The effect of aging on single muscle fibre and whole muscle contractile properties

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Nicole Mazara

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ABSTRACT

THE EFFECT OF AGING ON SINGLE MUSCLE FIBRE AND WHOLE MUSCLE CONTRACTILE PROPERTIES

Nicole Mazara
University of Guelph, 2018

Advisor(s):
Dr. Geoffrey A. Power
Dr. Stephen H.M. Brown

The aims of this thesis were to determine if whole muscle rate of torque development (RTD) and single fibre rate of force redevelopment (k*tr*) are lower in older adults compared to young adults, if k*tr* and RTD are associated with each other, and if k*tr* is affected by [Ca^{2+}] in humans. Knee extensor muscle strength, speed, power, and RTD were lower in old compared to young. Single fibre k*tr* was not different between young and old. There were k*tr*-RTD relationships trending toward significance in older TI fibres. Human single fibre k*tr* was also affected by [Ca^{2+}] such that with increasing [Ca^{2+}] k*tr* increased. In old single fibres, calcium sensitivity (pCa_{50}) was strongly associated with k*tr*, such that with increasing pCa_{50}, k*tr* increased. This work has confirmed the k*tr*-[Ca^{2+}] relationship in humans and found an emerging relationship between k*tr* and RTD in older adults.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE

RTD – rate of torque development

MVC – maximal voluntary contraction

Ca^{2+} - calcium

$[Ca^{2+}]$ – calcium concentration

k*tr* – rate of force redevelopment

P_o – maximal single fibre isometric tension

P – single fibre isometric tension

SF – specific force

V_o – maximal unloaded shortening velocity

LIST OF APPENDICES

1 Introduction

During the process of healthy adult aging, skeletal muscle and its functions become compromised, making it more difficult for older adults compared to young adults to do normal activities in their daily lives, such as getting out of a chair or righting oneself from a stumble. The loss of skeletal muscle mass and skeletal muscle strength, speed, and power – or performance – are key aspects contributing to difficulty in completing activities of daily living for older adults. The loss of muscle mass associated with adult aging, and not disease or disuse, is called *sarcopenia*, coined by Rosenburg (1987), and occurs at the rate of ~1-2% per year after age 60 (von Haehling et al. 2010). While the sarcopenic phenotype presents with muscle weakness, muscle strength loss (*i.e.* weakness) occurs independently, and more rapidly, than muscle mass loss, at ~2-3% per year after age 60 (Clark & Manini 2011; von Haehling et al. 2010). By definition, a sarcopenic older adult must have muscle mass ≥2 SD below the mean of a reference young adult population and a functional impairment such as low gait speed, *e.g.* walking speed <0.8 m/s on the 4 m walking test, however other functional tests can also be used for this diagnosis (Muscaritoli et al. 2010). While many older adults will not lose enough muscle mass or strength to necessarily become frail or sarcopenic by definition, their daily lives will still be hampered by the seemingly inevitable losses in muscle strength, speed, and power that come with age (Frontera et al., 2008; Goodpaster et al. 2006; Venturelli et al. 2015). The loss of muscle mass and rapid declines in strength and power in old age highlight the need for research investigating the mechanisms behind age-related changes in skeletal muscle performance.

Perhaps more important than an absolute loss in strength or speed, is the loss in rate of torque, or force, development (RTD) in whole muscle. Rate of torque development is lower in older adults compared to young (Izquierdo et al., 1999) as is the comparable measure, rate of force

development (Roos et al. 1999), so not only are older adults weaker, but the time it takes older adults to develop force from their muscle is slower than their young counterparts. This age-related slowing of RTD is a problem since there are many situations where developing force quickly is important, such as righting oneself from a stumble to prevent a fall or catching a railing on a slippery walkway. It has been suggested that the age-related declines in RTD may be partially due to age-related alterations in the neuromuscular system, such as a reduction in motor unit firing rate (Connelly et al., 1999). What research has shown in regard to the age-related impairment of the neuromuscular system is that older adults have fewer motor units than their young counterparts (Power et al. 2010), as well as a lower rate of activation (Reid et al. 2012). Structural degradation of the neuromuscular junction has been shown in humans (Oda 1984; Wokke et al. 1990) and animals (Jang & Van Remmen 2011), and assessments in aging muscle have shown neuromuscular junction instability (Bromberg & Scott 1994; Hourigan et al. 2015), which could interrupt neural input to the muscle and result in impaired RTD (Hunter et al. 2016). The neural changes with aging are prominent, however age-related RTD declines in whole skeletal muscle cannot be fully explained by only age-related changes in neural factors, as intrinsic contractile properties of skeletal muscle have also been associated with lower RTD in older adults (Klass et al. 2008).

Possibly, the explanation for age-related decrements in skeletal muscle performance and RTD development could become more complete by the addition of the cellular aspect of muscular contraction. Using single muscle fibre preparations from muscle biopsies offers an experimental design free of neural input. While the literature regarding changes in single muscle fibre measurements is moderately large, the collective results are inconsistent and inconclusive. Many studies show decreases in older adults compared to young in single fibre contractile measures such as single fibre force (P; maximal force [P_o]), specific force (SF; P/cross-sectional area [CSA]), and

unloaded shortening velocity (V_o) (D'Antona et al. 2003, 2007; Frontera et al. 2000a; Power et al. 2016), but there are a few that demonstrate no change (Miller et al. 2010) or even an increase in single fibre P_o, SF, and V_o from older adults (Trappe et al. 2003).

These global measurements of contractile activity (P_o, SF & V_o) in single fibres can be paralleled to strength and speed of whole muscle, and as such, there is also a single fibre parallel measure for whole muscle RTD. In single muscle fibres the measure is known as rate of force redevelopment (k*tr*), and it is used as a proxy for cross-bridge attachment rate (Brenner & Eisenberg, 1986). Briefly, a single fibre is activated to a plateau force, rapidly shortened to break bound cross-bridges, and then re-stretched to dissociate any remaining cross-bridge bonds and allow force to redevelop independent of calcium-dependent regulatory proteins (Brenner & Eisenberg 1986; Power et al. 2016). This measure is useful as it provides an environment where calcium saturation is maximal and actin-myosin binding sites are free from regulatory proteins blocking attachment, therefore we can attribute the rate of force development to the rate at which cross-bridges transition from detached non-force producing states to a force producing state. Research in animal models has shown that k*tr* is affected by calcium concentration ($[Ca^{2+}]$), such that a higher $[Ca^{2+}]$ elicits a higher k*tr*, however this relationship between k*tr* and $[Ca^{2+}]$ has not been shown in humans. It has been reported previously that calcium handling and sensitivity is altered in the skeletal muscle of older adults compared to young (Lamboley et al. 2010), thus it is important to investigate if the relationship between k*tr* and $[Ca^{2+}]$ is similar in humans as in animals, and if an altered calcium sensitivity in the single fibres of older adults compared to young affects k*tr*. To date, no studies have investigated changes in calcium sensitivity with aging in the context of k*tr*, highlighting an important gap in the field.

Additionally, few studies have sought to identify the relationship between the whole muscle and the cell (i.e. single muscle fibre), and how that relationship could change with aging. Specifically, the relationship between whole muscle RTD and single fibre k*tr* is unexplored as of yet, and this investigation could fill important gaps in the explanation of whole muscle performance declines during healthy, adult aging. As noted above, neural changes during adult aging do not explain the entirety of skeletal muscle performance declines, but with the addition of age-related changes in contractile properties of single fibres the picture could become more complete. Therefore, this thesis investigated how calcium concentration affects k*tr* of single fibres, how k*tr* of single fibres relates to whole skeletal muscle performance, specifically RTD, and how this relationship between single muscle fibres and whole muscle changes with age. The hypotheses were:

1. Increasing [Ca^{2+}] will cause an increase in k*tr* for single muscle fibers of both young and older adults, similar to animal models.
2. Single muscle fibres with a high calcium sensitivity will have a higher k*tr*.
3. The k*tr* of single fibres will be linearly related to whole muscle rate of torque development, such that a lower k*tr* will be associated with a lower rate of whole muscle torque development.
4. The relationship between k*tr* and whole muscle torque development will be weaker in older adults compared to young.

2 Literature Review

The proposed thesis needs a review of the literature in order to provide context and demonstrate the relevance of the project. The purpose of this thesis was to examine the relationship between whole skeletal muscle and single muscle fibres, specifically the relationship between RTD and k*tr*, and to determine if [Ca^{2+}] affects k*tr* in human single muscle fibres in a manner similar to animal models. Therefore, the aims of this literature review are:

1) to provide background information on age-related changes in skeletal muscle performance – strength, speed, power;
2) to provide background information on age-related changes in cellular contractile measurements – P_0, SF, V_0;
3) discuss RTD and k*tr* measurements in humans;
4) outline previous research that has sought to explore the relationship between whole muscle and single muscle fibres;
5) discuss the role of calcium in single muscle fibre contraction and how calcium handling changes with age.

2.1 Age-related Changes in Skeletal Muscle Performance

During healthy adult aging, deficits in skeletal muscle performance, which encompasses muscle strength, speed, and power, occur resulting in difficulty performing activities of daily living such as getting out of a chair, climbing stairs or lifting a laundry basket. Consistently, research has shown that compared to young adults both male and female older adults are weaker (*i.e.* lower absolute muscle strength) in the upper (Bazzucchi et al. 2004; Doherty et al. 1993; Frontera et al. 2000b; Hughes et al. 2001; Venturelli et al. 2015) and lower limbs (Bazzucchi et al. 2004; Frontera et al. 2000a, 2000b; Goodpaster et al. 2006; Hughes et al. 2001; McNeil et al. 2005; Venturelli et

al. 2015; Yu et al. 2007). Older adults also have a slower velocity of movement during maximal voluntary dynamic contractions (Dalton et al. 2012; Larsson et al. 1979); and are less powerful than young adults (Bassey et al. 1992; Petrella et al. 2005; Trappe et al. 2003). While active older adults have better outcomes on skeletal muscle performance tests relative to sedentary adults within the same age group, active older adults still show worse muscle performance relative to young adults (Zampieri et al. 2015). There are many driving factors to these muscle performance deficits with age, and I will focus on the neuromuscular and cellular system.

Skeletal muscle performance deficits with age have been partially explained by age-related declines in the neural and neuromuscular system. Reorganization of motor units and changes in innervation and the neuromuscular junction have been shown in aging muscle (Deschenes et al. 2010). This reorganization occurs when a motor neuron innervating a group of fibres dies (motor unit loss) during natural aging, and the fibres attached must either die or be reinnervated by a neighbouring motor neuron, and this reinnervation by neighbouring motor units results in larger but fewer motor units. Indeed, motor unit numbers can be estimated using an electrophysiological technique involving two size parameters: a compound muscle action potential (CMAP; represents the electrical size of a muscle) and the surface motor unit potential (SMUP; average electrical size of individual motor units), the SMUP is determined using both in-dwelling and surface EMG, and the ratio of CMAP:SMUP gives a motor unit number estimate (MUNE) for a muscle. This MUNE technique has been used in aging research and found that older adults have fewer motor units compared to young in the lower limb (Dalton et al. 2010; McNeil et al. 2005; Power et al. 2012). Motor unit loss is also supported by the age-associated fibre loss evidenced by fewer fibres in the vastus lateralis of older adults compared to young (Lexell et al. 1988), and the time to reinnervate likely results in atrophy, also supported by overall smaller fibre size in older adults (Lexell et al.

1988). Additionally, the structural degeneration of the neuromuscular junction with age has been shown in both humans (Bromberg & Scott 1994; Oda 1984; Wokke et al. 1989) and animal models (Cheng et al. 2013; Jang et al. 2010; Li et al. 2011; Valdez et al. 2010). Although structural degeneration does not always translate to functional impairment, degradations in neuromuscular junction function have also been found (Bromberg & Scott 1994) and these functional impairments have been implicated in the sarcopenic phenotype (Ibebunjo et al. 2013; Hunter et al. 2016), and age-related neuromuscular junction degradation can result in lower maximal force, RTD, and maximal power of skeletal muscle (Hunter et al. 2016). The degradation of the neuromuscular junction and motor unit loss are two key factors in muscle mass and strength declines (Rowan et al. 2011; Power et al. 2016), and while these neuromuscular factors aid the explanation of declines in muscle performance, there are still unexplained aspects as intrinsic contractile properties can also explain some of the variance in declines in muscular strength (Frontera et al. 2000), power (Straight et al. 2018), and RTD (Klass et al. 2008). To paint a clearer picture, researchers have turned to alterations in cellular muscle mechanics independent from the neuromuscular system, investigating age-related alterations of the cellular contractile apparatus, such as the disruption of force-producing characteristics; cross-bridge cycling; and calcium release and sensitivity, to additionally characterize performance declines in healthy adult aging skeletal muscle.

2.2 Age-related Changes in Cellular Contractile Properties

Single muscle fibre preparations offer a model free from neural input, allowing the investigation of purely cellular properties outside of voluntary control. There are three contractile measurements that are frequently used in aging literature: single fibre maximal force (P_0), maximal force/fibre CSA (termed specific force (SF)), and maximal unloaded shortening velocity (V_0). These contractile parameters differ based on the isoform of myosin heavy chain (MHC) present in

a single fibre. There are multiple MHC isoforms expressed in human skeletal muscle, but what I will discuss here are the major fibre types present in human skeletal muscle. MHC type I (TI) fibres are slow-type, high endurance fibres that are slower, produce less force, and less powerful than MHC type II (TII) fibres. There are TIIA and TIIX fibres within TII. TIIA are faster and stronger, fatigue more quickly than TI, and are recruited for rapid and/or explosive contractions. TIIX are the fastest, strongest, and fatigue the fastest compared to the other fibre types and are recruited for the most explosive contractions (Bottinelli et al. 1996). Fibres can also express multiple MHC isoforms, resulting in hybrid fibres, of which the most common types are I/IIA and IIA/IIX (Bottinelli et al. 1996), although other combinations are also possible.

2.2.1 Maximal Force

Single muscle fibre force is quantified as maximally calcium (Ca^{2+}) activated isometric force and is an indirect measurement of cross-bridge attachment and force per cross-bridge; a greater number of cross-bridges attached and a greater force per cross-bridge elicits a greater P_o than a fibre with less cross-bridges attached and force per cross-bridge. As whole muscle strength declines with aging, a logical conclusion to draw would be that single muscle fibre P_o would decrease, however there are differing results in the literature in both rodent models and humans.

In studies using rodent models, there is consistent evidence for lower P_o in old (~24-37 months) and young (~5-12 month) male rats across muscle and fibre types (Kim & Thompson 2013; Lowe et al. 2001; Thompson & Brown 1999; Thompson et al. 1998; Zhong et al. 2006), and between adult (~9-10 month) and old (~26-27 month) male mice in both the soleus (primarily TI) and extensor digitorum longus (EDL; primarily TII) muscles (Brooks & Faulkner 1988). However, when investigating muscle contractile properties in humans, studies involving human cross-sectional designs, there is strong support for lower P_o in old (~60-90 yr) compared to young

(~18-35 year) adults in both TI and TII fibres of the vastus lateralis (Frontera et al. 2000a; Larsson et al. 1997a, 1997b; Power et al. 2016), while others have reported weakness in only TII fibres (Yu et al. 2007). Still, others show no significant difference in P_o with age in TI or TII fibres (Hvid et al. 2011; Raue et al. 2009; Trappe et al. 2000; Venturelli et al. 2015) or even significantly higher P_o in older adults (Trappe et al. 2000; Yu et al. 2007 [TI fibres]).

Contrary to the cross-sectional study designs presented above, two longitudinal studies examining single fibre contractile properties are consistent. Frontera et al. (2008) completed a 9-year longitudinal investigation of older adults with a baseline age range of 62-81 years, and the returning cohort with an age range of 71-90 years. Both P_o and SF of TI fibres were trending towards an increase with age, with average values of 23% and 28% greater than baseline, respectively. In TIIA fibres, P_0 trended towards a significant increase with age, but there was no change in SF. Reid et al. (2014) completed a 3-year longitudinal study with a cohort of healthy, older (~75 years) and mobility-limited, older (~77 years) adults. In both cohorts, statistically significant increases in both P_o and SF were detected.

Overall, the results are consistently inconsistent, even when attempting to match for physical activity level and age. Cross-sectional studies have shown evidence for a degradation, maintenance, and improvement in single muscle fibre force in older adults compared to young. And yet longitudinally, there is evidence for a potential increase in older adults from ~60 years to ~80 years of age. The difference in results between study designs suggests that single fibre function is impaired in older adults compared to young, but that within old age remaining single fibres could get stronger and/or larger in a compensatory manner as more fibres are lost into very old age (>80 yrs), owing to the death of large remodeled motor units (Dalton et al. 2010; McNeil et al. 2005; Power et al. 2014). This compensatory effect theory has been posited to occur with respect to

muscle fibre size (Andersen 2003; Aniansson et al. 1992; Frontera et al. 2012; Rowan et al. 2010) and muscle fibre force (Frontera et al. 2008; Frontera et al. 2012), and likely occurs between the ages of 70 – 80 years.

2.2.2 Specific Force

Maximal isometric force is often reported normalized to CSA and is termed specific force (SF). Specific force is intended to account for force per unit of myofibrillar content and often the maximal P_0 value itself is not reported, as larger fibres typically produce more force than smaller fibres. As there is conflicting evidence for changes in single fibre size from young to older adults depending on fibre type and age group (Lexell et al. 1985; Miller et al. 2013; Nederveen et al. 2016; Venturelli et al. 2015), accounting for force relative to single fibre size is necessary to avoid a significant difference in fibre force only due to fibre size differences.

In animal models, the results remain unclear with studies showing an age-related decline SF in aged rats (~24-37 months) compared to young (~4-12 months) across muscles (Kim & Thompson 2013; Lowe et al. 2001, 2002; Prochniewicz et al. 2005; Thompson & Brown 1999; Zhong et al. 2006), significantly higher EDL SF and lower soleus SF (Degens et al. 1998; Li & Larsson 1996), or no difference in the soleus or EDL SF in rats aged 20-24 months relative to 3-6 month controls (Larsson et al. 1997b). In male mice aged 26-27 months, lower EDL SF, but no change in soleus SF compared to both young (2-3 months) and adult (9-10 months) groups was found (Brooks & Faulkner 1988). In humans, SF results, again, show similar inconsistencies with studies reporting lower SF with aging in TI and TII fibres (D'Antona et al. 2003; Frontera et al. 2000b; Larsson et al. 1997a, 1997b; Ochala et al. 2006, 2007; Power et al. 2016; Yu et al. 2007), lower SF only in TII fibres (Lamboley et al. 2015), and no significant differences (Hvid et al. 2011; Raue et al. 2009; Straight et al. 2018; Trappe et al. 2003; Venturelli et al. 2015). There are even

studies that have found significantly higher SF in older adults compared to young; Trappe et al. (2003) found significantly greater SF of TIIA fibres biopsied from the vastus lateralis of healthy older (~80 yrs) males compared to young (~25 yrs), but not in TI or in fibres from older females compared to young. Interestingly, another study that collapsed sex across groups still found a significantly greater SF in TIIA fibres in the older (61-75 yrs) group compared to young (Straight et al. 2018).

Something to consider when exploring contractile function is physical activity. It has been noted that physical activity has a protective effect against the deficits of aging at the whole muscle level, and investigations regarding the effect of physical activity on single fibre contractile function have been performed. D'Antona et al. (2007) sought to determine how differing levels of physical activity would change single fibre SF, and they recruited men who were sedentary, recreationally active, and endurance trained throughout the majority of their life (~73 yrs). Indeed, the researchers determined that lifetime physical activity levels correlated with single fibre SF, whereby the sedentary group showed significantly lower SF in TI and TIIA fibres of ~26% and ~21%, respectively, from young adults; the recreationally active group showed significantly lower SF in TI fibres and SF was lower, however not significantly, in TIIA fibres; and the endurance trained group showed small, non-significant decreases in both fibre types, although, this trend does not always hold true (Larsson et al. 1997a). However, a recent study investigating differences in single muscle fibre performance between a group of masters athletes and normal population older adults found that there were no differences between groups, so potentially life-long physical activity has a minimal protective effect on specific force in single fibres (Power et al. 2016a) but a robust effect on maintaining functional motor units and strength (Power et al. 2016b). This effect could be due to the maintenance of motor units in masters athletes as compared to the average older adult, which

causes the muscular tension to be distributed across more motor units/fibres. In the case of the average older adult, they have fewer motor units, and likely fewer single fibres, and so each unit/fibre is under greater tension, and therefore 'trains' harder. This potential harder training could be one reason why there seems to be no difference in the single fibres of masters athletes and recreationally active older adults.

