sigma (St. Louis, MO, United States).

## Data Processing and Statistics

Data were collected at 1 kHz with real-time software (600A, Aurora Scientific, Canada) and an A/D Interface (600A, Aurora Scientific, Canada). A custom-written MATLAB (MathWorks, Natick, MA, United States) script was utilized for data analysis. Unless stated otherwise, forces are expressed in absolute values (mN) or normalized to the individual maximum muscle force (\( F/F_0 \)). The shortening velocity is reported in relative units (\( \text{vel} \, L_0 = \text{s}^{-1} \)) or normalized to the fiber specific maximal shortening velocity (v/\( v_0 \)). Fiber lengths are expressed relative to the optimum fiber length (\( L_0 \)). Sarcomere lengths are expressed relative to the optimum sarcomere length (\( L_s/L_{S0} \)) or are reported in absolute values (\( \mu \text{m} \)). Mechanical work was calculated as the line integral of the changing force over the entire shortening.
distance during the SSCs and the pure shortening contractions and is expressed in relative values \((\frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}})\). The power output as a function of velocity \((P-v-r, \text{ orange solid line of Figure 2})\) was calculated based on the force-velocity-relation for shortening contractions \((F-v-r, \text{ blue solid line of Figure 2})\). Power is reported as relative values \((\frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}}) \cdot \frac{1}{\Delta t}\) or normalized to the maximal individual power \((P/P_0)\). The maximum mean power output \(P_0\) was 0.018 ± 0.005 \((\frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}}) \cdot \frac{1}{\Delta t}\) at 20% \(v_0\).

The power output of single skinned muscle fibers as a function of velocity calculated during isovelocity SSCs with varying velocities ranging from 30 to 85% \(v_0\) results from the work that was done within a particular time, delta [\(\Delta\)] t:

\[
\frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}} \cdot \frac{1}{\Delta t} = \frac{M}{M_{pool}}.
\]

To avoid ambiguity and misinterpretations of the terminology of “positive work” and “negative work” in the following text, they are defined as follows: positive work is performed when an active muscle shortens by internal forces. Negative work is performed when an active muscle lengthens by external forces (Cavagna et al., 1965).

All data are presented as mean ± standard deviation (SD) unless stated otherwise. To test whether the work (and power) significantly differs between the two conditions (control vs. Blebbistatin), a repeated-measures ANCOVA (factor 1 phase and covariate velocity) was used. Significant differences in the forces at the end of the stretch (control vs. Blebbistatin) were tested using a paired t-test. To test for differences in the energy recovery (defined as the ratio of elastic energy storage and release in the SSC; \(W_{con}/W_{ecc}\)) between both conditions, a paired t-test was used on data pooled across velocities in respective condition. To test whether the work and power output differ between the varying SSC velocities (30%, 60%, and 85% \(v_0\)), a two-way repeated-measures ANOVA (factor 1 velocity and factor 2 phase) was calculated. In case that the ANOVA demonstrated significant main effects, post hoc analyses were performed using the student’s t-test with Bonferroni correction. The statistical tests were likewise performed for the control and the Blebbistatin experiments and a comparison of both conditions. The level of significance was set at \(p < 0.05\). Statistical analyses were realized using SPSS 26 (IBM Corp., Armonk, NY, United States). The effect sizes of Cohen’s \(d\) were calculated as \(d = \frac{M_1-M_2}{S_{pool}}\), where \(M\) is the mean and \(S_{pool} = \sqrt{SD_1^2 + SD_2^2/2}\) (Cohen, 1988). The effect sizes were classified as small \((d = 0.2)\), medium \((d = 0.5)\), and large \((d = 0.8)\) (Cohen, 1988).

**RESULTS**

Significant differences between the control and the Blebbistatin condition \((p < 0.001)\) were observed for all parameters tested. Negative mechanical work during the stretch [control: \(-0.20 ± 0.01\) vs. Blebbistatin: \(-0.04 ± 0.01 \cdot \frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}}\)], positive mechanical work during shortening [control: \(0.03 ± 0.00\) vs. Blebbistatin: \(0.01 ± 0.00 \cdot \frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}}\)], negative power output during the stretch [control: \(-0.32 ± 0.13\) vs. Blebbistatin: \(0.07 ± 0.04 \cdot \frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}}\)], positive power output during shortening [control: \(0.04 ± 0.01\) vs. Blebbistatin: \(0.02 ± 0.01 \cdot \frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}}\)], and force at the end of the stretch were larger in the control condition (1.16 ± 0.20 \(F_0\)) compared with the Blebbistatin condition (0.42 ± 0.14 \(F_0\)). In contrast, energy recovery (\(W_{con}/W_{ecc}\)) was significantly lower in the control condition \((-0.15 ± 0.02\) compared with the Blebbistatin condition \((-0.25 ± 0.06)\).

