Effects of fiber type on force depression after active shortening in skeletal muscle

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Abstract

The aim of this study was to investigate force depression in Type I and Type II muscle fibers. Experiments were performed using skinned fibers from rabbit soleus and psoas muscles. Force depression was quantified after active fiber shortening from an average sarcomere length (SL) of 3.2μm to an average SL of 2.6μm at an absolute speed of 0.115 fiber length/s and at a relative speed corresponding to 17% of the unloaded shortening velocity (V_0) in each type of fibers. Force decay and mechanical work during shortening were also compared between fiber types. After mechanical testing, each fiber was subjected to myosin heavy chain (MHC) analysis in order to confirm its Type (Type I expressing MHC I, and Type II expressing MHC IId). Type II fibers showed greater steady-state force depression after active shortening at a speed of 0.115 fiber length/s than Type I fibers (14.5±1.5% versus 7.8±1.7%). Moreover, at this absolute shortening speed, Type I fibers showed a significantly greater rate of force decay during shortening and produced less mechanical work than Type II fibers. When active shortening was performed at the same relative speed (17% V_0), the difference in force depression between fiber types was abolished. These results suggest that no intrinsic differences were at the origin of the difference in force depression observed in Type I and Type II fibers when actively shortened at the same speed, but rather their distinct force-velocity relationships.

Introduction

The steady-state isometric force after active shortening of skeletal muscle is smaller than the purely isometric force at the same level of activation and the corresponding length. This phenomenon, referred to as force depression, was first described by Abbott and Aubert in 1952 and has been observed consistently in whole and isolated muscle preparations (Rassier and Herzog, 2004). The molecular mechanisms causing force depression remain unknown, but two primary mechanisms have been suggested. First, force depression has been associated with the development of sarcomere length non-uniformities upon active shortening (Morgan et al., 2000). According to this theory, force depression is caused by the development of large dispersions in sarcomere lengths during active shortening. Second, force depression has been associated with a stress-induced inhibition of cross bridges in the newly formed actin-myosin overlap zone following shortening owing to actin angular deformation (Marechal and Plaghki, 1979). According to this theory, active shortening causes a decrease in the probability of cross bridge attachment, thereby leading to a decrease in the proportion of force generating (strongly bound) cross bridges in the force depressed state at the same muscle length and overlap of thick and thin filaments compared to a purely isometric contraction.

Despite an abundance of information regarding the characteristics and mechanisms of force depression, there has been no investigation comparing force depression among skeletal muscle fiber types. Fast (Type II) and slow (Type I) muscle fibers have distinctly different force-velocity properties (Bottinelli et al., 1991), and thus force and work for shortening at a given speed differs substantially between fiber types. It has been shown that the magnitude of force depression is directly related to force and work performed during shortening (Corr and Herzog, 2005; Dargeviciute et al., 2013; Leonard and Herzog, 2005; Minozzo and Rassier, 2013).

The aim of the present study was to investigate force depression in Type I and Type II muscle fibers. Based on the acknowledged differences in force-velocity properties and transient forces between fiber types (Bottinelli et al., 1991), we hypothesized that force depression following active shortening at a given absolute speed is greater in Type II than Type I fibers but would be similar between fiber types for similar relative speed of shortening.

Materials and methods

New Zealand white rabbits were euthanized according to a protocol approved by the University of Calgary's Animal Care and Ethics Committee. Strips of soleus and psoas muscles were skinned and fibers were isolated and mounted between a force transducer and a length controller as previously described (Joumaa and Herzog, 2013; Toursel et al., 2000). SLs were measured using optical diffraction of a He-Ne laser beam. Fiber cross sectional area and volume were calculated assuming the fiber has a cylindrical shape. Experiments were performed at 15°C.

Maximal stress at an average SL of 2.6μm

Soleus (n=15) and psoas (n=22) fibers were activated at an average SL of 2.6μm in order to measure their maximal force at this length. Force was normalized to the fiber cross sectional area to obtain the maximal stress.

Active shortening contractions at a shortening speed of 0.115 fiber length/s

Soleus (n=15) and psoas (n=15) fibers were set at an average SL of 2.6μm and then passively stretched to an average SL of 3.2μm, held for 20 seconds and activated by changing the relaxing solution to a high calcium activating solution (Joumaa and Herzog, 2013). Fibers were then actively shortened to an average SL of 2.6μm at a speed of 0.115 fiber length/s, held isometrically until steady-state isometric force was reached, and then deactivated (Figure 1). After a rest period of 5 minutes, fibers were activated at an average SL of 2.6μm in order to measure their purely isometric reference force.