2.2.3 Shortening Velocity

Maximal unloaded shortening velocity is a measure of contractile speed that is dependent on cross-bridge detachment rate and is determined by the slack-test method wherein a fibre is maximally Ca^{2+}-activated and a series of shortenings (slacks) are introduced (Edman 1979). The time for force to redevelop after each shortening is determined, and V_0 is measured from the slope of the points; measured in muscle fibre lengths per second (FL/s). Considerably fewer studies have systematically investigated age-related differences in shortening velocity compared to the number of studies on single fibre strength. Based on older individuals whole muscle performance being slower than young, a slowing of contractile properties at the single muscle fibre level could also be expected, and the literature shows a general trend in that direction, however inconsistencies still remain.

In rat models, V_o appears to always be slower with age in TI fibres from soleus muscle (Degens et al. 1998; Li & Larsson 1996; Thompson 1998, 1999), and less consistently in TII fibres from the EDL and semimembranosus muscles (Degens et al. 1998; Prochniewicz et al. 2005). Other studies have found no significant differences in V_0 between age groups in TII fibres (Degens et al. 1998; Li & Larsson 1996; Zhong et al. 2006). Results from single fibres biopsied from human participants show a similar trend to animal models, with the majority of cross-sectional studies showing a consistent slowing in V_o with aging across fibre types of the vastus lateralis (D'Antona

et al. 2003; Larsson 1997a; Ochala et al. 2007; Power et al. 2016; Yu et al. 2007). A slowing of V_o in only TI (Korhonen et al. 2006; Krivickas et al. 2001), or no change in either fibre type (Raue et al. 2009; Reid et al. 2012; Trappe et al. 2003) have also been found. One longitudinal study that assessed V_o over ~3 years (Reid et al 2014) found that in both a healthy and limited mobility older population V_o significantly increased in both TI and TIIA fibres.

D'Antona et al. (2007) measured V_o groups with different levels of physical activity. No differences were found in the endurance trained elderly group or recreationally active elderly group (~73 yrs) compared with the young controls (~30 yrs) in TI fibres of the vastus lateralis, whereas a significant slowing was found in the sedentary elderly group (~73 yrs). However, in TIIA fibres a significant slowing was found across all elderly groups compared to the young control group. For comparison, the researchers added data from two elderly immobile participants (70 & 72 yrs) from a previous study (D'Antona et al. 2003) and found significantly higher V_o in both TI and TIIA fibres compared to young and all other old groups.

Currently, the conflicting results do not have an explanation, but it has been speculated that the dichotomous interaction between aging and disuse could be a factor, as well as different age ranges and physical activity levels of participants. As demonstrated by D'Antona et al. (2007), the retrospective data from the immobile participants show V_o being significantly higher, which is opposite to the typical trend. Indeed, it has been shown that after 17 days of bed rest V_o in healthy human TI soleus fibres increased (Widrick et al. 1997). These data suggest that shortening velocity is affected conversely by aging and disuse, which identifies need for screening and categorization of physical activity levels of participants to resolve discrepancies across studies. The disuse-aging paradigm likely has a strong influence on research results in this area. There is also large inter-individual variation in shortening velocity, as well as other single fibre measures, and so the

discrepancies in the literature could also be an artefact of individual variation washing differences out, then exacerbated by a low number of participants. While shortening velocity at the whole muscle level is slower in old as compared with young, differential effects of disuse on fibre type shortening velocity may be confounding findings at the cellular level.

2.3 Whole muscle and Single Fibre Rate of Force Development

Whole muscle strength, speed, and power are valuable measurements that give us insight toward the overall performance of older adults in their daily lives. However, those measures overlook one important aspect of contractile performance that is the rate at which force is developed, or how quickly an individual can contract their muscle to produce force. Rate of torque development (RTD) is important for actions that not only need force produced but need that force quickly to avoid a negative outcome (*i.e.* righting oneself from a stumble to avoid falling).

Research has shown that with age RTD is lower in many muscle groups, including the elbow flexors (Barry et al. 2005), plantarflexors (Bemben et al. 1991; Thelen et al. 1996; Thompson et al. 2014), dorsiflexors (Klass et al. 2008; Thelen et al. 1996), knee flexors (Thompson et al. 2013) and knee extensors (Ditroilo et al. 2010; Izquierdo et al. 1999; Thompson et al. 2013). These studies together report an average decline in RTD of ~40%, but only an average decline in maximal strength of ~25% in older adults compared to young. Many studies investigating changes in older adults skeletal muscle performance do not include RTD, and this is an important measure because a 75 year old adult may have the absolute strength to get out of a chair, but not a fast enough RTD to prevent themselves from falling. Indeed, two studies investigating differences in RTD between groups of older adults who had a history of falling (fallers) and groups who did not (non-fallers) found that the fallers had a much lower RTD than the non-fallers group (Bento et al. 2010; LaRoche et al. 2010). Therefore, measuring RTD in older adults could elicit a stronger

correlation with both decrements in physical function (*i.e.* activities of daily living) and potentially cellular contractile properties.

Rate of force redevelopment (k*tr*) in single muscle fibres is similar to the RTD measure in whole muscle, however k*tr* removes the effect of Ca^{2+}-dependent regulatory proteins. This k*tr* measure is important as it can be used as a proxy for rate of cross-bridge attachment, as the faster cross-bridges form to produce force, the greater the rate of force production becomes. The cycling of cross-bridges in skeletal muscle is what ultimately produces force, and the rate at which cross-bridges both attach and detach affects the rate of force production and relaxation, respectively. To describe the measure briefly, a fibre is maximally activated in a Ca^{2+}-containing solution, allowed to reach plateau force, then rapidly shortened to break cross-bridge bonds, the fibre is then re-stretched to the original length, dissociating any remaining cross-bridge bonds, and force is allowed to redevelop. The redevelopment of force occurs after Ca^{2+} saturation of the fibre and removal of the Ca^{2+}-dependent regulatory proteins from actin binding sites, therefore the rate of force development at this point can be reasonably attributed to only cross-bridge attachment rate. A recent study has shown that k*tr* is lower in older adults' single fibres compared to young, and that this slowing of k*tr* was also present in aged-match masters athletes alongside general population older adults (Power et al. 2016). To date, there were no other studies found investigating age-related changes in k*tr* in humans.

The rate of cross-bridge attachment does not necessarily affect maximal force output, but how quickly maximum force is reached. If at the cellular level, k*tr* is lower across fibres in older adults, then there could be a related slowing in RTD at the whole muscle level. A slowing of RTD seriously impairs an individual's ability to produce force quickly making them less able to recover from a trip and/or avoid falling. Therefore, this measure of single fibre force production paired

with whole muscle RTD may provide useful insight to performance of activities of daily living in older adults as compared with only single fibre force and whole muscle strength measurements.

2.4 Relationship between Whole and Cellular Muscle

Age-related alterations to single muscle fibre contractile properties are inconsistent; however whole muscle size, strength, speed, and power decrease consistently in older adults. There are few studies that have performed both whole muscle and single fibre performance tests and attempted to correlate the two in some way. A positive, non-linear relationship was reported between single fibre tension and whole muscle torque with aging (Frontera et al. 2000b), and another study reported that slower cross-bridge kinetics and lower P_0 in TIIA fibres predicted lower power in older adults (Miller et al. 2013). Straight et al. (2018) reported that a lower calcium sensitivity in TI single fibres was related to a reduced isokinetic power in the leg extensors of older adults. However, many studies do not show a clear relationship between fibre function and whole muscle function (Frontera et al. 2008; Reid et al. 2012, 2014; Venturelli et al. 2015). As mentioned previously, a theory posited for the vast inconsistency in single fibre properties of older adults is that a "compensatory" phenomenon is occurring, likely in an individual's late-seventies, in both fibre size (Aniansson et al. 1992) and contractile properties such as P_o (Frontera et al. 2008). As whole muscle mass/size decreases with age, the total number of muscle fibres also decreases, however the size and force of the remaining muscle fibres appears to be maintained or even increased in older adults over time or compared to the single muscle fibres of young adults, especially in TI fibres (Aniansson et al. 1992; D'Antona et al. 2003; Miller et al. 2013; Nederveen et al. 2016). This potential maintenance in performance of the surviving single fibres certainly indicates cellular adaptations to the loss of muscle fibres while the necessity to get out of a chair remains. Across investigations, shortening velocity is the only property that shows any measure of

consistency in both fibre types as compared with force and power with natural adult aging. At the whole muscle level, shortening velocity is reduced in old age (Dalton et al. 2010; McNeil et al. 2007; Petrella et al. 2005), and at the cellular level, V_0 is almost always reduced in healthy aging populations (D'Antona et al. 2003; Larsson 1997a; Ochala et al. 2007; Power et al. 2016; Yu et al. 2007). Therefore, there may be a correlation between whole muscle speed of contraction and cellular V_0 and/or cross-bridge attachment and detachment rates, as V_0 is dependent on rate of cross-bridge detachment (Piazzesi et al. 2007).

There are a few investigations that highlight some of the probable reasons for the disconnect between age-related changes in whole muscle performance and cellular contractile properties. A study comparing older adults (70 – 85 yr) with middle-aged (40 – 55 yr) adults showed non-significant impairment in whole muscle function (Reid et al. 2012). As most studies comparing whole muscle function between young and old show vast, and significant, impairments, this study comparing old to middle-aged adults clearly shows how gradual age-related decline in muscle performance is. On a cellular level, age-related changes most likely occur more subtly, and may be difficult to identify depending on age-range of participants, control group, or length of follow-up in a longitudinal design. Reid et al. (2012) also found no difference between age groups in either fibre type for P_0 and SF, again highlighting how slow moving age-related cellular alterations are. A young adult reference group would have been valuable to investigate changes from young to middle-aged, and finally to older adult. Another study comparing young adults with a mobile and immobile oldest old group (85 – 92 years) found that in the biceps brachii (mobile during wheelchair use) and the vastus lateralis (immobile during wheelchair use), there were no differences in P_0 between groups despite the large age difference (Venturelli et al. 2015). In the biceps brachii, it is possible that the maintained use across groups had a protective effect on both

whole muscle performance and single fibre P_0; however in the vastus lateralis, it could be that only examining single fibre P_0, other differences in single fibre contractile properties were overlooked. When comparing highly active masters athletes with community dwelling older adults, it was found that the athletes and general population older adults had similar single fibre function, suggesting that activity does not protect fibres of the vastus lateralis (Power et al. 2016). However, it could be the case that the relative decrease in activity over a lifetime is the issue; even master's athletes have had a relative decline in physical activity compared to their younger selves.

Importantly, the normal age-related loss in muscle mass likely does not have to progress to sarcopenia to impair the performance of activities of daily living (e.g. being able to get out of a chair, climb stairs, right oneself from a trip etc.), as demonstrated by the cross-sectional studies that determined skeletal muscle performance of healthy recreationally active older adults decline in comparison to young adults (Clark et al. 2011; Goodpaster et al. 2006; Petrella et al. 2010); while these studies did not diagnose sarcopenia it is highly unlikely that recreationally active older adults meet the diagnostic criteria for sarcopenia as it is quite extreme (muscle mass ≥2 SD below the mean of a reference young adult population and large functional impairment in some clinical physical assessment, most frequently gait speed <0.8 m/s [Muscaritoli et al. 2010]). Some of the work done in the cellular muscle mechanics field has been in non-sarcopenic older adults (Frontera et al. 2000a), and yet has still found age-related declines in single fibre properties that may be worsened by the progression of sarcopenia. Grouping older adult groups by age, physical activity, and/or the presence of sarcopenia could provide avenues to the elusive answer regarding age-related alterations of cellular muscle mechanics. Future work will be needed to identify the critical thresholds or appropriate tests at the cellular level to potentially identify those individuals at risk of future mobility impairment.

2.5 Role of Calcium in Cellular Muscle Contraction

To date, there are few studies that have investigated changes in calcium sensitivity in the context of aging in humans (Hvid et al. 2011; Lamboley et al. 2015; Straight et al. 2018). When investigating the effects of calcium on various aspects of single fibre contractile properties, a fibre is subject to different calcium concentrations [Ca^{2+}] in solution. Another way [Ca^{2+}] is presented is pCa (pCa = -$\log_{10}$[Ca^{2+}]), and this pCa value is simpler to work with than the very small absolute numbers of [Ca^{2+}] (*i.e.* if [Ca^{2+}] =0.00005 then pCa ≅ 4.3). Hvid et al. (2011) did an analysis of force-pCa curves before and after short-term limb immobilization in young and older men. There was no difference in calcium sensitivity between young and older men before the immobilization, but after the immobilization a decrease in calcium sensitivity was found in TI fibres compared to TIIA fibres from older men. In young men, TIIA fibres had a decreased calcium sensitivity compared to TI after immobilization. Lamboley et al. (2015) performed a more extensive analysis of calcium sensitivity using mechanically skinned fibres to keep the sarcoplasmic reticulum (SR) intact, and it was found that TII fibres had a lower calcium sensitivity in older adults than young. Recently, an investigation by Straight et al. (2018) has corroborated the findings above, showing lowered calcium sensitivity in both TI and TIIA fibres in older adults compared to young. The immobilization study results suggested that TI fibres are at a greater risk of lowered calcium sensitivity in older adults; however stated above there is evidence for lowered calcium sensitivity in both fibre types. Based on the converse effects that aging and disuse seem to have on single muscle fibre V_0, it is perhaps not surprising that a similar aging-disuse paradigm could exist for single fibre calcium sensitivity.

Lamboley et al. (2015, 2016) tested the storage and release of calcium from the SR in a series of experiments. It was found that in older adults endogenous and releasable calcium content

of the SR was reduced in both fibre types, and maximal uptake of calcium was reduced in TII fibres compared to young (Lamboley et al. 2015). In an examination of SR calcium loading, it was found that TI fibres of older adults have lower maximal storage, as shown by loading a greater percentage of calcium in the same amount of time relative to fibres from young adults. In terms of calcium release, both TI and TII fibres from older adults had a lower maximal release of calcium from the SR relative to maximal force elicited. Furthermore, evidence for greater leakage from the ryanodine receptors/calcium channels was found in TI fibres of older adults (Lamboley et al. 2016).

With so few studies in humans, evidence from animal models can be used as additional support. In aged rats a similar impairment in SR calcium release was found in the gastrocnemius muscle (Russ et al. 2011). Combined with the rodent model, the studies comparing young and older adults provide compelling evidence that calcium regulation, whether it be storage, release or sensitivity, is impaired with age. Calcium sensitivity would affect P_0, as it would affect the number of actin binding sites available for cross-bridge formation, and fewer cross-bridges attached would result in lower force production. Small decrements in sensitivity could result in large impairments during force production, which in turn would affect specific force and peak power dramatically.

Sensitivity to calcium also affects the rate of cross-bridge attachment, as measured by k*tr*, as shown in animal models (Brenner 1988; Metzger & Moss 1990; Wolff et al. 1995). These studies found that with increasing [Ca^{2+}], an increase in k*tr* occurs in skeletal muscle of both rabbits and rats (Brenner 1988; Metzger & Moss 1990), and cardiac muscle of mice (Wolff et al. 1995). Some of these studies performed experiments showing that while Ca^{2+} has an effect on k*tr*, it was independent of the influence Ca^{2+} has on the number of cross-bridge attachments (Brenner 1988; Metzger & Moss 1988), and it was then concluded that Ca^{2+} must be affecting the transition

from non-force generating (weakly-bound) to force generating (strongly-bound) cross-bridge attachments. This influence of Ca^{2+} on cross-bridge transitions (or turnover kinetics [Brenner 1988]) would then, in theory, cause more force generating cross-bridge attachments with greater amounts of Ca^{2+} and an increase in k*tr* (Metzger & Moss 1990; Wolff et al. 1995).

The effect of [Ca^{2+}] on k*tr* has not been examined in human skeletal muscle to date; however it is likely that the effect would be similar in humans as in animal models. If [Ca^{2+}] and k*tr* have the same positive, curvilinear relationship (*i.e.* increasing [Ca^{2+}] causes increasing k*tr* to a plateau) in humans as in animal models, it is likely that impaired Ca^{2+} handling/sensitivity in the single muscle fibres of older adults would have a negative effect on the Ca^{2+}-k*tr* relationship. Additionally, it has been shown in animal models that there is a greater proportion of weakly-bound cross-bridges in aged compared to young skeletal muscle (Lowe et al. 2001; Thompson et al. 2001; Zhong et al. 2006). The potential that aged human skeletal muscle has more weakly-bound cross-bridges similar to animal models, in addition to the impaired Ca^{2+}-handling/sensitivity, would suggest that the relationship between [Ca^{2+}] and k*tr* could be different between young and older single muscle fibres of humans.

3 Methods

3.1 Participants

Participants were recruited from the University of Guelph and surrounding communities. Ten healthy, young males (22-35yrs; 179±9cm; 84±12kg) and ten healthy, independently living, older males (60-81yrs; 174±6cm; 75±10kg) participated in neuromuscular measurements, physical function testing, and a muscle biopsy. Participants were excluded if they had a history of smoking (within 1yr), diabetes, uncontrolled hypertension, neuromuscular or vestibular impairments, chronic illness or disease, bleeding disorder, started new medication in the 3 months prior to visit 1, a hospitalization or surgery within 6 months prior to visit 1, use of a mobility aid or been using corticosteroid or androgen-containing compounds (within 1 yr). All participants had to be living independently and able to perform all activities of daily living without assistance. Written, informed consent was obtained from all participants, and all procedures and protocols were approved by the local Research Ethics Board and conformed to the Declaration of Helsinki.

3.2 Health/Physical Activity Assessment

A short health questionnaire was carried out by the researcher asking about any current medical conditions and family history to ensure the safety of participants during the study. A modified version of the Yale Physical Activity Survey (Appendix A; YPAS, Dipietro et al. 1993) was used to estimate physical activity of each participant for the 12 months prior to visit 1. I carried out the questionnaire as an interview, filling the questionnaire in in its entirety, and clarifying any aspect of the questionnaire for participants when necessary.

The initial sections of the interview were regarding activity levels in the last week, and if the last week was atypical (*e.g.* vacation, inclement weather, had a cold, etc.), then activity in a typical week in the last month. Total Time (TT; hrs/week) and estimated Total Energy Expenditure

(TEE; kcal/week) were calculated from these questions. The last sections of the interview were regarding activity, or lack thereof, in the last month, and if this time period was atypical the participants were asked to answer based on a typical month in the last few. The indices calculated from the monthly activity were: vigorous activity (VA), leisurely walking (LW), moving (Mov), standing (ST), and sitting (Sit). The Sit index was given a negative value, therefore a more negative value equated to greater amount of sitting. The activity index (AI) was calculated by summing the other indices together, a higher AI indicating greater overall activity.

3.3 Neuromuscular Measures

For neuromuscular assessment of the knee extensors, the left leg was used (barring orthopaedic impairments on that side, in which case the right leg was used). Torque was recorded by a HUMAC Norm dynamometer (CSMi Medical Solutions, MA). Hip angle was 110° and knee angle was set at 80° of knee extension (180° being a straight leg) for isometric contractions and the starting point for dynamic contractions. Dynamic knee extension range of motion (ROM) was 50°. Maximal voluntary contractions (MVC) were performed until participants reached ≥90% voluntary activation during two MVCs, as assessed by the interpolated twitch technique (ITT). During the MVC, participants were given real time visual feedback of torque production and verbal encouragement to ensure their maximal effort during each MVC. At least 3 min of rest were given between each MVC to mitigate fatigue. To measure RTD, two brief (<1 sec) explosive isometric contractions (instruction: “kick as fast and as hard as you can”) were performed consecutively followed by a 1 min rest; this was repeated twice for a total of 3 trials and 6 explosive contractions (Maffiuletti et al. 2016). Velocity was assessed by asking participants to kick “as fast as they can” through the 50° ROM twice consecutively, followed by a 1 min rest; this was repeated twice for a total of 3 trials and 6 unloaded isokinetic contractions. The dynamometer arm velocity was set to

500°/s, and as every participant was unable to reach that velocity, the contraction was as unloaded as possible. A power curve was created by having each participant contract through the 50° ROM at isotonic loads equivalent to 10, 20, 30, 40, 50, and 60% of their highest MVC. Two contractions were performed at each load.