**Control Condition: Effects of Velocity on Mechanical Work and Power Output**

The negative mechanical work during the stretching phase of the SSCs, when work is done on the muscle, increased significantly (+8.5 ± 2.5%, \(p < 0.001, d = 1.19, R^2 = 0.99)\) with increasing stretch velocity (Figure 3A and Table 1).

Negative work was significantly larger for fast \((-85%) \(v_0\) compared with moderate \((-60%) \(v_0\) stretching velocities (+4.1% ± 2.2%, \(p < 0.001, d = 0.61)\) yellow vs. red circles of Figure 3A) and for moderate compared with slow \((-30%) \(v_0\) stretching velocities (+4.3% ± 2.7%, \(p < 0.001, d = 0.63); red vs. blue circles of Figure 3A).

For the shortening phase of the SSCs, the positive mechanical work, when work is done by the muscle, decreased significantly

---

**FIGURE 2** | Representative force-velocity-relationship \((F-v-r)\) and power-velocity-relationship \((P-v-r)\) for soleus muscle. The blue solid line (and underlying individual data points) indicates the mean \(F-v-r\) for shortening contractions (positive velocities), while force declines as a function of shortening velocity (two fibers each from two rats and one fiber each from two other rats). The orange solid line indicates the mean \(P-v-r\) for shortening contractions. Power is maximum at intermediate velocities as relative values \((\frac{F}{F_0} \cdot \frac{\Delta L_S}{L_{50}}) \cdot \frac{1}{\Delta t}\) and is expressed in relative units \((\frac{M}{M_{pool}})\).
velocity. Power was significantly larger for moderate slow stretching velocities (+6.05; $p < 0.001$, $d = 1.19$, $R^2 = 0.96$) with increasing shortening velocity. Positive work was significantly smaller for moderate compared with slow stretching velocities ($-14.6\% \pm 4.2\%$, $p < 0.001$, $d = 1.28$; Figure 3B) and for fast compared with moderate shortening velocities ($-6.7\% \pm 5.6\%$, $p < 0.01$, $d = 0.51$; Figure 3B). The mean negative work output during muscle stretch was about seven times the amount of positive work during muscle shortening of SSCs (Table 1).

The negative power output increased successively with increasing stretch velocities ($+187.0\% \pm 6.7\%$, $p < 0.001$, $d = 12.88$, $R^2 = 0.99$) for the stretching phase of the SSC (Figure 3C). Negative power was significantly larger for fast compared with moderate stretching velocities ($+51.2\% \pm 3.2\%$, $p < 0.001$, $d = 6.05$; Figure 3C) and for moderate compared with slow stretching velocities ($+89.9\% \pm 4.9\%$, $p < 0.001$, $d = 9.12$; Figure 3C).

For the shortening phase of the SSCs, the positive power output increased significantly ($+110.7\% \pm 10.7\%$, $p < 0.001$, $d = 2.79$; Figure 3D) and for fast compared with moderate shortening velocities ($+35.7\% \pm 8.1\%$, $p < 0.001$, $d = 2.18$; Figure 3D).

The force-length traces of single muscle fibers are characterized by a steep rise in force in the early phase of active muscle stretching (red arrow in Figure 4), followed by a negative slope (up to about half the stretching time, Figure 4). Then the forces recovered relative to the initial drop in force to large parts toward the end of the stretch of the SSCs. The peak force during stretch increased with increasing stretch velocity ($+10.7\% \pm 4.1\%$, $p < 0.001$, $d = 2.49$). Furthermore, maximum forces were reached at longer sarcomere lengths with increasing stretching velocity ($+0.4\% \pm 0.2\%$, $p < 0.001$, $d = 3.00$; colored unfilled circles, Figure 4). During approximately 87% of the shortening phase, the highest forces were produced at the slowest shortening velocity (Figure 4, blue line).