Active shortening contractions at the same relative speed between Type I and Type II fibers

In order to perform active shortening at the same relative speed between Type I and Type II fibers, we measured the unloaded shortening velocity (V_0) for psoas (n=5) and soleus (n=5) fibers using the slack test method proposed by Edman (1979). Then we tested an additional group of psoas fibers in which we performed active shortening at the same relative speed (%V_0) as in soleus fibers. The unloaded shortening velocities were (mean±SEM) 0.67±0.09 fiber length/s and 1.77±0.03 fiber length/s in soleus and psoas fibers, respectively. The initial active shortening experiments were performed at a shortening speed of 0.115 fiber length/s corresponding to a relative shortening speed of 17% of V_0 in Type I fibers. Thus, seven additional psoas fibers were actively shortened at a relative speed of 17% of their V_0 corresponding to 0.3 fiber length/s.

Stiffness measurements

Fiber stiffness was obtained using a quick stretch-release protocol of 0.2% fiber length (Ford et al., 1981; Joumaa et al., 2012; Mansson, 1989; Rassier and Herzog, 2005) at a speed of 1 fiber length/s. Stiffness was measured once the isometric steady-state had been reached after active shortening, and during the purely isometric reference contraction.

MHC content

The MHC content was determined in each fiber after mechanical testing using SDS-PAGE gel electrophoresis on 4.5% and 7.5% acrylamide stacking and separating gels respectively (Toursel et al., 2000). The gels were run in a Biorad Mini-Protean III unit at a constant voltage of 73V for 40 hours at 4°C, stained with Coomassie Blue and scanned. Following electrophoresis, fibers were divided into two groups: Type I fibers expressing MHC I and Type II fibers expressing MHC IId.

Data analysis

Force depression: Force depression was determined as the percent difference between the steady-state isometric force following active shortening and the purely isometric reference force at 2.6μm average SL.

Stiffness (instantaneous stiffness): Stiffness was measured as the difference between the peak force reached after the quick stretch and the force immediately before the stretch divided by the amplitude of the stretch.

Rate of force decline during shortening: Force during shortening is characterized by a steep initial phase and a slow final phase. The initial and final phases were fitted by two linear least-squares regression functions. The slope of the initial steep phase of shortening, S1, was taken as an estimate of the rate of force decline during shortening. The slope of the final slow phase of shortening, S2, was taken as an estimate of the behaviour of the non-contractile (passive) element during shortening (Roots et al., 2007). S1 and S2 were normalized to the maximal isometric fiber force and compared between Type I and Type II fibers.

Mechanical work: Mechanical work during shortening was calculated by trapezoidal numerical integration of the force-length curve during the shortening phase. To compare work performed between fibers, the mechanical work was normalized to fiber volume.

Statistical analysis: All data reported are means±SEM. The Student's *t test* ($p<0.05$) was used to compare data between Type I and Type II fibers.

Results

Fibers were analysed for MHC composition (Figure 2). Thirteen fibers isolated from the soleus muscle and tested for force depression expressed MHC I and were grouped as Type I fibers. The remaining two soleus fibers expressed MHC IIa and were excluded from the analysis. Three psoas fibers were excluded from analysis because they co-expressed MHCs IId/IIb. The remaining psoas fibers tested for force depression (n=12 shortened at a speed of 0.115 fiber length/s, n=7 shortened at a relative speed of 17% V_0) expressed MHC IId and were labelled Type II fibers and analyzed.

The maximal stress at an average SL of 2.6μm for Type I fibers (151±14 kN/m^2) was not different from that of Type II fibers (159±13 kN/m^2).

Active shortening contractions at a shortening speed of 0.115 fiber length/s

Force depression in Type II fibers was higher than that of Type I fibers (14.5±1.5**%** versus 7.8±1.7**%**) when fibers were shortened at the same absolute speed of 0.115 fiber length/s. Type I fibers showed a steeper decay in force during shortening (Table 1, Figure 3), performed less mechanical work during shortening and showed smaller S2 than Type II fibers (Table 1).

Active shortening contractions at the same relative speed between Type I and Type II fibers

When Type II fibers were shortened at the same relative speed as Type I fibers (17% V_0), the difference in force depression between fiber types was abolished (4.1±1.8% in Type II versus 7.8±1.7% in Type I). Furthermore, the mechanical work and S1 were not different between fiber types (Table 1), but S2 was still smaller in Type I fibers compared to Type II

Force depression was correlated with stiffness depression (R^2=0.6, $p<0.05$, Figure 4) and S1 (R^2=0.4, $p<0.05$, Figure 5) across all fibers.