3.4 Voluntary Activation

Voluntary activation was assessed using the ITT. The femoral nerve was stimulated used a Digitimer DS7AH constant current stimulator (Digitimer Ltd., Welwyn Garden City, UK). The anode (Cleartrace 1700-030 ECG Electrode, ConMed, Utica, New York, USA) was placed over the inguinal triangle and the cathode was placed over the inferior gluteal fold. The cathode was a custom-made aluminum electrode pad (~6-8 cm width, ~8-10 cm length) wrapped in damp paper towel and covered in conductive gel. First, the level of current needed to deliver a supramaximal twitch was found by delivering increasing amounts of current until twitch torque plateaued, and then increasing the current by 10%. Then, during a MVC, a twitch was superimposed onto the torque plateau, and another twitch was delivered after the participant had relaxed fully (used as 'resting twitch torque'). A minimum of 90% activation was required for a trial to be considered a MVC. Voluntary activation was calculated by:

% Voluntary Activation = (1 – (interpolated twitch torque / resting twitch torque)) x 100

3.5 Physical Function Testing

Following the neuromuscular measurements, the physical function assessment was carried out. For one participant who was feeling tired, the physical function assessment was performed on a separate day. The assessment was modified based on the Senior Fit Test (SFT; Langhammer & Stanghelle 2015), using only 3 activities: 30 sec Chair Stand, Timed-Up-and-Go, and the 2-minute Step Test. The 30 sec Chair Stand consisted of participants getting in and out of a chair as many

times as they could in 30 sec with arms crossed over their chest; the test had to be done in a controlled manner, reaching full leg extension during the 'stand' phase and fully sitting down during the 'sit' phase (*i.e.* not hovering over the chair). The Timed-Up-and-Go consisted of participants rising from a chair, walking 2.4m and turning around, to then walk back and sit down; participants were asked to do this as quickly and as safely as possible. The 2-minute Step Test is a proxy for walking endurance and consisted of participants marching on the spot attempting to get as many 'steps' as possible in 2 min. For a 'step' to count, participants' knees had to be above a mark on the wall that is halfway between their iliac crest and lateral femoral epicondyle. These tests were chosen as they have lower body significance and both the neuromuscular testing and single muscle fibre experiments are of the quadriceps.

3.6 Neuromuscular Assessment Analysis

Peak MVC torque was taken at the highest point on the torque-time curve during the MVC with the highest torque and voluntary activation. To calculate the RTD of the isometric explosive contractions, the average slope of the torque-time trace was calculated during 5 different time epochs: 0-30 ms, 0-50 ms, 0-100 ms, 0-200 ms, and 0-500 ms. Onset occurred when the torque trace exceeded 3 standard deviations above baseline (Figure 1). An average of the 3 trials was used for each participant. Peak torque was the highest torque reached during the isometric explosive contractions used to calculate RTD. Torque for both the MVC and RTD calculation was low pass filtered with a cutoff of 8 Hz.

Peak velocity was taken as the highest velocity reached during the unloaded isokinetic trials, and peak acceleration of the isokinetic contractions was determined as the fastest instantaneous slope of the velocity trace. An average of the 3 trials was used for each participant. Velocity was low pass filtered with a cutoff of 8 Hz.

Power was calculated by multiplying the torque and velocity traces during the isotonic contractions at each of the loads (10, 20, 30, 40, 50, 60% of MVC) to get a power trace at each load. Peak power was taken as the highest value during the power trace that came before the end of the position trace, so as to avoid the artefact of the dynamometer hitting the end ROM. Torque at peak power was determined by finding the torque value corresponding with the peak power value, and velocity at peak power was found similarly by finding the velocity value corresponding

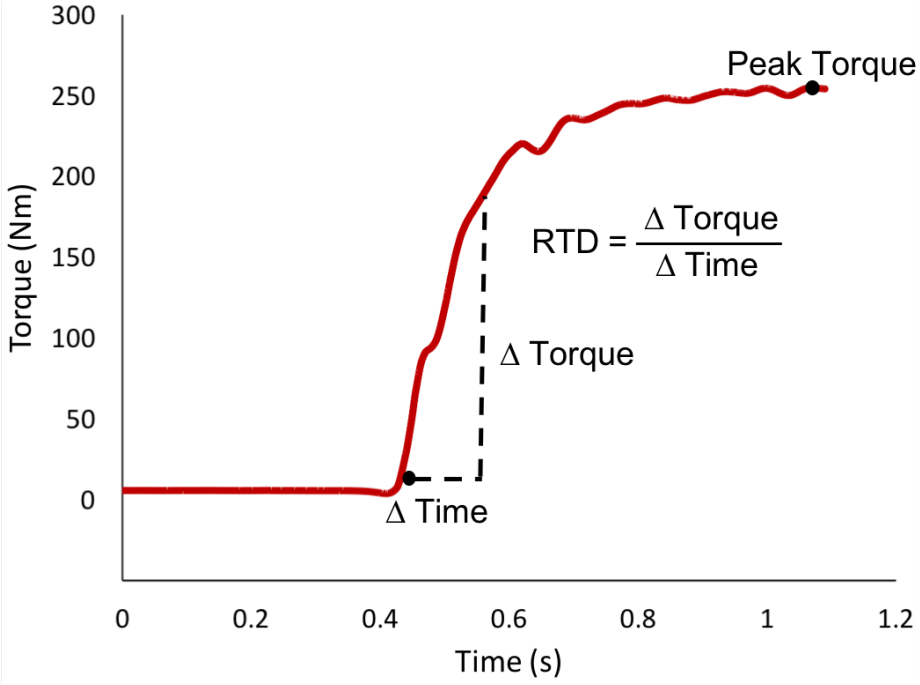

Figure 1: Representative raw torque-time trace used for the calculation of rate of torque development (RTD). Dashed lines illustrate the change in time (Δ Time) and the change in torque (Δ Torque).

to peak power. Peak RTD and peak acceleration were determined by calculating the highest instantaneous slope of the torque and velocity traces, respectively.

3.7 Single Fibre Contractile Experiments

3.7.1 Biopsy and Muscle Fibre Preparation

For the single muscle fibre experiments, a percutaneous muscle biopsy was taken from the *vastus lateralis* from each participant. The muscle sample was put into storage solution (relaxing-glycerol 50:50 with protease inhibitors) to permeabilize at 4°C for 24 hours. Then the muscle sample was then placed in fresh storage solution at -20°C for at least 1 week and up to 4 weeks. On testing days, the sample was placed in a petri dish with fresh storage solution, and a strip was cut from which to dissect single fibres. Single fibres were dissected underneath a binocular microscope and placed into relaxing solution for ~1 min. Fibres were transferred into a temperature-controlled chamber filled with relaxing solution and tied with nylon suture knots

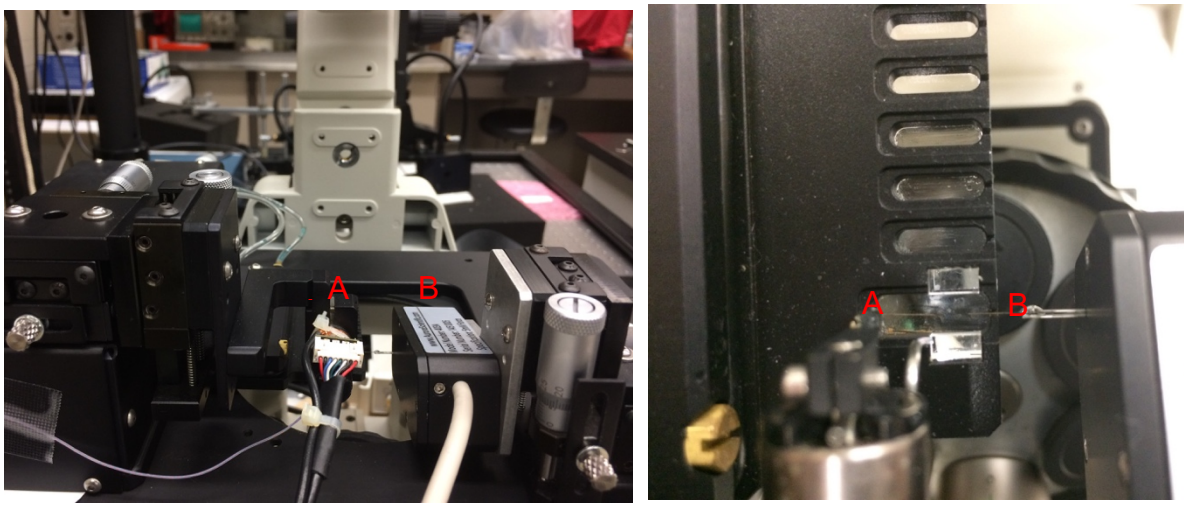

Figure 2: Left picture: force transducer (A) and length controller motor (B). Right picture: pin attached to force transducer (A) and pin attached to length controller (B).

between a force transducer (model 403A; Aurora Scientific, Toronto, ON, Canada) and a length controller (model 322C; Aurora Scientific, Figure 2).

3.7.2 Single Fibre Solutions

The storage solution, which was used for both storage and skinning of muscle samples, was composed of (in mM): KPr (170), EGTA (5), $MgCl_2$ (5.3), Imidazole (10), Na_2ATP (21.2), NaN_3 (1), Glutathione (2.5), Leupeptin (0.05), Glycerol (50% v/v).

The relaxing solution, which was used for dissection and single fibre experiments, was composed of (in mM): Imidazole (59.4), K.MSA (86), $Ca(MSA)_2$ (0.13), $Mg(MSA)_2$ (10.8), K_3EGTA (5.5), KH_2PO_4 (1), H_2O, Leupeptin (0.05), Na_2ATP (5.1).

The pre-activating solution was composed of (in mM): KPr (185), MOPS (20), $Mg(CH_3COOH)_2$ (2.5), ATP (2.5). All solutions were adjusted to a pH of 7.0 with the appropriate acid (propionic acid) or base (KOH).

The activating solutions were composed of the same ingredients in various amounts depending on how much Ca^{2+} was needed in each solution (Table 1). The composition of solutions was determined using a computer program that calculates the equilibrium concentration of ligands and ions based on published affinity constants. 250 units/ml of creatine phosphokinase was used in each activating solution. The pH of all solutions was adjusted to 7.0 with the appropriate acid (HCl) or base (Tris).

Table 1: Amount of each component of the activating solution for each pCa level (mM).

pCa	Ca^{2+}	Mg^{2+}	EGTA	MOPS	K^+	Na^+	CP	ATP
5.0	14.27	6.94	15	80	43.28	13.36	15	6.13
5.3	13.57	6.96	15	80	43.28	13.38	15	6.13
5.5	12.84	6.97	15	80	43.28	13.39	15	6.13

5.7	11.83	6.99	15	80	43.28	13.4	15	6.13
6.0	9.77	7.02	15	80	43.28	13.4	15	6.13
6.2	8.11	7.05	15	80	43.28	13.41	15	6.13
6.7	4.07	7.12	15	80	43.28	13.41	15	6.13

3.7.3 Experimental Protocol

The average sarcomere length (SL) was measured using a high-speed camera (Aurora Scientific, Aurora, ON) which was set at ~2.8µm to allow it to shorten to ~2.7µm, within the known optimal length range for force production of human quadriceps muscle (Walker & Schrodt 1974). Fibre length (L_o) was recorded, and fibre diameter were measured at three points along the fibre using a reticule on the microscope and micro-manipulators to move the fibre into place along the ruler, and cross-sectional area (CSA) was calculated assuming circularity. Chemical activation of single fibres was induced by transferring from the relaxing to pre-activating bath (reduced Ca^{2+}-buffering capacity), and then to an activating solution (Ca^{2+} and high ATP). A 'fitness' contraction was performed first in pCa 4.2 to ensure the ties were not loose and the fibre was in good condition. After the fitness test, SL was re-checked and, if necessary, re-adjusted to ~2.8µm.

To determine the force-pCa relationship, 7 pCa levels were used: 6.7, 6.2, 6.0, 5.7, 5.5, 5.3, 5.0. Single fibres were transferred to an activating bath, then allowed to develop force for ~30 sec. The highest force reached during the 30 s window was taken as P_o at each pCa level. To analyze the calcium sensitivity of each single fibre, P_o at each pCa level was normalized to the maximal force elicited by that fibre and the data was fit using a modified Hill equation programmed in

custom software (MatLab R2014b), where P/P_o is the relative force at a given pCa value and h is the Hill coefficient:

$$\frac{P}{P_o} = \frac{1}{(1 + 10 \text{ ^ } (-h \cdot (pCa_{50} - pCa))}$$

The pCa value at which 50% of maximal force was elicited (pCa_{50}) was used to determine differences in calcium sensitivity.

Once force was developed, a length step was induced to measure rate of force redevelopment (k*tr*). This was done by rapidly shortening the fibre with a ramp of 10 L_o/s by 15% of L_o and then a rapid (500 L_o/s) re-stretch back to L_o. The rapid shortening causes all cross-bridges to break, and then the re-stretch allows further dissociation of any remaining cross-bridges and redevelopment of force independent of Ca^{2+}-dependent regulatory proteins at L_o. A mono-exponential equation, $y = a (1- e^{-kt}) + b$, was fit to the redevelopment curve to determine k*tr*.

Instantaneous stiffness (*k*) tests were used to measure the proportion of attached cross-bridges during the k*tr* test, after force redevelopment. Instantaneous stiffness was assessed by inducing a rapid (500 L_o/s) stretch of 0.3% of L_o and dividing the change in force during the stretch by the length step.

Unloaded shortening velocity (V_o) was measured at the end of the experiment using the slack-test method. Three separate slack tests were performed on each fibre, shortening by 5%, 10%, and 15% L_o. The fibre reached plateau force and then the length step was performed rapidly, causing the force to drop to zero. Force then redeveloped over time proportional to the amount of shortening. The fibre was then transferred back to the relaxing bath and the length was returned to L_o and SL was re-checked and reset to ~2.8µm if necessary. The data resulting from the three slack tests were plotted and a linear regression was performed with the slope of the resulting line taken as V_0. All experiments were completed at 16°C.

3.7.4 Fibre Typing

Fibres were removed from the testing apparatus and placed into a homogenization buffer (containing 61 mM tris (pH 6.8), 11% (v/v) glycerol, 2.78 % (w/v) SDS, 5% 2-β-mercaptoethanol, and 0.02% (w/v) bromophenol blue) for fibre typing via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The 7% separating gel consisting of 2M tris HCL (pH 8.6), 50% glycerol, 10% sodium dodecyl sulfate (SDS), and 40% (w/v) acrylamide and N,N' -methylenebis-acrylamide with a monomer to crosslinker ratio of 37.5:1. The stacking gel consisted of 500 mM tris HCL (pH 6.7), 10% SDS, and 40% (w/v) acrylamide and N,N' -methylenebis-acrylamide with a monomer to crosslinker ratio of 37.5:1. Gels were run at a constant voltage of 50 V for approximately 40 hours at 4°C. Fibre TI and TII (IIA and IIX were not differentiated in this analysis) were determined by comparing to a standard protein ladder with known molecular

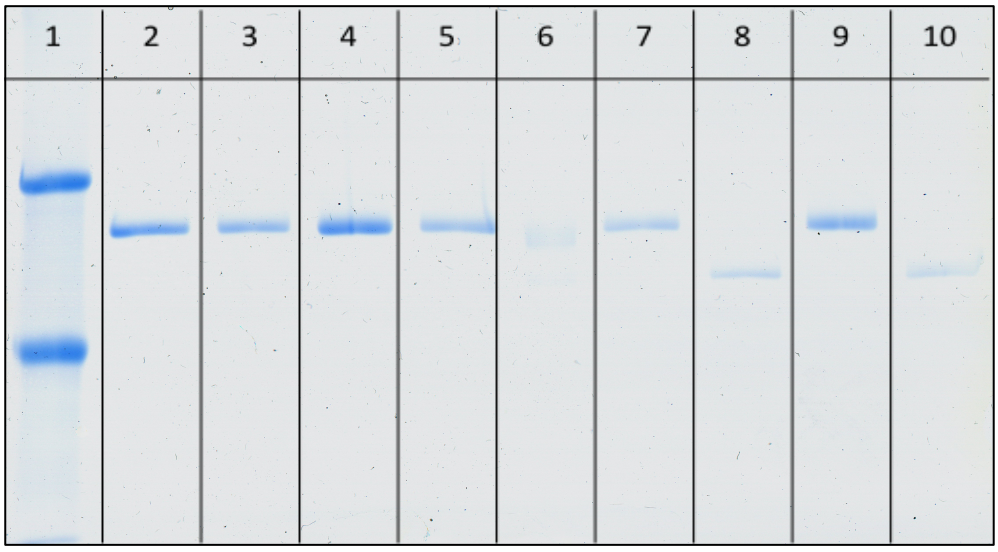

Figure 3: Representative picture of a gel. Standard protein ladder in lane 1; top band 250 kDa, bottom band 150 kDa. Lanes 2-5,7 & 9 represent TIIA fibres, lane 8 & 10 represent TI fibres. Lane 6 was a rat diaphragm homogenate.

weights (Figure 3). Fibres were in the buffer for at least 48 hrs at -20°C before typing, however some were stored in -80°C for up to 6 months.

3.8 Statistical Analysis

Independent samples t-tests were run between young and older adult groups to assess differences in the neuromuscular measurements: RTD, peak torque (PT), voluntary activation, peak velocity (PV), torque at PV, peak acceleration, peak power (PP), torque at PP, and velocity at PP. The YPAS results and physical function measures from the SFT were also compared using paired t-tests.

To determine differences in cellular measures (P_o, specific force, k*tr*, and stiffness), a 3-way repeated measures ANOVA was run with a Bonferroni correction, with age and fibre type as between groups factors and pCa level as the within group factor. To analyze differences in V_0 and pCa_{50}, a 2-way ANOVA for age and fibre type was used. Linear regressions were run between the calculated pCa_{50} and k*tr* values for both age groups and fibre types to determine if there was an association between Ca^{2+}-sensitivity and k*tr*.

To determine if there was a relationship between whole muscle RTD and single fibre k*tr*, linear regressions were performed. As each individual had multiple single fibres tested, fibres for one individual were averaged in order to create one data point to correlate with whole muscle RTD. Young type I fibres were averaged per participant and then correlated with RTD at 0-30ms, 0-50ms, 0-100ms, 0-200ms, and 0-500ms, and the same was done for young type II, old type I, and old type II. Additional correlations were performed with ages grouped within fibre type (*i.e.* young and old type I), and fibre types grouped (*i.e.* type I and II within young).

Significance was set at an alpha of 0.05 and all statistical analysis was done with SPSS (IBM SPSS Statistics Version 25). All data presented as mean ± SEM.

4 Results

4.1 Participant Characteristics

4.1.1 Anthropometrics

Due to difficulties with some single fibre experiments, 2 participants from each group were excluded, resulting in n=8 for both the young and old group. The young group was significantly different in age from the old group ($p < 0.05$), but height and weight were not significantly different ($p=0.23$ and 0.14, respectively; Table 2).

4.1.2 Physical Activity Survey

Physical activity over the last year, as measured by the YPAS, was not significantly different between the young and old groups for TT ($p=0.714$), estimated TEE ($p=0.973$), and the AI representing overall monthly activity ($p=0.117$). The indices in the YPAS represented different aspects of activity (VA, LW, Mov, ST) and inactivity (Sit). The VA, LW, and ST indices were not significantly different between young and old ($p < 0.05$), however the Mov index (movement throughout a typical day) was ~24% lower in the young group compared to old ($p < 0.05$) and the Sit index (time spent sitting in a typical day) ~30% lower in the young group compared to old ($p < 0.05$; Table 2).