**Blebbistatin Condition: Effects of XB-Inhibition on Work and Power as a Function of Velocity**

The XB-inhibitor Blebbistatin decreased the maximum isometric forces to 0.02 $F_0$ at 2.0 µm $L_s$ (Figure 5). Accordingly, eccentric and concentric forces during the SSCs in the Blebbistatin condition were decreased (Figure 5) in comparison to the control condition (Figure 4). Maximum forces were reached at the end of the stretch and increased with increasing stretching velocity (colored circles, Figure 5).

The negative work increased significantly with increasing velocities from slow to fast stretching velocities ($+48.8\% \pm 12.4\%$, $p < 0.001$, $d = 1.27$, $R^2 = 0.99$) during the stretching phase of the SSCs (cf. blue vs. yellow circles of Figure 6A and Table 2). Thus, negative work was significantly larger for fast compared with moderate velocities ($+17.0\% \pm 7.61\%$, $p < 0.01$, $d = 0.59$; yellow vs. red circles of Figure 6A) and for moderate compared with slow stretching velocities ($+27.6\% \pm 13.2\%$, $p < 0.001$, $d = 0.83$; red vs. blue circles of Figure 6A).

For the shortening phase of the SSCs, the mechanical work increased significantly with increasing velocity ($+70.7\% \pm 35.0\%$, $p < 0.001$, $d = 1.74$, $R^2 = 0.98$, Figure 6B). Thus, positive work was significantly larger for moderate compared with slow velocities ($+30.9\% \pm 24.1\%$, $p < 0.01$, $d = 0.89$, Figure 6B) and for fast compared with moderate shortening velocities ($+31.2\% \pm 18.4\%$, $p < 0.01$, $d = 1.02$). The overall negative work output during muscle stretch was about four times the amount of positive work during muscle shortening of SSCs.

The negative power output during muscle stretch of the SSCs increased significantly ($+293.5\% \pm 32.7\%$, $p < 0.001$, $d = 3.43$, $R^2 = 0.99$) with increasing stretching velocities (Figure 6C and Table 2). Negative power was significantly larger for fast compared with moderate stretching velocities ($+70.0\% \pm 11.1\%$, $p < 0.001$, $d = 1.78$, Figure 6C) and for moderate compared with slow stretching velocities ($+132.2\% \pm 24.0\%$, $p < 0.001$, $d = 2.79$; Figure 6C).

---

**FIGURE 3 | Control condition. Influence of varying stretch-shortening velocities on work and power during the lengthening phase (SSC\textsubscript{concentric}; negative work/power; A,C) and the shortening phase (SSC\textsubscript{ eccentric}; positive work/power; B,D) of SSCs, respectively. The gray dotted lines of the scatterplots shown in (A–D) indicate the individual paired data values, and solid gray lines indicate the mean values ($n = 14$ fibers from six rats). The blue circles indicate the SSC at ±30% $v_0$, the red circles at ±60% $v_0$, and the yellow circle at ±85% $v_0$. Work/power values are expressed in relative values (F/L vs. $v_0$) and $v_1$ vs. $v_0$ respectively. Asterisks mark differences in work/power with varying velocities in the intergroup comparison. Significance levels are marked as follows: **$p < 0.01$ and ***$p < 0.001$. The control condition refers to the experiments without XB-inhibition.**
For the shortening phase of the SSCs, positive power output increased significantly (+351.5% ± 92.7%, p < 0.001, d = 3.92, R² = 0.97) with increasing shortening velocity. Power was significantly larger for moderate compared with slow shortening velocities (+138.1% ± 43.9%, p < 0.001, d = 2.89; Figure 6D) and for fast compared with moderate shortening velocities (+90.7% ± 26.7%, p < 0.001, d = 2.24; Figure 6D).

**Contributions of ‘Isolated XB’ Force to Work and Power as a Function of Velocity**

To investigate the ‘isolated XB’ contributions to the total force response (Figure 7 and Table 3), Blebbistatin-suppressed forces (Figure 5) were subtracted from the total forces of control ramps (Figure 4) (see section “Calculations of XB- and Non-XB Forces”). Accordingly, differences in force, work, and power between control and Blebbistatin condition were referred to as ‘isolated XB’ forces, work, and power in the following.