Discussion

Our main finding in this study is that Type II fibers when actively shortened at the same absolute speed showed greater isometric steady-state force depression after active shortening compared to Type I fibers. However, the difference in force depression between fiber types was abolished when active shortening was performed at the same relative speed suggesting that no intrinsic differences were at the origin of the different force depression in Type I and Type II fibers, but rather their different force-velocity relationships.

The force-velocity relationship is markedly different between muscle fiber types (Bottinelli et al., 1991). Type I fibers have lower maximal shortening velocities (V_{max}) and a greater curvature of their force-velocity relationship compared to Type II fibers. When active shortening in the present study was performed at the same absolute speed (relative to fiber length) in Type I and Type II fibers, Type I fibers were shortening at a higher speed relative to their V_{max}, and thus produced less force during shortening than Type II fibers. A decreased force over the given displacement results in a lower mechanical work performed during shortening in Type I fibers as compared with Type II fibers. The increased rate of force decay in conjunction with the decreased mechanical work performed during shortening observed for Type I fibers provide quantitative support for the rationale that force and work during shortening are reduced in Type I compared with Type II fibers owing to their intrinsic force-velocity properties.

It has been found that force during the active shortening phase is highly correlated with the steady-state isometric force reached after shortening (Dargeviciute et al., 2013; Leonard and Herzog, 2005). Leonard and Herzog (2005) showed that force depression was the same when force during shortening was kept constant, even though the speed of shortening was changed substantially. Conversely, they found that force depression varied greatly when force during

shortening was altered while shortening speed was kept constant (Herzog and Leonard, 1997). Furthermore, since force depression is associated with the magnitude of shortening and force during shortening, it has been demonstrated that force depression is directly proportional to the mechanical work performed during shortening (Dargeviciute et al., 2013; Herzog and Leonard, 1997; Leonard and Herzog, 2005). Therefore, the lower force along with the decreased mechanical work performed by Type I compared with Type II fibers explains the decreased force depression in Type I compared to Type II fibers.

It has been hypothesized that since thin and thick filaments are slightly compliant (Goldman and Huxley, 1994; Kojima et al., 1994), when muscle is activated, mechanical stress causes a small elongation of the thin filament, possibly resulting in an angular distortion of the myosin binding sites on actin. This distortion may cause a decrease in the attachment rate of cross bridges and thus an inhibition of their formation in the newly formed overlap zone when muscle is then shortened and the stress in thin filaments is maintained. A decreased speed of shortening is associated with increased force, thus a potential increase in the deformation of actin and increased force depression. According to Marechal and Plaghki (1979), force depression "is precisely controlled by the mechanical parameters of the release", when the load - tetanic stress – is light, cross bridges are slightly inhibited and when the load is heavy, cross bridges are partially or completely inhibited. The relationship between force depression and stiffness depression found here (Figure 4) and by others (Minozzo and Rassier, 2013; Rassier and Herzog, 2004) supports the stress induced inhibition of cross bridge theory.

The sarcomere length non-uniformity theory could potentially explain the difference in force depression between fiber types when actively shortened at the same absolute speed. Because sarcomeres are assumed to be unstable on the descending limb of the force length relationship

(Hill, 1953) regardless of the fiber type, we suggest that sarcomere length non-uniformities develop in Type I and Type II fibers. To our knowledge, the amount of sarcomere length non-uniformities has not been compared between fast and slow fibers. However, if we assume that the development of sarcomere length non-uniformities increases during length changes when force is high, we might expect greater non-uniformities in sarcomere lengths in fast compared to slow fibers and thus a greater amount of force depression when shortening at the same absolute speed. However, experiments in single myofibrils and sarcomeres have shown that the development of sarcomere length non-uniformities is likely not the mechanism for force depression (Joumaa and Herzog, 2010; Trecarten et al., 2015).