Table 2: Anthropometrics (Anthro); age (year), height (cm), weight (kg). Physical activity survey (YPAS); total time (TT; hrs/week), total estimated energy expenditure (TEE; kcal/week); indices: vigorous activity (VA), leisurely walking (LW), daytime moving (Mov), daytime standing (ST), daytime sitting (Sit), total activity index (AI). Senior fit test (SFT); 30 sec chair stand (30CST), timed up and go (TU&G), 2 minute step test (2MST). Young (Y) and old (O). *significantly different from young, $p < 0.05$.

Anthro	**Age**			**Height**			**Weight**	
Y	26.4±1.5			179±3.3			83.9±4.2	
O	70.1±2.8*			174±2.0			74.8±3.4	
YPAS	**TT**	**TEE**	**VA**	**LW**	**Mov**	**ST**	**Sit**	**AI**
Y	23.7±5.5	105±21.4	35±2.9	18±2.1	6.4±0.35	4.9±0.51	-3.0±0.18	61.8±4.1
O	21.3±2.5	105±14	44±3.9	17±5.2	8.4±0.75*	4.9±0.74	-2.1±0.28*	71.9±6.5
SFT	**30CST**			**TU&G**			**2MST**	
Y	20.8±1.2			5.0±0.13			114±7.9	
O	17.2±0.72*			6.0±0.37*			101±6.6	

4.1.3 Functional Fitness Tasks

The modified SFT was used as a measure for functional fitness tasks in both the young and old groups. The young adults scored ~2 more stands during the 30 sec Chair Stand ($p < 0.05$) and were ~15% faster during the Timed-Up-and-Go ($p < 0.05$) compared to the old group. There was no difference between the young and old groups for the 2-minute Step Test ($p=0.27$; Table 2).

4.2 Neuromuscular measures

4.2.1 Peak Torque and Voluntary Activation

Peak MVC torque was ~37% higher in the young group than the old group ($p < 0.05$; Figure 4), and voluntary activation was not different between groups ($p=0.33$; Figure 4).

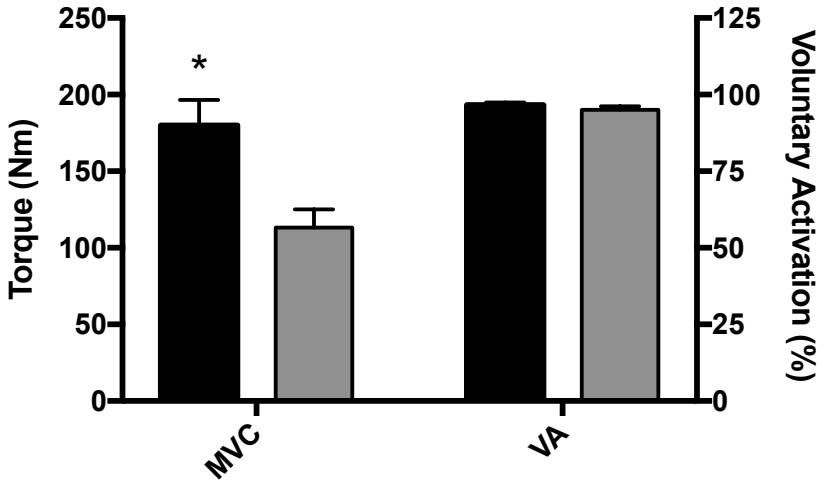

Figure 4: Peak torque from maximal voluntary contractions (MVC; Nm) for young (black) and old (grey) on the left Y axis. Voluntary activation (VA; %) during the MVCs for young (black) and old (grey), not significantly different from each other p=0.16. *significantly different from old, $p < 0.05$.

4.2.2 Rate of Torque Development

Peak torque during the explosive isometric contractions (that were used to determine RTD) was ~30% higher in young than old ($p < 0.05$; Figure 5). RTD was analyzed at different time epochs during the initial part of the contraction: 0-30 ms (T1), 0-50 ms (T2), 0-100 ms (T3), 0-200

ms (T4), and 0-500 ms (T5). There was no difference at T1 between the young and old groups (p=0.124). At T2, young RTD was trending to be higher than old RTD (p=0.067). Young RTD was ~43% higher than old at T3 and ~40% higher at T4 ($p < 0.05$). At T5, young RTD was again trending to be higher than old (p=0.071; Figure 5).

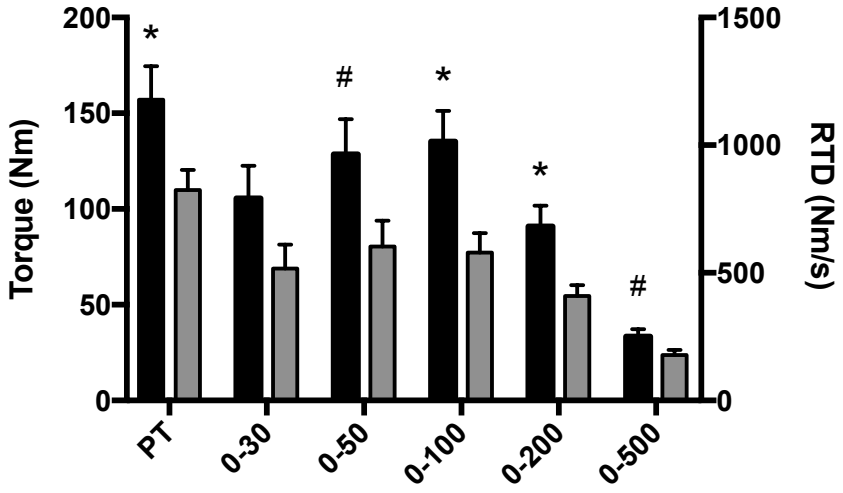

Figure 5: Peak torque (PT; Nm; left axis) and rate of torque development (RTD; Nm/s; right axis) for young (black) and old (grey). RTD measured at time epochs 0-30 ms, 0-50 ms, 0-100 ms, 0-200 ms, and 0-500 ms. #trending at $p < 0.1$, *significantly different from old, $p < 0.05$.

4.2.3 Peak Velocity and Power

The young group were ~11% faster during the unloaded isokinetic contractions compared to the old group ($p < 0.05$). Peak acceleration during the same contractions was also ~33% faster in young compared to old ($p < 0.05$; Figure 6).

Peak power was measured during isotonic contractions at 10, 20, 30, 40, 50, and 60% of MVC torque, and young were ~56% more powerful on average across isotonic loads compared to old ($p < 0.05$; Figure 7). Young and old both reached peak power at an isotonic load of 10% MVC. Torque and velocity at peak power was determined and young was ~42% and ~26%, stronger and faster than old at every load as well, respectively ($p < 0.05$; Figure 8). Both RTD and peak

acceleration were calculated at each isotonic load, and again, young had ~37% and ~45%, respectively, higher values compared to old ($p < 0.05$; Table 3).

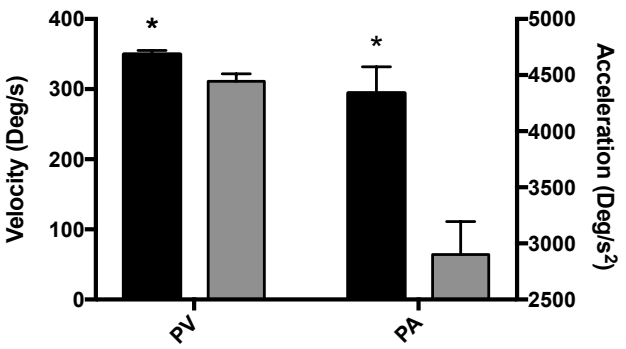

Figure 6: Peak velocity (PV; deg/s; left axis) and peak acceleration (PA; deg/s^2; right axis) during unloaded isokinetic contractions for young (black) and old (grey). *significantly different from old, $p < 0.05$.

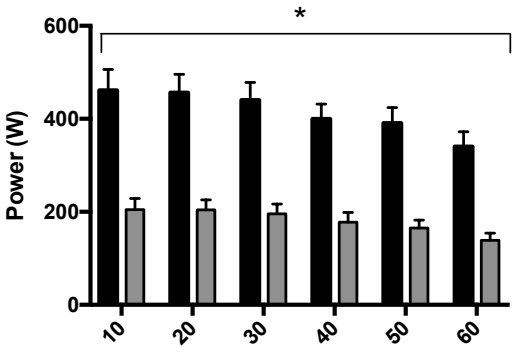

Figure 7: Peak power (W) for young (black) and old (grey) at isotonic loads 10, 20, 30, 40, 50 & 60% of maximal voluntary contraction. *significantly different from old, $p < 0.05$.

Table 3: Peak rate of torque development (RTD) and peak acceleration (PA) calculated at each isotonic load (10, 20, 30, 40, 50, 60% of maximal voluntary contraction). *significantly different from young, $p < 0.05$.

ISOT Load		10%	20%	30%	40%	50%	60%
RTD	Y	994.9±82	1174±82	1228±121	1369±170	1414±130	1325±161
	O	670.8±63*	762.1±82*	802.7±93*	801.3±83*	846.0±96*	806.3±98*
PA	Y	1829±205	1523±67	1307±76	1278±172	1125±135	914.9±162
	O	1015±87*	869.2±64*	807.1±71*	680.4±61*	579.3±53*	447.9±56*

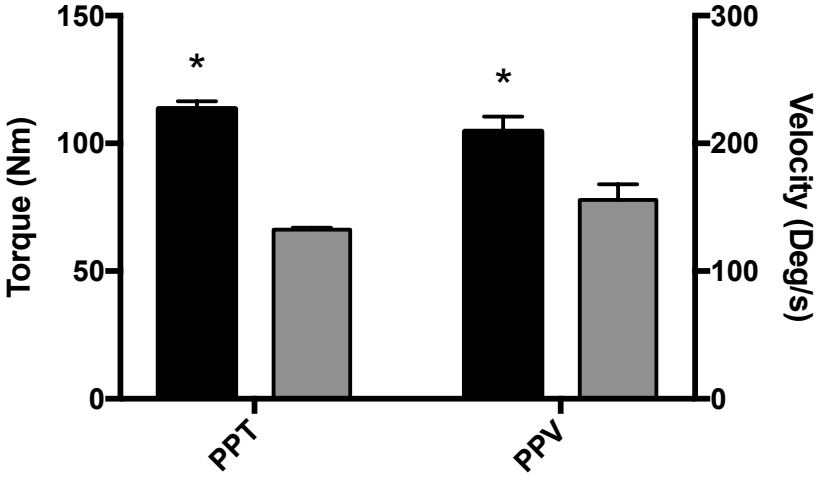

Figure 8: Average torque (PPT, Nm) and velocity (PPV, deg/s) at peak power across isotonic loads for young (black) and old (grey). *significantly different from old, $p < 0.05$.

4.3 Single Muscle Fibre Measurements

4.3.1 Single Muscle Fibre Characteristics

Of ~182 single muscle fibres (~88 from old and ~94 from young) that were tested, 83 fibres from young and 65 fibres from old were successful, resulting in a ~74% and ~88% success rate for young and old, respectively. However, due to experimental errors with the chemical solutions for single fibre testing, 4 participants' fibres had to be excluded based on the solutions the testing was completed in. Therefore, the following results are based on 67 fibres from young and 55 fibres from old. After fibre typing via SDS-PAGE, 27 TI and 40 TII fibres in the young group, and 42 TI and 13 TII fibres in the old group were identified. No hybrid fibres were identified in the successful tests (Figure 3). Single fibre CSA was not different within age groups (young, $p=0.44$; old, $p=0.19$) or within TI fibres from young and old ($p=0.11$). However, CSA was different within TII fibres between young and old ($p < 0.05$).

4.3.2 Isometric Single Fibre Contractile Properties

For ease of reading, the numerical differences reported in text are from tests at pCa 5.0, and values at the other pCa levels can be found in the figures. Young TII fibres had the highest P_o and were ~31% stronger than young TI, ~48% stronger than old TI, and ~43% stronger than old TII, and young TII had the highest force at pCa 6.2 and above ($p < 0.05$; Figure 9, 12 & 13). Young TI fibres were ~33% stronger than old TI and had higher force at pCa 6.0 and above ($p < 0.05$; Figure 11) but were not different from old TII ($p=0.233$). Old TI and TII fibres were not different from each other at any pCa level ($p=0.545$; Figure 10). There was a main effect of pCa on force for all groups ($p < 0.05$; Figures 9-13), such that with increasing [Ca^{2+}] (*i.e.* pCa 6.7 to 5), there was an increase in plateau force.

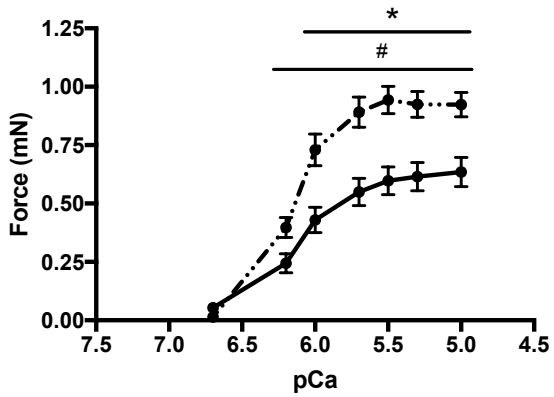

Figure 9: Average single muscle fibre plateau force at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (solid line) and young type II (dashed line). *significantly different from young type I, $p < 0.05$. #significant effect of pCa on plateau force for type I and type II, $p < 0.05$.

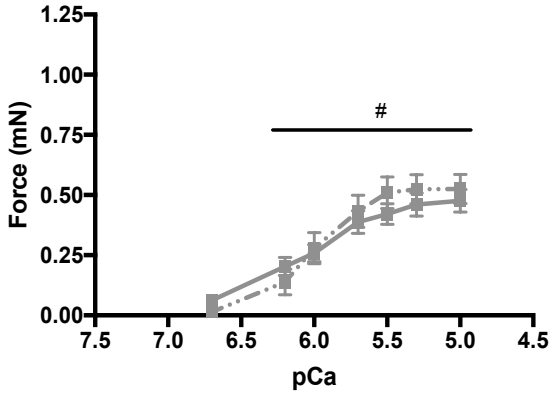

Figure 10: Average single muscle fibre plateau force at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for old type I (solid line) and old type II (dashed line). Not significantly different from each other, $p = 0.764$. #significant effect of pCa on plateau force for type I and type II, $p < 0.05$.

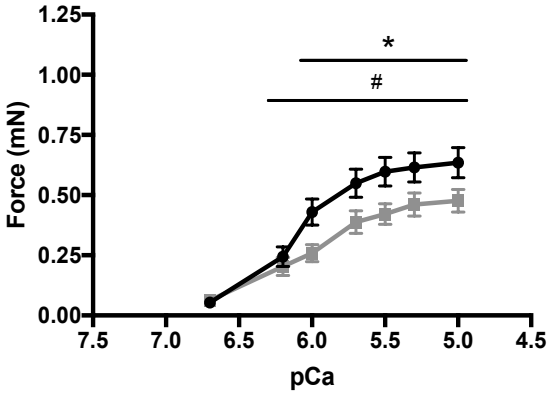

Figure 11: Average single fibre plateau force at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (black) and old type I (grey). *significantly different from old, $p < 0.05$. #significant effect of pCa on plateau force for young and old, $p < 0.05$.

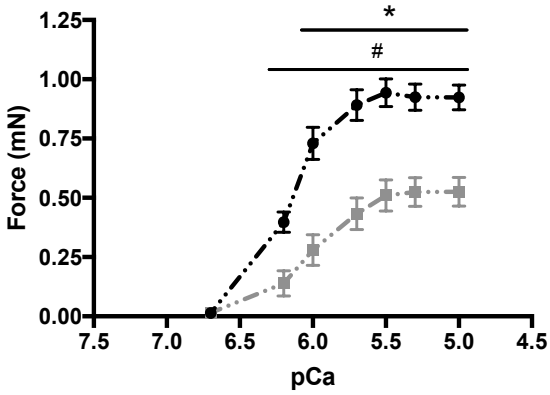

Figure 12: Average single fibre plateau force at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type II (black) and old type II (grey). *significantly different from old, $p < 0.05$. #significant effect of pCa on plateau force for young and old, $p < 0.05$.

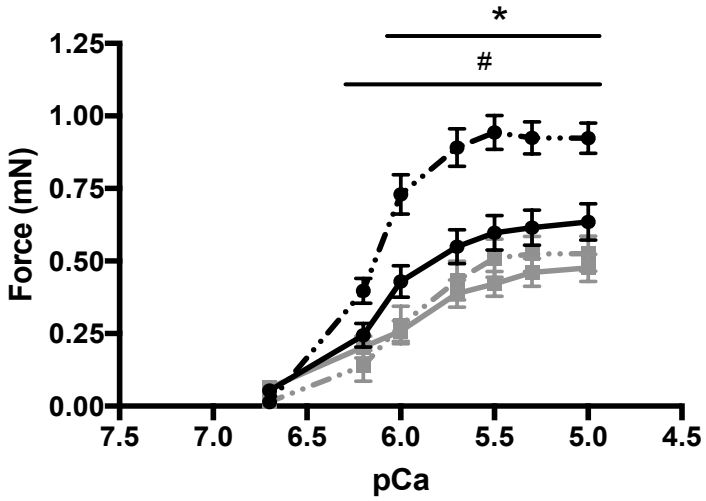

Figure 13: Average single fibre plateau force (P_0) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2 & 6.7) for each group: young (black circles), old (grey squares), type I (solid line), type II (dashed line). *significantly different from young and old type I, and old type II, $p < 0.05$. #significant effect of pCa on plateau force for all groups, $p < 0.05$.

When P_o was normalized to CSA to determine SF, young TII had a ~23% higher SF than young TI fibres ($p < 0.05$; Figure 14) but were similar to old TII ($p=0.273$; Figure 17). SF of young TI fibres and old TI fibres (Figure 16) were not different from each other ($p=0.419$). Old TI and TII fibres also had a similar SF ($p=0.190$; Figure 15). Again, there was a main effect of pCa on SF on all groups, such that with increasing $[Ca^{2+}]$, SF also increased ($p < 0.05$; Figures 14-17).

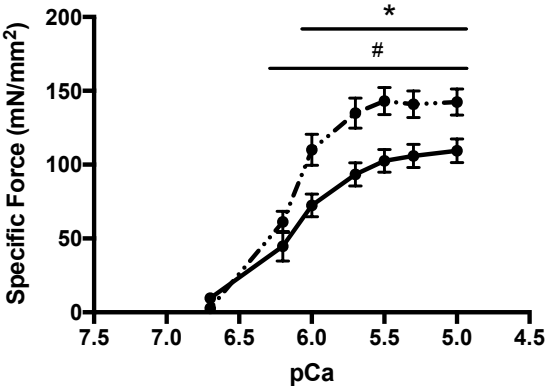

Figure 14: Average single fibre specific force (mN/mm^2) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (solid line) and type II (dashed line). *significantly different from type I, $p < 0.05$. #significant effect of pCa on specific force for type I and II, $p < 0.05$.

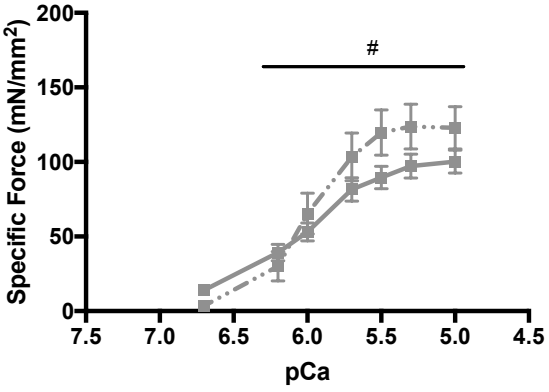

Figure 15: Average single fibre specific force (mN/mm^2) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for old type I (solid line) and type II (dashed line). Not significantly different from each other, $p = 0.287$. #significant effect of pCa on specific force for type I and II, $p < 0.05$.

