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The Effects of Ageing and Obesity on the Contractile Properties of Isolated Locomotory and Respiratory Skeletal Muscles

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The Effects of Ageing and Obesity on the Contractile Properties of Isolated Locomotory and Respiratory Skeletal Muscles

By

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Student:

Cameron Hill

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The Age & Gender-Specific Differences on the Mechanical Performance of Isolated Locomotory (EDL & Soleus) and Respiratory (Diaphragm) Skeletal Muscles Using the Work Loop Technique.

This is to certify that the above named student has completed the Coventry University Ethical Approval process and their project has been confirmed and approved as Medium Risk

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Dedication

I would like to firstly dedicate this work to my beautiful wife Gráinne Hill, for without her tireless love and support over the last four years I would never in my wildest dreams have imagined being in the position I am in today. Thank you for everything you do every day.

To my parents, Nicola Hill and Paul Adams, you have instilled principles into me at an early age that with hard work comes great rewards. Whilst you may not have a single clue what I do on a day-to-day basis, you have allowed me to follow my academic interests and hobbies to the fullest degree, supporting me every step of the way and for that, I am forever grateful.

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Abstract

Ageing is associated with a decline in skeletal muscle strength and power resulting in impaired *in vivo* locomotory and respiratory muscle function. It has been suggested that obesity in can exacerbate the reduction in skeletal muscle contractile function, though findings in older humans are equivocal. Ageing and obesity are associated with a significant decline in muscle quality in isolated skeletal muscles, though the capacity for producing and sustaining power, both concentrically and eccentrically, in isolated male and female locomotory and respiratory skeletal muscles is poorly understood. This is primarily due to the usage of contractility modes, such as isometric and isovelocity contractions, in isolated skeletal muscles poorly replicating *in vivo* contractile function. The work loop technique better replicates the *in vivo* contractile function of skeletal muscles by accounting for the power production during muscle shortening, and the passive forces during lengthening, thus providing a better model for examining isolated skeletal muscle contractile function. Using the work loop technique, this thesis examines the muscle-specific, sex-based differences in skeletal muscle ageing at multiple ages using males and females mouse skeletal muscles, and examines the effect of dietary-induced obesity in old age has on muscle function. The present work also outlines the differences in isometric force, concentric power across a range of contractile speeds and changes in eccentric power with increasing age. Between each study, absolute performance and performance normalised to muscle mass is calculated to provide an indication of changes in muscle quality. Finally, the ability of isolated muscles to withstand fatigue with age and obesity is determined. The results indicate that absolute concentric and eccentric power output and isometric force are well maintained with increasing age, with absolute power usually greater in males than females. When power output is normalised to muscle mass, there are few sex-based differences in the age-related decline in power output, though normalised performance in the oldest animals is worse for males than females. Furthermore, acute eccentric power output is well maintained with age, and older locomotor muscles are more fatigue resistant when fatigued eccentrically compared to young counterparts. Obesity in old age, however, does not further worsen locomotory performance normalised to muscle mass, nor

fatigue resistance, but is deleterious to diaphragmatic power. Increasing age results in greater body mass, with larger muscles of poorer quality. When considered *in vivo*, larger muscles of poorer quality contribute to an already elevated body mass and consequently may impair acute and sustained locomotor and respiratory function *in vivo*, where muscles of poorer quality are required to work against a greater bodily inertia. Although there is some evidence that obesity may accelerate the age-related decline in function, this was not uniform across all of the muscles assessed. As such, the functional impairments seen in the sarcopenic-obese populations is largely the result of weakened muscles moving and controlling a greater muscle mass.

Presentation of Results

Results of the present thesis have been presented at national and international conferences as follows:

Chapter 4 - The Sex-Based Differences in the Age-Related Changes in Isolated Locomotory (Soleus & EDL) and Respiratory (Diaphragm) Skeletal Muscle Contractile Function of CD-1 Mice.

- Poster presentation of full results; Hill, C., James, R. S., Cox, V. M. and Tallis, J. (2018) The Sex-Based Differences in the Age-Related Changes in Isolated Locomotory (Soleus & EDL) and Respiratory (Diaphragm) Skeletal Muscle Contractile Function of CD-1 Mice. [Poster]. Exhibited at the British Society for Research on Ageing (BSRA) 68th Annual Scientific Meeting in September 2018.

Chapter 5 - The Effect of Increasing Age on the Concentric and Eccentric Contractile Properties of Isolated Mouse Soleus and Extensor Digitorum Longus Muscles

- Oral presentation at the University of Westminster "Ageing & Metabolism in the Human" Translational Physiology Group Symposium in September 2017.

- Published; Hill, C., James, R. S., Cox, V. M. and Tallis, J. (2018) The Effect of Increasing Age on the Concentric and Eccentric Contractile Properties of Isolated Mouse Soleus and Extensor Digitorum Longus Muscles. *Journals of Gerontology, Series A. Biological Sciences and Medical Sciences*, 73(5): 579-587.

Chapter 6 - The Effects of Age and Dietary-Induced Obesity on the Contractile Function of Isolated Locomotory and Respiratory Skeletal Muscles

- Oral presentation of selected results at the British Heart Foundation physical activity and cardiovascular health meeting in October 2016

- Poster presentation of full results; Hill, C., James, R. Cox, V. and Tallis, J. (2017) Does fatness exacerbate frailty? The effects of age and dietary-induced obesity on the contractile function of isolated locomotory and respiratory skeletal muscles. [Poster]. Exhibited at 24th European Congress on Obesity, Porto, Portugal. *Obesity Facts*, 10(suppl 1): 1-274.
- Published; Hill, C., James, R. S., Cox, V. M. and Tallis, J. (2019) Does Dietary-Induced Obesity in Old Age Impair the Contractile Performance of Isolated Mouse Soleus, Extensor Digitorum Longus and Diaphragm Skeletal Muscles? *Nutrients*, 11(3): 505.

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Abbreviations

µJ – Microjoules

µm – Micrometres

ANOVA – Analysis of variance

ATP – Adenosine triphosphate

BM – Body mass

Ca^{2+} - Calcium ion

CF – Cycle frequency

CNS – Central nervous system

CSA – Cross-sectional area

DHPR – Dihydropyridine receptors

DXA – Dual x-ray absorptiometry

EDL – Extensor digitorum longus

EFAD – Essential fatty acid deficient

FPM – Fat pad mass

HFD – High-fat diet

HFL - High-fat lard

HFP – High-fat palm oil

Hz – Hertz

IL-6 – Interleukin-6

IMAT – Intramuscular adipose tissue

$kg.m^{-3}$ – Kilograms per cubic meter

$kN.m^{2}$ – Kilonewtons per square meter

L_0 – Pre-determined optimal length

LSHR – Last stimulation to half relaxation

mN - Millinewtons

mN.V – Millinewtons per volt

mW - Milliwatts

NAL – Nasoanal Length

OC – Old concentric

OE – Old eccentric

PO – Power output

PO-CF – Power output-cycle frequency

PPO – Peak power output

RC – Repeated concentric

RE – Repeated eccentric

ROS – Reactive oxygen species

S.E.M. – Standard error of the mean

SERCA – Sarco(endo)plasmic reticulum ATPase

SR – Sarcoplasmic reticulum

THPT – Time to half-peak tetanus

TNF-α – Tumor necrosis factor-alpha

V – Volts

V_0 – Maximum unloaded shortening velocity

V_{max} – Maximal shortening velocity

$W.g^{-1}$ – Watts per gram

$W.Kg^{-1}$ – Watts per kilogram

WL – Work loop

YC – Young concentric

YE – Young eccentric

Glossary of Terminology

Absolute isometric force
The absolute force produced by a muscle at a constant length.

Absolute power output
The maximal power produced by a muscle, but is not corrected for muscle mass, muscle cross-sectional area or body mass.

Cross-bridge kinetics
The interaction between actin and myosin during binding and detachment, and the resultant force production, is termed cross-bridge kinetics. Interchangeably known as cross-bridge cycling in the literature.

Eccentric muscular activity
Muscle activation during lengthening, via which work is absorbed by the muscle.

Fatigue resistance
The ability for skeletal muscles to maintain force or power during repeated bouts of concentric or eccentric muscle activity over an extended period of time. Also known as muscular endurance.

Isometric stress
Force produced per unit of muscle cross-sectional area. Provides an alternative measure of muscle quality.

Last stimulation to half relaxation (LSHR)
The time taken for an isolated muscle to reach half the peak isometric force following the last electrical stimulus. Also known as relaxation time.

Maximal strength
The maximal force produced against an external load in one single attempt.

Maximal torque
The maximal force produced around the rotational axis of a joint over a fixed distance and speed/rate.

Muscle quality
The amount of force or power produced relative to the size of the skeletal muscle, muscle quality defines the overall effect of changes in normalised contractile performance, namely *isometric stress* and *normalised power output*.

Normalised power output
The power output of the muscle, in milliwatts, is corrected to the muscle mass of a skeletal muscle to provide an alternative measure of performance relative to skeletal muscle size, or muscle quality.

Net work
The active work produced minus the passive work absorbed.

Obesity
Excessive fat accumulation that poses a risk to health. In humans, obesity is indicated by a body mass index (BMI=mass [kg]/height^2 [m^2]) ≥30. In animals, obesity is indicated by a Lee Index of Obesity (LIO=$\sqrt[3]{}$(mass [g])/(NAL [cm]) x 1000) ≥300 in 28-day old mice.

Tetanus
A sustained isometric muscular contraction in response to a high-frequency stimulation of an isolated skeletal muscle. A tetanus is characterised by the interaction between the maximal isometric force, and the time or rate of muscle activation and relaxation.

Time to half-peak tetanus (THPT)
The time taken for an isolated muscle to reach half the peak isometric force following the first electrical stimulus. Also known as activation time.

Work loop
The work loop technique measures the amount of work a muscle produces during concentric or eccentric sinusoidal wavelength changes, where the muscle is stimulated to produced force during shortening or lengthening. From work, power output can be calculated in absolute terms and normalised to muscle mass.

Chapter 1 - Introduction and Review of the Literature

1.1 - General Introduction and Thesis Outline

The life expectancy of the global population is rapidly increasing, with the World Health Organisation projecting a 200% rise in adults aged over 60 years living in our world between the years of 1970 and 2025 (Palus *et al.*, 2017). However, the number of co-morbidities associated with ageing is also expected to increase (Ethgen *et al.*, 2017), leading to a reduced quality of life and an excessive financial strain on healthcare providers (Caley and Sidhu, 2011). One important factor catalysing the age-associated increase in health risk is the age-related decline in muscle performance (Rosenberg, 1989). Poor muscle performance in older adults leads to a reduced capacity to perform tasks of daily living, increased fall risk and a sedentary lifestyle, thus causing susceptibility to health implications that arise from an inactive lifestyle (Faghri *et al.*, 2015). Skeletal muscle ageing is traditionally characterised as the progressive decline in skeletal muscle mass and contractile function with increasing age, leading to a loss of strength and power, which are critical for activities of daily living (Rosenberg, 1989). An age-related reduction in muscular strength has been shown to be a precursor for several co-morbidities including diabetes, coronary heart disease and overall mortality (Chen *et al.*, 2013; dos Santos *et al.*, 2017). Increasing evidence shows that an age-related decline in strength and power, rather than the loss of muscle mass, are the key determinants of increased mortality and poor quality of life in older adults (dos Santos *et al.*, 2017). In the literature, an age-related decline in contractile function without prevalent atrophy is described as dynapenia (Clark and Manini, 2008), whilst the combination of a low muscle mass and poor contractile function is described as sarcopenia (Rosenberg, 1989). A reduction in muscle mass typically begins after 50 years of age through to the end of life, though reductions can occur as early as 25 years of age (Lexell, 1995). The prevalence of sarcopenia, in Europe alone, is expected to increase by 72% by as soon as 2045 (Ethgen *et al.*, 2017). It is of importance to better understand the age-related decline in skeletal muscle contractile function to better improve morbidity and health-related outcomes.

An increase in age is also associated with an elevated body mass, which largely arises from increased storage of adipose tissue (Miard and Picard, 2008; Barzilai *et al.*, 2012). The incidence of obesity in older adults is increasing (Bowman *et al.*, 2017; Hamer and O'Donovan, 2017), with the prevalence of obesity doubling since 1980 in over 70 countries, with over 2 billion worldwide classified as obese (GBD 2015 Obesity Collaborators *et al.*, 2017). Of this population, the greatest incidence of obesity is found in women aged 60-64 years and men aged 50-54 years old (GBD 2015 Obesity Collaborators *et al.*, 2017). The synergistic effects of ageing and obesity can further exacerbate the prevalence of pre-existing comorbidities such as type 2 diabetes, cardiovascular diseases, metabolic syndrome, cancer, and has been proposed to exacerbate the age-related decline in muscle function (Chuang *et al.*, 2016; Bowman *et al.*, 2017; Tallis *et al.*, 2018). In the case of the latter, a low muscle mass and high fat mass may result in further limitations in locomotory and respiratory function, when compared to adults exhibiting only low muscle mass or high fat mass, due to a combination of an elevated bodily inertia and weaker skeletal muscles limiting normal physical functioning (Rolland *et al.*, 2009). The link between ageing and obesity in relation to skeletal muscle contractile function, however, is presently unclear.

Given the importance of ageing and obesity in relation to musculoskeletal health, *in vivo* studies examining the age-related and obesity-induced changes in maximal force, power and fatigue resistance have been undertaken in humans (Doherty, 2003; Fragala *et al.*, 2015). Assessments of isolated skeletal muscle have also been used to assess direct changes in muscle function with increasing age and following an obesogenic diet (Ballak *et al.*, 2014; Tallis *et al.*, 2018). *In vitro* studies offer an important advantage over *in vivo* assessments of muscle function, such as removal of any central nervous system effects, examination of specific skeletal muscles and a direct measure of muscle quality (muscle performance relative to tissue size). Although such studies have provided a valuable insight into specific muscle changes with increasing age the current body of literature is limited as little work has examined the sex-specific differences of isolated muscle performance across

multiple time points of an animal's life. Moreover, the majority of work has compared the isometric and concentric contractile properties of ageing skeletal muscle, with work examining eccentric muscle activity and obesity in old age lacking and ambiguous. There is a distinct lack of work that has measured the change in power output of isolated muscles with age, an important contractile property governing locomotory and respiratory function *in vivo* (Dickinson *et al.*, 2000), with much of the literature measuring age-related and obesity-related changes in isometric force production of isolated skeletal muscles (Pagala *et al.*, 1998; Moran *et al.*, 2005; Ballak *et al.*, 2014; Tallis *et al.*, 2018). Measures of isometric force have poor functional relevance to *in vivo* contractile dynamics otherwise examined via the work loop (WL) technique (James *et al.*, 1996). The present body of work uses the WL technique to better replicate the *in vivo* function of power producing skeletal muscles in ageing, obesity, and during eccentric muscle activity.

To address these important gaps in knowledge, the following three experimental studies have been conducted:

The Sex-Based Differences in the Age-Related Changes in Isolated Locomotory (Soleus & EDL) and Respiratory (Diaphragm) Skeletal Muscle Contractile Function of CD-1 Mice.

Only one study to date has examined the age-related changes in skeletal muscle power output using the WL technique (Tallis *et al.*, 2014). The first study aims to further the findings of Tallis *et al.* (2014) by determining the changes in muscle function and morphology of animals aged 3, 10, 30, 52 and 78 weeks of age to better identify the age-related onset of muscle atrophy of specific skeletal muscle. Each age was specifically chosen to represent a corresponding period in the human life span and is described in detail in section 3.1. The age-related changes in isometric properties, WL power and fatigue resistance are compared for a relatively slow (soleus) and fast (EDL) locomotory muscle phenotype, under voluntary control, in comparison with the diaphragm, a core muscle primarily under involuntary activation to maintain respiration. Moreover, this study also determines whether the age-

related decline in muscle function is significantly different between sexes. Evidence from *in vivo* studies suggests that the loss of absolute force and power, and force and power normalised to body mass, occurs faster in males than females in humans, though little is known as to what extent sex influences muscle mechanics at an isolated muscle level. Assessing the age, sex, and muscle-specific changes in morphology and function could further improve our understanding of the ageing process which can aid in the development of treatments and physical activity regimens which can offset the ageing process and enhance our quality of life.

The Effect of Increasing Age on The Concentric and Eccentric Contractile Properties of Isolated Mouse Soleus and Extensor Digitorum Longus Muscles

The aim of this study was to assess the age-related and muscle-specific responses to acute and sustained eccentric muscle activity and determine how this compares to concentric muscle activity. This was achieved by reciprocating the muscle length change and stimulation parameters which typically elicit maximal concentric power output, but during eccentric activity also, where the intention was to not deliberately cause contraction-induced injury upon the skeletal muscle as per previous studies. The WL model was used to examine whether soleus or EDL muscles are fatigued or damaged during repeated concentric and eccentric muscle activity, and whether these effects alter between young (10 weeks) and old (78 weeks) animals during the recovery of concentric power, and whether acute eccentric power changed with age for either skeletal muscle.

The Effects of Dietary-Induced Obesity on the Contractile Properties of Isolated Soleus, EDL & Diaphragm Skeletal Muscles from Aged CD-1 Mice

In comparison to the literature investigating age-related changes in isolated skeletal muscle function, there is a dearth of literature investigating the effects of obesity on isolated skeletal muscle function, with no work to date utilising older (~78 weeks old) skeletal muscles. Therefore, the aim of this study was to determine whether dietary-induced obesity in older animals significantly affected isolated

skeletal muscle function when compared to age-matched control animals fed a comparatively low-fat diet and whether any changes in muscle morphology and function were muscle-specific. By examining dietary-induced obesity in old age, an understanding on whether the quality of the skeletal muscle (i.e. force and power relative to muscle mass) was poorer in old obese skeletal muscles, and whether this will have a likely effect on *in vivo* locomotory and respiratory function.

A schematic of the thesis outline is shown in Figure 1.1.

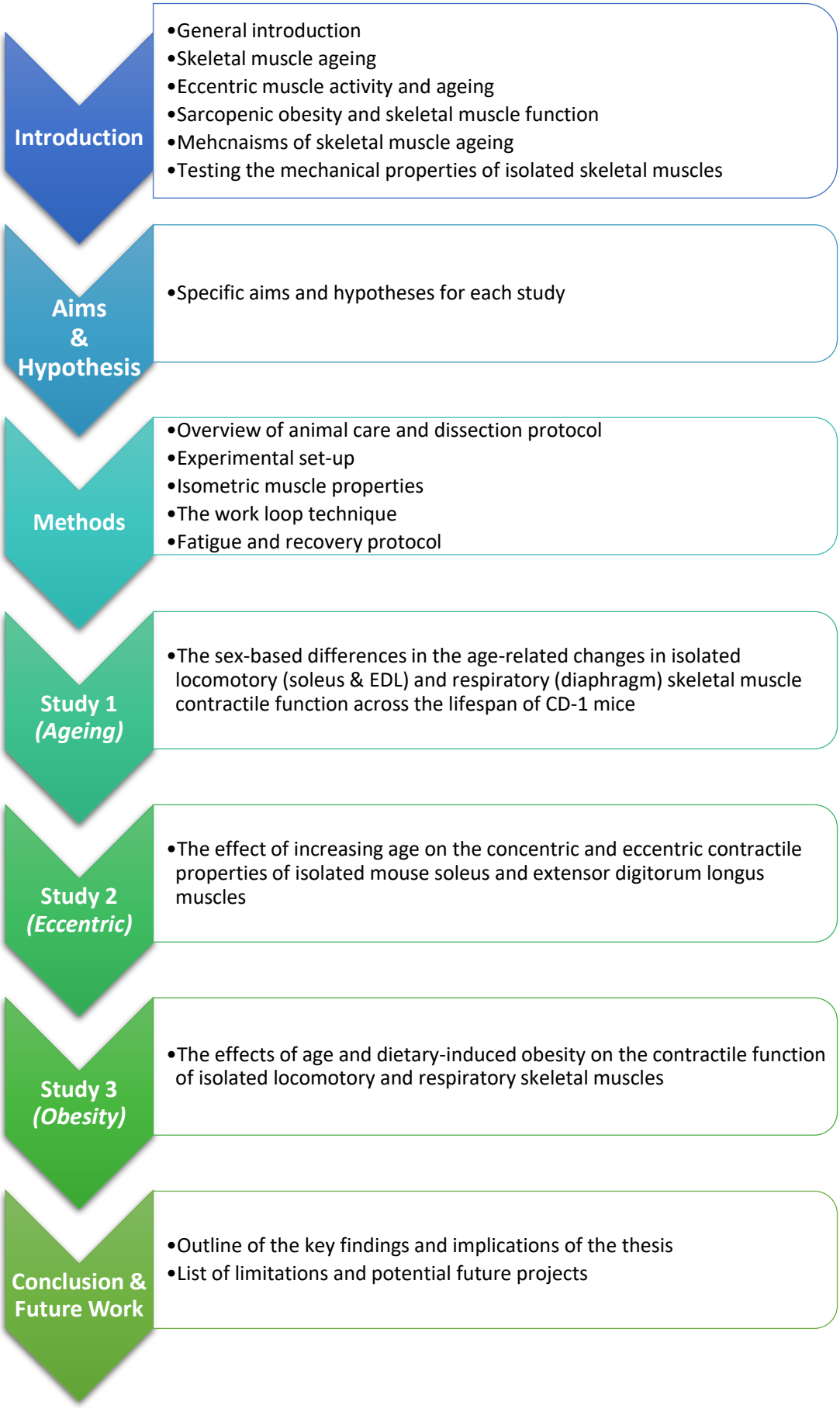


Figure 1.1 - A schematic outlining the chapters and contents of the thesis.

1.2 - Skeletal Muscle Ageing

1.2.1 - Changes in Skeletal Muscle Function with Age

As of 2017, it has been estimated that of the 66 million people in the United Kingdom, approximately 12 million of this population are aged 65 years or over (Office for National Statistics, 2018). The UK life expectancy is predicted to rise to 79.1 years for men and 82.8 years for women respectively, with a forecast that the number of individuals in the UK aged over 65 will increase to 17.3 million by 2035 (Office for National Statistics, 2018). With an increasing number of people over the age of 65, it is more important to understand the ageing process of skeletal muscle and how contractile function is altered. Skeletal muscle ageing is associated with debilitating factors in older adults such as reduced mobility (Marcus *et al.*, 2012), elevated fall risk (Muir *et al.*, 2010), reduced ability to perform activities of daily living (Janssen *et al.*, 2002), and as a consequence, a reduction in quality of life (Verlaan *et al.*, 2017) and an increase in mortality rates (Ethgen *et al.*, 2017).

An age-related reduction in skeletal muscle mass and function is commonly referred to in the literature as sarcopenia (Rosenberg, 1989). More specifically, sarcopenia is defined as the reduction in muscle mass, strength and a slowing of muscle contractile speed that occurs as a result of the ageing process (Rosenberg, 1997). Consequently, acute force, power and the ability of skeletal muscles to withstand fatigue are reduced in older, sarcopenic adults (Christie *et al.*, 2011). It is expected that by 2045 the number of adults aged 65 – 100 years old with sarcopenia will rise by 72.4% due to increased life expectancy (Ethgen *et al.*, 2017). Whilst evidence has traditionally shown a relationship between muscle size and muscle performance (Cruz-Jentoft *et al.*, 2010), work has shown that the age-related loss of contractile function occurs faster than the loss of muscle mass (Delmonico *et al.*, 2009) or even without prevalent muscular atrophy (Manini and Clark, 2012). The loss of force and power with age without muscular atrophy is known as dynapenia (Clark and Manini, 2008). Sarcopenia is the most commonly used term to describe skeletal muscle ageing, however, this term is generally misused when

describing age-related changes in skeletal muscle form and function. Dynapenia precedes sarcopenia, with substantial changes in contractile function occurring before the loss of muscle mass (Clark and Manini, 2008). However, it is currently unclear whether this differs substantially between males and females, and whether this occurs in a muscle-specific manner. This area of investigation is particularly important given that Lexell (1995) reported that muscle mass peaks as early as 25 years of age, with a 40% decline in muscle area between the ages of 20 and 80. However as age-related loss of strength and power is not attributable to a loss of muscle mass (Lynch *et al.*, 1999) this indicates that mechanisms other than muscle wasting must explain this earlier reduction in muscle function. From a functional perspective, it is reported that dynapenia, more so than sarcopenia, has a much greater impact on normal activities of daily living in older adults (Iwamura and Kanauchi, 2017).

This section will identify previous work investigating changes in skeletal muscle contractile function and morphology with age, both *in vivo* and *in vitro,* and will review the key mechanisms which contribute to reduced muscular performance with increasing age.

1.2.2 - The Effect of Age on Muscle Function In Vivo

1.2.2.1 - Strength & Power

A decrement in the strength and power output of skeletal muscle has been identified to occur as an inescapable result of the ageing process (Reid and Fielding, 2012). These measures of muscle performance are distinctly different and as such, it is important to first distinguish between the two. Muscular strength is defined as the maximum amount of static (isometric) force, where force is produced at a constant muscle length. Alternatively, strength is known as the dynamic force, or torque, a muscle can produce against an external load during a given movement (McMaster *et al.*, 2014). Muscular power is defined as the product of the force produced by a muscle multiplied by the velocity at which the muscle changes length (Power = Force x Velocity; Reid and Fielding, 2012). More simply, this indicates the rate at which work is performed (Rodgers and Cavanagh, 1984).

Changes in muscular strength and power are affected differently with increasing age, where the quantification of age-related changes in muscular power is more difficult to obtain than muscular strength (Earles *et al.*, 2001; Macaluso and De Vito, 2004). Assessment of muscular power is particularly important considering the greater functional need for power production versus static force production in everyday living such as locomotion, stair ascent and sit-to-stand movements (Foldvari *et al.*, 2000). Foldvari *et al.* (2000) assessed the relationships between muscle power and muscle strength in relation to their contributions to the self-reported functional status of older (mean age 74.8 ± 5.0 years) community-dwelling women. Peak power was computed as the product of force and velocity during chest press, upper back, leg press and hip abductor one repetition maximum test. Peak power was significantly better at predicting functional status and dependency compared to strength, thus highlighting the importance of measuring muscular power as opposed to muscular strength. Despite the associated difficulties, power output of the lower extremities has been successfully measured during isokinetic (i.e. isovelocity) contractions at varying angular velocities. Vertical jumps (Grassi *et al.*, 1991; Ferretti *et al.*, 1994; De Vito *et al.*, 1998) and sit-to-stand using force platforms (Gray and Paulson, 2014) have also been utilised to measure instantaneous muscular power in older adults.

By measuring these performance parameters in tandem with measurements of muscle morphology, the age-related declines in *in vivo* muscular function have been well documented. Table S1.1 outlines a selection of the studies that have investigated the effects of increasing age on muscular strength and power, the ability for skeletal muscles to withstand fatigue and, where reported, measures of muscle morphology.

There is a wealth of evidence that demonstrates a significant reduction in muscular strength with advancing age (Table S1.1). This reduction occurs in muscles of both the upper and lower extremities and is typically accompanied by a decline in muscle mass and muscle cross-sectional area (CSA) in the

oldest old adults (i.e. sarcopenia). Whilst several studies demonstrate a reduction in muscle size in line with strength (Young *et al.*, 1984, 1985; Overend *et al.*, 1992), other studies demonstrated a greater decline in muscular strength than muscle size with age in males and females (Frontera *et al.*, 2000; Klein *et al.*, 2001; Delmonico *et al.*, 2009). It is difficult to identify when the onset of sarcopenia occurs due to the variety of experimental approaches and the ages of the participants used, nor has any longitudinal study examined changes in muscle mass and function from adulthood to old age of a single cohort. Based on the evidence in table S1.1 where studies have examined muscle CSA, an age-related reduction in muscle CSA is typically observed by the 7th decade (Young *et al.*, 1984, 1985; Reed *et al.*, 1991; Overend *et al.*, 1992; Frontera *et al.*, 2000; Bazzucchi *et al.*, 2005; Delmonico *et al.*, 2009). Lauretani *et al.* (2003) reported little change in calf CSA for men and women up to 65 years of age, with a 10.2% and 4.9% reduction for men and women respectively aged 75-84 years, compared to participants aged 65-74 years. This is supported by Frontera *et al.* (2000) who reported a reduction in thigh muscle, leg extensor and leg flexor muscles by 14.7%, 16.1% and 14.9% respectively in a 12-year follow-up of men initially aged 65 years old. Irrespective of changes in muscle mass with age, a greater loss of muscle strength over size would indicate that muscle mass alone is not a good predictor of muscle function (Newman *et al.*, 2006).