Passive properties are different between Type I and type II fibers. Rabbit psoas muscle has shorter titin isoforms (two isoforms of molecular weights of ~ 3.3 and 3.4 MDa) compared to titin isoform in the rabbit soleus muscle (one titin isoform of a molecular weight of ~ 3.6 MDa) (Prado et al., 2005). Could this difference in titin isoforms be responsible for the difference in force depression observed at an average sarcomere length of 2.6 μm between Type I and Type II fibers? According to the primary mechanisms of force depression (i.e. the stress induced inhibition of cross bridges and the non-uniformity theory), passive force is not involved in force depression. However, Leonard and Herzog (2010) proposed a mechanism for residual force enhancement based on the interaction between (binding of) titin and actin upon muscle/fiber/myofibril activation. When the myofibril is stretched, its passive force is increased because the strain on titin is higher compared to a purely isometric contraction. Could titin's role in force depression mirror that of force enhancement? Indeed, Rode et al. (2009) suggested, based on a theoretical model using Hill-type muscle and incorporating titin as a "sticky spring", that titin might play a major role in force depression. Rode et al. (Rode et al., 2009) proposed

that titin attaches to the actin filament upon muscle activation. As a result, the length of titin's free spring length is dramatically reduced. This leads to an increased passive force when the sarcomere is stretched and a decreased passive force when the sarcomere is shortened. Based on this model, one might suggest that the shorter the titin isoform, the higher the reduction in force at steady-state following sarcomere shortening. Although many aspects of the "sticky-spring" mechanism should be carefully considered (i.e. the (non-) selective binding of titin to actin in long and short isoforms, the region of titin that binds to actin, the behaviour of titin during shortening, etc.), this mechanism could potentially explain the greater force depression observed in psoas Type II fibers compared to soleus Type I fibers. Titin contributions to force depression have also been proposed by Nishikawa et al. (2012) who suggested that titin might be responsible for force depression by a mechanism of winding and unwinding of titin onto the rotating actin filament. According to Roots et al. (2007), the contribution of the non-contractile (passive) element to force during shortening can be determined by S2 phase during shortening. S2 in Type I fibers was lower than that in Type II fibers suggesting that the passive element behaviour is different during shortening between fiber types. Passive force decreased at a higher rate during shortening in the Type II compared to Type I fibers. This finding agrees with the "sticky-spring" mechanism (Rode et al., 2009) for force depression in that short titin isoforms would be predicted to show increased rates of force reduction during shortening compared to long titin isoforms. Therefore, the diversity in titin isoforms between fiber types could potentially account for their differences in force depression.

In addition to their diversity in myosin heavy chain and titin isoforms, skeletal muscle fiber types also show numerous differences in protein structures (actin, troponin, tropomyosin, etc.) and functional properties (ATPase hydrolysis rate, cross bridge cycling rate, etc.). Therefore, it is not

clear whether force depression is independently related to structural differences between fiber types, or if fiber types show different force depressions exclusively because of the difference in their force-velocity relationships. In our tests at a fixed absolute speed of shortening, we found that force depression was greater in Type II compared to Type I fibers. Shortening at the same absolute speed resulted in Type I fibers shortening at a higher speed relative to their V_{max} and correspondingly a lower force than Type II fibers. In order to test if the difference in force depression between Type I and Type II fibers was an intrinsic property of fiber types, we determined the unloaded shortening velocity for each fiber type and force depression was then determined for conditions of the same relative speed of shortening for both fiber types. When the shortening speed relative to V_0 was the same for the two fiber types, the differences in force depression were abolished, suggesting that no intrinsic fiber type differences were the cause for the differences in force depressions observed when shortening at the same absolute speed. In the experiments performed at the same relative speed, S2 was lower in Type I fibers compared to Type II fibers, suggesting that the behaviour of the passive element during shortening is not responsible for force depression at the steady-state after shortening.

According to the stress-induced inhibition of cross bridge theory (Marechal and Plaghki, 1979), force production prior to active shortening is assumed to cause extension and distortion of the thin filaments. Thin filament distortion is thought to be proportional to its compliance; the higher the compliance, the higher the distortion (Herzog and Leonard, 1997; Kojima et al., 1994). When shortening occurred with similar forces between Type I and Type II fibers, force depression was also similar between fiber types. Thus it appears that the compliance of thin filaments of Type II fibers is similar to that of Type I fibers. This speculation is in accordance with previous findings (Galler and Hilber, 1998) showing that the variations in the structure of proteins between fiber

types are not sufficient to produce different compliances of the network of proteins within sarcomeres.