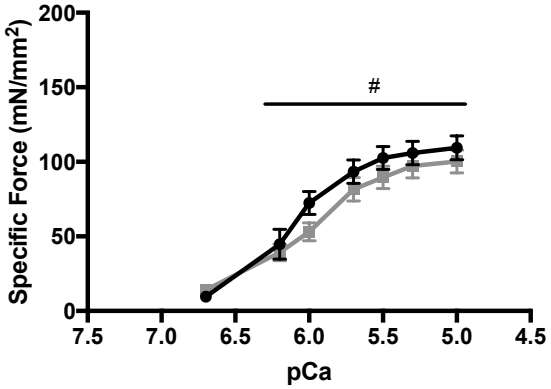

Figure 16: Average single fibre specific force (mN/mm^2) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (black) and old type I (grey). Not significantly different from each other, p = 0.321. $^{\#}$significant effect of pCa on specific force for young and old, p < 0.05.

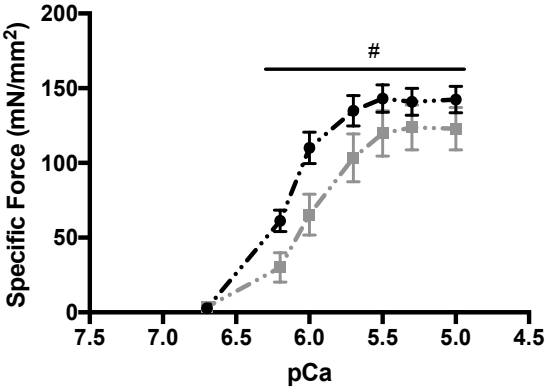

Figure 17: Average single fibre specific force (mN/mm^2) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type II (black) and old type II (grey). Not significantly different from each other, p = 0.115. $^{\#}$significant effect of pCa on specific force for young and old, p < 0.05.

4.3.3 Kinetic Single Fibre Contractile Properties

TII fibres were ~80% faster based on V_0 than TI fibres for both young and old ($p < 0.05$). Young and old TII fibres were not different from each other (p=0.665) nor were young and old TI fibres (p=0.902; Figure 18).

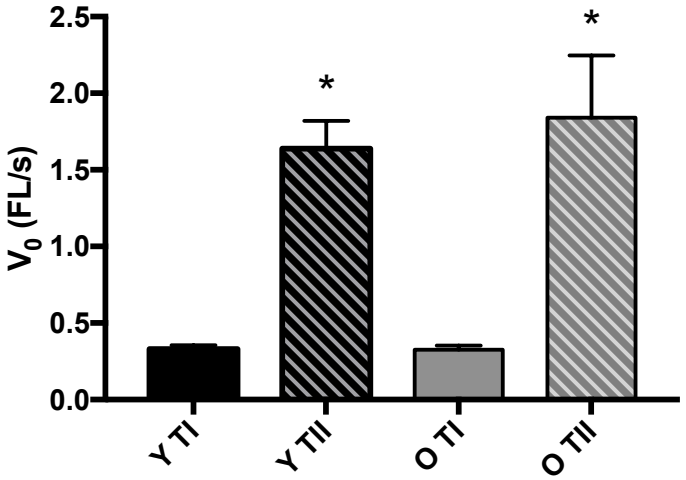

Figure 18: Maximal unloaded shortening velocity (V_0; FL/s) for young type I (Y TI), young type II (Y TII), old type I (O TI), and old type II (O TII) single fibres. Type II fibres had significantly faster V_0 than type I fibres. *significantly different from type I, p < 0.05.

Stiffness was measured at one time point preceding the k*tr* measure. Within age groups, stiffness was not different between TI and TII fibres ($p > 0.05$; Figures 19 & 20). However, within fibre type, young TI and TII were ~30% and ~45% stiffer than old TI and TII fibres, respectively ($p < 0.05$; Figures 21 & 22). There was also an effect of pCa on stiffness in all groups, such that stiffness increased with increasing [Ca^{2+}] ($p < 0.05$). At pCa 6.0 and above, young TI were stiffer than old TI fibres ($p < 0.05$), and at pCa 6.2 and above, young TII were stiffer than old TII fibres ($p < 0.05$; Figures 19-22).

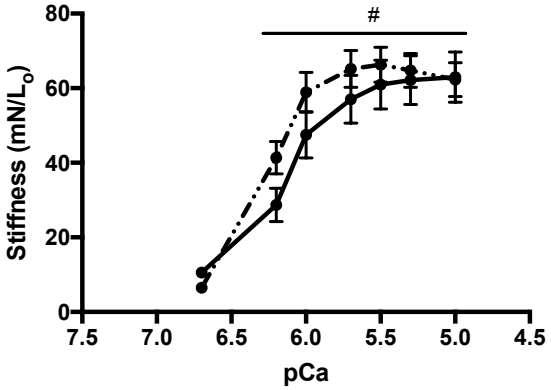

Figure 19: Average stiffness (mN/L_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (solid line) and type II (dashed line). #significant effect of pCa on stiffness for type I and II, $p < 0.05$.

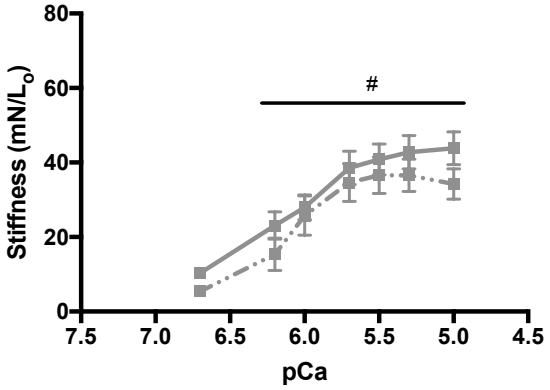

Figure 20: Average stiffness (mN/L_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for old type I (solid line) and type II (dashed line). #significant effect of pCa on stiffness for type I and II, $p < 0.05$.

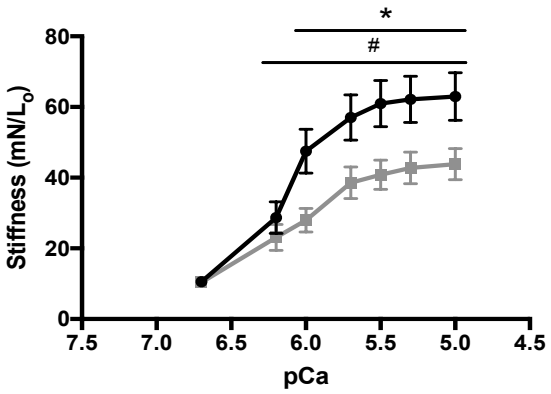

Figure 21: Average stiffness (mN/L_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (black) and old type I (grey). *significantly different from old, $p < 0.05$. #significant effect of pCa on stiffness for young and old, $p < 0.05$.

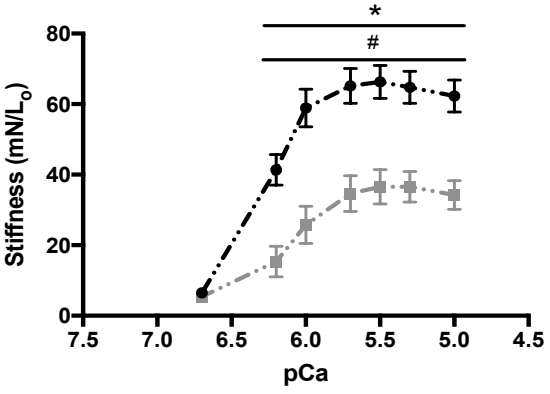

Figure 22: Average stiffness (mN/L_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type II (black) and old type II (grey). *significantly different from old, $p < 0.05$. #significant effect of pCa on stiffness for young and old, $p < 0.05$.

Rate of force redevelopment (k*tr*) was ~48% higher in young TII compared to young TI fibres and ~40% higher in old TII compared to old TI ($p < 0.05$). K*tr* was higher in TII compared to TI fibres at pCa 6.2 and above for both age groups ($p < 0.05$; Figures 23 & 24). Young and old TI fibres had similar k*tr* values at all pCa levels ($p=0.505$; Figure 25), as did young and old TII fibres ($p=0.909$; Figure 26). There was a main effect of pCa on k*tr* for all age groups ($p < 0.05$; Figures 23-26).

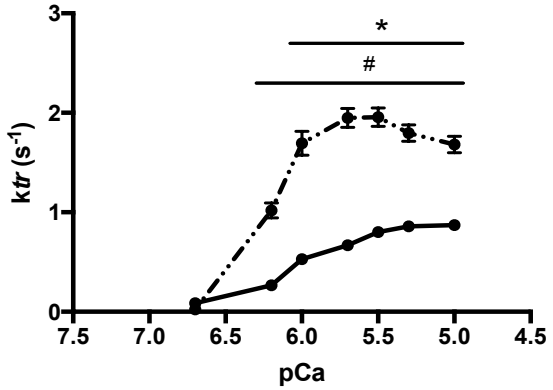

Figure 23: Average rate of force redevelopment (ktr; s^{-1}) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (solid line) and young type II (dashed line). *significantly different from type I, $p < 0.05$. #significant effect of pCa on ktr for type I and II, $p < 0.05$.

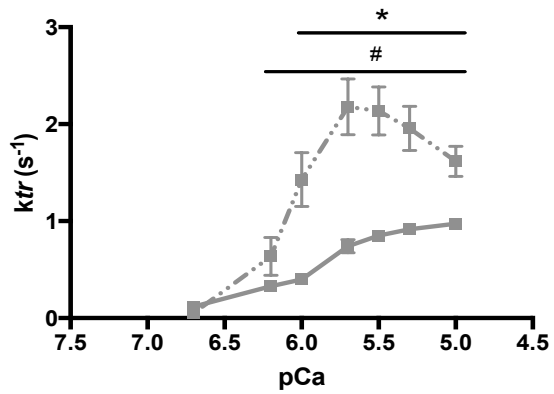

Figure 24: Average rate of force redevelopment (ktr; s^{-1}) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for old type I (solid line) and type II (dashed line). *significantly different from type I, $p < 0.05$. #significant effect of pCa on ktr for type I and II, $p < 0.05$.

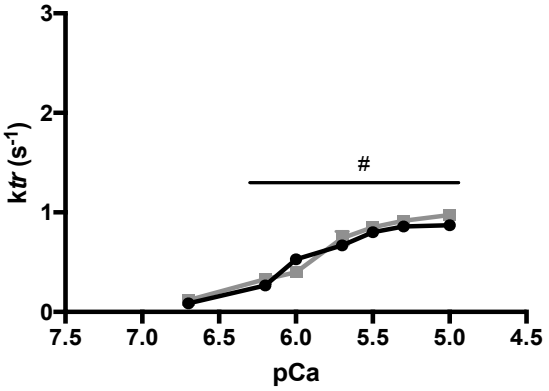

Figure 25: Average rate of force redevelopment (ktr; s^{-1}) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (black) and old type I (grey). Not significantly different from each other, $p = 0.505$. #significant effect of pCa on ktr for young and old, $p < 0.05$.

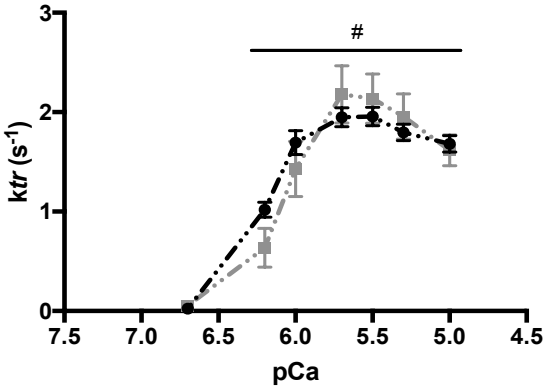

Figure 26: Average rate of force redevelopment (ktr; s^{-1}) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type II (black) and old type II (grey). Not significantly different from each other, $p = 0.909$. #significant effect of pCa on ktr for young and old, $p < 0.05$.

4.3.4 Calcium Sensitivity

Calcium sensitivity was determined by the pCa_{50} value of the Hill equation used to fit the force-pCa curve. Young TI and TII fibres had similar pCa_{50} values (6.01±0.043 vs. 6.06±0.035, p=0.45; Figure 27), as did old TI and TII fibres (5.91±0.039 vs. 5.91±0.052, p=0.941; Figure 28). Old TI fibres had a pCa_{50} shifted to the right, indicating a lower sensitivity, compared to young TI fibres, but it was not significant (p=0.095; Figure 29). However, old TII fibres had a pCa_{50} value significantly shifted to the right compared to young TII fibres ($p < 0.05$; Figure 30).

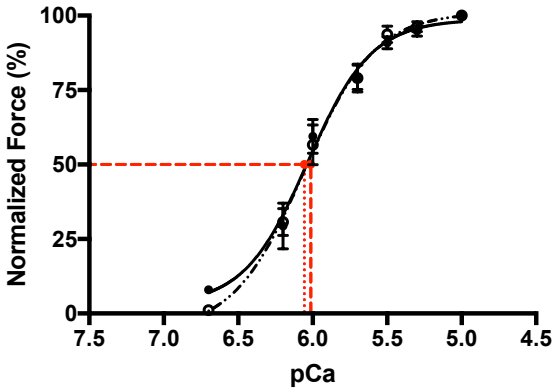

Figure 27: Normalized force (% of P_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (closed circles) and type II (open circles). Sigmoidal line of best fit for young type I (solid line) and type II (dashed line). Red symbols represent the calculated pCa_{50} value, type I = 6.01 (dashed line), type II = 6.06 (dotted line). Not significantly different from each other, p = 0.454.

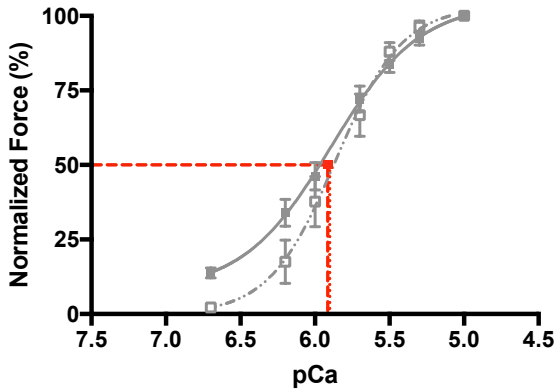

Figure 28: Normalized force (% of P_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for old type I (closed squares) and type II (open squares). Sigmoidal line of best fit for old type I (solid line) and type II (dashed line). Red symbols represent the calculated pCa_{50} value, type I = 5.91 (dashed line), type II = 5.91 (dotted line). Not significantly different from each other, p = 0.941.

4.3.5 Calcium Sensitivity and Rate of Force Redevelopment

Linear regressions were performed to determine a relationship between pCa_{50} and k*tr* for both age groups and fibre types. There was no relationship between pCa_{50} and k*tr* for young TI fibres (p=0.29; Figure 31A), but the relationship was trending toward significance for young TII

fibres (p=0.08; Figure 31B). There were significant relationships between pCa_{50} and k*tr* for both old TI and TII fibres (r^2=0.30, $p < 0.05$; r^2=0.44, $p < 0.05$, respectively; Figures 31C, 31D).

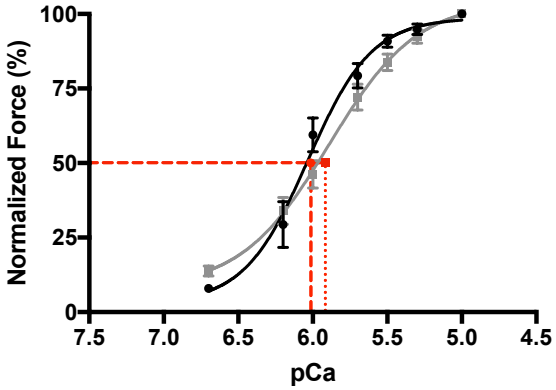

Figure 29: Normalized force (% of P_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type I (Y TI; black circles) and old type I (O TI; grey squares). Sigmoidal line of best fit for Y TI (black) and O II (grey). Red symbols represent the calculated pCa_{50} value, Y TI = 6.01 (black line), O TI = 5.91 (grey line). Trending towards significance, p = 0.095.

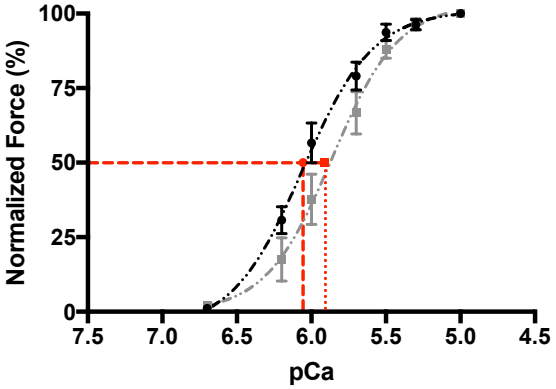

Figure 30: Normalized force (% of P_o) at each pCa level (5, 5.3, 5.5, 5.7, 6.0, 6.2, 6.7) for young type II (Y TII; black circles) and old type II (O TII; grey squares). Sigmoidal line of best fit for Y TII (black) and O TII (grey). Red symbols represent the calculated pCa_{50} value, Y TII = 6.06 (dashed line), O TII = 5.91 (dotted line). Significantly different from each other, p < 0.05.

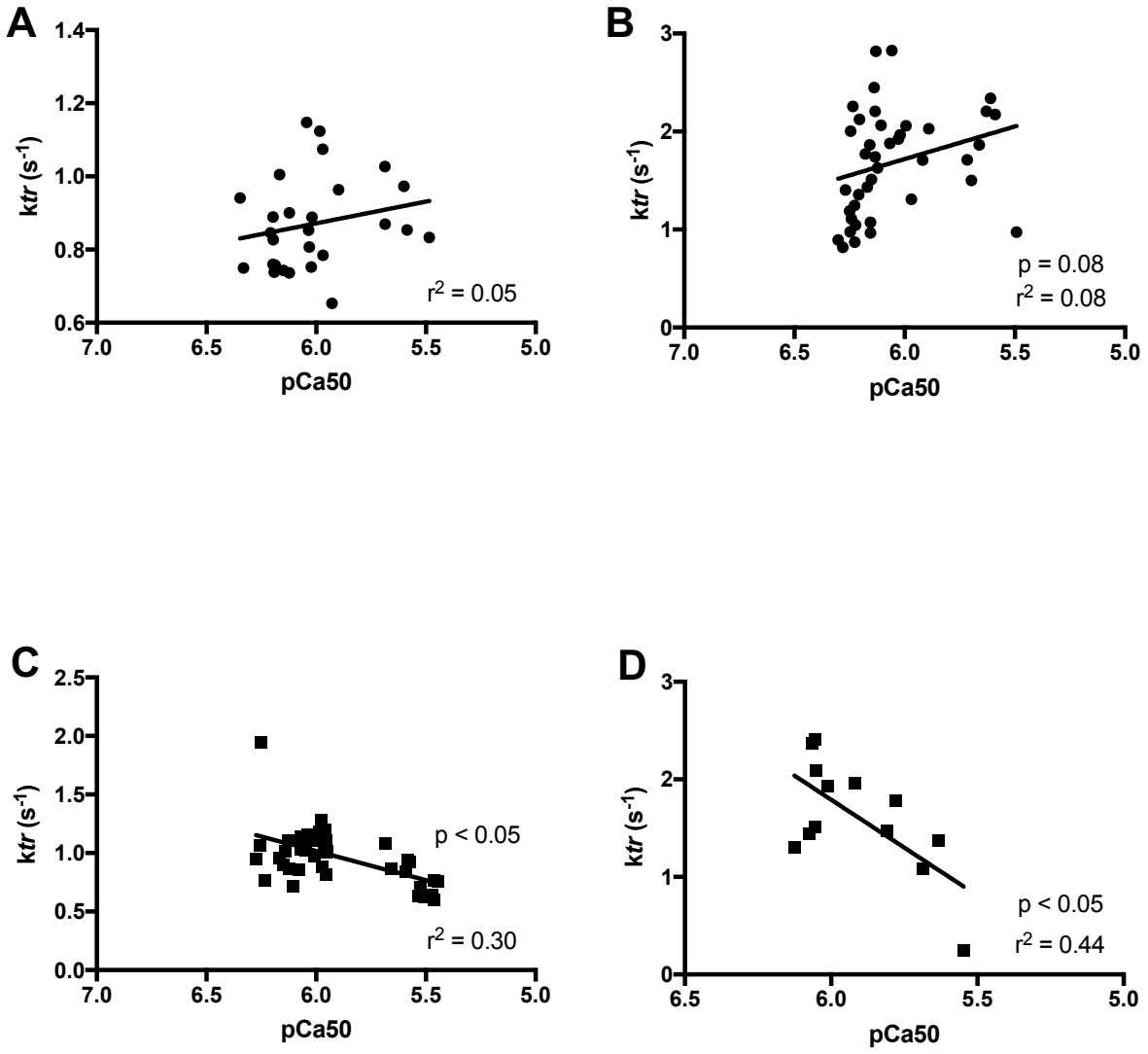

Figure 31: Relationships between pCa_{50} and ktr. Young TI fibres (A), young TII fibres (B), old TI fibres (C), and old TII fibres (D). No relationship for young TI ($p > 0.05$), relationship trending toward significance for young TII ($p = 0.08$); old TI and TII relationships are significant ($p < 0.05$), coefficients of determination (r^2) on graphs.