As with muscular strength, there is an age-related decline in muscular power (Skelton *et al.*, 1994; Metter *et al.*, 1997; De Vito *et al.*, 1998; Lauretani *et al.*, 2003; Macaluso and De Vito, 2003; Pojednic *et al.*, 2012; Edwén *et al.*, 2014). When compared to strength though, the loss of power exceeds that of strength in older adults (Skelton *et al.*, 1994; Metter *et al.*, 1997; Lauretani *et al.*, 2003; Macaluso and De Vito, 2003; Edwén *et al.*, 2014). For example, Metter *et al.* (1997) reported strength and power output 34% and 42% in men, and 32% and 46% respectively from the 20th decade to the 80th decade of life. Lauretani *et al.* (2003) observed a greater magnitude in decline between adults of the same age groups. The authors reported a decline of 60% and 57% in knee extensor torque for males and females respectively, compared with a 74% and 76% decline in knee extensor power output, with

Skelton *et al.* (1994) and Macaluso and De Vito, (2003) reporting similar findings for the musculature of the legs. A reduction in power before strength poses a concerning limitation to *in vivo* locomotory capacity in older adults due to the requirements of power rather than strength for locomotory function (Dickinson *et al.*, 2000).

Evidence indicates that the loss of strength in males is greater than that of females in absolute terms (Reed *et al.*, 1991; Skelton *et al.*, 1994; Lauretani *et al.*, 2003; Delmonico *et al.*, 2009; Edwén *et al.*, 2014). When measuring sex differences from a cross-sectional methodological approach, some studies demonstrate equal losses in absolute strength (Reed *et al.*, 1991; Lindle *et al.*, 1997; Lauretani *et al.*, 2003) and power (Metter *et al.*, 1997; Lauretani *et al.*, 2003), between males and females with age. Others show that the loss of strength (Metter *et al.*, 1997; Lynch *et al.*, 1999; Hughes *et al.*, 2001; Delmonico *et al.*, 2009; Dey *et al.*, 2009) and power (Skelton *et al.*, 1994; Edwén *et al.*, 2014) occurs faster in males than females with advancing age. However, the rate of loss of force and power varies between studies. For example, Hughes *et al.* (2001) found that men experienced a 12% loss in elbow extensor and flexor strength per decade compared to a 2% loss per decade in women. Edwén *et al.* (2014) found that for males the loss of power and force normalised to body mass occurred at a rate of $0.44 W.Kg^{-1}$ and $0.07 N.Kg^{-1}$ per year respectively whilst the loss of power and force normalised to body mass for females declined by $0.29 W.Kg^{-1}$ and $0.04 N.Kg^{-1}$ per year, respectively. Work by Edwén *et al.* (2014) also shows that the *in vivo* loss of power occurs faster than the loss of strength and it is proposed that the magnitude of loss is greater for men (Skelton *et al.*, 1994; Edwén *et al.*, 2014). Human work supports that ageing is concurrent with a decline in the force generating capacity of the muscle. However, there is further evidence to support that ageing is associated with a reduction in the shortening velocity (V_0) of skeletal muscles (Raj *et al.*, 2010), with the loss again faster in men than women (Edwén *et al.*, 2014). An age-related down and leftward shift (Figure 1.2.1) in the force-velocity relationship contributes to the faster rate of decline in muscle power output (Raj *et al.*, 2010), given that power is the product of force multiplied by shortening velocity.

Figure 1.2.1 - Ageing results in a downward and leftward shift in the force-velocity relationship (arrow 1) and a downward and leftward shift in the power-velocity relationship (arrow 2) in old adults (OA) compared to young adults (YA) (Raj et al., 2010).

Whilst absolute measures of strength and power are interesting, given that loss of contractility and functional capabilities exceed that which can be explained by reductions in muscle mass, it is important to assess muscle contractility in terms of muscle quality as opposed to muscle strength and size as independent variables. Muscle quality describes the total amount of force a muscle can generate per quantity of tissue (Lynch *et al.*, 1999), though few studies have measured this property of muscular function in an ageing model (Lindle *et al.*, 1997; Lynch *et al.*, 1999). The importance of measuring muscle quality, and the limitations to measuring muscle quality *in vivo*, is explained in detail in section 1.6.1.3. Few *in vivo* studies assessing muscle quality measure the force a muscle produces in relation to muscle CSA as determined by computed tomography (CT) scans or magnetic resonance imaging (MRI) scans (McGregor *et al.*, 2014). Alternatively, muscle quality can be determined by calculating force relative to muscle mass as determined by dual x-ray absorptiometry scanning (Cruz-Jentoft *et al.*, 2010). Whilst ageing research typically represents force and power in absolute measures, it is becoming more common to represent strength and power relative to muscle CSA as a

measure of muscle quality (Fragala *et al.*, 2015). Older adults who can maintain the same amount of force for a smaller muscle volume/quantity would be described as having good muscle quality, which allows for the more efficient functioning during everyday living as such muscle can produce the same force but has lower mass and inertia than a muscle of lower quality. A decline in muscle quality has been shown to occur in line with ageing (Lindle *et al.*, 1997; Lynch *et al.*, 1999) and appears to occur in a muscle-specific manner (Lynch *et al.*, 1999). When considering muscle quality and sex-based differences, the age-related decline in strength relative to muscle size occurs equally between men and women (Janssen *et al.*, 2000; Silva *et al.*, 2010). Doherty (2003) theorises that the loss of absolute force and power is faster for men than women because males start from a higher baseline at a younger age. Men generally experience greater declines in muscle CSA than women with advancing age, with a potential associated reduction in muscle quality (Hughes *et al.*, 2001) which may explain the greater reduction in power compared to women. However, this greater decline in power is likely due to male skeletal muscles being morphologically larger in terms of muscle CSA (Behan *et al.*, 2018).

The loss of skeletal muscle force and power does not occur in locomotor muscles only, where the decline in contractile performance occurs uniformly for all skeletal muscles in the human body. The diaphragm is a key regulator of respiratory function and, like locomotor muscles, is prone to an age-related reduction in contractile function (Polkey *et al.*, 1997). Diaphragm function is a significant contributor to the uptake and distribution of oxygen, where limited pulmonary function due to reduced diaphragm contractile function may predispose older adults to be at greater risk of ventilatory failure (Sharma and Goodwin, 2006). Diaphragm force production in humans has been shown to decline with age, with a 13-25% reduction in diaphragm force production in older adults (Tolep *et al.*, 1995; Polkey *et al.*, 1997), though there is a no evidence examining changes in diaphragmatic power in humans with age.

1.2.3 - Effects of Age on Muscular Endurance In Vivo

Whilst the age-related decline in muscular strength and power is well documented, findings in relation to the age-related decline in muscular endurance in humans are somewhat equivocal (Table S1.1). Muscular endurance can be defined as the ability of skeletal muscles to maintain force during repeated static activations or torque/power during sustained dynamic muscular contractions for an extended period of time (Kell *et al.*, 2001; Deschenes, 2004). Fatigue resistance is an important biomechanical parameter as normal everyday living requires skeletal muscles to be able to withstand fatigue in order to maintain locomotory and respiratory function (Dickinson *et al.*, 2000).

Whilst some studies have demonstrated an age-related decline in muscle endurance (Davies *et al.*, 1986; Sunnerhagen *et al.*, 2000; Izquierdo *et al.*, 2001) and poorer fatigue resistance for males than females (Davies *et al.*, 1986; Hicks and McCartney, 1996), some have shown no differences (Klein *et al.*, 1988; Bäckman *et al.*, 1995; Bemben *et al.*, 1996; Lindström *et al.*, 1997) or even an age-related increase in muscle endurance (Hicks and McCartney, 1996; Bilodeau *et al.*, 2001; Bazzucchi *et al.*, 2005). Discrepancies typically arise due to the different protocols used to measure fatigue (Deschenes, 2004), as well as the different ages, sex and muscle groups assessed.

1.2.4 - Dynapenia

Largely, the results outlined in the previous discussion have been attributed to sarcopenia. However, it is becoming increasingly common to consider that some of the age-related declines in muscle performance and associated loss of strength and power is independent of muscle wasting (Morley *et al.*, 2001). This has resulted in the more recent usage of the term dynapenia, literally meaning loss of power. This relatively new concept, first described by Clark and Manini (2008), should not replace the current understanding of sarcopenia, but should instead be used to supplement current knowledge. In humans, the loss of contractile function without prevalent atrophy has been well documented. Work by Delmonico *et al.* (2009) reported that the loss of strength over a 5-year period was on average

four times faster than the loss of muscle mass, irrespective of whether an adult gained or lost weight (Figure 1.2.2). This was coupled with significant declines in muscle quality and significant increases in intramuscular fat for men and women. Little evidence exists as to the age that sarcopenia and dynapenia begin and whether this differs between muscles and sex.

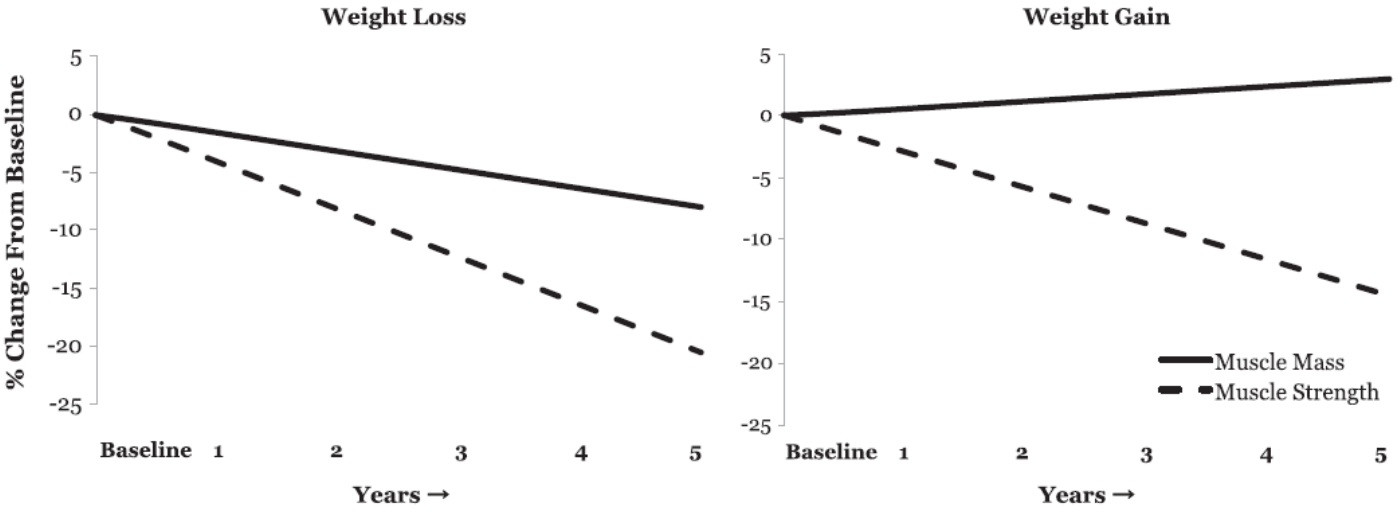


Figure 1.2.2 - The age-related decline in knee extensor strength (dashed) and quadricep femoris muscle mass measured by computed tomography in a 5-year longitudinal study. An increase in muscle mass (right panel) does not prevent loss of muscle strength indicating loss of muscle quality with age. (Data from Delmonico et al., 2009. Figure produced by Manini and Clark, 2012).

It is clear from the evidence presented that muscular ageing is a highly complex issue. There is robust evidence supporting both sarcopenia and dynapenia as key mechanisms for the age-related loss of muscle force via reduced contractile mass for the former and in the absence of reduced muscular atrophy for the latter (Seene and Kaasik, 2012). The magnitude of the effect of sarcopenia and dynapenia can be affected by several mitigating factors including sex, the age of the population assessed, the location and mechanical role of the skeletal muscle assessed, the predominant fibre type of the muscle, muscle recruitment and ageing of the central nervous system. These factors are covered

in detail in section 1.6.1, though all can be better controlled for via use of an isolated muscle model for examining muscle ageing.

1.2.5 - Examining Ageing Using Isolated Skeletal Muscle

As with *in vivo* assessments of muscle contractility, there is an abundance of literature which has investigated the effects of ageing on the contractile function of isolated skeletal muscles *in vitro*. The value of isolated muscle studies is explored in greater depth in section 1.6.1. In brief, *in vitro* testing can be used to test isolated muscles to determine the muscle-specific effects in response to a stimulus, in this instance ageing and obesity. This is important given skeletal muscles of different muscle fibre types and contractility modes are affected to different extents and are affected by age and obesity in separate ways as discussed in sections 1.2.5, 1.3.2, and 1.4.3 respectively. Prior *in vitro* research has typically employed isometric (constant length), isotonic (constant force) or isovelocity (constant velocity) contractions to assess strength and power in a young age group against an old age group, though such contractions poorly replicate *in vivo* muscle function (James *et al*., 1996).

When assessing muscle contractility and ageing *in vitro*, the soleus and EDL are the two most commonly assessed muscles, primarily due to their differing phenotype compositions and ease of isolation for use. When comparing the percentage of the different myosin heavy chain (MHC) isoforms, the soleus is predominantly composed of slower, less forceful type I fibre type (53.6% type I; 31.2% type IIA; 15.2% type IIX in 90-day old young adult C57BL/6 mice; Agbulut *et al.*, 2003) whilst the composition of the EDL is predominantly the faster and more forceful type IIB fibre type (3.9% type I; 9.3% type IIX; 86.8% type IIB in 90 day old young adult C57BL/6 mice; Agbulut *et al.*, 2003). The composition of the diaphragm is more mixed to cope with its mechanical role during respiration, where slow-twitch fibre recruitment is important for continual diaphragm activity during steady-state breathing, and recruitment of fast-twitch fibres for forceful expiration during exercise (15.6% type I;

6.2% type IIB, 34.6X type IIX, 43.6% type IIA in 90-day old young adult C57BL/6 mice; Agbulut *et al.*, 2003).

Muscles with a greater proportion of type I fibres will tend to have greater muscular endurance but generate less force (Costill *et al.*, 1976; Fink *et al.*, 1977; Saltin *et al.*, 1977) whilst muscles that possess a greater proportion of type IIA/X/B will produce more force per unit of muscle cross-sectional area (CSA) and power output relative to muscle mass, but will fatigue much more rapidly (Costill *et al.*, 1976). With these characteristics in mind, the soleus is important in maintaining posture and balance (Eston *et al.*, 1995) hence enhanced fatigue resistance is valuable in the successful completion of tasks in this muscle. On the other hand, the EDL is important for movements of the ankle via dorsiflexion, which contributes to the power required for locomotory activities such as running and jumping (Brockett and Chapman, 2016). Therefore, from an ageing perspective, it is interesting to assess these two locomotory muscles as each may respond differently to the ageing process, thus allowing for a closer assessment of whether muscle ageing is dependent on alterations of particular fibre types that would contribute significantly to the decline in muscular performance. In addition to examining the age-related changes in locomotory muscle function, examining the diaphragm provides an insight into the effects of ageing on respiratory muscle function. An impairment in the ability to uptake oxygen could contribute to other aspects of age-related diseases, such as impaired exercise capacity and substrate metabolism, and as such understanding the age-related decline in diaphragm contractile function could provide an insight into how the ability of this muscle to generate force and power is affected with age. In general, it is expected that muscles composed of primarily fast-twitch muscle fibres age faster than those composed of predominantly slow-twitch fibres, as characterised by a reduction force, mass, fibre type distribution (i.e. fast-to-slow transition) and smaller muscles CSA (Ballak *et al.*, 2014), though differences in methodological approach, animal strain, contraction type and ages examined means this is not always the case.

The remainder of this section shall compare the age-related changes in contractile function for single muscle fibres and whole skeletal muscles for the soleus, EDL and diaphragm, with an overview of the fatigue resistance of whole isolated skeletal muscles.

1.2.5.1 - Single Muscle Fibre Experiments of Locomotory Skeletal Muscles

Single muscle fibre testing can be used to assess age-related changes in contractile function, however, the evidence supporting an age-related decline in single fibre contractile function is equivocal. Ageing studies of single muscle fibres report a reduction in isometric force and stress (force/muscle cross-sectional area) of the soleus (Thompson and Brown, 1999; González *et al.*, 2000) and EDL (González *et al.*, 2000) with increasing age, whilst others reported no change in force or stress with increasing age for single soleus fibres (Kim and Thompson, 2012) or single EDL fibres (Brooks and Faulkner, 1994) (Table S1.2). González *et al*. (2000) also reported no age-related atrophy of the EDL despite a decline in force and stress, highlighting reduced mechanical performance as a reason for the decline in isometric stress. In addition to an age-related reduction in the force-generating capacity of single muscle fibres, measures of V_0 show equally equivocal findings, with Thompson and Brown (1999) and González *et al.* (2000) showing a significant slowing of V_0 of soleus and EDL fibres with age, whilst Kim and Thompson (2012) found no change in V_0 for rat soleus with age. However, absolute power declined significantly from young rats to aged rats, with an increase in absolute power from adult to aged rats (Kim and Thompson, 2012). Power normalised to fibre size also remained unchanged. This study is the first to demonstrate, at a single fibre level, that absolute measures of force and power are lost to a greater extent than normalised measures. Moreover, changes in absolute and normalised measures of force and power vary with age. Inconsistencies in these findings can be attributed to the rodent model used and the ages examined.

1.2.5.2 - Whole Muscle Experiments of Locomotory Skeletal Muscles

Whole muscle studies are beneficial for considering the age-related changes of contractile function as it allows for consideration of all contractile and non-contractile properties of the muscles, allowing for

a more relevant comparison to *in vivo* muscle function. Section 1.6.3.1 provides a more detailed discussion of the advantages and disadvantages of single fibre and whole muscle testing. Table S1.2 outlines the current work to date which has investigated the age-related changes in rodent skeletal muscle performance, for both isometric and dynamic muscular contractions of the soleus, EDL and diaphragm *in situ* and *in vitro*. *In situ* studies involve investigators anesthetising an animal and exposing a specific, intact muscle, which is stimulated with single or repeated stimuli isometrically or during shortening (Call and Lowe, 2016). As for *in vitro* investigations, the whole muscle is isolated from an animal with stimulations provided externally. Greater details of these methodological approaches are provided in section 1.6.

In general, as with human studies of ageing, as animals age the force and power output of the isolated skeletal muscle declines, though the magnitude of the effect is specific to the muscle and the contractile parameter examined. The proposed age-related reduction in force is ambiguous, with some reporting a reduction in both absolute force and isometric stress (force/muscle CSA) (Brooks and Faulkner, 1988; Phillips *et al.*, 1991; Kadhiresan *et al.*, 1996) whilst others report no change in either measure (Rice *et al.*, 2005; Kim and Thompson, 2012). Moreover, other authors have reported a decline in absolute force with no change in isometric stress (Pagala *et al.*, 1998; Lynch *et al.*, 2001; Graber *et al.*, 2015), whilst Tallis *et al.* (2014) reported a reduction in isometric stress, but no change in absolute measures of isometric force. The effects are likely to be muscle specific, with EDL absolute force and stress surprisingly better maintained with age than for the soleus (Lynch *et al.*, 2001; Moran *et al.*, 2005), which is surprising given the notion that faster fibres are more prone to muscular ageing (Miljkovic *et al.*, 2015). Sex may also play an influencing factor, with isolated EDL force and stress better maintained for males than females with advancing age (Chan and Head, 2010) despite *in vivo* evidence demonstrating otherwise (Reed *et al.*, 1991; Skelton *et al.*, 1994; Lauretani *et al.*, 2003; Delmonico *et al.*, 2009; Edwén *et al.*, 2014). Further work is required to better elucidate the sex-based differences in skeletal muscle function at an isolated muscle level.

Few studies have examined the effect of ageing on muscle power output. Generally, the literature demonstrates a reduction in absolute power and power normalised to muscle mass with advancing age (Brooks and Faulkner, 1991; Lynch *et al.*, 2001; Tallis *et al.*, 2014). Only Kim and Thompson (2012) reported a reduction in absolute power but maintenance of power relative to fibre size, though this may be due to the single soleus fibres being assessed compared to whole muscles as in previous studies, and usage of a different method to determine muscular power (Table S1.2). The effect of ageing on force and power is likely to be muscle specific and dependent on the contractile type. The loss of power exceeds the loss of strength in human studies of ageing (Metter *et al.*, 1997; Krivickas *et al.*, 2001; Raj *et al.*, 2010), however, the opposite is true of isolated EDL and diaphragm skeletal muscles (Tallis *et al.*, 2014). The loss of absolute and normalised power was greater in the soleus than EDL (Lynch *et al.*, 2001) which is contradictory to research examining isometric force where soleus force is well maintained compared to the EDL (Table S1.2). However, the power producing capabilities of EDL are better maintained than diaphragm with increasing age (Tallis *et al.*, 2014) indicating that the anatomical location and function of the muscle may play a role in the ageing response.

The studies of *in vitro* and *in situ* ageing show beyond doubt that ageing typically corresponds with a reduction in contractile function of whole locomotory skeletal muscles (Table S1.2), indicating that a reduction in contractile performance is not primarily due to deterioration of the central nervous system or neuromuscular junctions. A reduction in performance is largely mirrored by a decline in muscle mass and muscle CSA for the soleus (Brooks and Faulkner, 1988; Brown and Hasser, 1996; Pagala *et al.*, 1998; Lynch *et al.*, 2001) and the EDL (Gutmann and Carlson, 1976; Brooks and Faulkner, 1988; Pagala *et al.*, 1998; Lynch *et al.*, 2001; Graber *et al.*, 2015). However this is not always the case, with instances of a reduction in contractile performance, but not muscle mass in the earlier stages of an animals life (Phillips *et al.*, 1991; Brown and Hasser, 1996; Criswell *et al.*, 1997, 2003; Thompson and Brown, 1999; Moran *et al.*, 2005; Chan and Head, 2010; Kayani *et al.*, 2010; Tallis *et al.*, 2014).

1.2.5.3 - Respiratory Muscle (Diaphragm)

Despite a number of studies examining the contractile performance of the diaphragm in a young age group (Syme and Stevens, 1989; Yan *et al.*, 1993; Van Lunteren and Moyer, 1996; Ameredes *et al.*, 2000), the age-related changes in diaphragm contractility have been less extensively explored *in vitro* in comparison to locomotory muscles (Table S1.2). Unlike studies of locomotory skeletal muscles, studies of the diaphragm consistently report an age-related reduction in isometric stress (Zhang and Kelsen, 1990; Criswell *et al.*, 1997, 2003; Greising *et al.*, 2013; Tallis *et al.*, 2014; Elliott *et al.*, 2016) with only Lynch *et al.* (1997) reporting no changes in isometric stress and normalised power output of diaphragm strips from young to old age. Results from an isolated muscle model provide further support for a reduction in respiratory function and resultant respiratory problems in later life of older adults (Tolep *et al.*, 1995; Polkey *et al.*, 1997).

1.2.5.4 - Fatigue Resistance of Isolated Muscles

There are a limited number of studies that have investigated muscular endurance in an *in vitro* model (Table S1.2). Generally, an increase in age results in a maintenance of fatigue resistance (Pagala *et al.*, 1998; González and Delbono, 2001; Criswell *et al.*, 2003) or even an increase with age (Pagala *et al.*, 1998; Chan and Head, 2010), with only Tallis *et al.* (2014) reporting an age-related decline in the ability to withstand fatigue. Interestingly, studies which have observed a maintained or enhanced fatigue resistance in older skeletal muscles, the absolute force (Pagala *et al.*, 1998) and stress (Criswell *et al.*, 2003; Chan and Head, 2010) declines with age, which may provide an insight into the age-related changes in fibre-type composition of the skeletal muscle, where a fast-to-slow fibre shift results in weaker but more fatigue resistant skeletal muscles. Ageing has been shown to cause skeletal muscle fibres to shift to a more oxidative fibre type with advancing age (Alnaqeeb and Goldspink, 1987) with slower type I fibres being more fatigue resistant but less forceful than type II fibres, which have lower fatigue resistance but generate greater force. A further issue to consider is that a reduction in maximal isometric force in animals and humans may mean that the muscle is working closer to its maximal

force generating capacity during prolonged movement and as such it is likely to be difficult to maintain for prolonged periods, and in turn partially explains reduced endurance in older adults. The lack of consensus as to what defines fatigue resistance at the muscular level also makes comparisons between studies difficult due to the different methodologies employed and each author's interpretation as to what constitutes a significant reduction in fatigue resistance.

Due to the variety of methodological approaches employed in terms of muscles assessed (e.g. isometric, isovelocity and WL), animals examined, test temperature and age points used to determine young, adult, old populations, it is difficult to draw direct comparisons between studies. Such experimental approaches provide the main source of ambiguity when analysing the results of the age-related decline in muscle performance in sections 1.2.5.2, 1.2.5.3 & 1.2.5.4. Additionally, there is a surprising lack of work which has used more than three time points in an animals' lifespan, with many typically using two (young vs. old) age groups (Table S1.2). Further work is also required to examine the age-related changes in muscle function whereby the mode of contraction employed *in vitro* more closely replicates the dynamic activation patterns found during typical *in vivo* muscle activation, as opposed to "iso" contractile parameters. The WL technique has been previously used to provide a better examination of how skeletal muscle power is affected by age between locomotory and respiratory skeletal muscles in their ability to produce and sustain maximal concentric power during cyclic length changes (Tallis *et al.*, 2014). A detailed overview of the WL technique is provided in section 1.6.5.4.

1.2.6 - Assessment of Muscular Performance Using the Work Loop Technique

In the body of work by Tallis *et al.* (2014), absolute isometric force, isometric stress and power output normalised to muscle mass was assessed in isolated EDL and diaphragm from female CD-1 mice aged 3, 10, 30 and 50 weeks of age. By utilising the WL technique, Tallis *et al.* (2014) determined the age-related changes in contractile performance whilst more closely replicating real-world locomotory

function. Additionally, the *in vivo* physiological conditions were replicated *in vitro*, particularly by utilising a test temperature of 37°C, a much more physiologically relevant test temperature than the typical 20-25°C previously used (Table S1.2). The importance of test temperature in relation to muscle mechanics is discussed in greater detail section 1.6.4. One significant finding from this study was that the loss of isometric force (or strength) occurred faster than the loss of power. Secondly, the age-related decline in stress and power occurred with a concomitant increase in muscle mass and body mass with age, with each morphological measure peaking at 50 weeks of age. Additionally, the distribution of power relative to body mass in the 50-week old group indicated that the older, heavier animals may have poorer *in vivo* muscle performance due to the added load of the moving limb, and therefore may further limit locomotory capacity. Tallis *et al.* (2014) found muscle-specific differences in fatigue resistance during repeated WL's with the greatest fatigue resistance typically occurring at 3-weeks, for both EDL and soleus, and reduced by 50-weeks of age, likely due to older muscles producing less positive work as the fatigue protocol progressed, when compared to other ages, and greater negative, or eccentric, work through re-lengthening as fatigue progressed.