Why do Type I fibers exhibit less force than Type II fibers during shortening, while the opposite happens at the steady-state after shortening? Different mechanisms control transient muscle force during shortening and isometric force after shortening. Force decreases during active isokinetic shortening because of a decrease in the number of attached cross bridges and in the amount of force produced per cross bridge (Huxley, 1957). It is known that fast fibers have high rates of ATP hydrolysis and cross bridge attachment and therefore as filaments slide past one another at the same speed for fast and slow fibers, fewer cross bridges have the time to attach in slow fibers and thus force decreases more in slow compared to fast fibers (Barany et al., 1965). Force per cross bridge decreases during shortening because the distance "x" (Huxley, 1957) decreases when the filaments slide past one another. Since it has been suggested that the stiffness of the elastic region of the cross bridge might be higher in Type I compared to Type II fibers (Goubel and Marini, 1987; Pousson et al., 1991), the decrease in force for the same decrease in "x" distance would be higher in Type I compared to Type II fibers. Accordingly, the relatively lesser decrease in force during isokinetic shortening of Type II fibers compared to Type I fibers is likely due to a smaller decrease in the number of attached cross bridges and a smaller decrease in the force produced per cross bridge. On the other hand, in the isometric force depressed state, the primary mechanisms are, as discussed earlier, the stress induced inhibition of cross bridges (Marechal and Plaghki, 1979) and the non-uniformity theory (Morgan et al., 2000).

The biomechanical role of force depression during everyday movement is still unknown (Tilp et al., 2009). However, it has been speculated that active shortening and force reduction during shortening could reduce the work required to induce active lengthening (negative work) and thus,

increase the net work performed by a muscle over a full cyclic shortening/lengthening movement (Josephson and Stokes, 1999).

In line with the stress-induced theory of force depression, we show for the first time greater force depression in Type II fast muscle fibers compared with Type I slow muscle fibers when shortening was performed at the same absolute speed. When shortening speed was controlled and Type II and Type I shortened at the same relative speed, no difference in force depression was observed.

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Table 1

	Type I (n=13)	Type II (n=12)	Type II (n=7)
Absolute active shortening speed (fiber length/s)	0.115	0.115	0.3
Relative active shortening speed (% V_0)	17%	6.5%	17%
S1 (s^{-1})	-4.58 ± 0.46*	-2.18 ± 0.27	-4.01±0.18
S2 (s^{-1})	-0.06 ± 0.01*‖	-0.09 ± 0.01	-0.20±0.01
Mechanical work (kJ/m^3)	3.70 ± 0.25*	6.28 ± 0.87	3.99 ± 0.20

Mean values±SEM for S1, S2 and the mechanical work performed during shortening at the absolute and relative speeds. * indicates a significant difference of Type I fibers compared to Type II fibers shortened at the same absolute speed of 0.115 fiber length/s ($p<0.05$), ‖ indicates a significant difference of Type I fibers compared to Type II fibers shortened at the same relative speed of 17% V_0 ($p<0.05$).

Figure captions

Figure 1

Typical fiber response when passively stretched from an average SL of 2.6μm to an average SL of 3.2μm, activated, shortened to an average SL of 2.6μm and then deactivated. The noise indicates the time when the fiber was transferred between solutions. The sudden change in stress indicates the stretch-release cycle performed to measure stiffness. The grey force trace indicates the stress produced by the reference isometric contraction performed at the average SL of 2.6μm. (A stiffness test was also performed before active shortening but not used in the analysis).

Figure 2

Electrophoresis of MHCs. Lanes 1-3: fibers on which the mechanical measurements were performed. Lane 4: rabbit psoas muscle. Lane 5: rabbit muscles used as an electrophoretic marker. Fibers in lanes 1 and 3 expressed MHC IId and were identified as Type II. The fiber in lane 4 expressed MHC I and was identified as Type I.

Figure 3

Representative traces of the transient force changes during shortening of Type I and Type II fibers. Force decreases during shortening in two phases characterized by a steep initial decrease followed by a slower decrease in force fitted by two linear regressions (red and blue lines). The slopes of the steep and slow phases are respectively S1 and S2. Type I fibers showed a significantly steeper decay in force during the initial phase of shortening and lower S2 and force

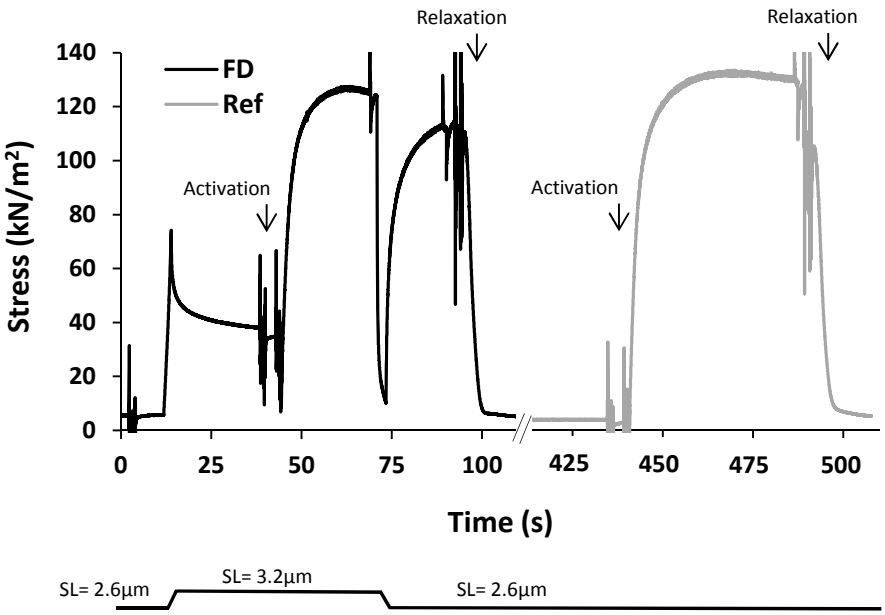
depression ($p<0.05$) than Type II fibers. Force depression was respectively 2 and 9% in Type I and Type II fibers shown in this figure.