4.4 Relationship between Whole Muscle and Single Fibres

Linear regressions were run to determine the relationship between RTD of the knee extensors and k*tr* of single muscle fibres from the *vastus lateralis*. The relationship between RTD and young TI or TII fibres was not significant at any of the RTD time epochs (Figures 32A-E & 33A-E). There was also no significant relationship between RTD and old TII fibres at any of the RTD time windows (Figure 35A-E). However, old TI fibres had a moderate strength relationship with trending significance with RTD T1 (r^2=0.48, p=0.055), RTD T2 (r^2=0.49, p=0.052), and RTD T3 (r^2=0.42, p=0.081). There was no significant relationship between old TI fibres and RTD T4 or T5 (Figures 34A-E).

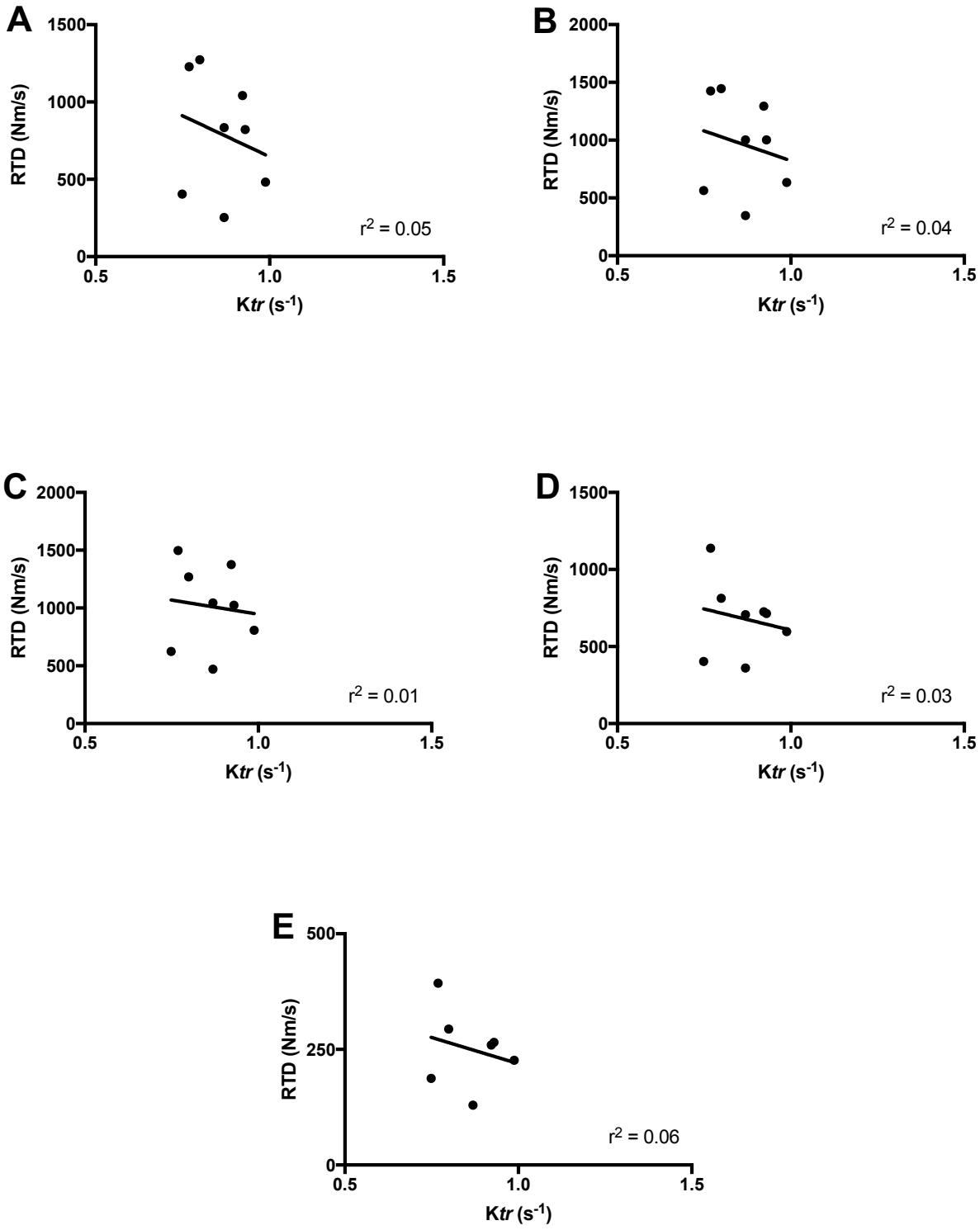

Figure 32: Relationships between rate of torque development (RTD) and ktr for young TI fibres. RTD 0-30ms (A), 0-50ms (B), 0-100ms (C), 0-200ms (D), 0-500ms (E). No significant relationships, $p > 0.05$. Coefficients of determination (r^2) on graphs.

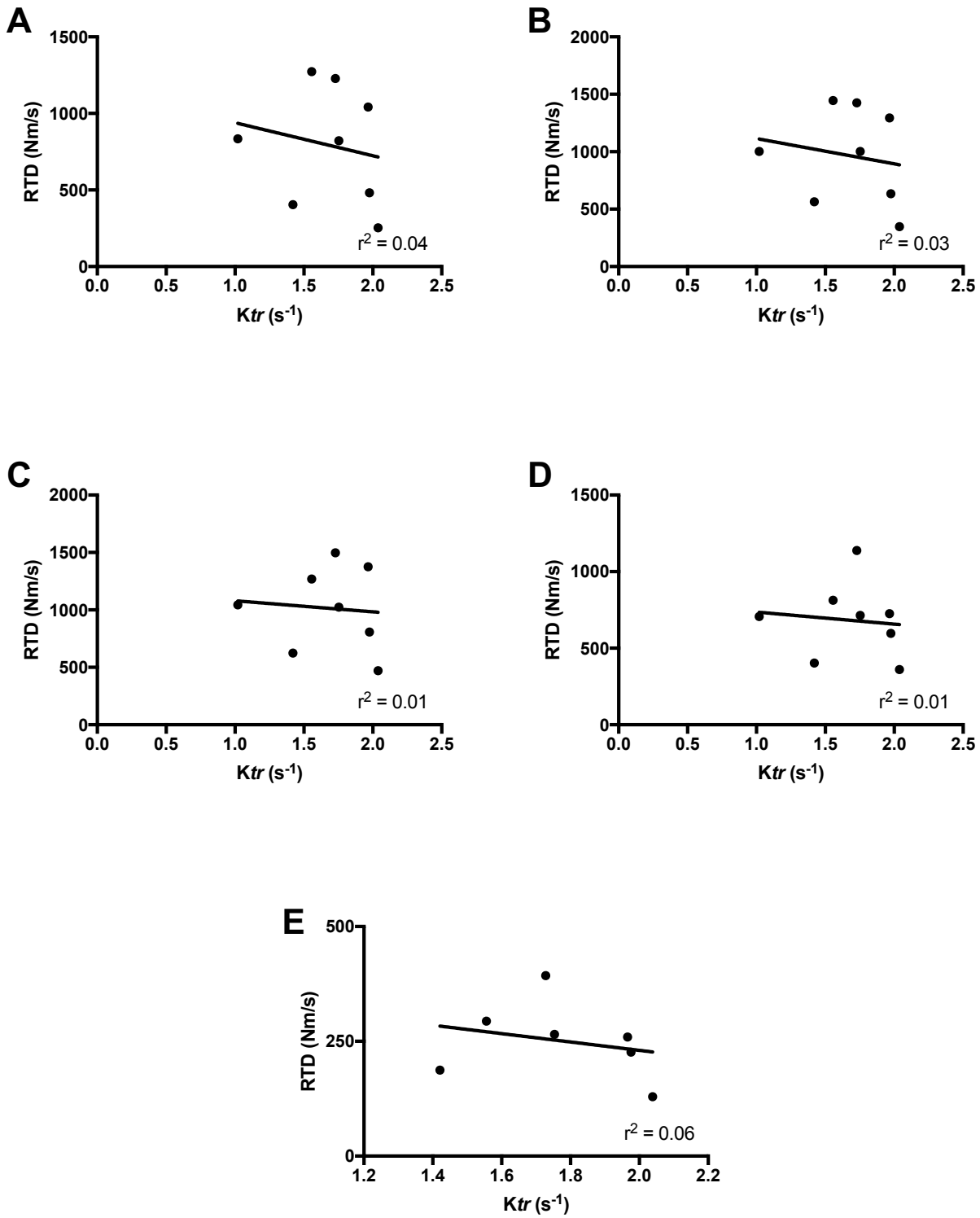

Figure 33: Relationships between rate of torque development (RTD) and ktr for young TII fibres. RTD 0-30ms (A), 0-50ms (B), 0-100ms (C), 0-200ms (D), 0-500ms (E). No significant relationships, $p > 0.05$. Coefficients of determination (r^2) on graphs.

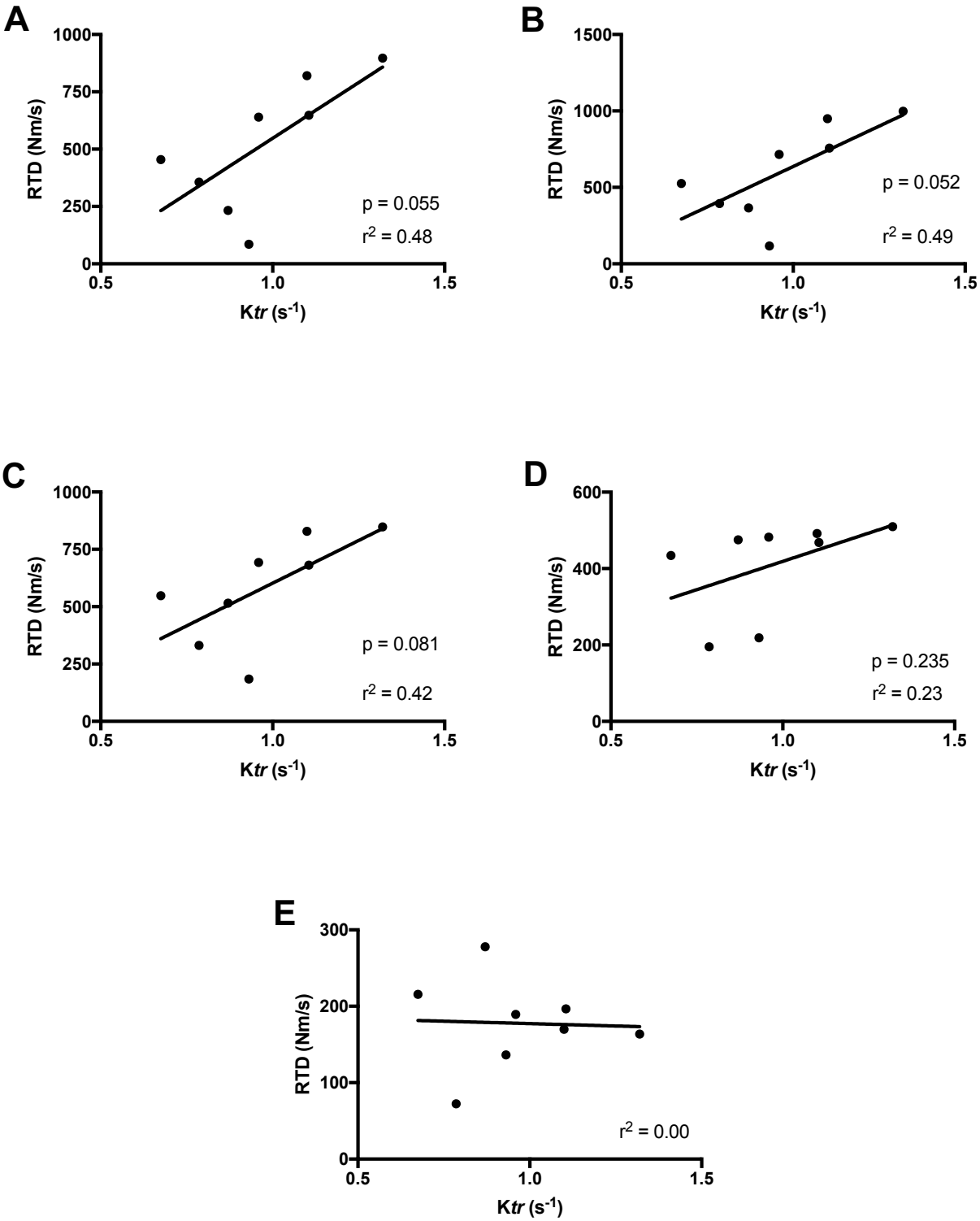

Figure 34: Relationships between rate of torque development (RTD) and ktr for old TI fibres. RTD 0-30ms (A), 0-50ms (B), 0-100ms (C), 0-200ms (D), 0-500ms (E). Moderate strength relationships in A-C trending towards significance, $p < 0.1$. No significant relationships in D or E, $p > 0.05$. Coefficients of determination (r^2) on graphs.

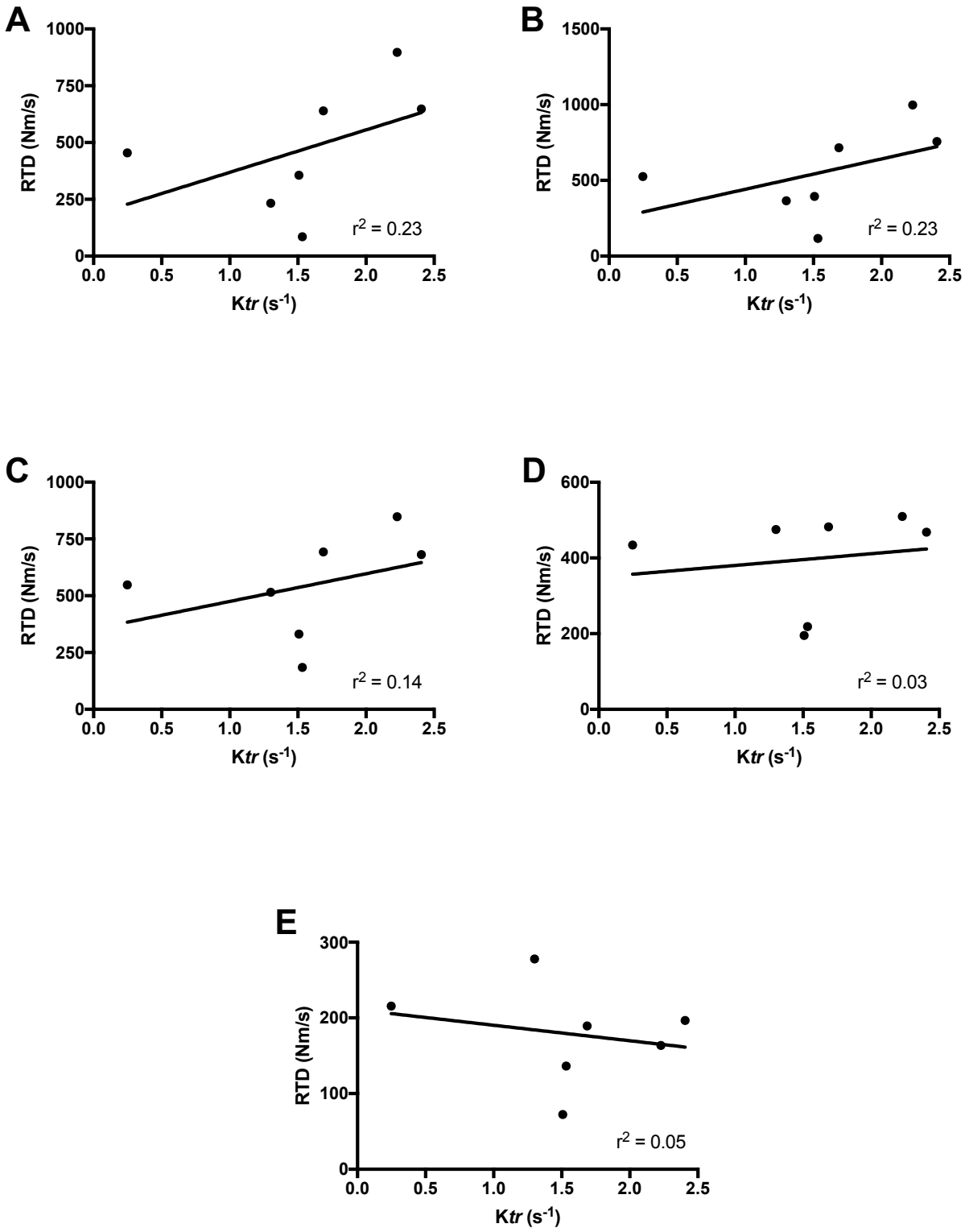

Figure 35: Relationships between rate of torque development (RTD) and ktr for old TII fibres. RTD 0-30ms (A), 0-50ms (B), 0-100ms (C), 0-200ms (D), 0-500ms (E). No significant relationships, $p > 0.05$. Coefficients of determination (r^2) on graphs.

5 Discussion

5.1 Summary of Findings

The aims of this thesis were to determine the effect of Ca^{2+} on the rate of force redevelopment (i.e., k*tr)*, in human single muscle fibres, and to explore the relationship between whole muscle RTD and single fibre k*tr* in healthy young and older adults. These aims were achieved by recruiting healthy, independently-living young and older adults to undergo whole muscle neuromuscular measurements and a muscle biopsy to test single muscle fibre contractile properties. We hypothesized that with increasing [Ca^{2+}] (*i.e.* pCa 6.7 to 4.2), k*tr* would increase, similar to the k*tr*-Ca^{2+} relationship found in animal models, and that single fibres with a higher Ca^{2+} sensitivity, measured by pCa_{50}, would have a higher k*tr* than fibres with a lower Ca^{2+} sensitivity. Additionally, we hypothesized that k*tr* would be linearly related to whole muscle RTD such that a lower k*tr* would be associated with a lower RTD, and that with age the relationship between whole and cellular muscle would be weaker; this would indicate an age-related disconnect between the cell and the whole muscle.

The results in this thesis were consistent with the first hypothesis, confirming that the k*tr*-Ca^{2+} relationship in human single fibres is similar to that found in animal models (Brenner 1988; Metzger & Moss 1988, 1990; Wolff et al. 1995), with an increase in [Ca^{2+}] causing an increase in k*tr* in both TI and TII fibres across age. Additionally, a relationship between pCa_{50} and maximal k*tr* was found in TI and TII fibres of older adults such that fibres which were more sensitive to Ca^{2+} had a higher k*tr*, confirming the second hypothesis. The Ca^{2+}-sensitivity and k*tr* relationship was not found in either TI or TII fibres of young adults. Overall, single fibre k*tr* was not related to whole muscle RTD at any time epoch for young TI or TII fibres or old TII. There were positive moderate-strong relationships in older adults between single fibre k*tr* and whole muscle

RTD at time epochs 0-30, 0-50, and 0-100 ms that were trending toward significance (r^2 = 0.48, 0.49, 0.42, respectively, p = 0.055, 0.052, 0.081, respectively) highlighting the role of 'intrinsic contractile properties' on early RTD (Anderson & Aagaard 2006). The lack of relationship between RTD and k*tr* in young and presence of a potential strong relationship in old TI fibres does not confirm the final hypothesis that with age the relationship between the whole and cellular muscle becomes weaker, as the only close to significant relationships were in the older adult group.