The work by Tallis *et al.* (2014) highlights a number of areas that have yet to be fully explored using the WL technique as the basis for investigation. One obvious area for investigation is the effect ageing has on the soleus to generate power. Previous isometric studies typically demonstrate that the soleus was more prone to a loss of absolute force and isometric stress than the EDL (Lynch *et al.*, 2001; Moran *et al.*, 2005). Given the importance of the soleus during *in vivo* locomotion (Eston *et al.*, 1995), an investigation into how acute and sustained power changes with age is warranted. The animals in Tallis *et al.* (2014) underwent no decline in muscle mass in the early stages of ageing despite a decline in muscle function, though many studies report significant reductions in locomotory muscle mass and function at much older ages (Gutmann and Carlson, 1976; Brooks and Faulkner, 1988; Kadhiresan *et al.*, 1996; Pagala *et al.*, 1998; Lynch *et al.*, 2001). The inclusion of animals older than 50 weeks of age may provide information on how WL power output and fatigue resistance is affected in older age and

whether changes are due to a reduction in muscle mass. Only one study to date has examined the contractile properties of whole isolated soleus and EDL with increasing age for males and females (Chan and Head, 2010), however, this study employs isometric contractions which are a poor indicator of *in vivo* contractile function (James *et al.*, 1996). Therefore, further work is required to better understand the sex-based differences in power output of isolated skeletal muscles with increasing age. Finally, ageing is associated with a slowing of V_0 and consequential shift in the force-velocity curve (Krivickas *et al.*, 2001; Raj *et al.*, 2010). Previous work using the WL has examined how contractile speed, or cycle frequency, affects power production, thus creating a power output-cycle frequency curve (Altringham and Young, 1991; James *et al.*, 2011). Applying this methodological approach to an ageing model could help determine whether changes in WL power are predominantly due to a change in optimal contractile velocity as a result of the slowing of muscle fibres, or whether a reduction in power is largely attributable to a reduction in the force-generating capacity of the muscle.

1.3 - Eccentric Muscle Activity and Ageing

During *in vivo* locomotion, skeletal muscles perform different roles in relation to their mechanical role, including eccentric activity (Dickinson *et al.*, 2000) with such a muscle activity affected by ageing (Hortobágyi *et al.*, 1995). Far less data examining ageing in relation to eccentric muscle function is available compared to the existing data examining age-related changes in force and power derived from isometric and concentric contractions (LaStayo *et al.*, 2003). Muscles that are active during lengthening are said to be acting eccentrically (LaStayo *et al.*, 2003). This type of muscle activity occurs to either decelerate a body in motion or to store mechanical energy in preparation for the next concentric contraction (LaStayo *et al.*, 2003). As muscles work as antagonistic pairs, eccentric contractions occur frequently during normal locomotion (Dickinson *et al.*, 2000), dynamic balance (Lindstedt *et al.*, 2002), stair descent (Andriacchi *et al.*, 1980; McFadyen and Winter, 1988) and during the transition from standing to sitting (Lovering and Brooks, 2014). Age-associated changes in the ability to perform eccentric activities ultimately contribute to the inability to perform everyday activities of daily living and may relate to an increased fall risk in older adults (LaStayo *et al.*, 2003). To accomplish these movements, eccentric muscle activity produces much higher forces compared to concentric contractions (Lindstedt *et al.*, 2001; Herzog, 2014). Eccentric activities can cause muscle fibre damage (Fridén and Lieber, 2001; Vissing *et al.*, 2008), leading to reduced muscular strength (Faulkner *et al.*, 1993) and delayed onset of muscle soreness (Byrne *et al.*, 2004). Given the potential for these debilitating factors to affect muscle performance and the regular occurrence of eccentric activity in activities of daily living, it is important to understand how ageing affects eccentric muscle activity.

1.3.1 - In Vivo Studies of Eccentric Muscle Activity

Compared to the numerous studies investigating changes in isometric and concentric muscle function with increasing age in older adults (Table S1.1), a comparatively smaller quantity of literature has investigated changes in eccentric muscle function (Table S1.3). This is due to the perceived high risk

associated with the high forces produced during eccentric muscle activity and the potential to cause damage (LaStayo *et al.*, 2003). Nevertheless, the studies which have assessed the effects of age on eccentric muscle function demonstrated a mixture of findings, with some showing that function is well preserved whilst others reported an age-related decline in eccentric function (Table S1.3).

1.3.1.1 - Changes in Eccentric Force and Torque

Whilst in some cases eccentric torque and force is well maintained with advancing age (Poulin *et al.*, 1992; Hortobágyi *et al.*, 1995; Porter *et al.*, 1997; Horstmann *et al.*, 1999; Klass *et al.*, 2005) or even increased (Phillips *et al.*, 1998), other studies showed an age-related decline in torque (Vandervoort *et al.*, 1990; Lindle *et al.*, 1997; Pousson *et al.*, 2001; Christou and Carlton, 2002; Delbaere *et al.*, 2003; Perry *et al.*, 2007) (Table S1.3). The discrepancies in results are likely due to differences in experimental approaches such as the angular velocity at which eccentric torque was measured, and the muscle groups assessed. For example, many studies examining eccentric torque use a slow and fast angular velocity, though the angular velocity in each study that depicts a fast and slow velocity differs between each study (Table S1.3). An unfamiliarity with producing eccentric force at fast angular velocities may further mask the age-related changes in eccentric torque. In cases where eccentric force was compared to concentric force, the magnitude of the decline in force in older adults was largely greater for isometric and concentric contractions compared to eccentric actions (Vandervoort *et al.*, 1990; Porter *et al.*, 1997; Pousson *et al.*, 2001; Klass *et al.*, 2005). This would indicate that age-related changes in muscle function are specific to the type of muscle action performed (Roig *et al.*, 2010). Furthermore, males lose eccentric force to a greater extent than females (Lindle *et al.*, 1997; Christou and Carlton, 2002) and in some cases was preserved for females compared to males (Klass *et al.*, 2005). However, this is not always the case with some studies showing no effect for sex (Pousson *et al.*, 2001; Delbaere *et al.*, 2003), with no differences in eccentric torque in older males and females (Pousson *et al.*, 2001; Christou and Carlton, 2002; Delbaere *et al.*, 2003).

A distinct lack of studies has examined eccentric muscle activity in relation to muscle quality. As previously discussed, preservation of muscle quality is key for normal locomotory function. Understanding how the quality of a skeletal muscle is affected in relation to eccentric muscle activity can provide a unique insight into the ability to perform and maintain eccentric muscle activity in older adults.

1.3.1.2 - Repeated Eccentric Muscular Activity

Many activities of everyday living require periods of sustained eccentric activity rather than single eccentric activation to successfully execute a required activity. As such, examining the fatigue response and consequential ability to recover force and power is important.

Investigations into the age-related alterations in sustained eccentric muscle activity are greatly under-researched. This is largely due to the negative association between prolonged bouts of eccentric work and increased susceptibility to skeletal muscle damage (LaStayo *et al.*, 2003; Lovering and Brooks, 2014), muscle soreness, and pain (Lovering and Brooks, 2014). This is despite the potential benefits of eccentric exercise in older adults for maintaining or improving muscle function (Narici *et al.*, 2014; Douglas *et al.*, 2017). Nevertheless, of the research to have examined the age-related ability to withstand the fatiguing effects of eccentric muscle actions, studies have typically examined eccentric exercise of specific limbs followed by recovery of eccentric force in the days following (Clarkson and Dedrick, 1988; Chen *et al.*, 2011). Very little work has measured the time-course of eccentric fatigue in older adults, whereby the rate in decline in eccentric force is compared to younger counterparts. Only Baudry *et al.* (2007) have measured the age-related changes in eccentric torque during an eccentric fatigue protocol. Torque during eccentric fatigue was maintained to a better extent than concentric torque for the younger participants. Older participants fatigued faster than younger adults during both concentric and eccentric fatigue, with concentric torque declining by 40.9% for younger adults and 50.2% for older adults ($P<0.05$) and eccentric torque by 27.1% and 42.1% ($P<0.01$) for young

and old adults respectively. However, there were no differences between contraction types for older adults.

No study has examined the age-related changes in eccentric power output with increasing age in humans. Moreover, eccentric torque has been measured as opposed to power, with power being of greater relevance for whole body locomotion than torque as explained in sections 1.2 & 1.6. The dearth in literature is largely due to the practicality of performing such experiments, as participants would have to perform plyometric actions which are associated with muscle damage in older participants that could cause unnecessary mobility limitations (Lovering and Brooks, 2014). One of the main limitations to these studies is the measurements of concentric fatigue *in vivo* as discussed in section 1.2.1, in that the elevated body mass at the same relative intensity may mask the true fatiguing effects on older, heavier adults. Moreover, the CNS may mask the time-course of fatigue, especially considering that Baudry *et al.* (2007) proposed that differences in fatigue were due to neuronal propagation.

1.3.2 - Animal Models

1.3.2.1 - In Situ

Investigators assessing eccentric muscle activity *in situ* firstly anesthetise an animal and expose a specific, intact muscle. Next, single or repeated isovelocity muscle actions are performed through lengthening of the muscle with activation occurring through stimulation of the supplying nerve (Call and Lowe, 2016). Many studies assessing age-related changes in eccentric muscle activity *in situ* purposefully caused contraction-induced injury to understand the mechanisms of damage and healing. As lengthening velocity has no effect on the force deficit following eccentric muscle activity (Lynch and Faulkner, 1998), research typically utilise large muscle length changes to ensure contraction-induced muscular damage occurs. In general, at smaller length changes (i.e. below 20% of muscle fibre length) there is no impairment in consequent recovery of force with age (Brooks *et al.*,

1995; Brooks and Faulkner, 1996) though this is not always the case, where Lynch *et al.* (2001) observed a significant reduction in old (26 months), but not young (6 months), rat EDL isometric force production following single eccentric muscle activations at 10% strain (see section 3.5.4.1 regarding strain) from optimal length (L_0). However, no differences in measures of isometric force were found using 5% strain between age groups (Lynch *et al.*, 2001), highlighting the importance strain magnitude has on eccentric force production, damage, and recovery of force (Choi, 2016). Usage of an *in situ* approach is advantageous as the recovery of muscle fibres and contractile function can be monitored in the weeks and months following injury. However, the CNS may mask the maximal eccentric force a muscle may produce, nor have *in situ* studies reported maximal eccentric force production or changes in eccentric force over time.

1.3.2.2 - In Vitro

In a subgroup of animals used by Brooks and Faulkner (1996), single permeabilised fibre experiments of the EDL from young and old rats were performed, where length changes of 5%, 10% and 20% from L_0 were imposed on each preparation. A strain of 5% caused no reduction in isometric force for either age group, though deficits increased significantly with increasing strain and to a greater deficit at 10% and 20% strain in older EDL fibres but not young fibres, which may indicate greater resistance to stretch as a mechanism of muscular damage. Lynch *et al.* (2008) reported similar findings in the assessment of single fibres from young (6 months) and old (26 months) male rats, in that there were no differences in isometric force from baseline or in old EDL fibres following single lengthening muscle actions by 5% of mean muscle fibre length (L_f), whilst at 10% and 20% of L_f the force deficit of older fibres was 9.0% and 20.2% lower respectively than baseline whilst young fibres declined by 5% and 14.9%.

In one regard, damaging eccentric muscle studies are advantageous as such protocols using large strains are reproducible for ensuring damage occurs (Call and Lowe, 2016). One limitation to previous

in vitro work is that replicating *in vivo* eccentric muscle activity through isovelocity activation is a poor estimate of *in vivo* muscle function as discussed in detail in section 1.6. Additionally, activities of daily living in older adults involving eccentric muscle activity can be performed without undue damage (Lovering and Brooks, 2014). Despite identifying that smaller strains of 5-20% of L_f are less damaging to skeletal muscles during eccentric activity (Brooks *et al.*, 1995; Brooks and Faulkner, 1996; Lynch *et al.*, 2008), the fatiguing effects of eccentric muscle activity in older skeletal muscles using these smaller strains is still poorly understood as these previous investigations use single lengthening muscle actions.

1.3.2.3 - Eccentric Activity and The Work Loop Technique

Implementing the WL technique can better address the limitations to measuring eccentric function via isovelocity lengthening actions given that repeated cyclical activity performed during the WL protocols are a better estimate of the dynamic pattern of activation observed during *in vivo* muscular activity (Tallis *et al.*, 2013, 2014, 2017). Furthermore, the WL technique allows for an assessment of the relative decline in eccentric power over time, allowing for a better understanding of the time-course of fatigue and potential for damage at a muscular level. The latter point is particularly pertinent as many previous studies have deliberately caused damage to skeletal muscles via repeated eccentric muscle activity, with no study to date examining the potential fatiguing effects of sustained eccentric muscle activity.

To date, no work has used the WL technique to examine the effects of age on eccentric fatigue of isolated skeletal muscles. Whilst Stevens (1996) and Choi and Widrick (2009) explored some of the responses during and following eccentric WL's in young mice, each study used large strains (±20%-±25% of L_f) which caused the muscle to be deliberately damaged. Work investigating the concentric power output of young (James *et al.*, 1996) and older (Tallis *et al.*, 2014) mouse skeletal muscles using the WL found that maximal power output typically occurred at a strain of 0.10 (±5% of L_0). Moreover,

recovery of force and power is unaffected following concentric fatigue using a strain of 0.10 compared to control values (Choi and Widrick, 2009). As muscles work as agonist-antagonistic pairs *in vivo*, a comparison of the response to eccentric muscle activity which reciprocates the optimal strain for concentric power production could provide a valuable insight into the nature of eccentric power production of isolated muscles without undue damage. Single fibre work reported no differences in force deficit during lengthening contractions at 5% of L_f during single isovelocity contractions (Brooks and Faulkner, 1996; Lynch *et al.*, 2008), though the combined effects of smaller strains during eccentric muscle activity and older skeletal muscles have yet to be explored in a WL model. Furthermore, the muscle-specific changes in eccentric muscle function have not been examined.

1.3.3 - Mechanisms of Muscular Damage and Fatigue Following Eccentric Activity

The time-course for muscular damage can be identified in a two-stage model. The first stage is the initial damage stage caused by the shear stress of eccentric muscle activity causing micro-damage to the skeletal muscles (Close *et al.*, 2005). This is attributed to damage to sarcomeres and connective tissue, as well as excitation-contraction coupling disruption (Close *et al.*, 2005; Gault and Willems, 2013). In the second stage, the damage caused by the shear forces are amplified in the 3-4 days following damage during the healing process causing a secondary reduction in force. This secondary loss of muscular function is largely due to inflammation and reactive oxygen species production limiting the healing process (Close *et al.*, 2005). Isolated muscle models are limited by the inability to examine the healing process in the days following damage. However, the initial damage stage is still important given that force declines and remains depressed following bouts of eccentric activity in older skeletal muscles (Zerba *et al.*, 1990; Brooks and Faulkner, 1996; Chan and Head, 2010). In consideration of this limitation of *in vitro* studies, only the initial stages of muscle injury, and why older muscles appear to be more susceptible to damage during this stage, shall be discussed.

1.3.3.1 - Sarcomere Damage

One of the primary explanations for a reduction in contractile function is overstretching of the muscles and high forces produced during eccentric muscular actions causing disruption of myofibrils (Gault and Willems, 2013). Stretch-induced damage of muscle fibres beyond optimal length is known as the popping-sarcomere hypothesis (Morgan, 1990). Early work by Katz (1939) using frog sartorius muscles showed that muscles yield and elongate at a high force and contractile velocity. This leads to a rightward shift in the force-length curve (see section 1.6.2) meaning myofibrils within the muscle no longer contract and are noncompliant thus producing passive force. This notion led to the belief that rapid, high force stretches cause some muscle fibres to be permanently stretched and potentially damaged. The earliest evidence suggesting structural damage of the contractile proteins following eccentric muscle activity demonstrated a broadening of Z lines and disruption of sarcomeres following repetitive stair descent in humans (Fridén *et al.*, 1981). During single active stretches of skeletal muscles, most actin and myosin filaments reinterdigitate to their normal resting state during muscle relaxation and are therefore undamaged (Allen, 2001). In isolated muscle models, older skeletal muscles undergoing maximal eccentric activity typically resulted in a 40% reduction in isometric force production (Zerba *et al.*, 1990; Brooks *et al.*, 1995; McBride *et al.*, 1995; Brooks and Faulkner, 1996; Chan and Head, 2010) which is largely explained by sarcomere damage. It has also been shown that connective tissue is able to withstand the myofibrillar stress associated with eccentric activity (McHugh, 2003) and therefore greater muscle stiffness may actually serve as a protective mechanism against contraction-induced damage (Kovanen *et al.*, 1984), much like an increased muscular collagen content and stiffness following a period of high-force resistance training (Mackey *et al.*, 2004).

1.3.3.2 - Excitation-Contraction Coupling Disruption

Work in humans has shown that exercise-induced muscle damage can lead to dilation of the sarcoplasmic reticulum (SR) and T-tubules which may in part disrupt the excitation-contraction coupling process and impair force production (Byrd, 1992; McCutcheon *et al.*, 1992). Warren *et al.*

(1993) were the first to utilise isolated muscles to examine the contribution of excitation-contraction coupling failure on impaired contractile function following eccentric muscle activity. Following eccentric stretching, 50mM of caffeine was added to the buffer solution to determine if caffeine could elicit a more forceful contractile response, with failure to do so indicating structural damage is of greater importance. Following ten eccentric, twenty eccentric and twenty isometric activations, isometric force declined by 20.0±2.3%, 42.6±4.2% and 3.9±2.4% of the previously obtained maximal force respectively. Direct application of caffeine was able to significantly recover force by an average of 118.4±8.6% of the post-injury force measure in the twenty eccentric contractions group, which was significantly greater than the other groups. These results indicated that damage as a result of prolonged eccentric muscle activity was due to impaired release of SR Ca^{2+}, though this was not due to sarcolemma injury due to change in resting membrane potentials of damaged fibres. Warren *et al.* (2001) also report that 57-75% of the reduction in isolated muscle force 0 to 3 days following *in vivo* damage protocols in young mice was attributable to excitation-contraction coupling failure in EDL with the remainder attributable to physical disruption of the musculature.

In ageing skeletal muscles, excitation-contraction uncoupling is a significant contributor to a reduction in muscular force and power output (Renganathan *et al.*, 1997). Despite this, it is still unclear as to why older skeletal muscles are more prone to single damaging bouts of eccentric activity, thus further work is required to determine whether older muscles are more susceptible to damage using more realistic length changes in an isolated muscle model, and the consequent recovery in contractile performance.

1.4 - Obesity and Muscle Function

The prevalence of obesity is quickly becoming epidemic, with the incidence of obesity has doubled in over 70 countries, resulting in over 2 billion individuals classified as obese (GBD 2015 Obesity Collaborators, 2017). Of this population, the greatest prevalence of obesity is found in adults aged 60-64 years for women and for men aged 50-54 years (GBD 2015 Obesity Collaborators, 2017). Coupling the increased proportion of people who are overweight or obese with the increased life expectancy of humans, it is becoming increasingly important to understand the implications of obesity in the elderly on health and the associated risk factors that could further impair skeletal muscle function.

1.4.1 - The Effect of Obesity in Older Adults on Muscular Function In Vivo

It is expected that obesity in older adults may further reduce the ability to generate force and power. This notion of sarcopenic obesity has been proposed as an additional comorbidity to sarcopenia in that the addition of obesity in old age further reduces skeletal muscle performance and increases the risk of developing associated comorbidities such as type 2 diabetes, and further increases all-cause mortality rates (Stenholm *et al.*, 2009; Cheng *et al.*, 2016). Functional capacity is also affected in old obese adults, where older obese adults are at a greater risk of falls and developing fractures (Himes and Reynolds, 2012; Huo *et al.*, 2016), have a lower gait velocity (Huo *et al.*, 2016), have greater frailty and a reduced ability to complete activities of daily living (Hirani *et al.*, 2017). The consequences of elevated fall risk and reduced functional capacity can significantly impact on quality of life. In comparison to sarcopenia and obesity in adult populations, investigations into how muscle strength, power, quality and fatigue resistance responds in sarcopenic obese populations are distinctly lacking, despite evidence of impaired functional capabilities in this specific population.

The studies of table S1.4 show that unlike sarcopenia and dynapenia, obesity in older adults has a variable effect on skeletal muscle strength and power. Compared with studies of younger obese populations (Maffiuletti *et al.*, 2013), studies of old obese populations demonstrate a reduction in

muscular strength (Hilton *et al.*, 2008; Stenholm *et al.*, 2009; Huo *et al.*, 2016) whilst others report no change (Miyatake *et al.*, 2000; Zoico *et al.*, 2004) or a muscle-specific increase in muscular strength (Rolland *et al.*, 2004) as seen in studies of obesity in young adults (Tomlinson *et al.*, 2016). Differences may arise due to the muscle group examined. Postural muscles are loaded via an increased fat mass whilst there is little loading on non-weight bearing muscles, such as those in the upper body. As such, it is unsurprising to see that there is no change (Miyatake *et al.*, 2000; Rolland *et al.*, 2004), or a reduction (Huo *et al.*, 2016) in hand grip strength of old obese adults compared to the increases in absolute strength of old obese adults of the lower musculature (Rolland *et al.*, 2004; Stenholm *et al.*, 2009; Tomlinson *et al.*, 2014).

The work which observed no differences or increases in muscle function typically compared overweight or obese populations which may explain the lack of differences in muscular strength (Miyatake *et al.*, 2000; Zoico *et al.*, 2004). Rolland *et al.* (2004) found that maximal isometric knee extensor and elbow extensor strength was higher than in the old lean group. This was coupled with a significantly greater fat mass, fat-free mass, and leg and arm skeletal muscle mass in the old obese populations compared to normal weight and lean. There was no indication however of the quantity of intramuscular adipose tissue (IMAT) within the skeletal muscle, which may affect muscle quality had strength been reported relative to muscle size.

Only one study to date has examined the effect of obesity on muscle power in older adults (Hilton *et al.*, 2008). In their study, maximal isometric strength and torque of plantar flexors and dorsiflexors were significantly lower in obese older adults compared to overweight older adults. When assessing absolute power, however, the magnitude of the difference between the overweight and obese groups was greater than that for muscular strength, indicating that as with ageing, the loss of absolute power is greater than that of strength. Moreover, muscle quality was significantly affected, with power relative to muscle volume also significantly worse in the obese group. The quantity of IMAT was

significantly higher in the old obese group, but no differences were observed in muscle and adipose tissue volume. This would indicate that the increased non-contractile mass may impair force production in older obese populations without affecting muscle size, meaning dynapenic obesity may be a significant contributor. This is particularly pertinent given that the ages of the adults in Hilton *et al.* (2008) are much younger than in other studies of obesity in older adults. Moreover, dynapenia has been shown to affect skeletal muscle mass and contractile function much sooner than sarcopenia, and therefore obesity may exacerbate mechanisms of dynapenia (Hilton *et al.*, 2008), but not sarcopenia (Stenholm *et al.*, 2009; Huo *et al.*, 2016).

1.4.2 - The Effect of Obesity on Muscular Endurance

Previous work in young mammals (Thomas *et al.*, 2014; Matsakas *et al.*, 2015) and fish (Seebacher *et al.*, 2017) has been performed to examine how obesity affects the ability to sustain locomotory function, though as with human studies, no work has been performed in old obese animals. In a young mouse population (7-10 weeks) provision of a high-fat diet (HFD) for 3-6 weeks was enough to significantly impair time to exhaustion during exhaustive treadmill running (Thomas *et al.*, 2014) and distance covered during treadmill running (Matsakas *et al.*, 2015). Whilst an *in vivo* animal model of examining locomotory function is valuable for providing an insight into how obesity negatively impacts on the ability to sustain locomotory function, it is difficult to discern whether changes in exercise capacity is a result of muscular fatigue, neural fatigue or limited respiratory and oxidative capacity as a result of a HFD, hence the requirement for *in vitro* analysis.

It appears that in ageing, the added influence of obesity may not further exacerbate absolute force of skeletal muscles, though does reduce muscle quality (Hilton *et al.*, 2008) and locomotory function (Stenholm *et al.*, 2009; Yang *et al.*, 2015; Hirani *et al.*, 2017) and tolerance to exercise in obese animals (Thomas *et al.*, 2014; Matsakas *et al.*, 2015). As with studies of ageing, difficulties arise in directly comparing results due to different ages assessed, determinants of body composition cut-offs and

muscle groups assessed. Adopting an *in vitro* approach can be beneficial for observing the muscle-specific effects of obesity on absolute and relative muscle force and power, and fatigue resistance to sustained activity without the elevated body mass affecting the rate of fatigue.

No previous study has examined the effects of obesity in older adults on the ability to withstand fatigue at a muscular level. Some work has demonstrated reduced functional capacity such as gait velocity (Huo et al., 2016) though no work has determined the ability for muscle of old obese adults to withstand the fatiguing effects of sustained bouts of muscle activity as has been done in studies of sarcopenia in older adults (Table S1.1).

1.4.3 - Isolated Skeletal Muscle Performance

It is difficult to directly compare the studies which have examined the effects of obesity on isolated skeletal muscle contractile performance. Much like with studies of skeletal muscle ageing, examining obesity in isolated muscle models is challenging given the differences in methodological approach, contractility mode, test temperature, diet duration and composition profoundly alter the outcome of a dietary protocol (Tallis *et al.*, 2018). An extensive overview of the effects of obesity on skeletal muscle contractile function can be found in Tallis *et al.* (2018). In brief, HFD's in rodents generally cause a significant increase in muscle mass via the ectopic accumulation of fat (Ayre and Hulbert, 1996; Shortreed *et al.*, 2009; Thomas *et al.*, 2014; Ciapaite *et al.*, 2015; Matsakas *et al.*, 2015; Eshima *et al.*, 2017), although the consequent effect on contractile function is muscle-specific and is dependent on several factors. Some studies have shown a reduction in tetanus stress for the soleus (Ciapaite *et al.*, 2015) and EDL (Matsakas and Patel, 2009; Eshima *et al.*, 2017; Tallis *et al.*, 2017). However, this is not always the case, particularly in studies comparing soleus and EDL contractile function, with Ciapaite *et al.* (2015) reporting reduced soleus tetanus stress and prolonged relaxation time following a HFD, but no effect for the EDL. By contrast, Tallis *et al.* (2017) reported directly opposing findings, with little effect of obesity on soleus absolute force and power producing capabilities, but a significant reduction

in EDL tetanus stress and power. This indicates that obesity is likely to have a greater effect on the quality of the contractile tissue rather than the absolute production of force and power, and may be related to the *in vivo* mechanical role and anatomical location of the skeletal muscle (Tallis *et al.*, 2018).

Tallis *et al.* (2017) also observed a significant reduction in diaphragm isometric stress and power output as a result of an obesogenic diet, though the ability to withstand fatigue was unaffected. The impairment in diaphragm isometric stress is likely to explain the reduced power output. From a functional perspective, the blunted diaphragm function is likely to contribute to other co-morbidities of obesity that further promotes a negative cycle of obesity (Parameswaran *et al.*, 2006).

To date, only one study has observed the effects of obesity on the contractile properties of skeletal muscles isolated from older animals (Bott *et al.*, 2017). At 20 weeks of age, twitch and tetanic isometric stress of the soleus and EDL isolated from male C57BL/6J mice was examined *in vitro*, with the same parameters tested following 13 weeks of a HFD and compared to age-matched controls. Whilst there was significant age-related atrophy of the soleus, this did not result in reduced twitch or tetanic force or stress. A HFD caused significant soleus hypertrophy, likely due to a loading effect of the elevated fat mass, though this did not corroborate with an improvement in soleus contractile function. As for the EDL, there were no age-related or dietary-induced changes in isometric stress compared to baseline or between groups, though twitch activation and relaxation times were significantly prolonged compared to baseline. However, a HFD promoted significant atrophy of type IIx and type IIb fibre CSA compared to age-matched controls, though ageing did not result in atrophy of the EDL fibres compared to baseline.