Figure 4

Force depression as a function of stiffness depression. There is a statistically significant correlation between force depression and stiffness depression. Type II-0.115 and Type II-17% V0 refers to Type II fibers shortened at a constant speed of 0.115 fiber/s and 17% V_0 respectively.

Figure 5

Force depression as a function of S1. There is a statistically significant correlation between force depression and stiffness depression. Type II-0.115 and Type II-17% V0 refers to Type II fibers shortened at a constant speed of 0.115 fiber/s and 17% V_0 respectively.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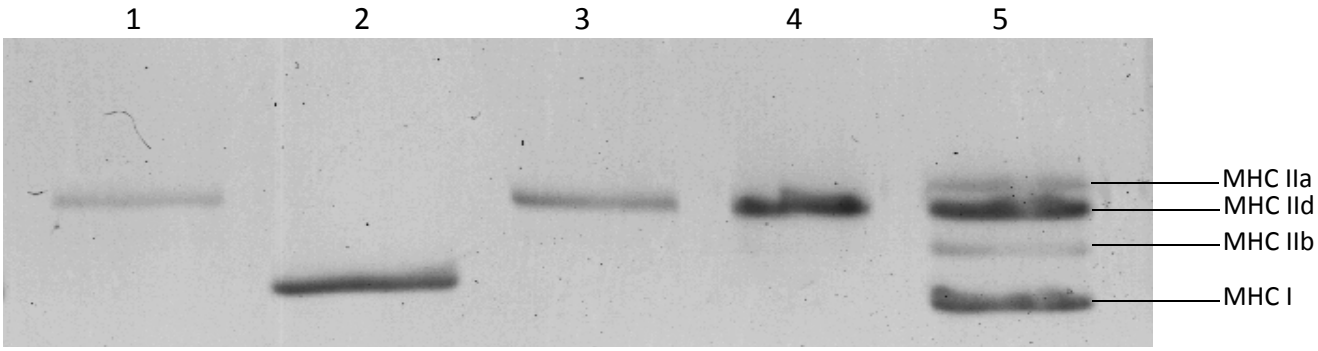
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MHC IIa
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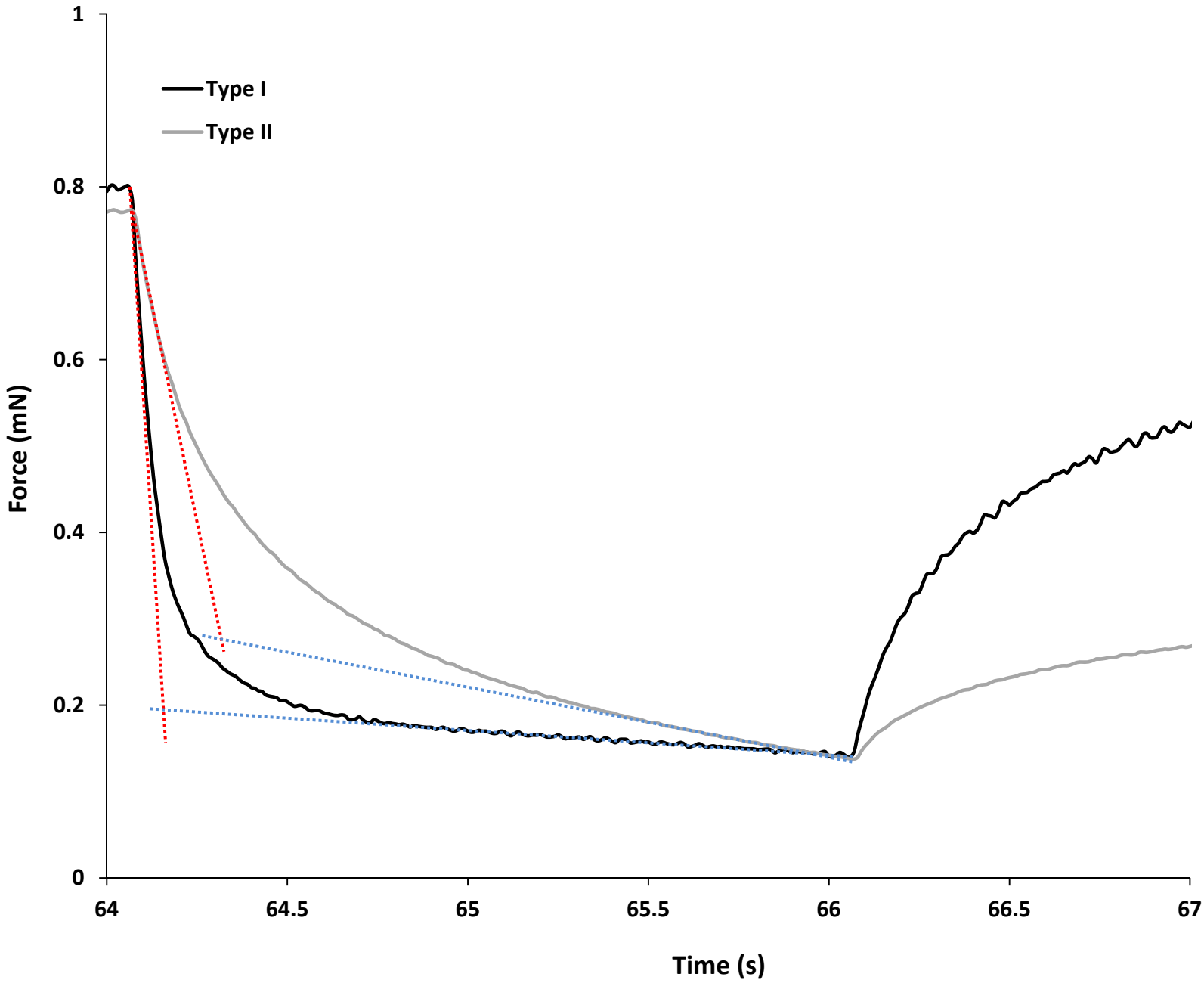