‘Isolated XB’ forces reached at the end of the stretches decreased with increasing stretching velocity (colored circles, Figure 7). During the stretch phase of the SSCs (cf. blue vs. yellow circles of Figure 8A) negative work performed by ‘isolated XBs’ did not change with increasing velocities (+0.6% ± 3.9%, p = 1.00, d = 0.06, R² = 0.39).

For the shortening phase of the SSCs, positive work of ‘isolated XBs’ decreased significantly with increasing velocity (−45.9% ± 7.6%, p < 0.001, d = 4.00, R² = 0.99, Figure 8B). Thus, mechanical work of ‘isolated XBs’ was significantly smaller for moderate compared with slow velocities (−26.9% ± 5.0%, p < 0.01, d = 2.33, Figure 8B) and smaller for fast compared with moderate shortening velocities (−25.8% ± 9.6%, p < 0.01, d = 1.67).

The negative power output of ‘isolated XBs’ during muscle stretch of the SSCs increased significantly (+166.0% ± 10.4%, p < 0.001, d = 8.22, R² = 0.99) with increasing stretching velocities (Figure 8C). Negative power of ‘isolated XBs’ was significantly larger for fast compared with moderate stretching velocities (+46.5% ± 4.6%, p < 0.001, d = 3.88; Figure 8C) and larger for moderate compared with slow stretching velocities (+81.6% ± 7.0%, p < 0.001, d = 6.20; Figure 8C).

For the shortening phase of the SSCs, the positive power output of ‘isolated XBs’ increased significantly (+43.2% ± 20.1%, p < 0.001, d = 1.86, R² = 0.95) with increasing shortening velocity. Power was significantly larger for moderate compared with slow shortening velocities (+33.0% ± 9.1%, p < 0.001, d = 1.98; Figure 8D). However, there was no change in positive power output of ‘isolated XBs’ for moderate compared with fast shortening velocities (+7.8% ± 13.9%, p = 0.611, d = 0.53; Figure 8D).

**DISCUSSION**

In contrast to our hypothesis, the power output during the shortening phase of the SSCs increased almost linearly (Figure 2, orange symbols) with increasing stretch-shortening velocity. This increase is contrary to the typical parabolic shape of the P-v-r for the same range of shortening velocities (Figure 2, orange
Control Condition: Influence of Muscle Fiber Kinetics on Mechanical Work and Power Output

Muscle Shortening

During the shortening phase of the SSCs, the positive work decreased (by 20%) with increasing velocities from 30 to 85% $v_0$ (Figure 3B). The $F$-$v$-$r$ can partly explain this decrease in work since forces decrease with increasing shortening velocity (Figure 2, blue line). Lower forces produced over the same shortening distance will result in decreasing work with increasing velocity. However, since power is work per unit of time, this $\approx$20% decrease in work is overcompensated by reducing the duration of the shortening phase (by 65%) from 30 to 85% $v_0$. Thus, despite a decrease in work, the power output during the shortening phase significantly increased with increasing ramp velocities (Figure 3D).

Muscle Stretch

For all tested velocities in our study, fiber kinetics was characterized by a steep rise in force during the early phase of the stretch, immediately followed by a relatively compliant transient phase until the stretching has stopped. The initial linear phase (Figure 4, red arrow) is biphasic with a steep force slope followed by a more shallow slope ($P_2$ transition; Figure 4). This observation is consistent with recent investigations of stretch-induced force responses (5% $L_0$ stretch amplitude) in intact and skinned muscle fibers (mammalian and amphibian) and over a wide range of velocities (Lombardi and Piazzesi, 1990; Burmeister Getz et al., 1998; Linari et al., 2003; Pinniger et al., 2006; Tomalka et al., 2020). Both force slopes mainly arise from XB characteristics and can be attributed to the extension of all attached myosin heads to actin (Pinniger et al., 2006). Remarkably, we observed a significant rightward shift of the

solid line), which is based on the hyperbolic shape of the $F$-$v$-$r$ (Hill, 1938). Accordingly, our main result does not comply with the cross-bridge theories of muscle contraction based on the interaction of the contractile proteins actin and myosin (Huxley, 1957). Based on the results from our experiments with the XB-inhibitor Blebbistatin, we suggest that the elastic protein titin plays a significant role in power output during SSCs. In the following, we discuss potential mechanisms that explain the increased power output during muscle shortening of SSCs.
The P2 transition (peak force ranging from 1.3 to 1.45 \text{F}) increases with increasing stretching velocity (Edman et al., 1978; Sugi and Tsuchiya, 1988; Pinniger et al., 2006). Thus, elastic energy stored in viscoelastic structures, such as titin, and Tsuchiya, 1996; Pinniger et al., 2006; Roots et al., 2007). This amount includes the contribution of XB-elasticity (2.2% of total energy storage, Linari et al., 2000) and the redistribution of XB-states (\approx 9.8% of total energy storage, Linari et al., 2003), while XB is pulled into states of higher energy during stretching.