No study has examined the effects of dietary-induced obesity on the contractile performance of skeletal muscles in an old age group. The animals in Bott *et al.*, (2017) are considerably young in the

lifespan of the C57BL/6 mouse, nor is an age-related reduction in contractile performance observed. Therefore, the study does not truly examine the synergistic effects of ageing and obesity. Considering that obesity exacerbates the age-related reduction of muscle quality *in vivo*, an examination of the direct effects of ageing and obesity on a muscular level is warranted.

1.5 - Mechanisms Causing Impaired Skeletal Muscle Contractile Function in Old Age

It is well established that as humans age, a loss of muscle mass occurs leading to a decline in muscle force and power (Rosenberg, 1989). However, other causes of muscular ageing, including central and peripheral nervous control, intrinsic muscle properties, hormonal status and dietary implications contribute to the decline in muscle performance with age (Doherty, 2003). The aforementioned causes which contribute to an age-related decline in skeletal muscle contractile function also share similar mechanistic pathways as obesity models, leading to poorer contractile function (Miljkovic *et al.*, 2015; Pérez *et al.*, 2016; Tallis *et al.*, 2018) and generally affect skeletal muscles composed of predominantly fast-twitch muscle fibres more so than predominantly slow-twitch muscles (Seene and Kaasik, 2012; Miljkovic *et al.*, 2015). This section shall examine the main mechanisms that contribute to the age-related decline in skeletal muscle contractile function, with reference to the mechanism that exacerbates ageing in obesity models. Figure 1.5.1 displays the contributing mechanisms that relate to a decline in muscle contractility, from the perspective of both sarcopenia and dynapenia.

Figure 1.5.1 - Overview of the interacting mechanisms which contribute to loss of muscle mass (sarcopenia) and muscle strength and power (dynapenia) in older adults (Seene and Kaasik, 2012).

1.5.1 - Denervation

It has been demonstrated that an increase in age results in a significant decline in the number of motor units supplying a muscle (Carlson, 2004). Electromyography recordings have shown a 25% decline in the number of functional motor units in small aged muscles of the hand and foot of those over the age of 60 (Stålberg and Fawcett, 1982). However, this decline in the number of motor units is counteracted by an increase in the size of the motor units in an attempt to maintain function. As older muscle fibres denervate, motor units remodel primarily through reinnervation to maintain axon supply to the muscle fibres (Carlson, 2004). However, the state of denervation-reinnervation results in net denervation and therefore loss of motor units and innervated muscle fibres (Deschenes, 2004). It is considered that the age-related loss of alpha-motoneurons that supply type II muscle fibres result in fibres which are incapable of activation and therefore a loss of function (Hashizume *et al.*, 1988; Lexell and Downham, 1992). In comparison, the motor unit area and number of type I fibres remain largely unaffected by age in human biopsy samples (Lexell, 1995) and rat gastrocnemius muscle (Kadhiresan *et al.*, 1996). In the latter case, however, whilst motor unit number decreased, the number of fibres per motor unit area increased 3-fold (Kadhiresan *et al.*, 1996).

Whilst denervation is an important cause of musculoskeletal ageing and the consequential loss of function in humans (Carlson, 2004), isolation of muscle from animals prior to testing, allows for the direct measurement of ageing and obesity on skeletal muscle function independent of the effects denervation may have on the force and power production of a muscle.

1.5.2 - Muscle Mass & Fibre Type Composition

The age-related loss in skeletal muscle mass is largely attributable to a reduction in fibre number and a reduction in fibre cross-sectional area (Deschenes, 2004; Degens, 2007). In humans, muscle mass has been shown to peak at 25 years of age (Lexell, 1995), with a small (10%) loss of muscle mass by the 5th decade and a more rapid loss of muscle mass beyond this to the 8th decade of life (Lexell, 1995;

Deschenes, 2004). A number of causes have been attributed to this age-related loss of muscle mass and size, including decreased protein synthesis, muscle disuse atrophy mechanisms and hormonal factors (Deschenes, 2004; Degens, 2007).

A reduction in the synthesis of myofibrillar proteins, with age and obesity, would explain the age-related loss in muscle function and mass (Navarro *et al.*, 2001). Both ageing and obesity have been described as causing a state of chronic inflammation, resulting in impaired muscle protein synthesis (Akhmedov and Berdeaux, 2013; Tallis *et al.*, 2018). Ageing, obesity, and their synergism leads to an upregulation of proinflammatory cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Whilst upregulation of IL-6 can be beneficial to the muscle by initiating myoblast proliferation in the process of skeletal muscle regeneration, particularly following injury (Otis *et al.*, 2014), maintaining a chronic state of inflammation can impair this process. For skeletal muscles to regenerate to maintain mass and therefore function, muscles must undergo a series of molecular events to promote tissue growth. Satellite cells are non-specialised stem cells found in a quiescent state between myofibre plasmalemma and basal lamina of the skeletal muscle (Mauro, 1961; Yablonka-Reuveni, 2011). Once stimulated, satellite cells proliferate and differentiate to form new myonuclei through the fusion of nuclei to form new skeletal muscle fibres (Verdijk *et al.*, 2014). With advancing age, satellite cell number and performance declines in a muscle-specific manner (Shefer *et al.*, 2006), where a reduction in the quantity or quality of satellite cells can limit myogenesis as new myonuclei cannot form, therefore leading reduced muscle mass (Le Grand and Rudnicki, 2007). An excessive body fatness results in an increase in adipose tissue accumulation, the site from which proinflammatory cytokines are secreted from (Hilton *et al.*, 2008). Therefore, there is an association between the quantity of subcutaneous fat and levels of circulating proinflammatory cytokines (Pinho *et al.*, 2017) meaning obesity in old age can further impair the muscle regeneration process at all stages of the regeneration cycle, from satellite cell proliferation through to differentiation and growth due to the elevation of circulating proinflammatory cytokines (Akhmedov and Berdeaux, 2013). One

example is an impairment in leptin release (Akhmedov and Berdeaux, 2013). Myogenic differentiation of proliferated satellite cells can be impaired by an increase in circulating TNF-α levels in old and obese individuals (Sishi *et al.*, 2011; Pérez *et al.*, 2016). Upregulation of TNF-α and another proinflammatory cytokine, interleukin 1 alpha, can also limit the growth of myoblasts, once differentiated, by inhibiting the activation of mechanistic target of rapamycin (mTOR) (Sishi *et al.*, 2011), an important anabolic pathway in the process of muscle growth (McCarthy and Esser, 2010). Despite the importance of proinflammatory cytokines for homeostatic maintenance of skeletal muscle, maintaining chronically elevated circulatory proinflammatory cytokines can be detrimental to muscle mass maintenance in old and obese populations. For example, increased circulation of TNF-α in mice expressing the TNF-α gene resulted in inhibited myoblast proliferation leading to significant atrophy of the gastrocnemius, soleus and plantaris muscles (Langen *et al.*, 2006). Moreover, local delivery of IL-6 for 14 days in young rats resulted in significantly impaired gastrocnemius growth, likely due to impaired satellite cell proliferation though this was not tested (Bodell *et al.*, 2009).

Maintaining a healthy balance of protein catabolism and synthesis is an important factor for muscle quality and quantity. Balagopal *et al.* (1997) reported a progressive age-related decline in myosin heavy chain synthesis; this is likely to reduce the muscle's ability to remodel contractile protein and is expected to contribute to the age-induced loss of muscle mass and strength. Welle *et al.* (1993) reported a one-third reduction in quadriceps strength between young and old subjects. This was consistent with a 21% reduction in muscle mass and was further supported by evidence highlighting a reduction in myofibrillar protein synthesis between young and old populations. An age-related reduction in mitochondrial protein synthesis may also contribute to a decrease in muscle mass and oxidative capacity (Rooyackers *et al.*, 1996).

Ageing is associated with a fast-to-slow shifting in fibre types, where type IIb fibres transition progressively towards a more oxidative fibre type (Deschenes, 2004). Whilst ageing skeletal muscle

fibres typically demonstrate a shift towards a more oxidative fibre type (Larsson, 1978; Klitgaard *et al.*, 1990; Coggan *et al.*, 1992), there is evidence to suggest obesity causes a shift to a faster fibre type in animals (Tallis *et al.*, 2018). The shift to a faster fibre type may partially explain the reduced fatigue resistance of human musculature given that fast twitch muscle fibres are less fatigue resistant. Moreover, fast twitch fibres have a reduced oxidative capacity due to fewer mitochondria available to oxidise fats, which may in part account for the continued accumulation of IMAT and subcutaneous fat. Rodent studies are somewhat more equivocal, with some showing no change in fibre type following dietary-induced obesity (Turner *et al.*, 2007; de Wilde *et al.*, 2008, 2009; Shortreed *et al.*, 2009) despite a reduction in contractile function (Tallis *et al.*, 2017), and others showing a muscle-specific (Trajcevski *et al.*, 2013) and sex-specific (DeNies *et al.*, 2014) shift to faster fibre types. The difference in results between human and rodent studies are likely due to experimental design, where controlling for diet and energy intake is more difficult in human than animal models (DeNies *et al.*, 2014). Little work has examined the alteration in skeletal muscle morphology, in terms of fibre type quantity and distribution, and the consequence this may have in old and obese skeletal muscles

1.5.3 - Changes in Excitation-Contraction Coupling and Ca^{2+} Handling

A muscular contraction is initiated by the release of Ca^{2+} from the sarcoplasmic reticulum (SR) which then binds to troponin-C to cause actin binding sites to be exposed to allow cross-bridge cycling and force production. The process via which Ca^{2+} is released is called excitation-contraction coupling (Ashley *et al.*, 1991). The age-related impairment of Ca^{2+} release from the SR of the muscle was first demonstrated by Delbono *et al.* (1995) where ageing caused a significant reduction in Ca^{2+} release from fast fibres of human quadriceps due to dihydropyridine-ryanodine receptor (DHPR) uncoupling. Similar findings have been observed in ageing rat soleus and EDL (Renganathan *et al.*, 1997) and ageing mouse flexor digitorum brevis muscles (Wang *et al.*, 2000) due to an increase in dysfunctional DHPR and ryanodine receptors. Given the recent evidence of reduced muscle quality independent of muscle wasting, impaired Ca^{2+} handling could be a significant contributor to the reduction in muscle quality

(Moran *et al.*, 2005; Chan and Head, 2010; Tallis *et al.*, 2014) due to an impaired Ca^{2+} release reducing Ca^{2+} availability for contractile proteins and consequently lead to a reduction in force and power. Impaired SR Ca^{2+} release could be muscle-specific, with Larsson and Salviati (1989) reporting a decrease in SR function and Ca^{2+} availability for single EDL fibres of 23-24-month-old rats, though no change in SR Ca^{2+} availability and Ca^{2+} pump activity was observed for the soleus. By contrast, Narayanan *et al.* (1996) found that Ca^{2+} uptake by the SR was significantly impaired in the soleus compared to the predominantly fast-twitch gastrocnemius, with impaired activation and relaxation times for the soleus. Impaired Ca^{2+} handling is not only likely to reduce the force-generating capacity of the skeletal muscles (Berchtold *et al.*, 2000), but is also likely to contribute to the age-related increase in activation and relaxation time of skeletal muscles (Xu and Narayanan, 1998; Tallis *et al.*, 2014). The protein sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) is an important regulator of Ca^{2+} resequestration into the SR and, therefore an age-related reduction in the activity of SERCA may have significant consequences for muscle contraction-relaxation dynamics. Work by Tallis *et al.* (2014) reported a significant reduction in SERCA activity, fatigue resistance and prolonged relaxation time for the EDL by 50 weeks of age. Interestingly though, the preservation of the quantity of SERCA mRNA implies that the activity of SERCA rather than quantity is most problematic during early ageing. The normal ageing process leads to excitation-contraction uncoupling through various mechanisms such as DRHP-RYR uncoupling and dysfunctional Ca^{2+} handling proteins such as SERCA. In an obesity model, Ca^{2+} handling can also be significantly impaired, which may help to explain the reduced force and power output of skeletal muscles and increased fatigability of isolated muscles (Bruton *et al.*, 2002; Schilder *et al.*, 2011; Funai *et al.*, 2013; Ciapaite *et al.*, 2015).

Force and power may be reduced in muscles isolated from obese animals due to a reduction of the TnT-3 isoform in some muscles (Schilder *et al.*, 2011). TnT-3 is the fastest isoform of troponin available for Ca^{2+} to interact with in the initiation of the cross-bridge cycle (Perry, 1998). A reduction in TnT-3 would reduce Ca^{2} sensitivity at a molecular level and as such may explain the reduced force-generating

capacity of faster skeletal muscle. For example, Funai *et al.* (2013) reported significantly reduced SERCA activity following a HFD protocol. Not only is reduced SERCA activity consistent with impaired force as shown in SERCA1-null mice (Pan *et al.*, 2003) and grip strength in HFD mice (Funai *et al.*, 2013), but it may also explain the prolonged activation time in obese skeletal muscles, particularly the soleus (Tallis *et al.*, 2017). As relaxation time during a WL fatigue protocol can increase with each contraction (Josephson, 1985; James *et al.*, 1996), a further inability to reuptake Ca^{2+} prior to the next contraction may explain the reduced fatigue resistance in the HFD soleus in Tallis *et al.* (2017), which may be exacerbated in old obese skeletal muscles. The combination of obesity in old age may further exacerbate dysfunctional excitation-contraction coupling and further impair force, power and fatigue resistance.

1.5.4 - Increased Connective Tissue and Intramuscular Fat

One mechanism attributed to skeletal muscle ageing is an increase in the non-contractile tissues, such as adipose tissue or collagen, within the skeletal muscle (Marcus *et al.*, 2010, 2012). An increase in non-contractile tissues within the skeletal muscles could increase muscle stiffness resulting in an unfavourable passive length-tension relationship. For example, Alnaqeeb *et al.* (1984) found a significant age-related increase in muscle stiffness of aged rat soleus and EDL muscles, due to an increase in thickness of the endomysium and perimysium and a greater total muscle collagen content.

Much like the mechanisms of sarcopenia and dynapenia, the mechanisms that describe the process of accumulation of lipids within skeletal muscles and consequent reduction in contractile function is multi-faceted, with various interlinking pathways. This section shall briefly summarise the mechanism of skeletal muscle fat accumulation and how ageing influences the storage of fat within skeletal muscles.

Obesity can be characterised by the excessive accumulation of lipids in non-adipose tissues, including skeletal muscles, in a phenomenon known as ectopic fat accumulation (van Herpen and Schrauwen-Hinderling, 2008), largely due to increased circulating lipids in obese individuals resulting in increased fatty acid uptake by skeletal muscles (Goodpaster and Wolf, 2004). In obese populations, when the adipocytes within adipose tissue reach their capacity to store lipids, fat storage consequently occurs ectopically in organs such as skeletal muscles. This process of lipid uptake and storage in skeletal muscles is known as myosteatosis (Miljkovic and Zmuda, 2010). Myosteatosis has two pathways which result in the ectopic accumulation of fat. In the first pathway, lipids accumulate between the muscle fibres and are stored as intermuscular adipose tissue (Goodpaster *et al*., 2000). Intermuscular adipose tissue is unlikely to significantly alter skeletal muscle mass due to fat accumulating extracellularly between myofibres as opposed to within the muscle fibres (Sinha *et al*., 2002, Boesch *et al*., 2006, Lee *et al*., 2012). The alternate pathway for myosteatosis is the accumulation of fat within the skeletal muscle fibres, known as intramuscular adipose tissue (IMAT) (Addison *et al*., 2014) which will increase whole skeletal muscle mass and ultimately impair contractile function and muscle quality (Fragala *et al*., 2015, Tallis *et al*., 2018).

Increased muscular fat could be a key contributor to age-related changes in skeletal muscle function. Whilst obesity has been identified as a key factor associated with ectopic accumulation of fat within skeletal muscles (Yang *et al.*, 2014), disuse can also result in fat accumulation, with a 4-week period of limb immobilisation in young healthy adults resulting in significant IMAT accumulation (Manini *et al.*, 2007). The key consideration in relation to skeletal muscle function is that an age-related increase in muscle non-contractile tissues usually results in greater skeletal muscle mass and CSA, thus reducing force generation per unit of muscle CSA and therefore reducing overall muscle quality. This has been shown to be the case in young, dietary-induced obese skeletal muscles (Ciapaite *et al.*, 2015; Matsakas *et al.*, 2015; Bott *et al.*, 2017; Tallis *et al.*, 2017) but has yet to be proven in older skeletal muscles.

Obesity may exacerbate the reduction in muscle quality in older populations given that ageing is associated with increases in non-contractile tissue mass. In previous work, computational modelling of the human gastrocnemius has been performed to estimate the contribution of increased IMAT to muscle quality and contractile performance of the computer-generated muscle belly in sarcopenic obese skeletal muscles (Rahemi *et al.*, 2015). In this work, gastrocnemius was modelled at differing quantities of IMAT, from 0% (control), 10% and 20% of total muscle belly volume. Isometric properties such as force and stress were simulated to provide an overview of how fat accumulation affects muscle contractile performance. Results indicated increasing levels of IMAT reduced muscle force and muscle quality due to increased muscle stiffness, with increased fat accumulation exacerbating the effect. However, no study to date has utilised biological tissue to examine the relationship between advancing age, increased IMAT and the effects on muscle force and quality.

1.5.5 - The Circulatory System

Chronic disuse of skeletal muscles and long periods of sedentary behaviour can impair cardiovascular function and therefore blood flow to working muscles during rest and exercise (Mechling and Netz 2009; Martini *et al*., 2017). However, the literature investigating muscle blood supply is conflicting, with many studies showing no change in muscle capillarisation with age (Aniansson *et al.*, 1981; Grimby *et al.*, 1982; Denis *et al.*, 1986; Jakobsson *et al.*, 1990; Chilibeck *et al.*, 1997; Kano *et al.*, 2002) and others showing a decline (Coggan *et al.*, 1992; Frontera *et al.*, 2000). Coggan *et al.* (1992) observed a ~25% reduction in capillary density in old men and women, with no sex differences observed. Sex-based differences became apparent when comparing capillary:fibre ratios due to the smaller fibre CSA of older females in this study. Human literature which has reported no overall changes in capillary density suggest that loss of capillary density with age could, in fact, be specific to fast-twitch muscle fibres (Proctor *et al.*, 1995; Kano and Sakuma, 2013). The fact there is greater evidence to support the maintenance of capillary density could indicate the body's desire to maintain homeostasis, as Möller and Sylvén (1981) reported where a slight increase in muscle myoglobin content was observed.

1.6 - Testing the Mechanical Performance of Isolated Skeletal Muscle

Isolated skeletal muscle methodologies offer several advantages over *in vivo* methods of assessing muscular contractility. This section will consider the value of an isolated muscle approach and outline the principle methodological approaches used for examining skeletal muscle contractility in the age-related changes in skeletal muscle function.

1.6.1 - Why Use Animals to Assess the Effects of Age on Muscle Performance?

To ascertain the contractile properties of skeletal muscles in models other than *in vivo*, it is common to utilise the muscles from animals rather than humans, particularly mammals such as mice, rats and hamsters (James *et al.*, 1995, 1996; Tallis *et al.*, 2012, 2013, 2014, 2017). The following section outlines many of the advantages of mammalian models for assessing muscle function compared to a human cohort in the examination of skeletal muscle ageing.

1.6.1.1 - Isolation of Specific Muscles

When considering *in vivo* assessments of muscle contractility in an older population, it is common to either test muscles of the lower body, such as the knee extensors and knee flexors, or those of the upper body, including elbow flexors, elbow extensors and forearm muscles recruited during hand grip dynamometry (Macaluso and De Vito, 2004). The key limitation here is that these are typically muscle groups of varying muscular phenotype. The quadricep femoris, for example, consists of four smaller muscles, the rectus femoris, vastus lateralis, vastus medialis and the vastus intermedius (Kary, 2010). In terms of muscle composition, the rectus femoris has a tendency for a greater proportion of fast-twitch muscle whilst the vastus medialis has a greater proportion of slow-twitch than fast-twitch muscle fibres (Jennekens *et al.*, 1971; Johnson *et al.*, 1973; Garrett *et al.*, 1984). As a result, it would be difficult to identify the fibre type specific effects of ageing and obesity, especially considering that ageing and obesity affects skeletal muscles in a muscle-specific manner (Table S1.1 - S1.4).

1.6.1.2 - Central Inhibition

In vivo assessments of muscle function rely upon voluntary activation of specific muscles. However, as muscle activation is under nervous control, the mechanisms which affect muscle contractility could be masked by the nervous system. For example, force inhibition could be due to poor functioning of the central nervous system (CNS), such as ineffective summation and propagation of nervous impulses prior to a contraction. Another mechanism is the protective neural mechanisms in place to protect the muscle from contraction-induced damage (St Clair Gibson *et al.*, 2001). Whilst this is good *in vivo* to prevent muscular damage, central inhibition limits the potential for skeletal muscles to reach their maximal force or power generating capacity that may be altered with ageing of the CNS. This means that determining whether *in vivo* changes in muscle performance are attributed to changes in neural control or skeletal muscle performance is difficult. By isolating a specific muscle *in vitro*, there is no control from the central nervous system as externally delivered electrical stimulations are manipulated and controlled by the investigator. Furthermore, any limitations to muscle contractility can be deduced as being intrinsic to the muscle and not through central inhibition.

1.6.1.3 - Muscle Quality

Muscle quality is defined as force or power production of a skeletal muscle relative to the size of the tissue (Fragala *et al.*, 2015). Measuring quality is important as during early ageing and obesity, skeletal muscles that are older or obese are larger but of a lower quality (i.e. less force per unit of CSA or lower power per unit of muscle mass), which has the same or lower absolute force or power as younger, leaner skeletal muscles (Tallis *et al.*, 2014, 2017, 2018). Ageing and obesity is associated with an elevated body mass, but poorer power output relative to the muscle size without a reduction in absolute force or power. (Tallis *et al*., 2014; 2017). One consequence of this is having to transport a larger body mass, which is concomitant with increasing age and obesity (Woo, 2016), despite producing the same amount of absolute force or power, and would be compounded further by reduced muscle quality. Another consequence is that the larger muscles will contribute further to the

elevated body mass and therefore increase the required force to overcome a greater bodily inertia (Tallis *et al.*, 2017, 2018). Measuring muscle quality *in vivo* is difficult due to the method of measuring muscle size, where previous studies have estimated muscle volume via computed tomography scans (Blimkie *et al.*, 1990), MRI (Hilton *et al.*, 2008) and ultrasound scans (Choi *et al.*, 2016). By isolating a skeletal muscle, muscle quality can be more accurately assessed as the whole muscle can be weighed, and therefore contractile performance can be expressed relative to whole muscle mass as opposed to muscle CSA (Tallis *et al.*, 2014, 2017). Expressing force or power relative to muscle CSA is beneficial as it can provide an indication of hypertrophy or atrophy. Moreover, normalising performance to muscle CSA allows for comparisons to previous studies which have used the same approach for normalising contractile performance. However, such an approach does not consider the replacement of contractile proteins with non-contractile mass and connective tissues with increasing age and obesity in rodents (Tallis *et al*., 2018) and humans (Addison *et al*., 2014; Fragala *et al*., 2015). By expressing power relative to whole muscle mass and relative to muscle CSA, a more comprehensive overview in the changes in muscle quality with age and obesity can be determined.

1.6.1.4 - Examining Fatigue Resistance In Vivo

Several limitations arise when considering the examination of skeletal muscle fatigue resistance *in vivo*. Human studies of examining fatigue in old age usually employ a variety of methodological approaches, such as the protocols used to determine the ability of a muscle to withstand fatigue, the duration of the fatigue protocol and the muscle groups tested (Deschenes, 2004). As such, making comparisons between studies is difficult (Table S1.1). Human studies of muscular endurance are also limited by the fact that fatigue resistance of the muscle cannot be accurately measured *in vivo*. Older adults, who tend to have an elevated body mass and poorer muscle quality than younger counterparts, would have to overcome a greater force to overcome the inertia of the moving limb and as such are likely to appear to fatigue faster irrespective of exercise intensity. By using an isolated muscle model, a standardised approach can be used to allow for better comparisons between studies and between

different conditions within a study. Moreover, any changes in fatigue resistance are due to changes at the skeletal muscle level as the central nervous system has been isolated, thus eradicating the central fatigue effect.

1.6.1.5 - Nutritional Profile

From a nutritional perspective, particularly in relation to chapter 6 (sarcopenic obesity), the diets provided to mice can be more closely controlled in comparison to humans. During any exercise and health studies involving humans, it is common for researchers to ensure their participants consume the same standardised diet to ensure each participant consumes equal calories and macronutrient quantities which could otherwise impact upon results through metabolism of macronutrients (Ziogas and Thomas, 1998). Despite employing an appropriate methodological approach prior to exercise testing, it is difficult to determine and control the chronic nutritional profile of a volunteer, nor is it always possible to ensure the correct quantities of macronutrients are consumed. During research where *in vitro* techniques are employed, a consistent lab diet is provided to the animals which contains the same relative quantities of calories, macronutrients and micronutrients. Whilst the amount of food that animals eat cannot easily be controlled for, the nutritional profile is consistent for all mice thus reducing the potential impact diet may have on muscle function during *in vitro* muscle investigations.

1.6.2 - Testing Skeletal Muscle Contractility In Situ

The assessment of skeletal muscle contractility *in situ* involves the isolation of specific muscles whilst conserving the tendon origin, nerves, and blood supply in the organism (Croes and von Bartheld, 2007). Testing *in situ* is beneficial to those who wish to understand the integrated mechanisms that contribute to the contractile properties of specific skeletal muscles. Moreover, measurements of muscle contractility can be made over days and weeks in the same animal. During *in situ* protocols, the animal is first anaesthetised, then the target muscle and the nerve controlling this is surgically exposed, and an electrode capable of electrical stimulations is attached (MacIntosh *et al.*, 2011; Hakim

et al., 2013). Care is taken not to sever any of the blood supply to the muscle to ensure the supply of oxygenated blood is maintained to prevent the build-up of an anoxic core (Croes and von Bartheld, 2007). One end of the limb muscle is attached to a force transducer to allow for measurements of force produced during a muscular contraction whilst the leg is stabilised. The exposed muscle is kept warm at a physiologically relevant temperature of 37°C by placing the animal on an animal heating platform to ensure the circulating blood does not cool during experimentation (MacIntosh *et al.*, 2011). Furthermore, the muscle is submerged in either oxygenated Krebs-Henseleit solution which mimics blood plasma (Brown & Hasser, 1996) or mineral oil (Degens and Alway, 2003; Kung *et al.*, 2014) where either substance is warmed and maintained at 37°C to prevent heat loss (MacIntosh *et al.*, 2011). When an electrical stimulation is provided to a single nerve via the electrode, a specific muscle is therefore activated and consequently contracts to allow for the measurement of force. Whilst *in situ* testing offers many benefits, it is still difficult to truly isolate the contributing mechanisms that can otherwise affect force production during muscular contractions as the nerves remain intact. Moreover, typical anaesthetics used have been shown to significantly impair muscle contractile function (Ingalls *et al.*, 1996). Should the degree of anaesthesia vary between or within experiments then the magnitude of the effects on the muscle's performance will consequently be affected. As such, *in vitro* methods of assessing muscle contractility have been utilised to remove the influence of the CNS during a muscular contraction to allow for a closer assessment of the mechanical properties of isolated skeletal muscles.

1.6.3 - Testing Skeletal Muscle Contractility In Vitro

Studies which investigate biological matter outside of their normal context in an externally controlled environment are said to be *in vitro* investigations. In the case of skeletal muscles, this is usually performed using whole muscle (Moorwood *et al.*, 2013; Tallis *et al.*, 2015), fibre bundles (Park *et al.*, 2012) or single fibres (Squire, 1997).