5.1.1 Neuromuscular Measures

Importantly, the young and older adult groups I tested for this project were significantly different in age, and were activity-matched, based on scores from the YPAS (Table 2). The groups being activity-matched is important because it has been shown that physical inactivity may have an effect on single muscle fibre contractile properties that is different from the possible effect of aging (D'Antona et al. 2003, 2007; Miller et al. 2013). Therefore, in having groups that differed in age only, and not activity, we can be more confident that any differences between groups in both whole and cellular muscle were due to the natural process of aging. The only indices where young and old differed were the Mov (amount of time on feet moving throughout a day) and Sit (amount of time sitting throughout the day), where old seemed to move significantly more and sit significantly less (Table 2). However, this is easily explainable by my thesis' young population consisting primarily of students who spend most of their days sitting at a desk or lab bench, and these indices did not have an effect on estimated energy expenditure (TEE) or time spent being physically active (TT).

Despite the young and old group being activity-matched in their day to day life, there were still differences found in the neuromuscular measurements performed. Maximal isometric strength (tested using an MVC with ITT) was ~37% higher in the young compared to old group, while

voluntary activation was high (~96%) and the same across groups. These maximal torque measures are similar to previous studies that have reported knee extensor strength of older adult males being ~45% (Dalton et al. 2012, 2015), ~38% (Frontera et al. 2000), ~35% (Hvid et al. 2013), ~31% (Suetta et al. 2009), and ~44% (Yu et al. 2007) weaker than young adult males. The optimal knee angle for knee extensor torque production is ~130° of extension (or 50° of flexion [Haffajee et al. 1972]) and the knee angle used in this study was 80° of extension positioning the knee extensors to be at a longer length and less optimal position for torque production, therefore the absolute torque reported in this thesis was lower than data from the aforementioned studies.

Peak velocity of the unloaded contraction was 11% faster in the young group compared to old, similar to ~7% slowing from young to old found previously (Larsson et al. 1979). Another group reported a lower V_o of ~20% and ~18% in velocity from young to old in an unconstrained velocity task, however these tasks were performed at a load of 20 and 15% MVC, and so a direct comparison is not possible (Dalton et al. 2012, 2015; respectively), however we can compare to the isotonic load condition in this thesis at 20% MVC where we found a 22% slowing in velocity of old compared to young (unreported). Most studies do not report unconstrained isokinetic velocity values, but rather, velocity values determined from contractions performed with some amount of load, and because of this, there were limited studies (one in this instance) that a direct comparison can be made. However, the main objective of recording velocity was to confirm that my project's older adult population was consistent with the weaker, slower, and less powerful phenotype that is often reported in aging studies on whole muscle.

Age-related power loss was also comparable with other studies reporting power loss from ~35-60% (Bassey et al. 1992; Dalton et al. 2012, 2015; McNeil et al. 2007; Petrella et al. 2005; Trappe et al. 2003), and my study having an average power loss of 56% across %MVC loads. Two

other studies found similar decrements in power of ~30 and ~45% (Dalton et al. 2012; Trappe et al. 2003), however power was tested isokinetically, so a direct comparison of absolute power loss cannot be made. This thesis reported that an isotonic load of 10% MVC elicited the highest power from both the young and old group, which is different from other studies that have reported maximum power in young adults at an isoinertial load of ~40-50% MVC and in older adults at ~30-40% MVC (Petrella et al. 2005). The difference between my study and others is likely because there are inherent limitations of isotonic contractions on a dynamometer, which are the compliance in the system and the inconsistent load on the dynamometer arm throughout a range of motion. However, the outcome of the power measurement was not to determine where peak power occurs, but whether or not absolute peak power is different between the old and young groups, and we found that it was. As both young and old groups were tested on the same system, with the same limitations, we can confidently conclude that absolute power was lower in old compared to young.

Overall, RTD was ~40% lower in the old group compared to young. These results are similar to others that have reported peak RTD during knee extensor isometric contractions (Izquierdo et al. 1999; Roos et al. 1999). In this thesis, RTD was split into time epochs (0-30, 0-50, 0-100, 0-200 & 0-500 ms) as it has been shown previously (Andersen & Aagaard 2006) that different points in the torque-time curve are associated with different aspects of contraction (*i.e.* intrinsic contractile properties vs. voluntary strength); and this novel methodology to analyze RTD could bring out interesting differences between young and old RTD at different time points. When split into time epochs, the only statistically significant differences were at 0-100 and 0-200 ms (~43 and 40% lower in old, respectively), while 0-50 and 0-500 ms were trending toward significance (~38 and 29% lower in old, respectively). A reason for the similarity in 0-30 ms RTD could be based on a previous study reporting that early RTD (<75 ms) is associated with intrinsic

contractile properties (twitch RTD, specifically) of the muscle, while late RTD (>75 ms) is highly associated with maximal voluntary strength (Andersen & Aagaard, 2006). This thesis reported much lower values for maximal voluntary strength in older adults compared to young, and it follows that late RTD (>75 ms) was significantly lower in old as well. It is possible that young and old adults had similar twitch RTD of the knee extensors, potentially explaining the similarity in early RTD (<75 ms), however it has been previously reported in older men that twitch RTD of the plantar flexors is significantly lower than young men (Wallace et al. 2016); therefore it is more likely that experimental error played into the lack of difference between young and old at the initial time epochs as the dynamometer may have too much compliance to be accurate in the initial slope of the torque-time curve. Further studies investigating differences in early phase RTD and knee extensor twitch RTD between young and old are needed.

5.1.2 Single Fibre Measures

As expected, single fibre P_o was found to be higher in TII than TI fibres of young adults, but this fibre type difference was not apparent in fibres of old adults. When comparing within fibre type, maximal single fibre force was ~33 and 43% higher in TI and TII fibres, respectively, of young compared to old. The magnitude of this difference was similar to that found in previous studies reporting young single fibres being ~30 – 50% stronger than old (Frontera et al. 2000a; Larsson 1997a; Power et al. 2016). While the age-related differences in P_o support some studies, there is still conflicting evidence with studies finding no difference (Hvid et al. 2011; Straight et al. 2018; Trappe et al. 2003) or significantly greater P_o (Trappe et al. 2003 [female TI]; Yu et al. 2007 [male TI]) with age.

When fibre CSA is taken into consideration by calculating SF, the differences between young and old fibres are mitigated in both TI and TII, however young TII fibres are still ~23%

stronger than young TI fibres, whereas old TI and TII fibres remain not different. In the present study, there seems to be maintained 'muscle quality' with age (*i.e.* force per unit contractile material), and this has been corroborated by others (Hvid et al. 2011; Straight et al. 2018; Trappe et al. 2003). However, conflict remains in the literature as other studies report a lower old SF compared to young (D'Antona et al. 2003, 2007; Frontera et al. 2000a; Hvid et al. 2013; Larsson et al. 1997; Ochala et al. 2006, 2007; Power et al. 2016; Yu et al. 2007). In the present study, it seems that the lower force between young and old is largely driven by differences in CSA, but to what degree SF, a relative measure of cellular force, is important for whole muscle function is unknown. For the purpose of comparing skeletal muscle performance, absolute strength loss is more pertinent than SF, as an individual still requires an absolute amount of force to rise from a chair, for example; and as we know older adults lose muscle fibres (Lexell et al. 1988), I would argue that the absolute loss of force is more significant than the 'maintained muscle quality' found here. Specific force should still be considered in this type of research, but it is a far more significant metric when it is, in fact, lower with age, therefore showing a detriment in the contractile material present in the single muscle fibres of older adults. Potentially, this muscle quality degradation does not manifest measurably until much later in life (~80 yr), which could explain the difference between the reported no change in this thesis and other studies reported an age-related weakness in SF, as the majority of the studies reporting this lower SF have an older average age, smaller age gap, and/or an older 'youngest' participant.

It is well-known that TII fibres are stronger than TI, even when CSA is accounted for (Bottinelli et al. 1996), however, in this study, the P_o and SF of older adults' TI and TII fibres were not different from each other, unlike young TI and TII, which suggests an age-related problem with absolute force production that is exacerbated in TII fibres, and not driven by differences in

CSA. Indeed, other studies have reported similar TII-targeted weaknesses in both P_o (Trappe et al. 2003 [in old females]; Yu et al. 2007) and SF (Larsson et al. 1997; Lamboley et al. 2015), and one of the first proponents of this age-related selective TII degradation was regarding fibre CSA (Lexell et al. 1988). Selective TII fibre atrophy has been attributed to denervation caused by fast type motor unit death (Lexell & Downham 1991), and this has been corroborated by fast type muscles, such as tibialis anterior, showing an age-related decrease in motor units (McNeil et al. 2005) while the soleus, a slow type muscle, maintains motor unit number (Dalton et al. 2008). However, recent research in animal models speculates that denervation causes co-expression in skeletal muscle (Rowan et al. 2011, 2012) which actually conceals TI fibre atrophy and denervation, and co-expression has been found in elderly muscle previously (Andersen et al. 1999), suggesting that TII fibres may not be selectively affected by atrophy. However, the similarity between TI and TII P_o and SF in old single fibres, definitely shows some kind of targeted TII deficit in contractile material, and I speculate it has much to do with TII fibres being used less in old age as fast type motor units must be recruited less frequently due to a lack of rapid and/or explosive movements completed by older adults.

Shortening velocity was not different between young and old TI fibres or young and old TII fibres, furthermore TII fibres were ~80% faster than TI fibres regardless of age. Similar V_o between age groups in my study was unexpected as many previous studies show consistent slowing in V_o in old compared to young (D'Antona et al. 2003; Larsson 1997a; Ochala et al. 2007; Power et al. 2016; Yu et al. 2007). However, D'Antona et al. (2007) found that physical activity has a protective effect on the V_o of TI fibres, and it is possible that because most of my group of older adults were still highly active, V_o was protected from aging by physical activity. Krivickas et al. (2001) also found a lower TIIA V_o, but not TI, however physical activity of the participants was

not indicated, although the average age was slightly older than in my study (74 yr vs. 70 yr). A report by Reid et al. (2012) found no change in V_o, but had compared older adults to a middle-aged group, and given that aging is a gradual process, it is likely that comparing to a young group could have resulted in significant differences. Another study reported sprint trained athletes with an age range of 53-77 years and found V_o was protected in TII fibres but was different from young in TI, likely as they were sprinters and would be using TII fibres consistently and significantly (Korhonen et al. 2006 . Lastly, a study with very old participants (average age ~80 yr) who were sedentary reported no change in V_o between young and old regardless of fibre type (Trappe et al. 2003). However, D'Antona et al. (2003) found that, while recreationally active older adults V_o is lower, in immobile older adults (wheelchair bound), V_o actually was higher, and a similar disuse-V_o phenomenon had been found in young adults undergoing a disuse protocol (Widrick et al. 1997, 1999). This aging-disuse paradigm makes it very important to report physical activity and mobility of the older adult population being studied to enable the accurate interpretation of changes, or lack thereof, in single fibre V_o between young and older adults.

Instantaneous stiffness (*k)*, was significantly lower in old compared to young in both TI and TII fibres, indicating a lower proportion of cross-bridge attachments in old vs. young. Instantaneous stiffness was measured by a quick stretch, and approximates proportion of cross-bridge attachments, regardless of whether those cross-bridges are weakly- or strongly-bound (Colombini et al. 2005). In a PhD thesis from Marquette University, similar results were found when measuring stiffness; old single fibres had lower peak stiffness than young (Nelson 2014), although a slightly different method was used to obtain this estimation of bound cross-bridges (Campbell & Moss 2003). One other study that used a quick stretch stiffness test in young and old human single muscle fibre found there was no age-related difference in *k* in 'slow type' fibres, but

a lower P_o (Power et al. 2016). A similar *k* between young and old, with lower force in old, suggests similar cross-bridges attached with a greater proportion of weakly-bound cross-bridges; and previous research in animal models has shown that in aged rat single fibres there are more weakly- than strongly-bound cross-bridges at maximal activation (Lowe et al. 2001; Thompson et al. 2001; Zhong et al. 2006). Miller et al. (2013) did not find a difference in proportion of strongly-bound cross-bridges between young and old humans, using a different method than in the animal models cited above, however with the inconsistency in single fibre literature it is likely that different populations of older adults may have a higher proportion of weakly-bound cross-bridges compared to others. In this thesis, a lower *k* and lower maximal force in old single fibres likely indicates that at maximal activation there are both fewer cross-bridges attached and more weakly-bound attachments.

Intriguingly, while a difference in *k* was found between young and old, there was no difference in k*tr* within fibre type between young and old. In the same thesis mentioned above, a pilot investigation was performed with ~7 and 22 fibres from young and old, respectively, and found that k*tr* was not different with age (Nelson 2014), whereas Power et al. (2016) found significantly lower k*tr* in old single fibres compared to young. The average age of the older adult group in Power et al. (2016) was older (~78 yr) than Nelson (2014) and my study (~70 yr), which could explain the opposite results in *k* and k*tr* found in Power et al. (2016) vs. Nelson (2014) and my thesis.

While few, if any, other studies have examined k*tr,* other studies have done similar studies of cross-bridge kinetics in humans. Ochala et al. (2007) performed a 'quick-release' experiment whereby a fibre is maximally activated, rapidly shortened, and then force is allowed to redevelop at the slightly shortened length (0.15% shorter); then the $t_{1/2}$ of force redevelopment is calculated,

which has similarities to k*tr* in that $t_{1/2}$ is a measure of how quickly force redevelops, although under slightly shortened conditions. This group found that $t_{1/2}$ did not differ between old and young TI fibres, but $t_{1/2}$ was significantly longer (indicating slower force redevelopment) in old TII compared to young TII fibres. Miller et al. (2013) used sinusoidal length changes to relate sinusoidal analysis to specific steps of the cross-bridge cycle and found longer myosin attachment time and reduced rates of myosin transition from weakly- to strongly-bound states in older women, but not older men, compared to young.

Little is known about the effect that physical activity/inactivity or aging has on k*tr*, or other measures relating to cross-bridge kinetics, in human single muscle fibres, and these first reports are only scratching the surface of this measure, and the underlying cross-bridge attachment k*tr* pertains to. Physical activity seems to not have a protective effect on k*tr* as Power et al. (2016) did not find a difference in k*tr* between age-matched recreationally active older adults and masters athletes. This being said, Nelson (2014) and my thesis had older adult participants that were very active and no difference in k*tr* was found. Although, Ochala et al. (2007) did find a slowing of cross-bridge kinetics, and the older participants were not very active (< 1 hr walking/day), and Miller et al. (2013) had a group of less active older participants, activity matched to the young group, and did not find slowing of cross-bridge kinetics in older men but did in older women. Taken together, these studies indicate that investigating the effect of sex and a spectrum of inactivity to high activity has on k*tr* and other measures of cross-bridge kinetics would help to further the understanding of this cross-bridge based mechanism.

There remains the need for an explanation as to why Power et al. (2016) and myself found opposing results in the age-effect on k*tr* and *k* despite near identical methodologies. While Power et al. (2016) did not report physical activity levels, I do not believe physical activity is the driving

factor for the difference in results. The two major differences between Power et al. (2016) and my thesis are age of the older adult group, as mentioned earlier, and fibre grouping procedure. I used gel electrophoresis to group the single fibre population into fibre types (TI, TII, hybrid) based on myosin heavy chain (MHC) isoform, whereas Power et al. (2016) employed a binning procedure based on mechanical V_o using fibres only with a V_o <0.5 L_o/s. This V_o binning method has a known error rate of ~7% for slow type fibres (Claflin et al. 2011), and could have placed TII fibres with slower V_o into the 'slow type' group, and conversely, not analyzed TI fibres that fell beyond the 0.5 L_o/s cut off. While it is possible that a mechanical measure of speed could more accurately represent 'fast cross-bridge kinetics' (*i.e.* high k*tr*) as compared to a molecular measure of fibre type (MHC), it is unknown if V_o is associated with k*tr*. In young single fibres, contractile parameters, such as V_o, P_o, P_o/SF, are highly dependent on MHC isoform (Bottinelli et al. 1996), but it is unknown if MHC isoform remains the most important factor in contractile performance of old single fibres. I used the V_o binning procedure to check if my results would change significantly and found that old 'slow type' (V_o <0.5 L_o/s) fibres had a k*tr* significantly greater than young 'slow type', however k*tr* remained similar between young and old 'fast type' fibres. In terms of fibres changing groups (*i.e.* from TI to 'fast type'), only 2 young and 2 old TI fibres were put in the 'fast type' bin, while all TII fibres remained in the 'fast type' bin. Additionally, I ran linear regressions between V_o and k*tr* and did not find any associations between the measures in either age group or fibre type. Though it is impossible to say if using MHC isoform to group fibres would have changed the results in Power et al. (2016), based on my mini-analysis experiment, I do not believe the difference in fibre binning is greatly influencing the disparity in our results, and it is more likely that the ~8 year age gap is the driving factor.

Calcium sensitivity measured by pCa_{50} was not different between TI and TII within age group; however, within TI, a lower pCa_{50} in old compared to young was trending ($p = 0.095$) towards significance, and there was significantly lower (~2.5%) pCa_{50} in old TII fibres compared to young (mean $pCa_{50} = 5.91$ vs. 6.06, respectively). This lowered Ca^{2+} sensitivity in old TII fibres was also found by another group with a ~0.8% decrease in old compared to young ($pCa_{50} = 5.91$vs. 5.96, respectively; Lamboley et al. 2015), and a more recent publication found that pCa_{50} was lowered in old single fibres by ~2.4 and 1.2% in TI and TII fibres, respectively (Straight et al. 2018). Two other studies by the same group testing age-related differences in Ca^{2+} sensitivity in humans did not find a difference in pCa_{50} for either TI or TII fibres (Hvid et al. 2011, 2013). Although the absolute differences between average pCa_{50} for young and old were similar between the above studies (~0.8 – 2.5%), no statistically significant differences were found by Hvid et al., likely because of high between subject variability. Both studies done by Hvid et al. (2011, 2013) had a younger average age (~66, ~67 yr) and tested only males, while the others had an average age of ~68 and 70 years and tested males and females together (Straight et al. 2018; Lamboley et al. 2015, respectively). Lamboley et al. (2015) did not test for sex differences, however Straight et al. (2018) did not find a sex effect on pCa_{50}. The present study had an older average age of ~70 years, but also tested only males. All studies reported moderate-high levels of physical activity and activity-matching between young and old groups. Therefore, it is likely differences in age of older participants and methodology driving the conflict in results regarding the effect of age on pCa_{50}.

As mentioned above, the hypothesized k*tr*-Ca^{2+} relationship in human single fibres was confirmed and is similar to that found in animal models (Brenner 1988; Metzger & Moss 1988, 1990; Wolff et al. 1995), with an increase in $[Ca^{2+}]$ causing an increase in k*tr*. This relationship

was the same in all groups regardless of age or fibre type. A similar k*tr*-Ca^{2+} relationship between animal and human models would indicate a similar mechanism underpinning the influence of Ca^{2+} on k*tr*, and that is the affect Ca^{2+} has on the transition from weakly- to strongly-bound cross-bridge attachments (Metzger & Moss 1990; Wolff et al. 1995). These cross-bridge state transitions affect k*tr* directly, while k*tr* appears to be independent of the absolute number of cross-bridge attachments or myosin binding sites available (Brenner 1988; Metzger & Moss 1990; Wolff et al. 1995). Recently, it has been shown that myosin has a 'mechanosensing' property which causes a conformational change in myosin heads allowing more to become available to form bonds with actin based on the amount of mechanical tension on the fibre, translated to the myosin filament (Linari et al. 2015). So called 'high load' contractions cause enough tension on the fibre to significantly increase the number of myosin heads available to form cross-bridge bonds; and while this is a new finding that has not been tested in humans or with regards to Ca^{2+}, it is possible that because more Ca^{2+} causes an increase in single fibre force, therefore mechanical tension on the myosin filament, it could be another mechanism by which skinned single fibre k*tr* is sensitive to the amount of Ca^{2+} in solution.