Accordingly, more than 80% of energy storage cannot be explained by XB mechanisms, particularly since attached XB detaches quickly from actin filaments (Huxley and Simmons, 1971), and their stored elastic energy is lost (Bosco et al., 1982; Wilson et al., 1991). Consequently, non-XB structures, such as titin, may store and release most of the elastic energy during the SSCs’ eccentric and concentric phases, respectively, thereby increasing power output during the shortening phase (section “Blebbistatin Condition: Influence of Non-XB Structures on Mechanical Work and Power Output”).

‘Isolated XB’ Forces During the SSC
Based on the assumption that muscle force during a stretch is the sum of XB- and non-XB forces (Nocella et al., 2014; Tomalka et al., 2017, 2020), and that a high proportion of XB-based forces is switched off in the presence of Blebbistatin, subtraction of forces in the Blebbistatin condition from the forces in the control condition leads to XB-forces (Figure 7).

Large parts of the stretch (\approx 2.1–2.4 \text{\mu m} L_0) show XB forces clearly below 1.0 F/F_0. This might be explained by muscle “give” since a fraction of XB is torn off due to initial stretch. After the initial peak, the XB force continuously decreases until the end of the stretch for each stretch velocity (unfilled colored circles, Figure 7). Interestingly, in the second half of the stretch (2.2–2.4 \text{\mu m} L_0), where an almost regular XB-cycling may be restored due to the constant stretching velocity, forces are lower (p < 0.001) for highest stretch velocity (85% v_0, yellow line, Figure 7) compared with the slowest stretch velocity (30% v_0, blue dashed line, Figure 7). This contrasts with our typical understanding of the eccentric F-v-r, where forces increase with increasing negative velocities and plateau at a certain threshold (Joyce et al., 1969; Haeufle et al., 2014). One possible reason why this behavior is not yet mentioned in the literature might be that the decreasing XB contribution with increasing stretch velocity is overcompensated by an increasing non-XB contribution (Figure 5) to generate enhanced muscle fiber force as found in the control condition (Figure 4, second half of the stretch). However, this reasoning requires the basic assumption that Blebbistatin completely inhibits XB contributions to force production, which is further discussed below.

In the SSCs’ shortening phase, and in line with the F-v-r for shortening contractions (Hill, 1938), higher forces were produced at lower shortening velocity (30% v_0, Figure 7, blue dashed line) compared with higher shortening velocity (85% v_0, Figure 7, yellow dashed line). Consequently, the ‘isolated XB’-based work decreased with increasing shortening velocity.
TABLE 2 | Descriptive statistics and pairwise comparisons of work and power values obtained during the Blebbistatin experiments.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Mean differences</th>
<th>SD</th>
<th>95% Confidence interval of the difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$W_{\text{ecc}}$ 30% $v_0$</td>
<td>−0.032</td>
<td>0.010</td>
<td>−0.008*</td>
<td>0.003</td>
<td>0.005 − 0.012</td>
</tr>
<tr>
<td>$W_{\text{ecc}}$ 60% $v_0$</td>
<td>−0.041</td>
<td>0.010</td>
<td>−0.007*</td>
<td>0.005</td>
<td>0.003 − 0.012</td>
</tr>
<tr>
<td>$W_{\text{ecc}}$ 85% $v_0$</td>
<td>−0.048</td>
<td>0.014</td>
<td>−0.016*</td>
<td>0.006</td>
<td>0.010 − 0.021</td>
</tr>
<tr>
<td>$W_{\text{con}}$ 30% $v_0$</td>
<td>0.007</td>
<td>0.002</td>
<td>0.002*</td>
<td>0.001</td>
<td>−0.004 − 0.001</td>
</tr>
<tr>
<td>$W_{\text{con}}$ 60% $v_0$</td>
<td>0.010</td>
<td>0.002</td>
<td>0.003*</td>
<td>0.002</td>
<td>−0.005 − 0.001</td>
</tr>
<tr>
<td>$W_{\text{con}}$ 85% $v_0$</td>
<td>0.012</td>
<td>0.003</td>
<td>0.005*</td>
<td>0.002</td>
<td>−0.007 − 0.003</td>
</tr>
</tbody>
</table>