In brief, an animal with a set of predetermined desirable properties (e.g. specific age) is selected and humanely sacrificed. A specific muscle is then isolated and prepared for assessment of its mechanical properties. In the case of a mammalian muscle, the isolated muscle would be placed in a chamber of circulating, oxygenated, Krebs-Henseleit solution (Table S1.2). Mechanical properties are then examined using a variety of contractile methods.

1.6.3.1 - Comparing Whole Muscle Testing Against Single Fibre Testing

Once isolated from an organism, muscles can be prepared further to assess muscle bundles or single muscle fibres (Brooks and Faulkner, 1994; Thompson and Brown, 1999; González *et al.*, 2000; Kim and Thompson, 2012, 2013). Assessing bundles of muscles or single, intact fibres can be beneficial to further isolate mechanisms that contribute to a muscular contraction and can allow for fibres of consistent fibre type to be used along with the removal of the effects of connective tissue. Initially, muscles are isolated, trimmed and, in the case of single fibres, are chemically skinned using a glycerinated skinning solution (Stienen, 2000). Single fibres are teased away from the prepared bundle, mounted on the testing apparatus and assessed for their mechanical properties (Thompson, 1999; Thompson and Brown, 1999). This method of testing skeletal muscle can be particularly advantageous for quantifying the actomyosin cross-bridge interactions (Brooks and Faulkner, 1994). As this type of testing occurs in a Ca^{2+} controlled environment (Thompson and Brown, 1999), changes in force production at the cross-bridge level can be determined without the influence of Ca^{2+} release and reuptake, which can limit force production (Metzger and Moss, 1987).

The diffusion pathway for oxygen is much smaller when single fibres and bundles are used due to a smaller quantity of tissue being assessed. As such, this prolongs the quality of the muscle for many hours, once isolated (González *et al.*, 2000). Larger muscles are at a greater risk of developing an anoxic core due to the greater diffusion pathway (Barclay, 2005), hence in whole muscle experiments muscles from mice are usually used, rather than rats.

Single fibre experiments are not, however, without their limitations. One of the main aims of this thesis is to simulate *in vivo* conditions to allow for a functional comparison between results found *in vitro* and *in vivo*. Using single muscle fibres is not representative of *in vivo* function as the majority of the contractile mass is removed for analysis, therefore the whole muscle is not stimulated as would be the case *in vivo*. Moreover, fat and collagen accumulation and within skeletal muscles occurs within the muscle belly, so removal of this by isolating single fibres may not provide an appropriate appraisal of the influence of obesity on mechanical function due to the removal of connective tissue which affects contractile function (Tallis *et al.*, 2017). To simulate the muscular actions found *in vivo* using isolated muscles, it is, therefore, more appropriate to utilise whole skeletal muscles as opposed to single fibres or bundles of fibres.

1.6.4 - Simulating In Vivo Conditions Using Isolated Muscles

Many experiments, where *in vitro* analysis of skeletal muscles have been used, have failed to closely replicate *in vivo* conditions, particularly in the case of experimental temperature. Arguably the most important external factor to be controlled, test temperature has previously been shown to have the greatest and most significant impact on the ability of muscles to perform work (James, 2013). In many early studies, a temperature of ~25°C has been typically used to prolong the time-course over which the muscle is viable, once removed from the animal, as metabolism is reduced at these temperatures (Lüttgau and Oetliker, 1968; Allen *et al.*, 2008). However, usage of such a low temperature in comparison to a temperature more indicative of *in vivo* conditions has been shown to significantly impair contractile function. For example, the examination of the iliotibialis isolated from the frog *Xenopus tropicalis* revealed a negligible effect on the force-generating capacity of the muscle at 24°C and 32°C. However, there was a significant increase in power output and in the ability to sustain power output at 32°C than at 24°C (James *et al.*, 2012). Inorganic phosphate accumulation during fatigue is a significant contributor to local muscular fatigue, impairing force during fatigue via a reduction in SR CA^{2+} release and myofibrillar calcium sensitivity (Allen *et al.*, 2008; Fitts, 2008). Increased fatigue

resistance with increased test temperature is likely due to the increased test temperature enhancing force production and suppressing the force depression caused by high inorganic phosphate concentrations (Coupland *et al.*, 2001; Debold *et al.*, 2004). The core body temperature in mice (McLaren, 1961) and humans is ~37°C (Mackowiak *et al.*, 1992), and as such previous work investigating ageing, where a test temperature of ~25°C has been used (Table S1.2) may not be assessing the true maximal contractile performance. To allow for a more realistic simulation of *in vivo* muscle action, more recent work utilising mouse skeletal muscle has typically utilised a temperature of 37°C (James *et al.*, 1996; Tallis *et al.*, 2012, 2013, 2014, 2017). It is also important to note that whilst 'warmer is better' is a useful generalisation, hyperthermia will damage the contractile proteins and cellular processes due to reactive oxygen species accumulation (Allen *et al.*, 2008) and as such tissue viability is likely to deteriorate more rapidly compared to cooler test temperatures.

1.6.5 - Measuring Contractile Parameters of Whole Isolated Skeletal Muscle

There are various modes via which skeletal muscles can be activated, all of which have been examined in whole isolated skeletal muscles. These modes of muscular activity must be critically analysed to determine the most appropriate mode of assessing muscular function which can closely replicate *in vivo* muscular function.

Typically, assessments of isolated muscle contractility have been conducted via isometric, isovelocity and isotonic modes of contraction under concentric and eccentric conditions.

1.6.5.1 - Isometric Muscular Contractions.

Isometric muscular activity is where force is generated via stimulation of the muscle whilst held at a constant length, with fascicle shortening and an increase in pennation angle occurring as a result (Kawakami *et al.*, 1998). Isometric contractions are used primarily to determine the absolute force production of a skeletal muscle and the quality of the skeletal muscle (force per unit of muscle CSA). During *in vitro* experiments investigating muscle contractility, it is commonplace to perform isometric

twitch and tetanus contractions to not only determine maximal muscle force production but to optimise muscle length and stimulation parameters prior to commencing further assessments (James *et al.*, 1996; Tallis *et al.*, 2012, 2013, 2014, 2017) (Chapter 3).

1.6.5.1.1 - The Twitch Response

An isometric twitch contraction is a contractile response that is elicited from a single stimulation (Figure 1.6.1) (MacIntosh *et al.*, 2006). The small period of time between the stimulation and force generation is known as the electromechanical delay (latency period) (Hufschmidt, 1985) and occurs due to sequential biochemical events required to cause muscle activation. When considering a twitch response, only a single stimulation occurs resulting in a small release of Ca^{2+} from SR. As a result, only a small amount of actin binding sites are freed so cross-bridge formations are limited, consequently force production is small. A multitude of factors can influence the magnitude of the twitch response, including the rate of Ca^{2+} release, troponin saturation with Ca^{2+} and the resultant speed of the cross-bridge cycle. The aforementioned factors are greatly influenced by muscle fibre type (Figure 1.6.1), and temperature (MacIntosh *et al.*, 2006).

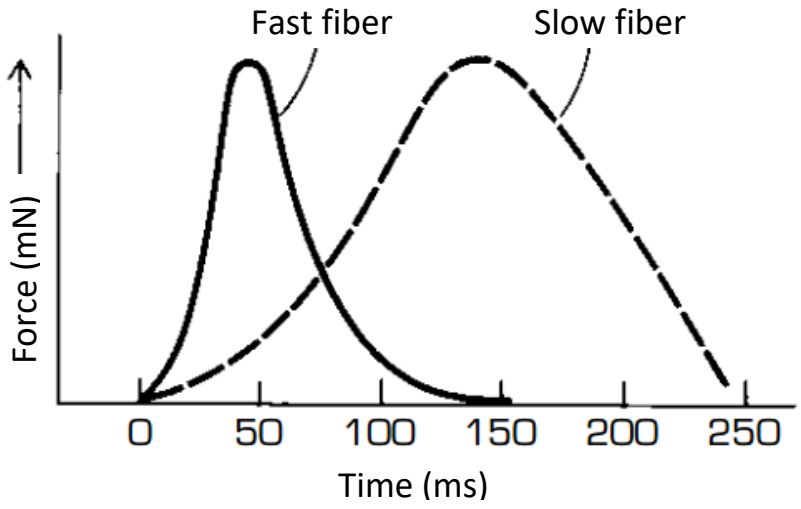


Figure 1.6.1 - The effect of muscle fibre type on time (ms) to the achievement of peak force and full relaxation during an isometric twitch. In a predominantly fast-twitch muscle (solid line) peak force and consequent relaxation is achieved much faster than in a slow twitch muscle (dashed line). (Adapted from Matthews, 2003).

The length of the muscle can greatly influence the resultant isometric response. A force-length curve can be produced to determine the optimal length of the muscle to evoke maximal twitch force. Gordon *et al.* (1966) were the first to describe the force-length relationship in isolated frog skeletal muscle fibres. If a muscle is too short, the muscle has less available actin binding sites for myosin due to overlapping actin filaments resulting in reduced cross-bridge formation to produce force (Matthews, 2003) (Figure 1.6.2 A&B). Conversely, if a muscle is overstretched whilst determining the optimal length, the overlap between thin (primarily actin) and thick (primarily myosin) filaments decreases leading to reduced cross-bridge formation (Figure 1.6.2D). A continual increase in length leads to no overlap of thick and thin filaments and therefore no active force development (Figure 1.6.2E). Therefore, peak force occurs at an optimal muscle fibre length (Figure 1.6.2C) (Silverthorn, 2013).

Figure 1.6.2 - The force-length relationship and the effect on sarcomere length in a crimped (A & B), optimal (C) and overstretched (D & E) state. (Based on Gordon et al., 1966; adapted by Silverthorn, 2013).

1.6.5.1.2 - The Tetanus Response

A tetanus response is achieved via a series of stimulations that result in a much greater amount of force production than a twitch contraction. Performing an isometric tetanus *in vitro* can determine the maximal amount of force a muscle can produce whilst held at a constant length. However, during a tetanus, muscles can be activated maximally or submaximally *in vitro* by altering the rate at which stimulations are provided to a muscle (Vassilakos *et al.*, 2009; Tallis *et al.*, 2012). The interaction between stimulation frequency and force generation is known as the force-frequency relationship and is a useful measure of how electrical activity *in vivo* affects muscle recruitment and therefore force production. Stimulation frequency is altered to promote a greater tetanic response until peak force is achieved or can be lowered to promote a submaximal contraction. Submaximal contractions occur as

the muscle partially relaxes before the next stimulation as the stimulations are not close enough together. This type of tetanus is also known as an unfused tetanus response. In terms of contractile mechanics, the unfused nature of the stimulations indicates the reuptake of some Ca^{2+} to the SR prior to the next stimulation. This causes a lower net concentration of Ca^{2+} to be present in the muscle myoplasm and subsequently exposed actin binding sites. As a result, the total force production is lower.

To maximally activate muscles *in vitro*, each single stimulation occurs close to the previous stimulation not allowing time for the muscle to relax. This is known as a fused tetanus response (Figure 1.6.3). In mechanical terms, the greatest possible amount of Ca^{2+} is released so more cross-bridges can form. The stimulation frequencies used to elicit a maximal tetanic response are muscle specific, with the amount of force produced dependent on the concentration of Ca^{2+} released from SR. This likely varies due to fibre type and muscle function, with the Ca^{2+} release and reuptake faster in predominantly fast-twitch skeletal muscles than predominantly slow-twitch skeletal muscles.

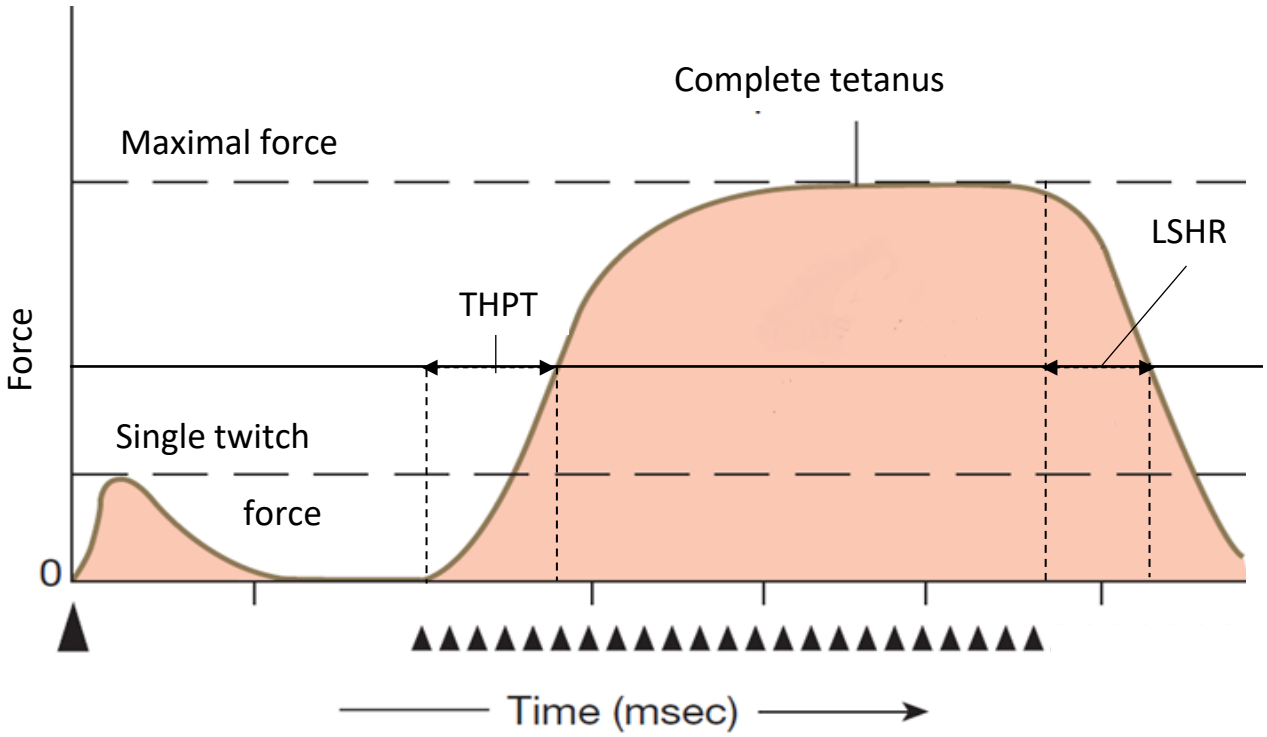


Figure 1.6.3 - A comparison of the force produced in a typical single twitch and fused tetanus response. Each black triangle represents a single stimulation and the dashed line represents the maximal force for each response. (Adapted from Silverthorn, 2013).

The rate of Ca^{2+} release and uptake from the SR determines the speed of activation and relaxation of a muscle. The time to half-peak tetanus (THPT) and last stimulus to half relaxation (LSHR) can be determined using isometrics assessments. These measures are important for indicating activation and relaxation kinetics, or the ability to release and reuptake Ca^{2+}, which have significant consequences for the power producing capabilities of skeletal muscle, with faster release and reuptake resulting in a greater, more rapid force response. Muscle fibre type is the key limiting factor to activation and relaxation dynamics of skeletal muscle, with type II fibres demonstrating faster activation and relaxation dynamics than the slower type I fibres (Tallis *et al.*, 2012, 2013, 2014).

By measuring the maximal amount of force produced during twitch and tetanus muscular contractions, it is possible to calculate the stress (force divided by cross-sectional area) of a muscle.

Muscle stress is an indicator of the quality of the isolated muscle. Whilst direct measurement of force allows for an assessment of absolute isometric force, stress considers the physiological cross-sectional area (PCSA) of the muscle, which can be derived from muscle length, muscle mass and an assumed muscle density.

Use of isometric assessments has greatly contributed to our understanding of the contractile function of skeletal muscles *in vitro*. However, this method is limited when considering its application and validity in relation to *in vivo* contractile mechanics. Isometric contractions do occur during human locomotion; however, many skeletal muscles perform work and in turn generate power via a range of cyclical length changes. In order to perform work, a muscle must undergo passive re-lengthening following shortening in preparation for the subsequent contraction to follow. James *et al.* (1996) determined that usage of isometrics as a method of testing underestimated likely force production and activation and relaxation kinetics during cyclical length changes when compared to force kinetics obtained using the WL technique.

1.6.5.2 - Isotonic Testing

An important measure in muscle mechanics is muscle shortening velocity and how this impacts on force generation. One way of measuring shortening velocity is via usage of isotonic (constant force) muscle contractions to assess the force-velocity relationship. The force-velocity relationship describes the relationship between the speed at which a muscle can shorten and the resultant force it can generate and vice versa (Askew and Marsh, 1998). From a methodological perspective, the muscle is initially optimised in terms of length and stimulation frequency via isometric contractions until the force generated is constant and maximal (F_0). From here, after-loaded shortening contractions can be implemented to determine the force-velocity relationship (Marsh and Bennett, 1986; Askew and Marsh, 1998). This involves stimulating the muscle with isometric tetani using a specific stimulation frequency thus controlling the amount of force that can be generated. Once the desired force is

generated, this is “clamped” and the muscle is then allowed to shorten against this resistive force. The shortening velocity is then recorded. This protocol is then repeated at differing predetermined loads to generate a scatter plot of force against velocity. The force-velocity relationship of a muscle can then be expressed via fitting a line to the data via a method such as Hill's (1938) equation. This is expressed as follows (Equation 1.6.1):

$$(F + a)(V + B) = (F_0 + a)b$$

*Where: **F** is the tension (or load) in the muscle, **V** is velocity of a contraction, **F_0** is the maximum isometric tension (or load) generated in the muscle, **a** is coefficient of shortening heat, **b = a· V_0/F_0**, where **V_0** is the maximum velocity, when **F = 0**.*

Equation 1.6.1 - Hill's (1938) equation for calculating the force-velocity relationship of skeletal muscles.

Hill’s equation generates a hyperbolic curve (Figure 1.6.4). The most important aspects of the force-velocity curve are the curvature of the line and the muscles maximum shortening velocity (V_{max}) (Barclay and Lichtwark, 2007). Figure 1.6.4 also shows that velocity is inversely proportional to force, or as velocity decreases, force increases and vice versa. In relation to the sliding filament theory, if velocity is too great, force generation is impaired as the filaments slide over one another too quickly for many cross bridges to be formed.

Figure 1.6.4 - The typical force-velocity relationship of fast-twitch glycolytic fibres from the iliofibularis muscle of the lizard Sceloporus occidentalis. (Marsh and Bennett, 1986; Adapted by Askew and Marsh, 1998).

For each data point, force can be multiplied by velocity to determine power output so that the relationship between contractile velocity and power can be determined. From figure 1.6.5, the inverted U-shaped traces indicate the force at which power is maximal. By factoring in these as a whole component, force generation and the speed at which the muscle contracts for the given amount of force must be optimal to elicit maximal power.

Figure 1.6.5 - Example of a force-power output curve for the EDL (A) and soleus (C) isolated from male C57BL/6 mice. Power is calculated as the product of force (mN) at a given percentage of maximal force production (P_0) and the maximal shortening velocity of the muscle at a % of P_0 (Graber et al*., 2015).*

Whilst force-velocity (Figure 1.6.4) and force-power output (Figure 1.6.5) relationships give us useful information about the intrinsic mechanical properties of muscle they are generated under artificial experimental conditions that do not simulate likely *in vivo* conditions. The muscle must re-lengthen prior to the next contraction, with re-lengthening requiring mechanical energy input, or negative work (Abbott *et al.*, 1952), that is not considered during isotonic testing. When considering power output, isotonic testing overestimates measures of power, with power obtained via isotonic testing double that obtained via the work loop technique. James *et al.* (1996) suggested a set of conditions that must occur for this to be possible. The muscle must first activate and relax instantaneously prior to the next cyclical contraction. The mechanics of Ca^{2+} release and uptake instantly limit this as a condition. A muscle must also be able to maintain optimal velocity and force during shortening for maximal power

output to be obtained each time. However, as previously highlighted, this is simply not achievable *in vivo* due to the varying velocities and forces generated during cyclical movement.

1.6.5.3 - Isovelocity Testing

Isovelocity testing is another means of assessing the force-velocity relationship of skeletal muscle Whilst isotonic testing measures velocity at a known, clamped force, isovelocity testing involves measuring force production at a known and constant rate of shortening or lengthening. One way is to utilise the slack-test method (Edman, 1979), where the muscle is shortened without an added load, otherwise known as maximal unloaded shortening velocity. Another method is the step-release protocol, where the muscle is initially activated through an isometric tetanus stimulation until peak force is achieved when at this point the muscle is shortened to a new length resulting in a rapid decrease in muscle length and force. This is then followed by shortening at a constant known velocity which allows for force generation to be maintained at the lower level of force reached at the end of the rapid shortening (Lou *et al.*, 2002). Whilst isovelocity testing has contributed to our pool of knowledge regarding muscle contractility, like isotonic testing, this mode of contraction does not consider the contributing dynamic conditions under which muscles normally operate *in vivo*.

These 'iso' forms of testing have allowed for a greater understanding of the underlying characteristics that predominantly affects the mechanical properties of skeletal muscle and the mechanisms under which they operate, namely length, force and velocity. These methods of assessing muscles have been utilised to better understand the effects of ageing (chapter 4 & 5) and obesity (chapter 6) on isolated skeletal muscle contractility. Whilst these 'iso' methods have demonstrated the relationship between these mechanical characteristics and improved our understanding of how these relationships affect muscle contractility and human movement, they poorly contextualise the actual mechanical relationships found during *in vivo* function. Whilst indeed some muscles do operate under these conditions, such as isometric activity of the muscles involved in maintaining posture and stabilisation

whilst standing (De Troyer, 1983; Loram *et al.*, 2004), dynamic changes in force, length and velocity must be considered in tandem during human movement (Josephson, 1985; Dickinson *et al.*, 2000).

More recently, the work loop (WL) technique has been employed as a means of more closely replicating *in vivo* conditions when assessing muscle contractility *in vitro*. This method addresses the main drawbacks found with 'iso' modes of contractions and can more accurately simulate the physiological conditions found *in vivo*.

1.6.5.4 - The Work Loop Technique

This thesis primarily employs the WL technique as the method of better understanding the mechanics of skeletal muscle in relation to *in vivo* contractility. As muscles work in antagonistic pairs, they must undergo cyclical length changes at a range of speeds and muscle lengths in order to produce work for locomotory actions (such as walking, running and swimming; (Dickinson *et al.*, 2000) and respiratory motions (diaphragm contraction during inhalation and relaxation during exhalation). Because of this technique, it is possible to quantify the net amount of work (the total net energy generated during muscular contractions) and therefore power that is produced by individual muscles when undergoing active length changes.

The first studies to have utilised the WL technique as a means of simulating cyclical length changes was through the investigation of asynchronous flight muscles of insects (Machin and Pringle, 1959, 1960; Machin *et al.*, 1962). This work was further enhanced by Josephson (1985) who added phasic stimulation to produce synchronous muscular contractions i.e. one phase of stimulation producing one full WL cycle.

The WL method works by delivering length changes in conjunction with phasic electrical stimulation. This involves a pattern of muscle lengthening from the optimal length, shortening and re-lengthening

back to the optimal starting length (Figure 1.6.6). This alteration in length is known as a change in strain, which is optimised to maximise net work when the power producing ability of the muscle is of interest. Many WL studies have used sinusoidal length changes, either to simulate *in vivo* length change or as a convenient strain pattern when *in vivo* strain is unknown. As discussed, muscles *in vivo* undergo varying complex length changes. For example, when the isolated semimembranosus muscle from the toad *Bufo americanus* was activated cyclically, the proximal and distal end of the muscle strained in opposite directions for up to 34% of the WL cycle, indicating that the adjacent muscle segments operate differently in mechanical terms by working at different regions of their force-length and force-velocity relationships (Askew *et al.*, 1997; Ahn *et al.*, 2003). Sine waves have been identified as a good, easy to implement, generalisation of muscle length changes found *in vivo* (James *et al.*, 1995, 1996) whilst being able to offer a direct comparison between different muscles due to the same mode of action. During these length change cycles, external electrical stimuli can be provided just as the muscle reaches its maximal length, ensuring that the muscle is fully activated at its greatest length and that the stimulation occurs through some of the muscle shortening phase to generate positive work (Figure 1.6.6). This is then followed by a period of largely passive re-lengthening where no stimuli are provided.

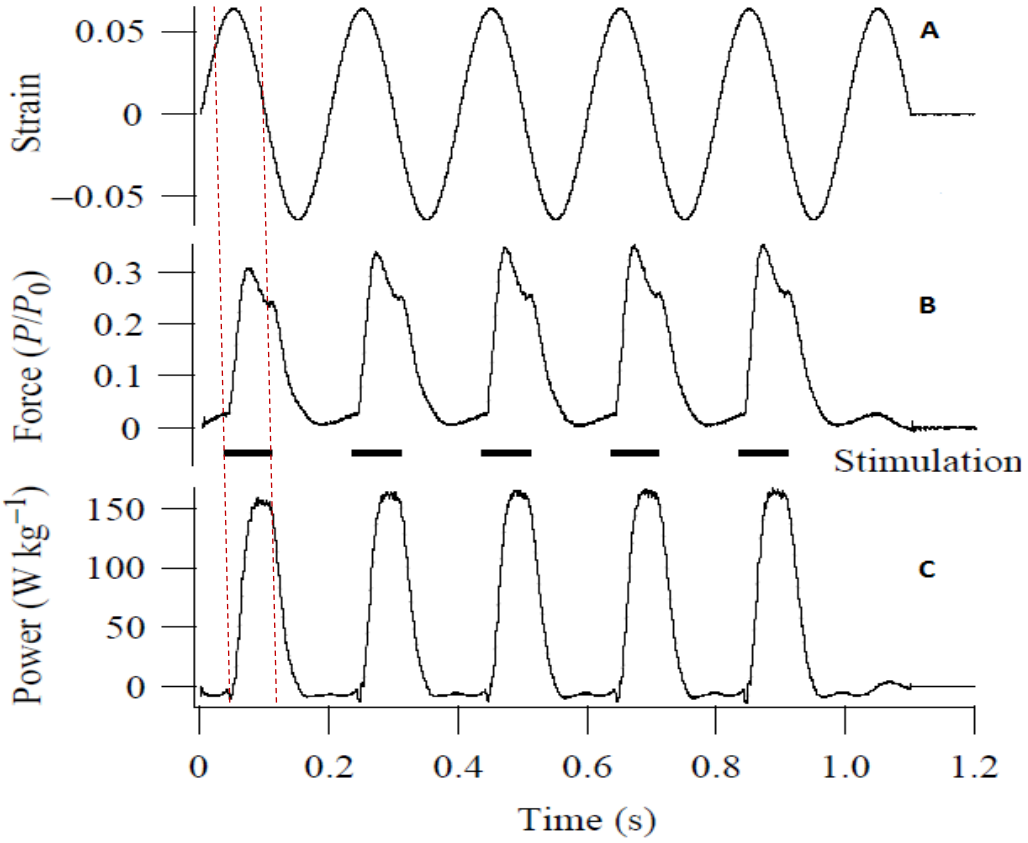


Figure 1.6.6 - Changes in muscle strain with the implementation of sine waves (A). Changes in force during WL cycles. Black horizontal dashes indicate periods of electrical stimulation, where the stimulation occurs as the muscle reaches its greatest length and is maintained during muscle shortening. This is accompanied by a rapid increase in force and a respective decline during muscle shortening and consequential lengthening (B). The changes in muscle power output during WL cycles in relation to force and length changes (C). (Adapted from Askew et al., 1997).