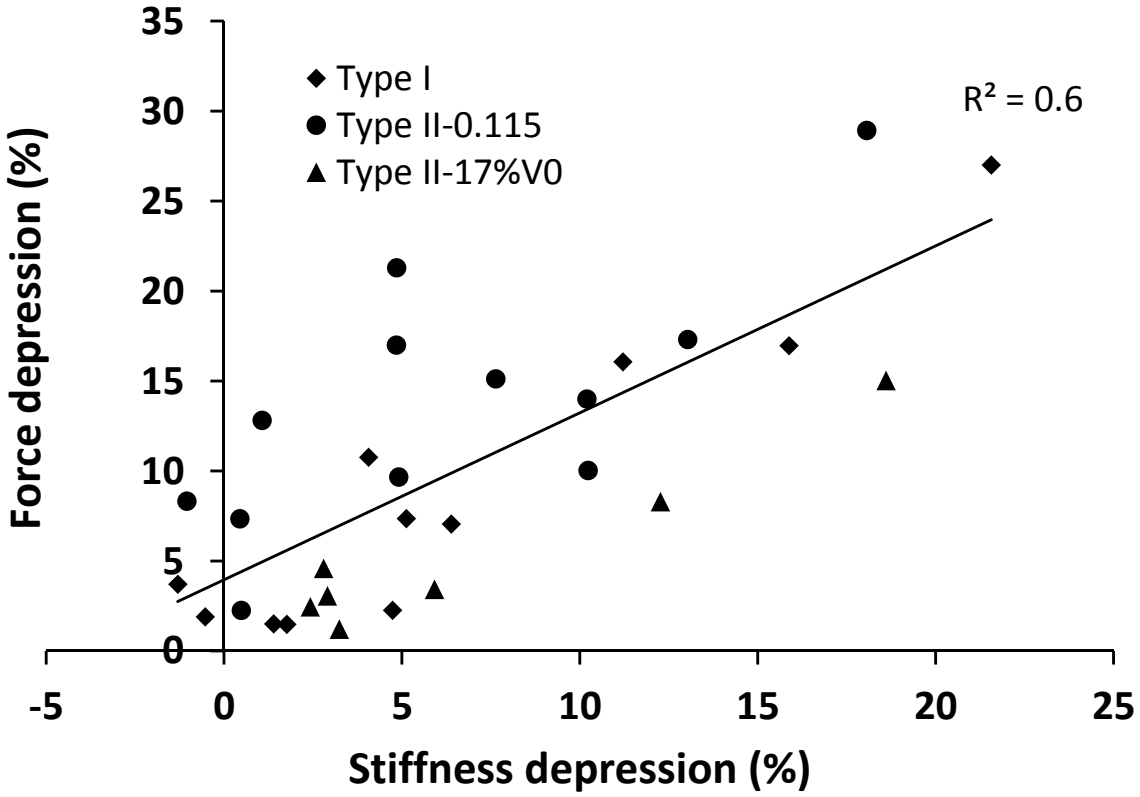
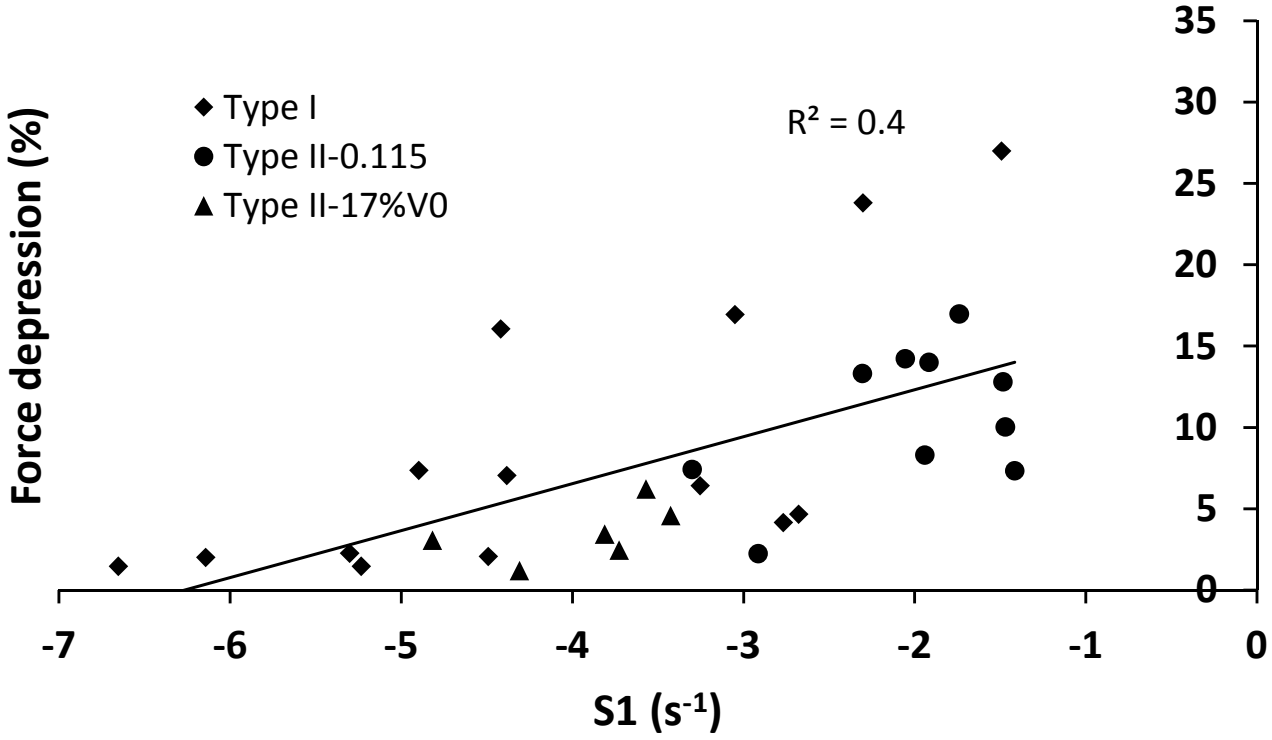
MHC IIb
MHC I


1
0.8
0.6
0.4
0.2
0
Force (mN)
Type I
Type II
64
64.5
65
65.5
66
66.5
67
Time (s)


35
30
25
20
15
10
5
0
-5
0
5
10
15
20
25
Force depression (%)
Stiffness depression (%)
Type I
Type II-0.115
Type II-17%V0
R² = 0.6


Type I
Type II-0.115
Type II-17%V0
R² = 0.4
Force depression (%)
S1 (s-1)
35
30
25
20
15
10
5
0
-7
-6
-5
-4
-3
-2
-1
0