**Power Output**: The power output ($P_{\text{power}}$) was calculated as $P_{\text{power}} = F \cdot \frac{\Delta L}{\Delta t}$, where $F$ is force, $\Delta L$ is displacement, and $\Delta t$ is time. The mean difference in power was calculated as $P_{\text{mean}} = \frac{P_{\text{power}}}{\Delta t}$ for each condition.

**Work Output**: The work output ($W_{\text{work}}$) was calculated as $W_{\text{work}} = F \cdot \Delta L$. The mean difference in work was calculated as $W_{\text{mean}} = \frac{W_{\text{work}}}{\Delta t}$ for each condition.

---

velocity (Figure 8B). However, the power produced by XB increases with increasing shortening velocities (Figure 8D). This increase in power output can be explained by calculated XB forces (Figure 2, gray symbols) slightly above the $F-v$-r (Figure 2, blue line). Multiplication of these slightly higher forces with the respective shortening velocity results in the observed XB-based power output ($P = F \times v$) (Figure 8D). One possible explanation for the slightly enhanced XB forces (Figure 2, gray symbols) might be that too little non-XB forces were subtracted from control forces. Titin–actin interaction might be enabled (at least partially) by strong XB-binding (Leonard and Herzog, 2010; Powers et al., 2014; Tomalka et al., 2020). A reduced amount of strong XBs in the Blebbistatin condition might reduce non-XB-based forces and, thus, explains the slightly overestimated XB forces (Figure 2, gray symbols).

However, it cannot be taken for granted that Blebbistatin completely eliminates XB-based force production, since Blebbistatin [and similar drugs as butanedione monoxime (BDM) (Rassier and Herzog, 2004; Rassier, 2008) and benzyltoluene sulfonamide (BTS) (Roots et al., 2007)] seems to affect the contractile apparatus in a complex manner (Minozzo and Rassier, 2010; Månsson et al., 2015). There are indications that Blebbistatin leads, among other things, to a considerable reduction of $v_0$ under certain conditions (Stewart et al., 2009; Rahman et al., 2018). Furthermore, with Blebbistatin the relative force enhancement increases during the ramp stretch of 3–5% $L_{50}$ (Pinniger et al., 2006; Minozzo and Rassier, 2010). An effect that is explainable by the potential influence of an increased population of weakly bound XBs, which are suggested to contribute to an increase in stiffness and non-XB-based force while strained during muscle stretch (Pinniger et al., 2006; Rassier, 2008; Minozzo and Rassier, 2010; Rahman et al., 2018).
Regardless of the effect of Blebbistatin on the contractile apparatus, a contribution of weakly bound XBs to force during the stretch (Rassier, 2008; Minozzo and Rassier, 2010) seems to be likely only for small stretch amplitudes (>1.5% L0) (see section "Muscle Stretch"). For rather extensive ramp amplitudes of 17% L0, as used in this study, weakly bound XBs rapidly detach (Schoenberg, 1985; Bagni et al., 2002). Thus, it seems unlikely that weakly bound XBs are primarily responsible for the observed significant peak forces at the end of the lengthening phase of the SSCs.

However, our approach does not clearly separate XB- and non-XB contributions. Accordingly, other inhibitors such as BTS or alternative approaches like the depletion of non-XB structures (e.g., selective digestion of titin by trypsin; Higuchi, 1992) should be considered for further studies attempting to separate XB- and non-XB contributions (Iwamoto, 2018; Ma et al., 2018).