By plotting muscle strain (length change of the muscle ÷ initial length) against force (or stress), a concentric, anticlockwise WL trace can be formed (Figure 1.6.7) (Josephson, 1985, 1993). The area within this WL is the net work of the length change cycle and is the sum of the active, positive work and the passive, negative work.

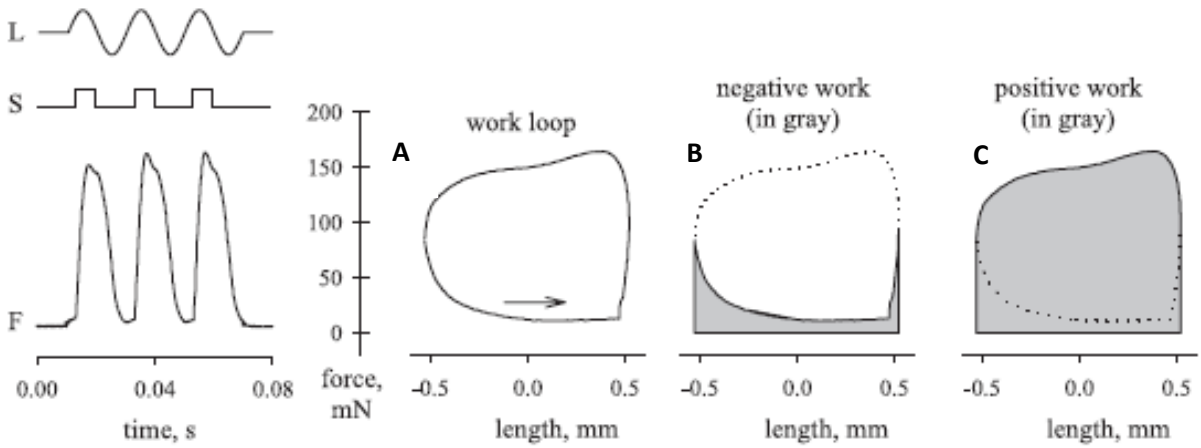


Figure 1.6.7 - Example of a concentric WL generating net positive work (A). Sinusoidal length changes are imposed on a muscle (L), with stimulation periods (S) optimised to stimulate through shortening. Force production (F) is plotted against muscle length to form a WL. The arrow indicates the direction of the WL. Length 0.0mm indicates starting muscle length. Shaded areas represent the positive (C) and negative work produced (B), with each calculated as force x length during shortening (positive) and lengthening (negative). Net work is then defined as the positive work minus the negative work, or the area within the loop (A). (Choi and Widrick, 2009).

As the net work (Joules) and the length change duration (s) is known, dividing work done by the time taken can provide a value for power ($J.s^{-1}$ or Watts) (Josephson, 1993). This can be normalised to muscle mass, thus quantifying the amount of power generated per quantity of tissue ($Watts.kg^{-1}$) or to body mass to provide an indication of power relative to animal size. Changes in cycle frequency (CF) essentially alter the shortening velocity and hence force and work production. So as per the force-velocity relationship, net work is inversely proportional to CF. When CF and net work are multiplied, power output is determined and the relationship between CF and power output can be plotted, following an inverted-U trend, or more simply, a power output-cycle frequency curve (PO-CF; Figure 1.6.8). Whilst construction of PO-CF curves has been performed in mammalian skeletal muscle such as the diaphragm, EDL and soleus (Altringham and Young, 1991; James *et al.*, 2011), no work to date

has produced a PO-CF curve in relation to ageing and obesity. This could be valuable considering the possibility for the optimal CF to shift to elicit maximal power output in response to each experimental condition.

Figure 1.6.8 - Example of a power output-cycle frequency curve for the soleus and EDL isolated from the Djungarian hamster, Phodopus sungorus, during control conditions and daily hibernation (torpor). Power is expressed relative to muscle mass, using cycle frequencies ranging from 1Hz to 10Hz for soleus and 2Hz to 15Hz for EDL (James et al., 2011).

1.6.5.5 - Assessment of Muscular Fatigue

Isometric contractions have been employed, not only to measure isometric force and stress but also, to measure the ability of the muscle to withstand fatigue during repeated isometric contractions. This is achieved by subjecting an isolated preparation to repeated contractions with short periods of rest between each period of stimulation, with measurement of the rate of fatigue or time-course of fatigue (Zhang and Kelsen, 1990; González and Delbono, 2001) or time to decline to 50% of the pre-fatigue maximal isometric force (Pagala *et al.*, 1998). However, as identified by Tallis *et al.* (2014) measuring

fatigue resistance via repeated isometric contractions is a poor indicator of dynamic skeletal muscle action *in vivo* (Josephson, 1985). To counteract this, recent work has assessed the fatigue resistance of isolated muscles using the WL technique (Tallis *et al.*, 2012, 2013, 2014, 2017). Work by Askew *et al.* (1997) utilised mouse soleus muscle to assess the effects of cycle frequency on fatigue development during repeated WL cycles. The most notable changes during the fatigue protocol was a reduction in force over time, a slowing in muscle relaxation and a change in the force-velocity relationship. These changes in the mechanical properties with fatigue were identified by changes in the WL shapes, coupled with an increase in negative work and decrease in positive work, at different cycle numbers (Figure 1.6.9).

In terms of the WL shape, the most distinguishable change is the reduction in the area of the WL and thus decreased net work, during the onset of fatigue (Figure 1.6.9). This reduction in net work can be attributed to a lower peak force and the ability to maintain force during muscle shortening. As fatigue develops, a figure of eight shaped WL may eventually develop, whereby during part of the cycle positive work is produced and in another part of the cycle negative work. Fatigue beyond this will generate fully negative WLs as the muscle will generate greater force through lengthening than shortening whilst undergoing the same length change cycle.

Figure 1.6.9 - Changes in the WL shape of the mouse soleus muscle in a series of WLs at cycle frequencies 2Hz (A) and 6Hz (B) (Askew et al., 1997).

1.6.6 - Methodological Approach of This Thesis

Whilst all the 'iso' parameters described have provided a valuable insight into how skeletal muscles contract, usage of the WL technique is currently the most reproducible *in vitro* technique to gain insight into *in vivo* performance of skeletal muscle in mammals. A combination of isometric muscle contractions and the WL technique shall be utilised in the present thesis. Isometrics, whilst limited in their *in vivo* application, allows for comparisons to previous studies examining age-related changes in isometric force and stress.

In this thesis, the soleus, EDL and diaphragm shall be assessed due to the two locomotory muscles having been the most commonly assessed muscles used in *in vitro* muscle contractility work and these muscles being of differing phenotype; predominantly slow and fast-twitch respectively (Tallis *et al.*, 2012, 2013, 2017). As for the diaphragm, this is utilised to represent the effects age and obesity may have on muscles of different anatomical location and function (Tallis *et al.*, 2014). With the above methods being employed, an understanding of the contractile properties of specific muscles can be performed to identify changes in skeletal muscle contractile function, and the influence of co-morbidities, and how this may relate to whole body muscle performance.

Chapter 2 - Research Questions and Hypotheses

The rationale for each study is provided in brief in section 1.1 of the introduction, with a more detailed discussion of each study rationale in the introduction and each corresponding chapter. Below, a series of research questions and hypotheses are provided for each experimental chapter.

Study 1 (Chapter 4) - **The Sex-Based Differences in the Age-Related Changes in Isolated Locomotory (Soleus & EDL) and Respiratory (Diaphragm) Contractile Function of CD-1 Mice.**

This study sought to address the following questions:

1. Are the age-related changes in the contractile performance of isolated skeletal muscle-specific and sex-specific?
2. Does ageing result in a shift in the optimal velocity for producing power and to what extent does ageing cause a decline in power?
3. When does the muscle-specific and sex-specific onset of dynapenia and sarcopenia occur?
4. Does the loss of absolute force and power exceed the loss of relative force (stress, force per unit of muscle cross-sectional area) and power (power relative to muscle mass and body size)?
5. To what extent does dynapenia (loss of contractile performance) and sarcopenia (loss of contractile performance along with a reduction in muscle mass) contribute to the skeletal muscle ageing process, and how is this affected by sex and the muscle examined?
6. Does the decline in isometric stress occur to a greater magnitude than that of muscle power, and does this change with increasing age?
7. What is the effect of increasing age on the fatigue resistance of isolated soleus, EDL and diaphragm muscles for males and females?

Hypotheses

1. As with human and animal models of muscular ageing, it is expected that younger males will produce greater absolute and normalised force and power than females. However, the loss of

absolute force and power from peak maturity and onwards will occur at a faster rate for males than females, with a faster and earlier decline for the EDL compared with the soleus and diaphragm due to greater atrophy of fast-twitch fibres with age, and a shift from a fast-to-slow twitch phenotype.

2. A shift towards a slower cycle frequency for optimal power output is expected for EDL due to a fast-to-slow shift in muscle fibre type that is more likely to occur for the EDL than with the soleus and diaphragm due to composing of predominantly fast-twitch muscle fibres.
3. As EDL muscle mass increased in the study by 50 weeks of age in the study by Tallis *et al*. (2014) by 50 weeks of age for isolated EDL, a loss of muscle mass is still unlikely by 78nweeks of age for soleus and EDL. This is due to muscle atrophy not typically occurring until the final 25% of an animal's lifespan, with a 50% mortality rate for 78-week-old CD-1 mice. Should there be a loss of muscle mass, the loss is expected to be greater for males than female skeletal muscles due to males typically having greater muscle mass, so there is greater scope for mass to be lost.
4. It is expected that absolute performance to be better maintained in old age rather than muscle quality due to intrinsic mechanisms, such as excitation-contraction coupling and increased non-contractile mass contributing to a decline in quality rather than a loss of absolute function due to age-related increases in whole muscle mass (Tallis *et al*., 2014).
5. As with Tallis *et al.* (2014), it is expected that the age-related loss of isometric stress will occur to a greater magnitude than the loss of normalised WL power output.
6. The loss of muscle function will occur before the loss of muscle mass for all muscles of both sexes in the first instance, with the magnitude of the decline likely to occur greatest for the EDL of males. By 78 weeks of age, a loss of muscle mass may have occurred, which will consequently lead to an acceleration in the decline of force and power.
7. The effect of age on fatigue will be muscle specific. Generally, older muscles will be less fatigue resistant than younger muscles when normalised to muscle mass, though sex-differences may

not be apparent given the current evidence examining sex-based differences in muscle fatigue of isolated skeletal muscles (Chan & Head, 2010). Moreover, the EDL will be most susceptible to an age-related change in fatigue resistance compared with soleus and diaphragm.

Study 2 (Chapter 5) - **The Effect of Increasing Age on the Concentric and Eccentric Contractile Properties of Isolated Mouse Soleus and Extensor Digitorum Longus Muscles.**

This study sought to answer the following questions

1. Does increasing age affect the absolute eccentric power and eccentric power normalised to muscle mass of isolated locomotory muscles?
2. is there a difference in the time-course of fatigue during repeated concentric and eccentric work loops, and are the difference muscle-specific and age-specific?
3. Does sustained concentric and eccentric activity impair the consequent ability to recover concentric power in older skeletal muscles?

Hypotheses

1. Absolute and normalised eccentric power will not change with age, though concentric power will be significantly lower for the soleus and EDL.
2. Older skeletal muscles are likely to fatigue faster during concentric and eccentric fatigue than younger skeletal muscles.
3. Older skeletal muscles will not recover power as rapidly as younger animals, with concentric power following sustained eccentric activity likely to remain depressed to a greater extent than young skeletal muscles during recovery. Previous work has reported that older skeletal muscles are more susceptible to contraction-induced damage during repeated lengthening protocols. As such, recovery following sustained eccentric work loops is likely to be more impaired in older skeletal muscles

Study 3 (Chapter 6) - **The Effects of Age and Dietary-Induced Obesity on the Contractile Function of Isolated Locomotory and Respiratory Skeletal Muscles.**

To answer the following questions

1. To what extent does dietary-induced obesity affect the morphology of older animals?
2. Does a high-fat diet cause a reduction in absolute measures of force and power, and muscle quality compared to control animals?
3. Will a high-fat diet result in a reduced fatigue resistance for older isolated skeletal muscles?

Hypotheses

1. Consumption of a high-fat diet will result in significantly greater body mass, fat mass, and muscle mass compared to age-matched control animals.
2. A high-fat diet will cause a significant reduction in absolute and relative mechanical performance in a muscle-specific manner, with force of faster muscles impaired to a greater extent due to the mechanistic similarities between ageing and obesity that results in poorer contractile function in faster skeletal muscles.
3. Fatigue resistance of slower muscles is likely to be impaired to the greatest extent than faster muscles as with younger studies of obesity.

Chapter 3 - General Methods

3.1 - Animals

The ethical committee at Coventry University approved the use of animals for use in these projects. White male and female mice (strain CD-1, Charles River Harlan Laboratories, UK) were purchased at ages 3-9 weeks and kept in-house to mature at Coventry University. Each sex was divided into single-sex cages of 8-10 based on their target age for each experimental group. Each cage was exposed to 12-hour light-dark cycles at 50% humidity and an ambient temperature of 25°C without access to running wheels. All mice were provided with a standard lab chow diet (SDS Rat and Mouse No.1 Maintenance, LBS Biotech, Hookwood, UK) (SDS RM-1 M). Mice had access to food and water *ad libitum* throughout the duration of the studies. A qualified vet examined the animals at regular intervals and those that were deemed unhealthy were removed. Unhealthy animals were identified by visually apparent symptoms such as unusual growths/tumours or dragging of the hind limbs. If upon sacrifice internal complications such as growths were identified, then the animal was discarded, and the muscle not utilised for experimentation. The exact numbers excluded in each study are provided in each respective chapter.

Many previous studies examining age-related changes in skeletal muscle contractile performance typically utilise inbred strains, such as the C57BL/6J mouse and the F344 rat (Table S1.2). Using inbred strains for such purposes are useful as each animal is genetically homogenous, so the consistency of the genetic background is greater than that of outbred strains, such as the CD-1 mouse. However, the CD-1 strain was selected as studies have found that the CD-1 strain is outbred enough to display a genetic heterogeneity similar to those found in humans (Rice and O'Brien, 1980; Aldinger *et al.*, 2009), with the aim of the thesis to replicate *in vivo* conditions found in humans as closely as possible. Further to this, Miller and Nadon (2000) suggest that the outbred strains, such as the CD-1, are better suited to ageing studies than inbred strains due to a greater longevity and fewer health complications.

For study one, mice were aged to 3, 10, 30, 52, and 78 weeks prior to experimentation. In study two, mice were aged to 10 weeks and 78 weeks whilst in study three mice aged to 79 weeks of age. No data exists to depict the representative ages of CD-1 mice to humans, however, Flurkey *et al.* (2007) mapped the representative ages of the inbred C57BL/6J strain to humans and as such the representative ages are used as a proxy for the CD-1 mouse. For study one, a 3-week age group, representing adolescence in humans, was included to demonstrate the rate of ageing from a young age group to what has been deemed as peak sexual maturity at 10 weeks of age, with this age group representing adulthood in humans (Tallis *et al*., 2014). A 30-week old age group, representing the start of middle-aged humans, was used to determine whether there was a linear decline in contractile performance between 10 weeks and 52 weeks of age. A 30-week-old age group was only included for males as data already exists for females, with little changes in contractile performance observed (Tallis *et al*., 2014). Whilst a 50-week old age group was used in Tallis *et al.* (2014) a 52-week (12-month) old age group was used in study one to represent a mature adult population. Beyond these age groups, a 78-week (18-month) old male and female group were used to represent an older population, where a 50% mortality rate for female CD-1 mice occurs at 78 weeks of age (Navarro *et al.*, 2002). Many studies having previously utilised a 20-24-month age group to represent a very old population when investigating muscle contractility *in vitro* (Thompson and Brown, 1999; González *et al.*, 2000; González and Delbono, 2001; Lynch *et al.*, 2001; Moran *et al.*, 2005; Chan and Head, 2010; Graber *et al.*, 2015). The current work attempted to replicate this by using a 24-month-old age group. However, by 2 years of age, the majority of the animals had died, with only 4 female mice and no male mice surviving.

Once animals had reached their target age in all studies, each age group was utilised within 14 days to minimise the effect of the age group being used continuing to age. The 3-week age group were used within 7 days of arrival to the animal unit.

3.2 - Skeletal Muscle Dissection and Preparation

Mice were sacrificed by cervical dislocation and then weighed to determine body mass (BM) (1475 MP 8-2, Sartorius, Göttingen, Germany). For study three, nasoanal length (NAL) and body circumference were quickly recorded following sacrifice to later calculate Body Mass Index (BMI) and Lee Index of Obesity (LIO), common non-invasive measures of obesity in rodents (Bernardis and Patterson, 1968; Sjögren *et al.*, 2001). The soleus and EDL were utilised to determine whether the effects of age and obesity differed between locomotory muscles of different fibre type and function (soleus 53.6% type I, 31.2% type IIA, 15.2% type IIX; EDL 3.9% type I; 9.3% type IIX; 86.8% type IIB in 90 day old adult C57BL6/J mice; (Agbulut *et al.*, 2003). In chapters 4 and 6, the diaphragm was also used to determine whether this respiratory muscle is affected in a different manner by age and obesity.

3.2.1 - Soleus and EDL Dissection

Following cervical dislocation, the hind limbs were skinned and removed. The limb with the required muscle was pinned out whilst submerged in fresh and frequently changed (once every 5 minutes) refrigerated (~4^{o}C) oxygenated (95% O_2: 5% CO_2) Krebs-Henseleit solution of composition (mM) NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; CaCl 2.54; pH 7.55 at room temperature (James *et al.*, 2005). The EDL or soleus from the right hindlimb was used to examine skeletal muscle contractility whilst the EDL or soleus from the left limb was rapidly removed, dabbed on tissue paper, flash frozen in liquid nitrogen and kept in a -80°C freezer (Forma 900 Series, Thermo Scientific, Ohio, USA). The EDL and soleus isolated for contractility measures were isolated with the tendon still attached at the distal end and the tendon and a small piece of bone left attached at the proximal end of the muscle. The distal tendon was then wrapped with an aluminium foil T-clip as close to the muscle as possible (Figure 3.1) (Tallis *et al.*, 2012, 2013, 2014).

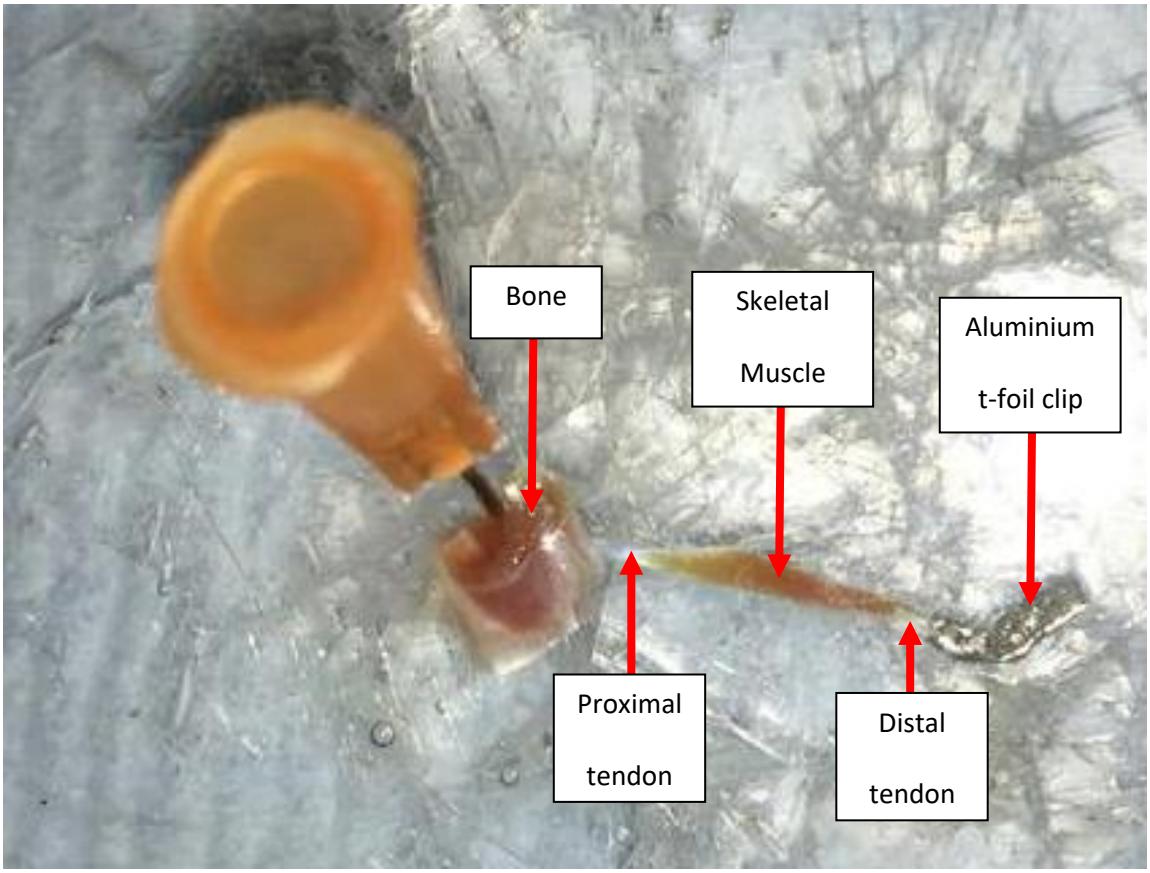


Figure 3.1 - A diagram which displays an isolated locomotory muscle (soleus) which has been clipped and is ready for the isometric and WL protocol. The bone at the proximal end and the clip at the distal end are placed into crocodile clips in the rig. This muscle is approximately 9mm long.

3.2.2 - Diaphragm Dissection

As with the locomotory muscles, following cervical dislocation the animal was skinned with the entire thoracic cavity removed, ensuring that the diaphragm and ribs connecting to it were intact and undamaged. Following removal of the internal organs and the skin, the ribs and diaphragm were pinned out so that the diaphragm sheet was above the sternum. In this position, a portion of the diaphragm no wider than two ribs was isolated from the right-hand side with ribs attached at one end and an aluminium foil clip was placed around the central costal tendon (Tallis *et al.*, 2014) (Figure 3.2). Extra care was taken to ensure that the muscle fibres of the diaphragm were equally aligned in the clip and that all the fibres were under the same amount of tension due to the implications of the force-

length relationship (Gordon *et al.*, 1966). The left-hand portion of the diaphragm was snap frozen in liquid nitrogen and stored in a -80°C freezer for later biochemical analysis.

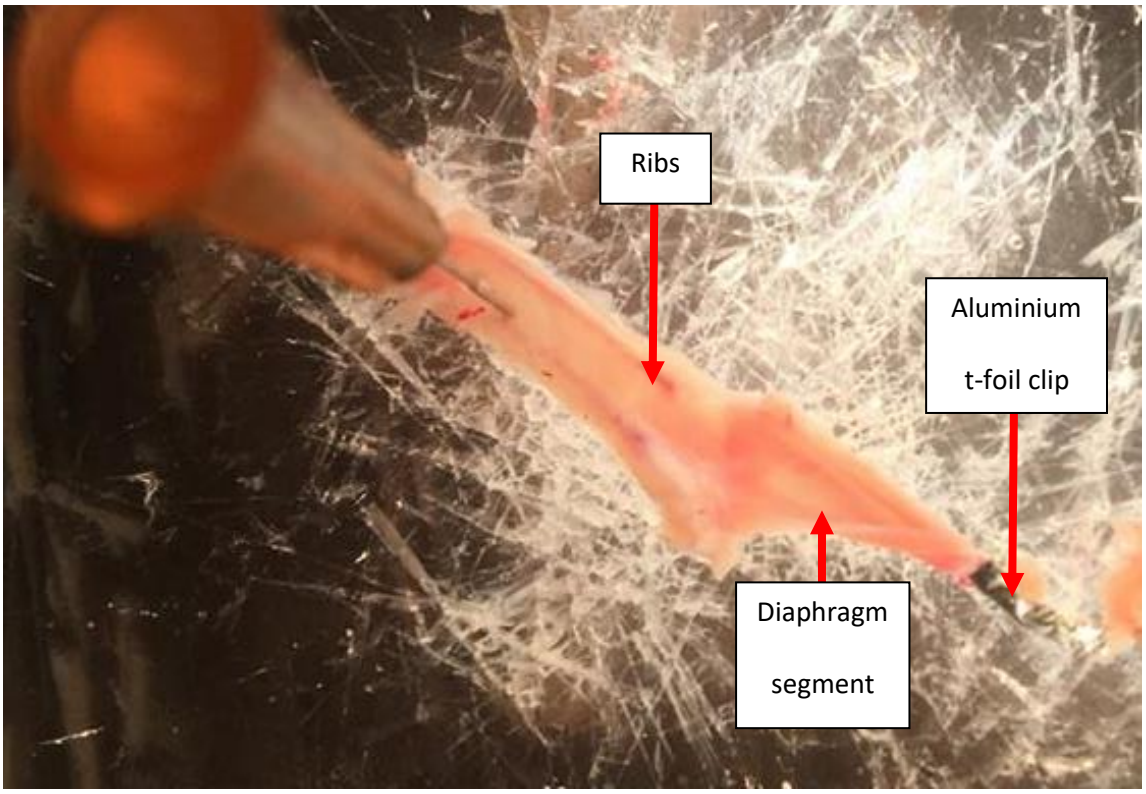


Figure 3.2 - A clipped segment of diaphragm with the clip wrapped around the central tendon.

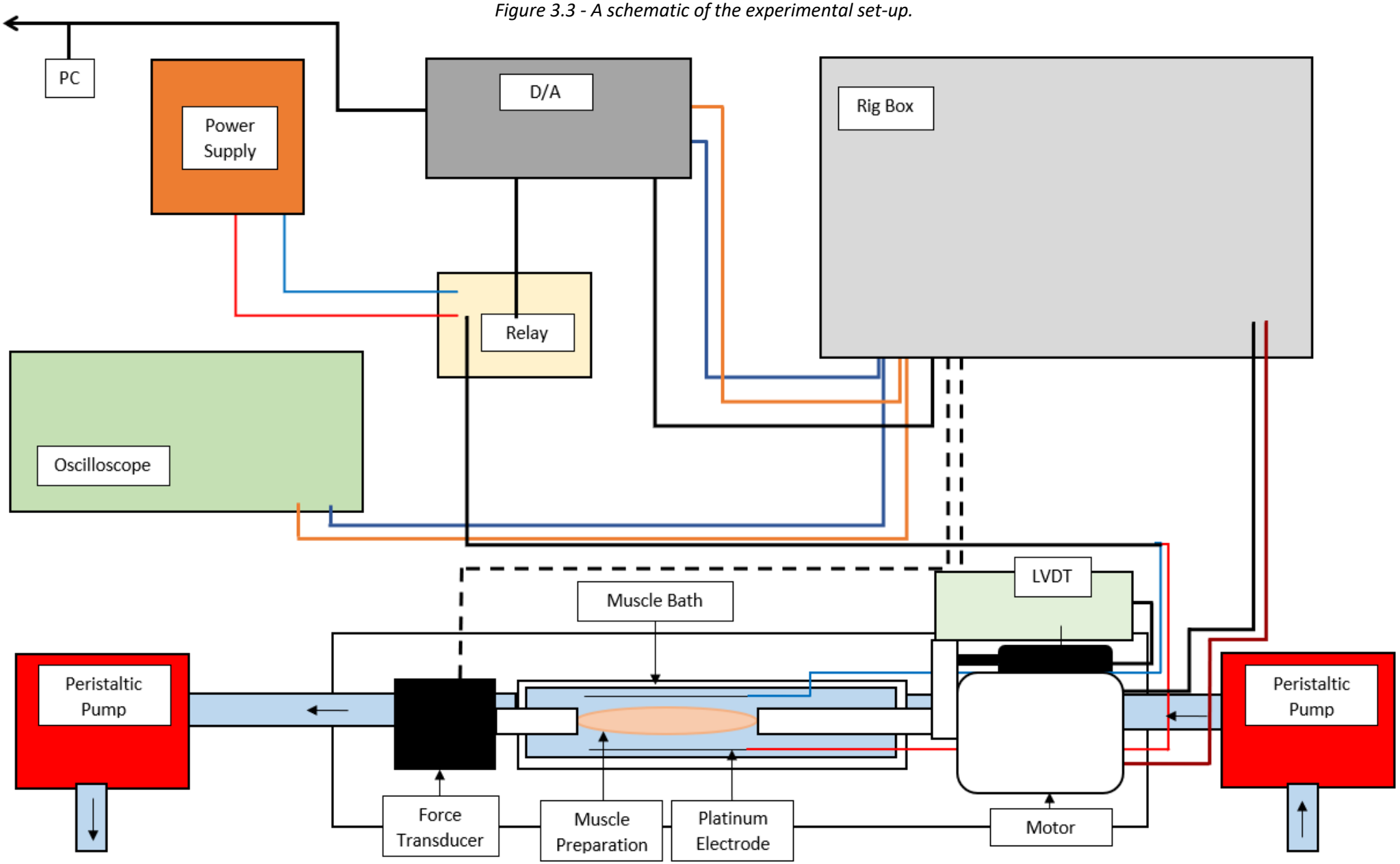
Once the target muscle had been isolated and placed into the rig, the white gonadal adipose tissue from around the pelvic region of the mouse was removed in its entirety, placed in a weighing boat and weighed on an electronic balance (B204-S, Mettler-Toledo, Zurich, Switzerland). The gonadal fat pad mass (FPM) (g) was recorded, with the fat pad and remaining carcass then safely discarded. Isolation and weighing of gonadal FPM was only performed in study three.