Following the relationship found between k*tr* and [Ca^{2+}], it seemed probable that a higher pCa_{50} would be associated with a higher maximal (pCa 5.0) k*tr* value. In fact, it was found that pCa_{50} and maximal k*tr* have little association in either TI or TII single fibres from young adults, but there are significant moderate-strong associations in TI (r^2=0.30, $p < 0.05$) and TII (r^2=0.44, $p < 0.05$) single fibres from older adults. In older adults' single fibres calcium sensitivity explains ~30 and 44% of the variance associated with k*tr* in TI and TII fibres, respectively. The lack and presence of a relationship between pCa_{50} and k*tr* in young and old, respectively, was an unexpected result. It is unclear why Ca^{2+} sensitivity would be associated strongly with k*tr* in older adults

(irrespective of fibre type), but not in young. No other studies have attempted to associate pCa_{50} with k*tr*, and so I can only speculate as to the reason for this result. It has been shown that old single muscle fibres have impaired calcium handling (Lamboley et al. 2015; 2016), so it is possible that altered sensitivity in the population of old fibres has a greater effect on k*tr* than in young where Ca^{2+} handling and kinetics are not impaired. Additionally, as Ca^{2+} has a governing effect on the transition from weakly- to strongly-bound cross-bridge attachments, a greater proportion of weakly-bound cross-bridges in old compared to young single fibres could cause Ca^{2+} sensitivity to be far more important a factor in the measure of k*tr*. This idea also gains support from the possibility that aged human single fibres are similar to aged animal models with a higher proportion of weakly-bound cross-bridges than young (Thompson 2009), and while Miller et al. (2013) did not find an age-related change in proportion of strongly-bound cross-bridges, they did find a slowed myosin transition from weakly- to strongly-bound states. Therefore, age-related impaired Ca^{2+} handling and sensitivity could play a role in both a higher proportion of weakly-bound cross-bridges and slowed phase transitions between weakly and strongly bound myosin states, consequently impacting k*tr* and resulting in a strong association between pCa_{50} and k*tr*.

5.1.3 Single Fibre and Whole Human Relationship

While the mechanisms underpinning k*tr* and its potential age-related changes were sought after in this thesis, it was also a goal to investigate the relationship between single fibre k*tr* and whole muscle RTD. As mentioned above, relationships between k*tr* and RTD at any time epoch were not apparent in young TI or TII fibres or in old TII fibres, however associations between old TI fibres and RTD at 0-30, 0-50, and 0-100 ms were trending toward significance. It was surprising that the trending relationships were in old, as my initial expectation was that a relationship would exist in young and old, and if any relationship were to be weaker it would be in older adults, but

the opposite seems to be coming out of this work. There are many factors contributing to the voluntary RTD measure from central drive to cross-bridge cycling, and so it is not overly surprising that strong associations were not present. Furthermore, with only 8 participants in each correlation, I was underpowered to see significant relationships emerge, and it is likely that with a greater n, the k*tr*-RTD relationships would gain significance. Indeed, other studies have found significant relationships between other whole muscle and single fibre contractile properties with larger sample sizes (Straight et al. 2018; Yu et al. 2007).

A possible explanation for an emerging k*tr*-RTD relationship in older adults is that the neural system responsible for skeletal muscle contraction may degrade at a faster rate than the cellular system in old age, which could cause the cellular system to have a greater influence on whole muscle contraction than in young. Therefore, k*tr* in old TI fibres, which are suggested to be somewhat protected from neuromuscular alterations, may be responsible for more of the variance in RTD than in young. Some evidence in this regard are the consistent findings that with age motor unit number estimates decline (Dalton et al. 2010; McNeil et al. 2005; Power et al. 2012), motor units reorganize and innervation changes (Deschenes et al. 2010), and the neuromuscular junction degrades structurally (Bromberg & Scott 1994; Oda 1984; Wokke et al. 1989) and functionally (Bromberg & Scott 1994; Hepple & Rice 2016; Ibebunjo et al. 2013); and in contrast, cellular changes are inconsistent showing degradation, no change or improvement in various contractile properties of single fibres in older adults. Strong evidence for cellular compensatory is showcased in the few longitudinal studies performed. Frontera et al. (2008) found trending increases in P_o in TI and TIIA fibres after ~9 years (~71 to 80 yr) and Reid et al. (2014) found significant increases in both P_o and V_o after ~3 years in TI and TIIA fibres (~77 to 80 yr). Far more longitudinal studies need to be completed investigating a variety of single fibre contractile properties in order to draw

more conclusions regarding the interplay between the neural, neuromuscular, and cellular systems of skeletal muscle contracting in old age.

The trending relationships were at 0-30, 0-50, and 0-100 ms, with the 0-30 ms relationship being the closest to significance (p=0.052). It makes sense that if any of the k*tr*-RTD relationships would be significant, it would be with the early time epochs of RTD because of the association between early RTD (<75 ms) and twitch contractile properties (Andersen & Aagaard 2006). While the associations between early vs. late RTD and involuntary vs. voluntary contraction were completed in young adults, it is likely that these associations still hold true in older adults. It is feasible that had the n in this thesis' older adult group been higher, the k*tr*-RTD relationships would have been significant. Further research should be done in this regard.

5.2 Limitations

Over the course of my thesis, there were many experimental limitations. First, the old group had a large age gap (60 – 81 yrs) of ~20 years, which is an issue as it has been noted that a lot can change between the ages of 60 and 80 (Frontera et al. 2008; Reid et al. 2014), and about half of them were relatively young for an aging study (60 – 70 yrs). Other research has suggested grouping participants into old and 'oldest old' groups (D'Antona et al. 2007; Power et al. 2016; Venturelli et al. 2015) because of differences that have been found at varying stages of the aging process. Unfortunately, I lacked widespread access and time to reach the 'oldest old' individuals in the community, and so were not as exclusive with age as we could have been. With this issue, we also lacked absolute number of participants (n=4 <70 yr; n=4 >70 yr) to have more than one old group, and as such could be washing out differences with our large age range. Potentially, if I had an old group with an average age closer to or above 80 we would have found differences between young

and old in single fibre SF, V_o, and/or k*tr*, and perhaps more pronounced differences in RTD and single fibre P_o.

Additionally, the older group was highly physically active and ended up having similar amounts of self-reported physical activity compared to the young group, who regularly engage in weight and endurance training, as well as recreational sports. While this is an advantage as it makes the possibility of aging and disuse counteracting each other low (*i.e.* aging-disuse paradigm in V_o), this population of older adults is not necessarily representative of the average older adult, and without a reference population of lower physical activity, conclusions cannot be drawn regarding high physical activity protecting against negative aspects of aging. Ideally, older groups that have differing amounts of physical activity (*i.e.* sedentary, recreationally active, highly active) would be used for comparison. Additionally, physical activity data was self-reported with no objective measure corroborating it, such as accelerometry. Ideally, having something like accelerometry is beneficial to ensure the most correct physical activity data.

Only 13 TII fibres from older adults were successful during testing and analysis, and there were 42 successful TI fibres from older adults, which is more than triple the number of TII. Contrast this to the number of 27 TI and 40 TII fibres that were successful in young, and it is evident that there is something happening to the TII fibres in older adults. A similar number of total fibres was tested in the young and old groups by the same researcher, and yet only ~24% of fibres in old were TII whereas in young ~60% of the fibres were TII, and a major roadblock is that fibre typing can only be done after mechanical testing. While the low number of TII fibres from old is a limitation in comparing groups, it also offers some anecdotal insight into mechanisms of age-related skeletal muscle degradation.

I had to exclude a number of participants from the study because of experimental errors with the chemical solutions used for single fibre testing. Initially, we were using certain recipes for the pre-activating and various pCa solutions, however I found that I was losing force very quickly during contractions and over the course of a mechanical test. After some readings and meetings with other professors, my colleagues and I decided to adopt pCa recipes from Todd Gillis (PhD, University of Guelph, *e.g.* Gillis et al. 2007). These solutions solved my force loss problems, and the majority of single fibres lasted through the 12-14 contractions, but I had to exclude the initial 2 young participants. However, the last 4 participants in the study had to be excluded due to problems with solutions, that we think was an issue with our storage solution. After the skinning and storage procedure, the muscle samples could be described as "fraying", were very fragile, had excessively low P_o, and would break easily during testing. For these reasons, the data from these participants could not be trusted and we made the decision to exclude.

The number of participants in this study (n = 8 young, 8 old) was similar to other studies examining similar outcome measures (Lamboley et al. 2015; Power et al. 2016; Straight et al. 2018), however as this study sought to perform regression analysis between single fibre and whole muscle neuromuscular data, a higher n (15 young and 15 old) was initially sought. Due to recruitment difficulties, participant attrition, and experimental error, we were 7 participants short in each group, and as the general rule is a sample size of ~15-20 per independent variable in the analysis the relationship analysis must be interpreted with caution. Keeping this in mind, a recent publication came out noting that a minimum sample size of 2 per variable accurately estimated regression coefficients, standard error, and confidence intervals (Austin & Steyerberg 2015), so while our n was below the accepted guideline, we were well above the minimum needed to accurately perform a regression analysis.

One major limitation in this field of work, I believe, is the lack of a standardized methodology for single fibre experiments. There are numerous ways to perform single fibre contractile experiments that use different recipes for solutions, skinning procedures, storage protocols for the fibres, and a different amount of time that testing these single fibres is allowable. Additionally, different labs test 'baseline' single fibre properties at different temperatures, which can make comparing and contrasting results difficult, as it is known that temperature has a large effect on parameters such as P_o and V_o (Bottinelli et al. 1996; Coupland et al. 2001). At this point, it is unknown the effect of these different methodologies on single fibre results, and if they could disparately affect young and old single fibres. With the consistently inconsistent nature of single fibre literature, a need is certainly highlighted for a reliable, standardized method to assist in untangling the effect aging has on single fibre contractile properties.

5.3 Future Directions

Future work aiming to further our understanding of cellular mechanisms underpinning age-related skeletal muscle degradation should aim to test distinct age groups within an older adult population, such as 'old' and 'very old' groups. Aging is a continuous, gradual process, which happens at slightly different rates for everyone, therefore there may be differences that our measures are not sensitive enough to pick up between adults of 25 years and 65 years but comparing adults of 25 years to 80 years could result in measurable differences. Indeed, in other research regarding age-related changes in skeletal muscle function, it has been noted that changes become more evident around 75 – 80 years (D'Antona et al. 2007). Furthermore, we are also missing a continuum of aging by testing groups with large age ranges (~20 yr), and testing a young adult (18 – 30 yr), middle-aged (40 – 55 yr), older (60 – 74 yr) and very old (> 75) could uncover how the single fibre contractile properties change across the lifespan, changing positively or

negatively at different time points due to aging, illness, disuse, training or any number of other lifestyle factors.

This area of age-related changes in single fibre contractile property is severely lacking longitudinal research. Currently, there are only two studies that have attempted to complete longitudinal experiments, with varying times between measurements, and both have found trending or significant increases in single fibre P_o between time 1 and time 2 in older adults (Frontera et al. 2008; Reid et al. 2014). This is definitely a gap in the literature that needs to be filled, as longitudinal research is advantageous to investigate cause and effect and mechanisms behind changes in single fibre contractile properties across the lifespan.

Another aspect to be focused on is the influence of differing physical activity levels in older adult populations. Previous work on age-related change in k*tr* compared highly active master's athletes with a group of recreationally active older adults, and high amounts of physical activity did not have a protective effect on k*tr* (Power et al. 2016). However, recreational activity may be enough to protect against changes in k*tr* that could occur in sedentary/immobile individuals, but without a sedentary and/or immobile group to compare to, we do not have information on the spectrum of activity/inactivity. Comparing highly active, recreationally active, sedentary, and/or immobile would add to the understanding of the effect of inactivity and aging on one aspect of cross-bridge interaction (rate of cross-bridge attachment, k*tr*). Additionally, if a group does not have the resources to collect participants in all physical activity levels, at the very least a good effort should be made to have similar physical activity among all participants to mitigate a potential wash-out effect due to high between subject variance. For example, there has been previous work done on physical activity influence on single fibre V_o, and it was found that sedentarism/disuse actually increases V_o whereas 'pure aging' (*i.e.* maintained physical activity) slows V_o (D'Antona

et al. 2007), so grouping sedentary/immobile individuals with those of high activity could result in a net 'no change' in V_o and incorrect conclusions could be drawn.

There are, again, inconsistent results when it comes to sex differences in single fibre literature. Some literature finds sex differences are significant (Krivickas et al. 2006; Miller et al. 2013), while others do not (Straight et al. 2018; Trappe et al. 2003) and still others group males and females together without testing for a sex effect (Lamboley et al. 2015). I think a problem that exists with the uncertainty of a sex effect is sample size, as most of the studies do not have an equal or large (>8) number of both male and female participants. Another issue is that most studies only examine one or two single fibre contractile parameters, leaving many contractile parameters, such as k*tr*, *k*, and isotonic power, out of the sex difference investigation. Certainly, a direction for the future would be to systematically test for sex differences with a larger sample size and variety of contractile parameters.

5.4 Conclusions

There is a positive relationship between k*tr* and $[Ca^{2+}]$, such that as $[Ca^{2+}]$ increases k*tr* does as well, and this is similar to the k*tr*-pCa relationship shown in animal models. There is also a strong positive relationship between pCa_{50} (calcium sensitivity) and maximal k*tr* in TI and TII fibres of older adults, however this association does not exist in young adult single fibres, and it is likely that the impaired Ca^{2+}-handling and sensitivity in older single fibres is driving the stronger association. Future work is needed to explore the relationship between impaired Ca^{2+}-handling/sensitivity in old single fibres and cross-bridge kinetics. As old single fibres were weaker and had a lower proportion of attached cross-bridges, there is evidence for a decline in cellular function. Contrary to expected, there was not a relationship between whole muscle RTD and single fibre k*tr*, except for trending relationships between older adult early phase RTD and TI single fibre

k*tr*. As there are many factors influencing voluntary RTD, the influence of the single cell will be relatively small. A larger sample size is needed for possible whole muscle and single cell relationships to emerge and to investigate how they change with age.

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APPENDIX A

Yale Physical Activity Survey

*as adapted from: DiPietro, L., C.J. Caspersen, A.M. Ostfeld, and E.R. Nadel. A survey for assessing physical activity among older adults. Med. Sci. Sports Exerc., Vol. 25, No. 5, pp. 628-642, 1993.

Date: _____ Time: _____

This questionnaire gives a good understanding of the physical activity patterns of older adults, and will be used to investigate if our research population differs in their physical activity during everyday life.

I am going to ask you a few questions about the activities that you usually perform, as part of your daily routine and also for leisure. There are no right or wrong answers. Your name will not be used in the results, therefore the information that you give me will be confidential and only used for research purposes.

SB: _____

Name: _____

Adresss 1: _____

Address 2: _____

Telephone number: _____

Date of birth: _____

Age: _____

Date of interview: _____

Part 1

We are interested to learn about the types of activities which are part of your regular routine. I am going to show you lists of common types of physical activities. Please tell me how much time (in minutes or hours) you spent during the past week.

Activity	**Time**		**Intensity Code**
Work	**Hours**	**Minutes**	
Shopping (e.g. grocery, clothes)			3.5
Stair climbing with a load			8.5
Laundry: Unloading/loading machine Hanging, folding only Washing by hand	 _____ _____	 _____ _____	 3.0 3.0 4.0
Light housework: tidying, dusting, sweeping, collecting garbage in home, polishing, ironing			3.0
Heavy housework: vacuuming, mopping, scrubbing floors and walls, moving furniture, boxes, garbage bins			4.5
Food preparation: chopping, stirring, moving about to get food items and pans			2.5

Food service: setting table, carrying food, serving food			2.5
Dish washing: clearing table, washing/drying dishes, putting dishes away			2.5
Light home repair: small appliance, light home maintenance			3.0
Heavy home repair: painting, carpentry, washing/polishing car			5.5
Other:			
Yard Work	**Hours**	**Minutes**	
Gardening, pruning, planting, weeding, digging, hoeing			4.5
Lawn mowing (walking only)			4.5
Clearing walks/driveways: sweeping, shoveling, raking			5.0
Other			

Care taking	**Hours**	**Minutes**	
Older or disabled person (lifting, pushing wheelchair			5.5
Child care (lifting, carrying, pushing stroller)			4.0
Exercise	**Hours**	**Minutes**	
Brisk walking			6.0
Pool exercises, stretching, yoga			3.0
Vigorous calisthenics, aerobics			6.0
Cycling			6.0
Swimming (laps only)			6.0
Other			
Recreation	**Hours**	**Minutes**	
Leisurely/slow walking			3.5

Needlework: knitting, sewing, needlepoint, etc.			1.5
Dancing: line, ballroom, tap, square etc.			5.5
Bowling			3.0
Golf			5.0
Racquet sports: tennis, squash			7.0
Billiards			2.5
Other			

Part two

I would now like to ask you about certain types of activities that you have done during the past month. I will ask you about how much vigorous activity, leisurely walking, sitting, standing and some other things you usually do.

1. About how many times during the month did you participate in **vigorous** activities, that lasted at least **10 minutes** and caused large increases in breathing, heart rate, or leg fatigue, or caused you to perspire?

	Score
Not at all (go to Q3)	0

1-3 times per month	1
1-2 times per week	2
3-4 times per week	3
5 + times per week	4
Refused	7
Don't know	8

Frequency score _____

2. About how long do you do this vigorous activity/ies each time?

Not applicable	0
10-30 minutes	1
31 – 60 minutes	2
60 + minutes	3
Refused	7

Vigorous activity index score:

Don't know	8

Duration Score _____

Weight: 5

3. Think about the walks you have taken in the past month. About how many times per month did you walk **for at least 10 minutes** or more **without stopping** which was not strenuous enough to cause large increases in breathing, heart rate, or leg fatigue or cause you to perspire?

Not at all (go to Q5)	0
1-3 times per month	1
1-2 times per week	2
3-4 times per week	3
5 + times per week	4
Refused	7
Don't know	8

Frequency score _____

4. When you did this walking, for how many minutes did you do it?

Not applicable	0
10-30 minutes	1
31 – 60 minutes	2
60 + minutes	3
Refused	7
Don't know	8

Duration Score _____

Weight: 4

5. About how many hours per day do you spend moving around on your feet while doing things? Please report only the item that you are **actually moving**.

Not at all	0
Less than 1 hour per day	1

Leisurely walking index score:

1 to 3 hours per day	2
3 to 5 hours per day	3
5 to 7 hours per day	4
7+ hours per day	5
Refused	7
Don't know	8

Moving score _____

Moving Index Score

Weight: 3

6. Think about how much time you spend standing or moving around on your feet on an average day during the past month. About how many how per day do you **stand**?

Not at all	0
Less than 1 hour per day	1

1 to 3 hours per day	2
3 to 5 hours per day	3
5 to 7 hours per day	4
7+ hours per day	5
Refused	7
Don't know	8

Standing score _____

Weight: 3

7. About how many hours did you spend sitting on an average day during the past month?

Not at all	0
Less than 3 hours	1
3 hours to less than 6 hours	2
6 hours to less than 8 hours	3
8 + hours	4
Refused	7
Don't Know	8

Sitting Score _____

Weight: 1

Sitting Index Score

8. About how many stairs do you climb up **each** day? (let 10 steps = 1 flight)

_____

9. Please compare the amount of physical activity that you do during each season of the year with the amount of activity you just reported for a typical week in the past month?

	A lot More	Little More	Same	Less	A lot Less	Don't know
Spring	1.30	1.15	1.00	0.85	0.70	-
Summer	1.30	1.15	1.00	0.85	0.70	-
Autumn	1.30	1.15	1.00	0.85	0.70	-
Winter	1.30	1.15	1.00	0.85	0.70	-

Seasonal adjustment score = sum of all seasons/4 = _____