**Blebbistatin Condition: Influence of Non-XB Structures on Mechanical Work and Power Output**

Linari et al. (2003) observed an increase in energy storage with increasing stretch velocity in frog muscle fibers and suggested that non-XB structures may be responsible for this observation. Recently, there is increasing support that contributions of non-XB structures, such as titin, are at least partially responsible for the observed SSC-effect on the muscle fiber level (Fukutani et al., 2017b; Fukutani and Herzog, 2019, 2020b; Tomalka et al., 2020). It is essential to separate XB and non-XB contributions to total muscle force to approach the physiological mechanisms. Our Blebbistatin experiments, which suppress XB contributions, confirm these recent observations on the importance of non-XB structures for the stretch phase of SSCs and point to a velocity dependence of the SSC-effect in the control condition.

In the presence of Blebbistatin, when actin–myosin cycling is negligible, we found a quasi-linear force response during the SSCs’ stretch phase (Figure 5) with no muscle “give” upon active stretching (cf. section "Muscle Stretch"). Therefore, the increasing forces with increasing stretch velocities (Figure 5) point to higher loading of non-XB structures. This increased loading of titin or other non-XB structures with increasing stretch velocity contributes to increased energy storage during the stretch phase of SSCs, associated with amplified negative work and power (Linari et al., 2003; Pinniger et al., 2006; Tomalka et al., 2017).

Consequently, when a spring recoils, stored elastic energy is recovered in the shortening phase of SSCs and thus contributes to the observed performance amplification (Figures 3, 6), which was also suggested by Lindstedt (2016) and Hessel et al. (2017). However, significantly more work is done on the muscle upon active muscle stretching (negative work) than work generated by the muscle during the shortening phase of SSCs (positive work), typical for viscoelastic materials. Viscoelastic titin behavior during SSCs has been reported in previous studies (Bianco et al., 2007; Chung et al., 2011; Herzog et al., 2014) and may be attributed to titin’s mecano-structural properties. Thus, at low stretch velocities, temporary energy storage in viscoelastic elements leads to a significant reduction of muscle fiber’s negative power (Figures 3C, 6C), which agrees with Roberts and Azizi (2010).

Previous work showed that maximum eccentric forces in the XB-inhibited conditions are enhanced by 11-fold than passive experiments (without Blebbistatin and calcium) at 85% v0 (Tomalka et al., 2020). This enhancement is partly attributed to

---

**Table 3**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Lower</th>
<th>Upper</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>W50 30% v0</td>
<td>0.163</td>
<td>0.015</td>
<td>--</td>
<td>--</td>
<td>0.007</td>
</tr>
<tr>
<td>W50 60% v0</td>
<td>0.165</td>
<td>0.014</td>
<td>--</td>
<td>--</td>
<td>0.003</td>
</tr>
<tr>
<td>W50 85% v0</td>
<td>0.162</td>
<td>0.017</td>
<td>--</td>
<td>--</td>
<td>0.002</td>
</tr>
<tr>
<td>W60 30% v0</td>
<td>0.026</td>
<td>0.003</td>
<td>--</td>
<td>--</td>
<td>0.012</td>
</tr>
<tr>
<td>W60 60% v0</td>
<td>0.019</td>
<td>0.003</td>
<td>--</td>
<td>--</td>
<td>0.005</td>
</tr>
<tr>
<td>W60 85% v0</td>
<td>0.014</td>
<td>0.003</td>
<td>--</td>
<td>--</td>
<td>0.007</td>
</tr>
<tr>
<td>W50 30% v0</td>
<td>0.012*</td>
<td>0.001</td>
<td>--</td>
<td>--</td>
<td>0.010</td>
</tr>
</tbody>
</table>

XB contributions were calculated by subtraction of forces obtained during Blebbistatin condition from forces during control condition (section "Calculations of XB- and Non-XB Forces"). Work/power values ± SD are expressed in relative values (P_S · S0 · F · c · min⁻¹) and (P_S · S0 · F · c · 1 s⁻¹), respectively. W, mechanical work; P, power output; ecc, eccentric phase during active muscle stretch; con, concentric phase during active muscle shortening; %v0, percentage of maximum contraction velocity; ns, not significant. *The mean difference is significant at the 0.05 level.
Energy Recovery