3.3 - Experimental Set-Up

Mechanical performance of skeletal muscle was assessed using a custom-built system (Figure 3.3) whereby stimulation parameters and length were altered to determine maximal isometric force production as well as dynamic power output via the WL technique. Following dissection, the muscle was placed in a bath with circulated oxygenated Krebs-Henseleit solution maintained at 37±0.2°C. Crocodile clips were used to attach the muscle, via the aluminium t-foil clips or bone, to a force transducer (UF1, Pioden Controls Ltd., UK) at one end and a motor arm (V201, Ling Dynamic Systems, UK) at the other. The position of the motor arm, and therefore muscle length, was detected via a Linear Variable Displacement Transformer (LVDT) (DFG5.0, Solarton Metrology, Bognor Regis, UK). The force produced by the muscle was measured by the force transducer. The signals from the force transducer and LVDT were delivered via a custom-built rig box, where the signals were processed and amplified, to a digital storage oscilloscope (2211, Tektronix, Marlow, UK) to provide a rapid visual representation of force production and length changes. This information was also delivered to the PC, via a data acquisition board (KPCI3108, Keithley Instruments, Ohio, USA), which ran the custom written Testpoint software (Testpoint, CEC, Massachusetts, USA) which controlled the stimulation and length change parameters delivered to the muscle. The experimental length and stimulation variables, including stimulation frequency, length change cycle frequency (CF), strain and stimulation burst duration, were changed by the user via Testpoint software. The length and stimulation parameters used were muscle-specific; greater detail of this, in relation to each experiment, is explained in later chapters. Testpoint also provided the calculated net total work done for each WL in microjoules (μJ). This was achieved by the programme plotting force produced against change in length during muscular lengthening and shortening resulting in a plot of a WL, where the area of the plot corresponds to the calculated net work performed (Josephson, 1985). Force and length data were sampled at a rate of 10 kHz. The starting length of the muscle was manually changed, with an alteration in the distance between the force transducer and the motor arm changing the physical

length of the muscle in the bath. Peristaltic pumps either side of the rig maintained the flow of the oxygenated Krebs-Henseleit solution into the bath. The Krebs-Henseleit solution was kept at a physiologically relevant temperature (37.0±0.2°C) where a reservoir of ~500ml of the solution was placed into a heater/cooler bath (Grant LTD6G, Grant Instruments Ltd., Shepreth, UK) in order to keep the temperature within the aforementioned range. At any one time, ~30ml of the solution was in circulation around the system and in the muscle bath**.** This temperature was used as it is reported that the temperature of mouse muscles *in vivo* is ~37°C (Mackowiak *et al.*, 1992). The temperature of the Krebs-Henseleit solution in the bath was constantly measured with a digital thermometer (Checktemp C, Harvard Apparatus, UK), with the temperature of the water bath adjusted accordingly to maintain the temperature range. The muscle was electrically stimulated via parallel platinum electrodes in contact with the solution with stimulation amplitude (12V-18V) altered by a benchtop power supply (PL320, Thurlby Thandar Instruments, Huntingdon, UK). Each stimulation signal from the computer caused the relay to switch to open, allowing the power supply to deliver an electrical stimulus.

Figure 3.3 - A schematic of the experimental set-up.



3.4 - Calibration of Equipment

It is important that the equipment was calibrated at regular intervals to ensure that the force, length and work being recorded were the true values and not due to a recording error because of uncalibrated equipment. The two components of the experimental set-up which were calibrated were the force transducer and the LVDT.

3.4.1 - Calibration of the Force Transducer

The force transducer was calibrated regularly by hanging known weights from it. Once the weights were applied, the magnitude of the force was measured on the oscilloscope and the data plotted as per Figure 3.4. Masses ranged from 2g to 10g and was performed in a random order. Calculating the slope and the intercept of this data allowed for the calculation of the force transducer calibration (in $mN.V^{-1}$).

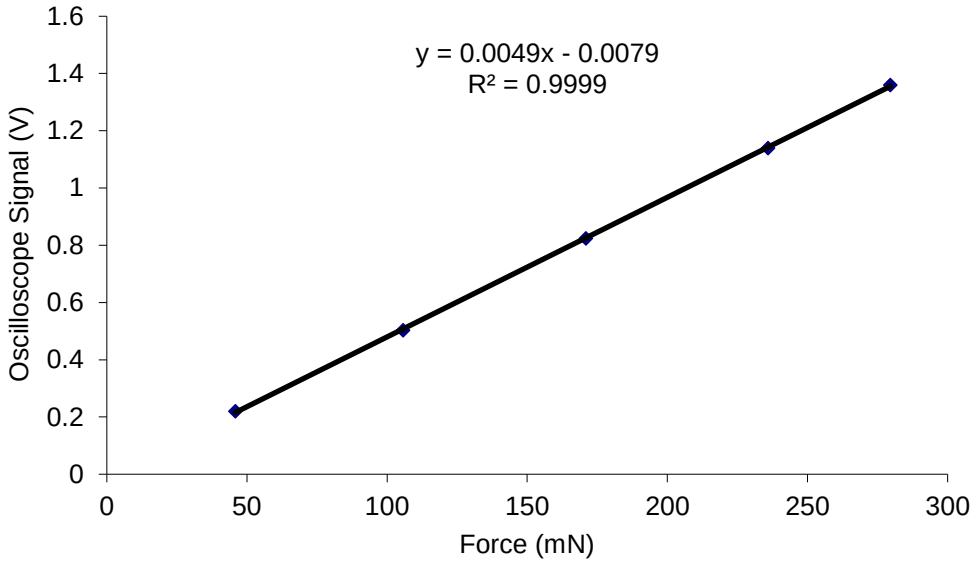


Figure 3.4 - The output for the calibration of the force transducer. x intercept = the force produced by the known masses (dependent variable); y = the voltage recorded on the oscilloscope (independent variable).

3.4.2 - Calibration for Length Changes from L_0

An item (usually a small screw) was placed into the crocodile clip attached to the motor arm to act as a referent point. A strain which elicited an expected length change was input into Testpoint and performed continual sinusoidal wavelength changes whilst measuring the total number of units the object moved from the initial starting position via an eyepiece graticule fitted to a microscope (a). This represented the total length change value from L_0. The magnitude of the sinusoidal wavelength change was measured on the oscilloscope in Volts (b). This represents the voltage change which occurs during the sinusoidal waveforms and is equivalent to the physical length change from L_0. A physical measurement of the item in the motor arm (c) was then made in millimetres and measured as units under the eyepiece graticule (d). The equation to calculate the length calibration is as follows (Equation 3.4):

$$[(c/d) * a]/b = \text{length calibration; mm/V},$$

Where - a = units moved on graticule during sinusoidal waveforms; b = delta voltage of sinusoidal wavelength changes; c = physical length of the object measured; d = length of the object derived from the graticule.

Equation 3.1 - The equation used to calibrate the experimental set-up in terms of the length calibration value to ensure accurate muscle length measurements were achieved.

3.5 - Mechanical Measurements of *In Vitro* Skeletal Muscle Performance

Once the muscle was placed in the rig and attached to the crocodile clips, the muscle underwent a 10-minute equilibration period to allow for the muscle to adapt to the new environment. In the case of these studies, the muscle preparations then underwent a series of both isometric and dynamic (WL) assessments during concentric (Chapters 4, 5 & 6) and eccentric (Chapter 5) muscular activity.

3.5.1 - Twitch Stimulations

Following 10 minutes of stabilisation of the preparation, muscles were stimulated to produce a series of twitch responses. Firstly, muscle length was gradually increased by physically lengthening the muscle by changing the distance between the crocodile clips. Twitch stimulations were performed at each new length until twitch force plateaued despite an increase in length. Next, stimulation voltage (typically 12-16V for soleus, 10-16V for diaphragm and 14-18V for EDL; whereas a pulse width of 1.2ms and stimulation amplitude of 160mA was fixed) was increased until the highest twitch force was achieved. Again, this was determined as the point where twitch force plateaued/decreased despite an increase in stimulation amplitude. Should force decrease following an increase in the physical length or stimulation amplitude, the length was returned to the previous length or stimulation value which elicited the higher amount of twitch force. The maximal twitch force was then recorded.

3.5.2 - Measuring Muscle Length (L_0)

Once physical length and stimulation amplitude were optimised for the EDL and soleus, muscle length was measured using an eyepiece graticule attached to a dissection microscope and was defined as L_0. The mean muscle fibre length was calculated as 75% and 85% of the measurement obtained via the eyepiece graticule for the EDL and soleus respectively, as measured and determined by James *et al.* (1995). For the diaphragm, the muscle was measured directly using the eyepiece graticule as no estimation exists, for the fibre:muscle length ratio, due to different sections of the diaphragm being taken for each dissection (Tallis *et al.*, 2014).

3.5.3 - Tetanus Stimulations

Once maximal twitch force was determined, tetanus stimulations were then provided to the muscle to determine maximal tetanic force. These were performed using the muscle length and stimulation parameters that previously elicited maximal twitch force. The stimulation frequency, or the rate at which electrical stimulations were delivered to the muscles, was altered until maximal force was produced. This typically ranged from 120-140Hz for the soleus and diaphragm, and 200-220Hz for the EDL. The duration of the electrical stimulation, or burst duration, was 350ms for soleus, 250ms for diaphragm and 250ms for EDL, as per previous research (Tallis *et al.*, 2012, 2013, 2014, 2017). These remained unchanged during the assessment of tetanic force. 5 minutes of rest were imposed between each tetanus stimulation to allow for sufficient recovery of the muscle prior to the next burst of stimulation (Tallis *et al.*, 2013, 2014). Once peak isometric force was determined, a final tetanus stimulation, using the first stimulation frequency tested, was performed prior to the WL protocol to monitor changes in performance over time. Using the trace of isometric force from tetanus stimulations on the oscilloscope, measurements of peak tetanic force, activation time (THPT) and relaxation time (LSHR) were performed (James *et al.*, 2004; Seebacher and James, 2008; Tallis *et al.*, 2012, 2014; Higgins *et al.*, 2013; James, 2013). These are common measures used to assess the speed of activation and relaxation, and therefore indicate rates of Ca^{2+} release and subsequent uptake by the SR (Tallis *et al.*, 2013, 2014) and can also provide an indication into cross-bridge efficiency during muscle activation and relaxation.

3.5.4 - The Work Loop Protocol

Once the isometric properties of the muscle had been optimised, a 5-minute rest was imposed before the assessment of muscular power using the WL technique. This technique measures the ability of specific isolated muscles to generate force whilst undergoing cyclic length changes (Josephson, 1985; James *et al.*, 1996) and as a result, calculates the power output of the muscle. Testpoint can derive average net work due to the ability to utilise instantaneous force and velocity values to calculate work.

As per section 1.6.5.4, this technique considers both the passive and active properties of the muscle during cyclical muscular contractions or the positive and negative work produced during a contraction (Josephson, 1985). The muscle was held at the previously determined L_0 with the stimulation amplitude and frequency parameters derived from the isometric contractions employed. Each muscle was subjected to four sinusoidal length changes per set of WL measurement, with a 5-minute rest period imposed between each set of WL's to ensure sufficient recovery (Tallis *et al.*, 2014, 2017). Length changes were performed via the motor arm, where electrical stimulations were initiated before the muscle was at its greatest length and ceased before its shortest length during a WL (James *et al.*, 1996). In this work, a range of speeds of contraction, known as cycle frequencies (CF) was assessed for each muscle to determine the effect age and obesity had on muscle mechanics at faster and slower speeds of contraction. Each stimulation variable and its implication on contractile properties, net work and WL shape is outlined below.

3.5.4.1 - Strain

Strain is defined as the change in length during cyclical contractions from the L_0 determined during twitch contractions. The value for strain and the resulting total length change was determined by the value input into the Testpoint software. In relation to the WL, the skeletal muscle initially passively lengthens from L_0 to its maximal length. Just prior to the muscle reaching its maximal length, electrical stimulation is delivered to the muscle for a specific burst duration. The timing at which the electrical stimulation is provided prior to the muscle reaching its maximal length is defined as the phase, which is discussed in greater detail in section 3.5.4.3. The electrical stimulation continues to be delivered during muscular shortening, ceasing prior to the muscle reaching its shortest length, before being re-lengthened back to L_0 (Figure 1.6.7). As an example, a strain of 0.10, or ±5% length change from L_0, infers the muscle will increase in length from L_0 by 5%, shortens by 5% back to L_0 and shortens a further 5% from L_0 to its shortest length, and then re-lengthens by 5% back to the predetermined L_0. The strain was altered to ensure maximal net work was achieved at a given cycle frequency.

3.5.4.2 - Burst Duration (ms)

The time period during which a muscle is actively stimulated during shortening by the parallel platinum electrodes is known as the burst duration. This period of electrical stimulation causes the release of Ca^{2+} from the SR and initiates the formation of cross-bridges. Like strain, it is important to achieve an optimal burst duration to ensure maximal Ca^{2+} release during muscle shortening for maximal force development and thus work. Under-stimulation due to a small burst duration will reduce force production. Likewise, over-stimulation of the muscle would result in the muscle being stimulated during muscle re-lengthening, resulting in greater eccentric work due to greater resistance to muscle re-lengthening as the muscle is too active during lengthening, therefore increasing negative work thus reducing net work. All changes in burst duration were implemented to ensure optimal force production during muscle shortening in order for maximal net work to be achieved. In relation to WL shapes, burst duration was reduced if the WL shape resembled an "infinity" sign (Figure 3.5A) or if the shape sloped downwards during muscle re-lengthening (Figure 3.5B). Burst duration was increased if the shape sloped downwards during shortening (Figure 3.5C). Typical burst durations for each muscle at 10 weeks of age are provided in table 3.1.

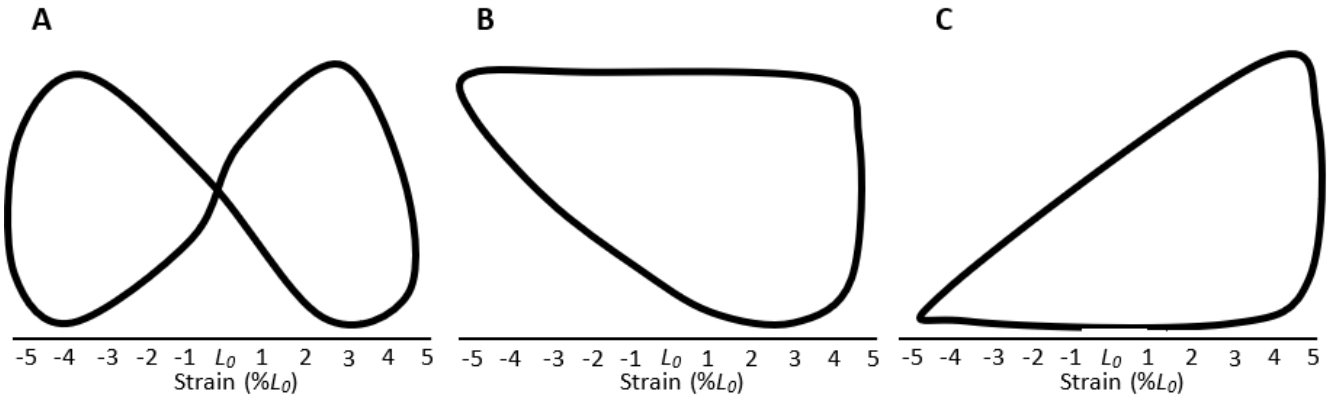


Figure 3.5 - Typical concentric WL shapes if a muscle has been over stimulated due to burst duration being too large (A & B) or under stimulated due to burst duration being too small (C). The expected WL shape for a concentric contraction is provided in figure 1.6.7.

3.5.4.3 - Phase (ms)

To ensure that the muscles were active during shortening, a negative value for phase (ms) was used to initiate the electrical stimulation at a set time before the muscle reached its maximal length. For example, a phase shift of -10ms for soleus ensured that stimulation began 10ms prior to the muscle reaching its greatest length. The stimulation would consequently be provided throughout the shortening phase just prior to the muscle reaching its' shortest length to reduce negative work. Other phase shift durations were -5ms for diaphragm and -2ms for EDL; these durations ensured that the muscle was active at the start of the muscle shortening in order to achieve maximal net work, and have been used in previous work (Tallis *et al.*, 2012, 2013, 2014). Stimulation starts before the muscle starts to shorten to account for the latency period during which the electrical stimulation causes a cascade of events, during activation, throughout the skeletal muscle resulting in Ca^{2+} release and consequent force production. As such, although electrical stimulation starts during muscle lengthening, negative work is minimised.

3.5.4.4 - Cycle Frequency (Hz)

The rate at which each muscle undergoes sinusoidal length changes during the WL technique is determined by the CF. The soleus, EDL and diaphragm produce peak power at different optimal shortening velocities due to variation in fibre phenotypes. As a result, each muscle generates greater power at an optimal CF where the actin and myosin filaments bind at a contraction speed where the optimal velocity of shortening occurs. Lower CF's result in greater net work due to greater cross-bridge formations but cause the muscles to contract at a slower speed resulting in reduced power output as power output = CF × work per cycle. By contrast, higher CF's cause the muscles to contract at faster speeds, but net work is lower due to fewer cross-bridge formations. It is therefore important to identify and use the optimal CF to generate the true maximal power output of a skeletal muscle. Prior *in vitro* research has shown that V_{max} is reduced in older age groups (Thompson and Brown, 1999; Kim and Thompson, 2013), whilst some demonstrate no change with age (Brooks and Faulkner, 1988, 1994; Kim and Thompson, 2012). Therefore, a range of CF's was used to determine whether the CF that elicited maximal power output changed with age and obesity and if there was an overall shift towards a slower, optimal CF with age and obesity. Some work utilising the WL technique has previously assessed mouse muscle power output across a range of CF's (James *et al.*, 1995, 1996, 2011; Askew and Marsh, 1998) allowing for the formation of a parabolic power output-cycle frequency (PO-CF) curve where power output was plotted against CF for each muscle (Figure 3.7) though no work has investigated this in relation to increasing age or obesity.

In previous research, power output was greatest using CF's of 5Hz, 7Hz and 10Hz and typically using burst durations of 65ms, 55ms and 50ms for soleus, diaphragm and EDL respectively with a strain of 0.10 usually eliciting maximal power output in each muscle using the aforementioned parameters (Altringham and Young, 1991; James *et al.*, 1995, 2004; Tallis *et al.*, 2012, 2013, 2014). These parameters are optimal in a young age group and were subject to change in older age groups. These CF's with their constituent parameters were used first to optimise the muscle to determine the

maximal power output of the muscle. On some occasions, the strain and burst duration were altered slightly based on the WL shapes as previously mentioned in order to ensure maximal net work was achieved at each CF.

Muscles then underwent WL's across a range of CF's as outlined in table 3.1, with burst duration and strain altered at each CF to elicit maximal WL net work. The general trend was that as the CF decreased, optimal strain and burst duration increased and vice versa (James *et al.*, 1996). As for the first CF's (5Hz, 7Hz & 10Hz), burst duration and strain were altered based on WL shape to ensure maximal net work. The order in which each CF was tested was determined at random using an online number generator. This was employed to remove an order effect for each muscle. The only exception was for the slowest CF's (2Hz, 3Hz and 4Hz for soleus, diaphragm and EDL respectively) which were used last as the slow speeds, with longer burst durations, may damage the fibres of the muscles.

Due to the build-up of an anoxic core over time (Barclay, 2005), drops in performance are observed in skeletal muscles tested *in vitro*. For example, Tallis *et al.* (2014) observed an average 13.8% and 15.4% decline from the maximal power output obtained in the EDL and diaphragm respectively over a time-course of 180 minutes from the start of the WL protocol. Therefore, in chapters 4 and 6, control WL stimulations were performed every 2-3 sets of WLs, to monitor the changes in tissue viability over time. A control set of WLs' was performed using the parameters that elicited the greatest net work at CF 5Hz, 7Hz and 10Hz for the soleus, diaphragm and EDL respectively every 2-3 CF's once maximal net work had been achieved for each CF. This also allowed for later corrections to be made in order to show the true maximal net work at each CF that may otherwise be masked by the decline in muscle quality. This has been performed in previous work as a means of measuring changes in muscle quality over time (James *et al.*, 1996; Tallis *et al.*, 2014). The correction factor is described in greater detail in section 3.6.

		Cycle Frequency (Hz)										
Muscle	*Parameter*	2	3	4	5	6	7	8	10	12	14	16
Soleus	Burst Duration (ms)	245	150	92	65	52	35	24	11	-	-	-
	Strain	0.13	0.12	0.11	0.10	0.09	0.08	0.07	0.06	-	-	-
EDL	Burst Duration (ms)	-	-	110	-	75	-	65	50	32	24	16
	Strain	-	-	0.13	-	0.12	-	0.11	0.10	0.09	0.08	0.07
Diaphragm	Burst Duration (ms)	-	180	140	92	78	55	48	24	15	-	-
	Strain	-	0.14	0.13	0.12	0.11	0.10	0.09	0.08	0.07	-	-

Table 3.1 - The burst durations and strains typically used for each muscle at each cycle frequency which elicited maximal net work during the WL protocol. Where a CF is not tested for a muscle a dash (-) represents this. These values are only typical values used for a young (aged 10 weeks) age group. Based on WL shapes, these values were subject to change at other age groups. A greater description of the parameters employed for the older age groups is provided in chapter 4.

3.5.4.5 - Fatigue Protocol

Following the final control set of WLs', a 10-minute rest was implemented to allow for the muscle to recover. Following the 10-minute rest, muscles underwent 50 consecutive WL's to assess the fatigue resistance of muscles using the stimulation and length change parameters which were utilised as controls. As the muscles fatigue, the net work from the WL declines over time due to a reduced ability to generate force during muscle shortening and increased eccentric work during muscle re-lengthening (Vassilakos *et al.*, 2009). The net work for every second WL was recorded until the 50th WL. Tallis *et al.* (2013, 2014) have utilised this protocol to investigate the fatigability of isolated soleus, EDL, and diaphragm muscles, and the ability of the muscle to recover from a continuous period of sustained work.

3.5.4.6 - Recovery Protocol

For the recovery element of the protocol, each muscle underwent 30 minutes of recovery immediately after the fatigue run, with four WL's performed after 10, 20 and 30 minutes of recovery respectively. These time points were used to map the rate of recovery for each muscle (Tallis *et al.*, 2014). Following the final WL, the muscle was removed from the bath for weighing and freezing. Frozen tissue samples were stored for any future biochemical analyses.

3.5 - Tissue Mass Measurements and Dimension Calculations

Following experimentation, the muscle was taken from the bath, and the tendons were then removed ensuring the muscle was left intact. The muscle was blotted on absorbent paper, to remove any excess Krebs-Henseleit solution, and placed on an electronic balance (Mettler-Toledo B204-S, Zurich, Switzerland) to determine the wet muscle mass of the muscle to the nearest 0.00001g. The muscle was then placed in a labelled Eppendorf tube and frozen in liquid nitrogen. Mean muscle cross-sectional area was calculated from the mean fibre length (muscle sample length for diaphragm) at optimal isometric force generation, muscle mass and assumed muscle density of 1060kg.m^{-3} (Méndez

and Keys, 1960). Using muscle density, muscle CSA (m^2) was calculated as muscle mass divided by the product of muscle density and mean muscle fibre length. Absolute force (mN) was calculated as the product of force (V) and the force calibration value, calculated in section 3.4.1 (mN/V). Isometric stress ($kN.m^2$) was calculated as absolute force divided by mean muscle cross-sectional area. As the third WL tended to provide the greatest net work at each CF, this value was used to calculate PO. Therefore, the net work from the third WL was used to represent the true capacity of the muscle to produce power. Absolute power output (mW) was calculated as the product of maximal net work calculated by the Testpoint system and CF, with power output normalised to muscle mass ($W.kg^{-1}$) calculated as absolute power divided by the muscle mass.

The total duration of the experiment, from the point of cervical dislocation to the final set of WLs' of the recovery period, was ~190 minutes in length for the studies in chapters 4 & 6, and ~105 minutes in length for the study of chapter 5.

3.6 - Correction Factor

Control WL contractions were performed at regular intervals to monitor the rate of decline of work performed over time (chapter 4 & 6). The quality of a dissected muscle will decline mainly due to the build-up of an anoxic core in the muscle (Barclay, 2005). It is therefore important to correct the work completed at each CF to gain a true representation of the maximal power output of the muscle at each CF. To calculate the rate of decline and constituent corrected work, the control contraction which elicited the greatest work represented 100%. From this, the changes in muscle performance were calculated as a percentage between each control, assuming a linear change in performance between each control. The work performed at each CF was then divided by the calculated percentage and multiplied by 100 to determine the actual work performed. For example, if the muscle quality declined from 100% by 4% over 4 WL runs muscle quality between each control was assumed to have declined by 1% per contraction. By the fourth contraction, the work performed is 96% of the true value. So, the work done at that CF is divided by 96 and then multiplied by 100 giving the true work done at that CF. A comparison of an uncorrected and corrected power output x CF curve is provided in figure 3.6.