The energy recovery ($W_{con}/W_{ecc}$; calculated by dividing the work output obtained during the shortening phase of the SSC by the work done during the lengthening phase) significantly decreases in the control condition with increasing velocity (from 17.4% at 30% $v_0$ to 12.8% at 85% $v_0$). This decrease is partly due to the XB’s dissipative properties, as dissipation increases with velocity. Thus, inhibiting XB-cycling by Blebbistatin leads to an increase in energy recovery. The ratio ($W_{con}/W_{ecc}$) is higher in the Blebbistatin condition compared with the control experiments. Interestingly, in the Blebbistatin condition, the energy recovery ($W_{con}/W_{ecc}$) tends to increase slightly with increasing velocity (from 23.9% at 30% $v_0$ to 26.4% at 85% $v_0$, ns). This increase suggests that titin can be mechanically understood as a spring in series with a damper (Millard et al., 2019; Brunello and Fusi, 2020; Powers et al., 2020). For such a spring-damper system, at a higher velocity, due to the serial damper, the spring is stretched with higher force (and can store more energy in the spring element) and thus release more energy when it is shortened. Although the force at the end of the stretch in the Blebbistatin condition is only about a third of the force in the control experiments (cf. Figure 4 vs. Figure 5), the work during the XB-inhibited shortening phase of SSCs (at 85% $v_0$) reached nearly 50% of the work reported in the control condition. This finding implies a comparatively high energy recovery by titin.

Implications for in vivo Muscle Action

Despite clear evidence of the SSC-effect across all structural muscle levels (for a recent review, see Groeber et al., 2019), the contraction modalities (such as, e.g., the stretch-shortening amplitude and contraction velocity) might have important implications on experimental findings of comparable studies in the literature. In general, there are conflicting results regarding the occurrence of active SSCs in the muscle fascicles themselves during in vivo human movements. Depending on movement tasks studied, SSCs without (Cronin and Finni, 2013; Lai et al., 2015; Aeles and Vanwanseele, 2019) and with fascicle stretch (Ishikawa et al., 2005; Rubenson et al., 2012; Nikolaidou et al., 2017) have been reported. To date, no mechanism exclusively explains the SSC-effect and there likely is an interaction of mechanisms at different structural levels, with a dominance, e.g., depending on involved muscle (group) or movement dynamics. The transfer of experimental in vitro findings on in vivo SSC-effects should be considered with caution; however, the existence of an SSC-effect on the fiber level cannot be neglected. Since many (cyclical) everyday movements occur at submaximal muscle activation levels (Groeber et al., 2019), future studies should be done in skinned muscle fibers at different calcium concentrations to better understand the meaning of the SSC-effect in in vivo situations.

CONCLUSION

In the present study, we found work and power amplification in the shortening phase of SSCs in control- and Blebbistatin conditions. Interestingly, (i) this SSC-effect is velocity-dependent...
since the power output increases with increasing velocity. (ii) The energy recovery (ratio of elastic energy storage and release in the SSC) is higher in the Blebbistatin condition compared with the control condition. This amplified energy recovery in the Blebbistatin condition can be explained by the viscoelastic properties of the non-XB structure titin.

This SSC-effect study promotes a basic understanding of human locomotion since SCCs are part of the most basic, everyday-type of muscle contraction. The separation of XB- and non-XB structures is of primary importance to give a more detailed understanding of the potential involvement of viscoelastic elements, such as titin, working as an energy-storing spring during lengthening contractions and SCCs. This information is required for the improvement of muscle models (Heidlauf et al., 2016; Tahir et al., 2018; Seydewitz et al., 2019) as well as for improved predictions by multi-body models (Röhrle et al., 2017; Haeufle et al., 2020) concerning, e.g., movement control and efficiency of locomotion.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The authors would like to thank Annika Klotz for technical help with Figure 1A.

REFERENCES


Cornachione, A. S., and Bassier, D. E. (2012). A non-cross-bridge, static tension is present in permeabilized skeletal muscle fibers after active force inhibition approved according to the regulations of the German Animal Protection Law (Tierschutzgesetz, §4 (3); Permit Number: 35-9185.81/0491) by the Regierungspräsidium Stuttgart, Department of Landwirtschaft, Ländlicher Raum, Veterinär- und Lebensmittelwesen.


Linnar, M., Caremari, M., Peverio, C., Brandt, P., and Lombardi, V. (2007). Stiffness and fraction of Myosin motors responsible for active force in permeabilized...


Tomalka, A., Rode, C., Schumacher, J., and Siebert, T. (2017). The active force–length relationship is invisible during extensive eccentric contractions in...
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Copyright © 2021 Tomalka, Weidner, Hahn, Seiberl and Siebert. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.