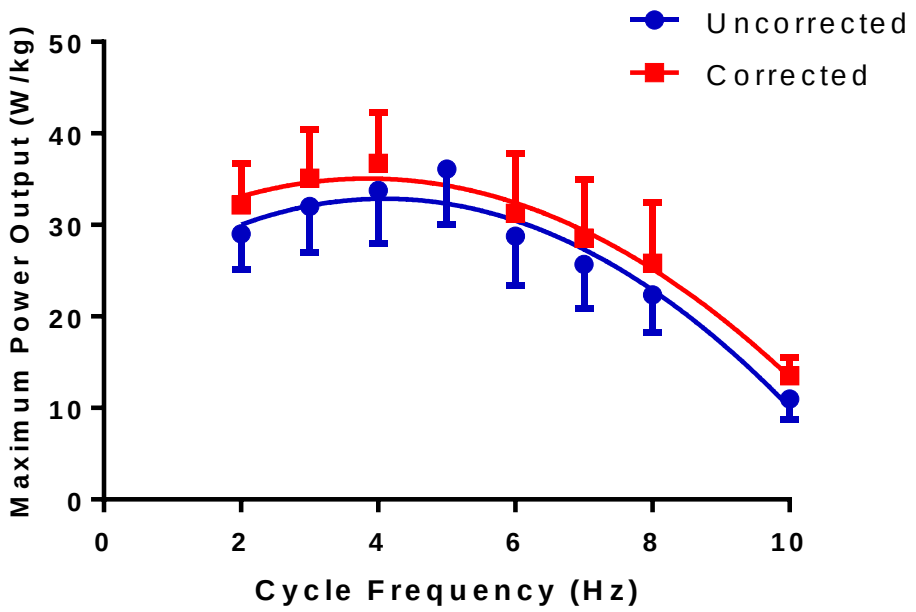


Figure 3.6 - A power output-cycle frequency curve for soleus muscle (n=8). This highlights the effect of correcting the net work at each cycle frequency over time to present the true power output of the muscle. Blue circles represent the uncorrected power output whilst the red squares show the true power output at each CF following correction. No correction was performed at 5Hz as this was the control CF and is, therefore, only blue in colour.

3.7 - Statistical Analyses

Due to the different methodological approached used for each experimental chapter, the details of the statistical analyses used are provided in detail for each experimental chapter.

Chapter 4 - The Sex-Based Differences in the Age-Related Changes in Isolated Locomotory (Soleus & EDL) and Respiratory (Diaphragm) Skeletal Muscle Contractile Function of CD-1 Mice.

4.1 - Abstract

In isolated skeletal muscles, little is currently known about the sex-based, muscle-specific occurrence of sarcopenia and dynapenia, and the consequential effects on contractile performance. The present study uses the work loop technique to examine the ageing related changes in skeletal muscle contractile performance of isolated soleus, EDL and diaphragm for males and females at various time points of the animal lifespan. Measurements of animal morphology, absolute and normalised force, absolute and normalised power across a range of contractile velocities, fatigue resistance and recovery were compared in male and female mice aged 3, 10, 30 (male only), 52, and 78 weeks old. Ageing results in increased body mass, soleus & EDL muscle mass and absolute force and power of locomotor muscles up to 52 weeks of age, where males were heavier and more powerful than females. The loss of muscle quality exceeded the loss of muscle mass, where isometric stress and normalised power peaked at 10 weeks and progressively declined with age to similar levels for males and females. By 78 weeks of age, however, muscle quality was significantly worse for males than females for all skeletal muscles. Ageing did not cause a shift towards a slower contractile velocity to maintain power output. Poorer fatigue resistance was only observed in female diaphragm, whilst recovery of power for soleus and EDL was unaffected by age whereas EDL recovery was more variable. Acute and sustained *in vivo* locomotor performance is likely to be poorer in males than females due to muscles of poorer quality working against a greater bodily inertia than in females.

4.2 - Introduction

Studies of musculoskeletal form and function in humans demonstrate an age-related reduction in strength (Young *et al.*, 1984, 1985; Overend *et al.*, 1992; Goodpaster *et al.*, 2001) and power (Skelton *et al.*, 1994; Lauretani *et al.*, 2003; Pojednic *et al.*, 2012; Edwén *et al.*, 2014), with the loss of power exceeding that of strength (Metter *et al.*, 1997; Krivickas *et al.*, 2001; Raj *et al.*, 2010) and the loss of absolute force and power, and force and power relative to body mass and muscle volume, occurring to a greater magnitude for males than females (Skelton *et al.*, 1994; Edwén *et al.*, 2014). The age-related reduction in contractile performance is associated with poorer locomotory capabilities, independence, greater all-cause mortality and a lower quality of life (Bassey *et al.*, 1992; Horner *et al.*, 2011; Volaklis *et al.*, 2015; Tsekoura *et al.*, 2017; Celis-Morales *et al.*, 2018). Coupled with the ever-increasing financial burden on public health services in the treatment of age-related muscle disorders and their co-morbidities (Sousa *et al.*, 2016), there are a number of factors that provide motivation for furthering our understanding of the age-related changes in skeletal muscle morphology and contractile function to improve quality of life for the older population.

Studies of skeletal muscle ageing are extensive in the assessment of *in vivo* human skeletal muscle contractile function in males and females (Table S1.1), with studies of isolated skeletal muscle contractile function using young and old rodents largely reciprocating findings of *in vivo* research (Table S1.2). Whilst human studies of skeletal muscle ageing have been valuable in furthering our understanding of age-related changes in skeletal muscle contractile function, there are several limitations to utilising such an approach. Firstly, there is a dearth of literature that has examined changes in muscle quality, which describes contractile performance relative to tissue mass, in comparison to studies of absolute changes in strength and power (McGregor *et al.*, 2014). Measuring muscle quality is important as ageing is associated with an increase in intramuscular adipose tissue, consequently increasing muscle mass and overall body mass (Marcus *et al.*, 2010; Addison *et al.*,

2014). These larger muscles produce the same or lower absolute force for an increased mass, thus increasing the effort to overcome a larger bodily inertia, potentially contributing to increased fatigue, and elevating the overall metabolic cost of maintaining a larger skeletal muscle mass (Fragala *et al.*, 2015). Measuring muscle quality of specific skeletal muscles is also difficult due to the difficulties of accurately measuring muscle quality *in vivo* (Fragala *et al.*, 2015; Tallis *et al.*, 2018), and as such the muscle-specific nature of skeletal muscle ageing is poorly understood in humans. Of the studies to have measured the age-related changes in skeletal muscle quality, such work expresses force relative to muscle CSA (Cruz-Jentoft *et al.*, 2010; McGregor *et al.*, 2014) or body mass (Edwén *et al.*, 2014), with the loss of function exceeding that of muscle size (Clark and Manini, 2008). Measuring whole muscle mass, however, is a more accurate method of assessing muscle quality (Tallis *et al.*, 2018). Another limitation is that denervation of muscle fibres occurs with increasing age also, contributing to a reduction in contractile performance, and consequently may mask the ageing effect at the skeletal muscle level (Carlson, 2004).

By utilising an isolated muscle approach, the confounding effects of examining ageing in humans can be overcome. However, previous studies of isolated skeletal muscle ageing are also flawed in their methodological approach. Most notably, there is a significant dearth in the literature examining age-related changes in power output (Tallis *et al.*, 2014). Determining power output from isometric and isovelocity contractions is a poor indicator of *in vivo* muscle function, where dynamic activation of muscles during power production is required for locomotion and respiration rather than static strength (Dickinson *et al.*, 2000). Usage of the WL technique provides a more accurate replication of *in vivo* muscle function (Josephson, 1985; James *et al.*, 1996) though only one study to date has examined the age-related changes in isolated skeletal muscle contractile function using the WL technique (Tallis *et al.*, 2014). There is also a distinct lack of studies examining sex-based differences in isolated contractile function, with only one study to date which ahs compared the age-related changes in isometric force of isolated male and female EDL muscles (Chan and Head, 2010). Finally,

no study to date has measured age-related changes in power output across a range of cycle frequencies (CF's).

Ageing is associated with a downward and leftward shift in the force-velocity relationship (Raj *et al.*, 2010) but does not cause a leftward shift in the force-power curves with increasing age (Graber *et al.*, 2015). Tallis *et al*. (2014) used a fixed CF in the examination of WL power output for isolated EDL and diaphragm, despite it being possible for a shift towards a slower contractile velocity with increasing age in order to maintain optimal power output. Construction of a power output-cycle frequency (PO-CF) curve (James *et al.*, 2011) can aid in determining whether there is a downward and leftward shift in the PO-CF curve with increasing age and whether the optimal CF required to elicit maximal power is altered by age. As the speed of contraction is fixed, maximal shortening velocities of skeletal muscles cannot affect the power produced and therefore a leftward shift in the PO-CF will be due to fibre-type shifting towards a more oxidative state (James *et al.*, 2011).

The current study furthers the work conducted by Chan & Head (2010) and Tallis *et al.* (2014), in which it is the first to simultaneously consider the age-related changes in absolute and normalised force and power to determine changes in muscle quality, the muscle-specific responses to ageing, the interaction between power output and contraction velocity, and whether sex has a significant influence on mechanical performance of isolated muscles at multiple ages. An examination of the predominantly slow-twitch soleus morphology and contractile performance was performed, in addition to the predominantly fast-twitch EDL and the diaphragm (respiratory muscle of a mixed phenotype), to compare the age-related changes of this locomotory skeletal muscle with another locomotor muscle as is common with *in vitro* ageing research (Table S1.2). The *in vivo* anatomical location, function and fibre composition means it is likely that these skeletal muscles will age at different speeds compared to one another.

It is expected that with increasing age beyond peak physiological maturity (i.e. 10 weeks), the loss of muscle quality shall exceed that of absolute force and power as with human studies of ageing due to a maintenance of muscle mass but a poorer contractile tissue quality. Ageing is likely to result in a downward shift in the PO-CF curves normalised to muscle mass in line with an age-related decline in muscle quality. The effects of ageing will be muscle-specific, with the soleus and diaphragm likely to be affected by ageing to a lesser extent than the EDL due to their *in vivo* mechanical roles and fibre type profile.

4.3 - Methods

A more detailed account of the methodological approach is provided in chapter 3.

4.3.1 - Animal Information

White male and female CD-1 mice were purchased at aged 3-9 weeks old and allowed to mature in-house at Coventry University. At 9-10 weeks, male mice were later housed in groups of 3-4 to minimise the fighting that was observed due to the inability to maintain a colony hierarchy in each cage. Female mice housing remained unchanged throughout. The only exception was for mice aged 3 weeks, which were used within one week upon arrival at Coventry University. All other groups were allowed to mature to the following ages prior to experimentation: 10 weeks, 30 weeks (males only), 52 weeks and 78 weeks (1.5 years). A justification for use of these age groups is provided in section 3.1.

10 animals in total were excluded due to illness. Upon sacrifice, certain ailments were identified, including cancerous growths, typically around the gonadal area near the hind limbs (n=6), extremely swollen and inflamed livers (n=3) and severe malnourishment (n=1).

4.3.2 - Muscle Isolation and Preparation

Once the animals had reached their target age, mice were selected and were sacrificed by cervical dislocation and weighed to determine body mass (BM). At room temperature (~22°C), the segment of animal that contained the target muscle was skinned, rapidly isolated and placed in chilled (~5°C), oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution.

A single muscle from each single mouse was utilised for muscle mechanics. The target muscles from the right hindlimb were prepared for mechanics measurements. Aluminium foil T-clips were wrapped around the distal tendon of each locomotory muscle to avoid tendon slippage during muscular contractions (Tallis *et al.*, 2014). A small piece of bone was left at the proximal end of the muscle to allow for the muscle to be anchored in the experimental rig. Whole diaphragm muscle was removed

from the animal, with only the ventral segment of the costal diaphragm used to assess mechanical performance. Aluminium foil T-clips were wrapped around the central tendon with two ribs at the opposing end of the diaphragm segment left intact to anchor the muscle in the rig. Once dissected, muscles were placed in a flow-through chamber filled with oxygenated Krebs-Henseleit solution heated to and maintained at 37±0.2°C.

4.3.3 - Isometric Contractions

All muscles underwent a series of twitch activations in order to optimise muscle length (L_0) and stimulation amplitude, as per sections 3.5.1 and 3.5.2. The physical length and stimulation amplitude (14-20V for EDL, 12-17V for soleus and 12-18V for diaphragm; stimulation current 160mA, 1.2ms pulse width) were altered until maximal isometric twitch force was achieved. The physical length at which peak twitch force was achieved was defined as L_0. The stimulation amplitude required to achieve peak twitch force for all muscles was unaffected by age ($P>0.67$) and sex ($P>0.92$). The maximal tetanic force was measured by subjecting the EDL and diaphragm to a 250ms burst of electrical stimulation and the soleus to a 350ms burst of electrical stimulation. The frequency at which the stimulations were provided was altered until peak tetanic force was achieved. This was typically 120-140Hz for soleus, 120-150Hz for diaphragm and 200-220Hz for EDL. The duration of muscle activation and relaxation was measured as time to half peak tetanus (THPT) and last stimulus to half tetanus relaxation (LSHR) respectively. 5-minutes of rest were imposed between each tetanic stimulation in order to allow for sufficient recovery.

4.3.4 - Work Loop Protocol

Each muscle was held at the previously determined L_0 and the stimulation amplitude and frequency that resulted in maximal tetanic force were implemented. Using the WL, sinusoidal length changes were implemented to examine power output across a range of contractile velocities (cycle frequencies), outlined in detail in section 3.5.4. Initially, a cycle frequency of 10Hz for EDL, 7Hz for diaphragm and 5Hz for soleus was used as these cycle frequencies typically elicited maximal power

output in previous research for locomotory (James *et al*., 1995, Tallis *et al*., 2014) and respiratory (Altringham & Young, 1991; Tallis *et al*., 2014) skeletal muscles. A strain of 0.10 (±5% length change from L_0) was typically used for all muscles at the aforementioned cycle frequencies, with phasic bursts of electrical stimulation provided per sinewave for durations of 50ms, 55ms and 65ms to the EDL, diaphragm and soleus respectively. Each work loop was performed every 5-minutes to allow for sufficient recovery. Net work was determined across a range of cycle frequencies in order to produce a power output-cycle frequency curve (PO-CF) (James *et al.*, 1995, 1996, 2011; Askew and Marsh, 1998). Cycle frequencies utilised ranged from 4-16Hz for EDL, 3-12Hz for diaphragm and 2-10Hz for soleus and the order in which each CF was tested in was selected at random. Length change and stimulation variables were altered via the Testpoint software at each cycle frequency until peak net work was achieved. Generally, as cycle frequency increased, strain and burst duration decreased and vice versa, with strain and burst duration altered to elicit maximal power output at a given CF. Whilst burst duration to elicit maximal power did not change with age, 52 week and 78-week-old muscles tended to require a smaller strain at each CF. For example, a ±5% length change from L_0 (strain 0.10) at 5Hz was optimal for 10-week-old soleus, but occasionally a ±4% (strain 0.08) length change from L_0 was optimal for 52 week and 78-week-old soleus. Control sets of WLs using the parameters that elicited maximal net work for the first cycle frequencies (10Hz, 7Hz and 5Hz) were performed after every three cycle frequencies and following the final cycle frequency to monitor each muscles ability to produce power over time. Following the final control stimulation, each muscle underwent 10-minutes of rest prior to the fatigue run.

4.3.5 - Fatigue Resistance and Recovery

In order to determine the fatigue resistance of each muscle, 50 consecutive WL cycle were provided to each muscle using parameters implemented during the control stimulations (I.e. 5Hz, 7Hz and 10Hz for soleus, diaphragm and EDL respectively). The net work of every second WL was recorded until a

significant decline and plateaux in net work had occurred or until net negative work was produced (Tallis *et al*., 2014).

The ability of each muscle to recover from the fatigue run was monitored for 30-minutes immediately following the fatigue run. Every 10-minutes, one set of WL cycles were provided to each muscle and net work was recorded. This was directly compared to the pre-fatigue maximal power output.

On average, the experimental protocol for each muscle was ~190 minutes from the moment of cervical dislocation to the final WL performed 30-minutes after the fatigue run. Tissue viability declined by an average of 14% for all muscles prior to the fatigue run and is in keeping with previous research of isolated skeletal muscles of similar length (Tallis *et al.*, 2012, 2013, 2014). Section 3.6 describes the process for correcting the change in quality, that is mainly attributed to the build-up of an anoxic core (Barclay, 2005).

At the end of the experiment, the muscle was detached from the rig, tendons and excessive fluid removed, then weighed in order to calculate isometric stress ($kN.m^2$) and normalised muscle power ($W.kg^{-1}$). Section 3.5 describes the calculation of force, stress and WL power output.

4.3.6 - Statistical Analyses

All data are presented as mean ± S.E.M calculated in Excel (Excel 2016, Microsoft, Washington, USA). The level of significance was set at $P<0.05$ for all analyses. Tests for homogeneity of variance were conducted on all data to determine parametric or non-parametric analysis. All data were analysed in SPSS (SPPS, v22.0, Chicago, IL, USA) and was analysed using a two-factor analysis of variance (ANOVA) with sex and age set as the fixed factors. Independent variables included animal and muscle morphology (body mass, soleus and EDL muscle mass, muscle length & muscle CSA) and isometric properties for each muscle (absolute twitch and tetanus force, twitch and tetanic stress, THTP, LSHR).

A three-factor ANOVA was used to determine significant changes in soleus & EDL absolute power output, and normalised WL power output for all muscles. Absolute and normalised power were set as the dependent variables, whilst, sex, age and cycle frequency set as the fixed factors. For all two-factor and three-factor ANOVA's, differences between sex and age groups were examined by single-factor ANOVA's when an interaction was observed to examine main effects. To calculate the magnitude of differences in absolute and normalised power output for age and sex, the percentage decline in power output for each cycle frequency was combined and an average calculated to provide mean percentage decline for each muscle at each age group.

Muscle fatigue and recovery of power were examined using a repeated measures three-factor ANOVA to determine if sex, age and time affected the maintenance in muscle power during repeated WLs over time and whether the muscles were able to recover WL power 30 minutes following the fatigue protocol. Should a main effect for age or sex be observed for the analyses of fatigue, a single factor ANOVA was run for each muscle of each sex to determine whether age affected time to reach 50% of pre-fatigue maximum and whether this was affected by sex.

For all ANOVA's, Tukey's *post hoc* analyses were used should significant differences be present.

The truncated product method (Zaykin *et al*., 2002) was used to analyse the distribution of *P*-values to provide a *P*-value for each group of multiple hypothesis tests to assess whether these values were biased via multiple hypothesis testing. The truncated product method *P*-value was <0.001, demonstrating that the results were not biased based on multiple hypothesis testing.

4.3.7 - Interpretation of Figures

To visually demonstrate age-related and sex-based differences in animal morphology and contractile parameters, a common symbols approach, using the English alphabet has been adopted; an approach

based on the figures within Tallis *et al.* (2014). Males are represented by the blue letters whilst females are represented by green figures. Should a letter of a single colour match another letter of the same colour within a figure, this indicates a significant ($P<0.05$) age-related difference. For example, in figure 4.1A, there is a blue letter "a" at 3 weeks and 10 weeks of age, indicating a significant difference in body mass for males at these ages. Should there not be a common symbol between ages, then no significant ($P>0.05$) difference were observed. For example, in figure 4.1A, there are no matching letters for both males (blue) or females (green) at 52 weeks and 78 weeks of age, indicating no further increase in body mass is observed with age in this case.

Black ovals and the "%" symbols are used to represent sex-based differences in a particular parameter at a given age. For example, in figure 4.1A, there are 2 black ovals around the data points at 3 weeks and 10 weeks of age, indicating males were significantly heavier than females at this age, but no differences were found at any other ages. Likewise, for figure 4.5B, the % symbol next to 10 weeks in the key indicates female EDL generated significantly greater absolute power at 10 weeks when compared with males.

4.4 - Results

4.4.1 - Animal Morphology

Ageing resulted in a significant increase in mean animal body mass (Figure 4.1A; P<0.001), where males were significantly heavier than females (P<0.001), and a sex*age interaction observed (P=0.001). Peak body mass occurred at 52 weeks of age for males and females. Males were 35% and 21% heavier than females at 3 and 10 weeks of age respectively (P<0.001), but not at 52 or 78 weeks of age (P>0.30). No differences in male body mass were observed between 30, 52 and 78 weeks of age (P>0.81), though female body mass tended to be lower by 8% at 78 weeks of age compared with 52-week-old animals (P=0.06).

Figure 4.1 demonstrates age-related changes in soleus (B) and EDL (C) muscle mass. For soleus, muscle mass significantly increased with age (Figure 4.1B; P<0.001), peaking at 52 weeks of age. No differences in muscle mass were observed between 30 weeks and 52 weeks for males (P=0.91) nor between 52 weeks and 78 weeks for either sex (P>0.28). Male soleus muscle mass was significantly heavier in males than females (P=0.002). Male soleus muscle mass was 55% heavier than females at 3 weeks of age (P=0.001), but no differences were observed at all other ages (P>0.12). EDL muscle mass was also significantly altered by age (P<0.001), where ageing caused an increase in EDL muscle mass, peaking at 52 weeks of age. Whilst no further decline was observed in males from 52 weeks to 78 weeks (P=0.18), 78-week-old female EDL muscle mass was 15% lower than at 52 weeks (P=0.004). Male EDL muscle mass was significantly heavier than females (P<0.001), where male EDL muscle mass at 3 weeks, 52 weeks, and 78 weeks was 41%, 25% and 31% heavier than females respectively (P<0.002). No difference in EDL muscle mass was found between males and females at 10 weeks of age (P>0.10).

Muscle length was significantly affected by age for both soleus and EDL ($P<0.001$ in both cases), though there was no effect for sex for either muscle ($P>0.09$ in both cases). A sex*age interaction was found for the EDL ($P=0.003$) but not the soleus. Muscle length was smallest at 3 weeks of age for both soleus and EDL for both sexes ($P<0.001$). No differences in muscle length were found between all remaining ages for both muscles and sexes ($P>0.07$).

No such comparisons of diaphragm muscle mass or muscle length can be made due to different sections of the costal diaphragm from each animal used for each preparation.

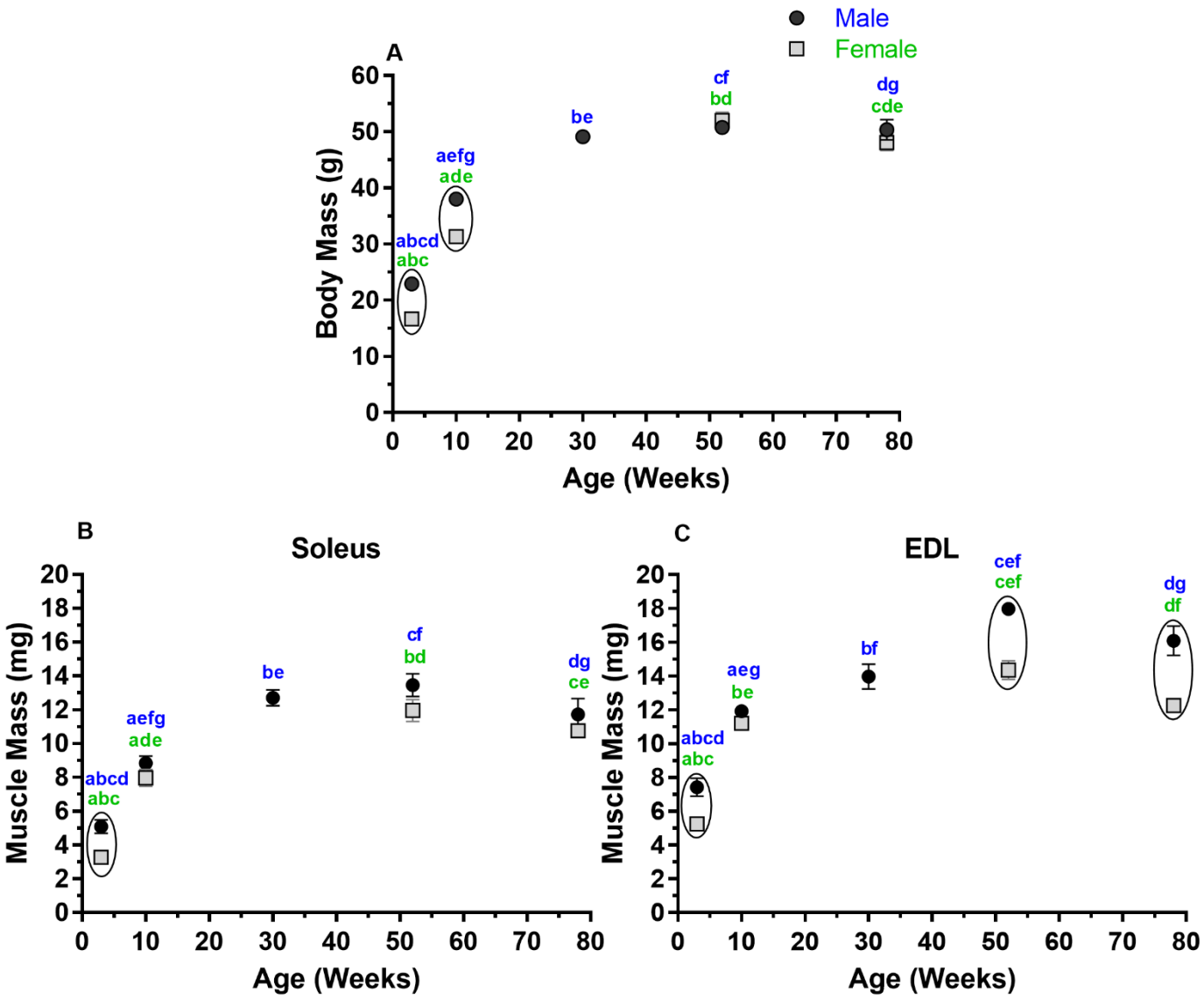


Figure 4.1 - Age-related changes in animal body mass (A), and soleus (B) and EDL (C) muscle mass, for males and female CD-1 mice aged 3, 10, 30 (male only), 52 and 78 weeks old. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by common symbols; blue symbols for males and green symbols for females. Black ovals represent significant (P<0.05) sex-based differences in body mass and muscle mass at each age group.

4.4.2 - Twitch and Tetanus Force and Stress

Ageing resulted in significant reductions in twitch force and twitch stress for all muscles (Figure 4.2 A-E; P<0.001), with soleus and EDL twitch force significantly greater in males than females (Figure 4.2 A&B; P<0.008), though no differences in twitch stress was observed between males and females for all skeletal muscles (Figure 4.2 C-E; P>0.48). Maximal twitch force peaked at either 30 weeks or 52 weeks of age for males and females but did not decline further with age (P<0.08). Maximal twitch stress peaked at 10 weeks of age for all muscles of both sexes, declining significantly with age for male soleus and EDL (P<0.001), and for female diaphragm (P<0.01). There was no sex*age interaction for EDL twitch force or twitch stress (Figure 4.2D; P>0.16) nor diaphragm twitch stress (Figure 4.2E; P=0.49). However, there was a significant sex*age interaction for soleus twitch force (Figure 4.2A; P=0.05) and twitch stress (Figure 4.2C; P=0.002). Males produced greater twitch force at 3 weeks of age for EDL (Figure 4.2B; 71%; P=0.004) and 10 weeks for soleus (Figure 4.2A; 38%; P=0.004).

Tetanus force was significantly affected by age for soleus and EDL (Figure 4.3 A&C; P<0.001), though there were no sex-based differences in soleus and EDL tetanus force (Figure 4.3 A&C; P>0.20). There was no sex*age interaction for the EDL or soleus tetanus force (Figure 4.3 A&C P>0.06). Soleus tetanus force peaked at 30 weeks for males and declined significantly by 29% by 78 weeks of age (P=0.001) whereas female soleus tetanus force peaked at 78 weeks of age. Peak tetanus force for the EDL occurred at 10 weeks for females and 52 weeks for males and declined significantly by 78 weeks of age for both sexes (P<0.001).

Tetanus stress was significantly affected by age for all skeletal muscles (Figure 4.3 B, D&E; P<0.001). Sex-based differences were observed for the EDL (Figure 4.3D; P=0.02), but not for the soleus or diaphragm (Figure 4.3 B&E; P>0.10). A sex*age interaction was observed for the soleus (Figure 4.3; P=0.02) but not for the EDL or diaphragm (Figure 4.3; P>0.52). Tetanus stress peaked at 10 weeks of age for all muscles, declining significantly by 78 weeks of age (P<0.0.001). There was no significant

reduction in tetanus stress from 52 weeks to 78 weeks for all skeletal muscles of both sexes ($P>0.23$ in all cases).

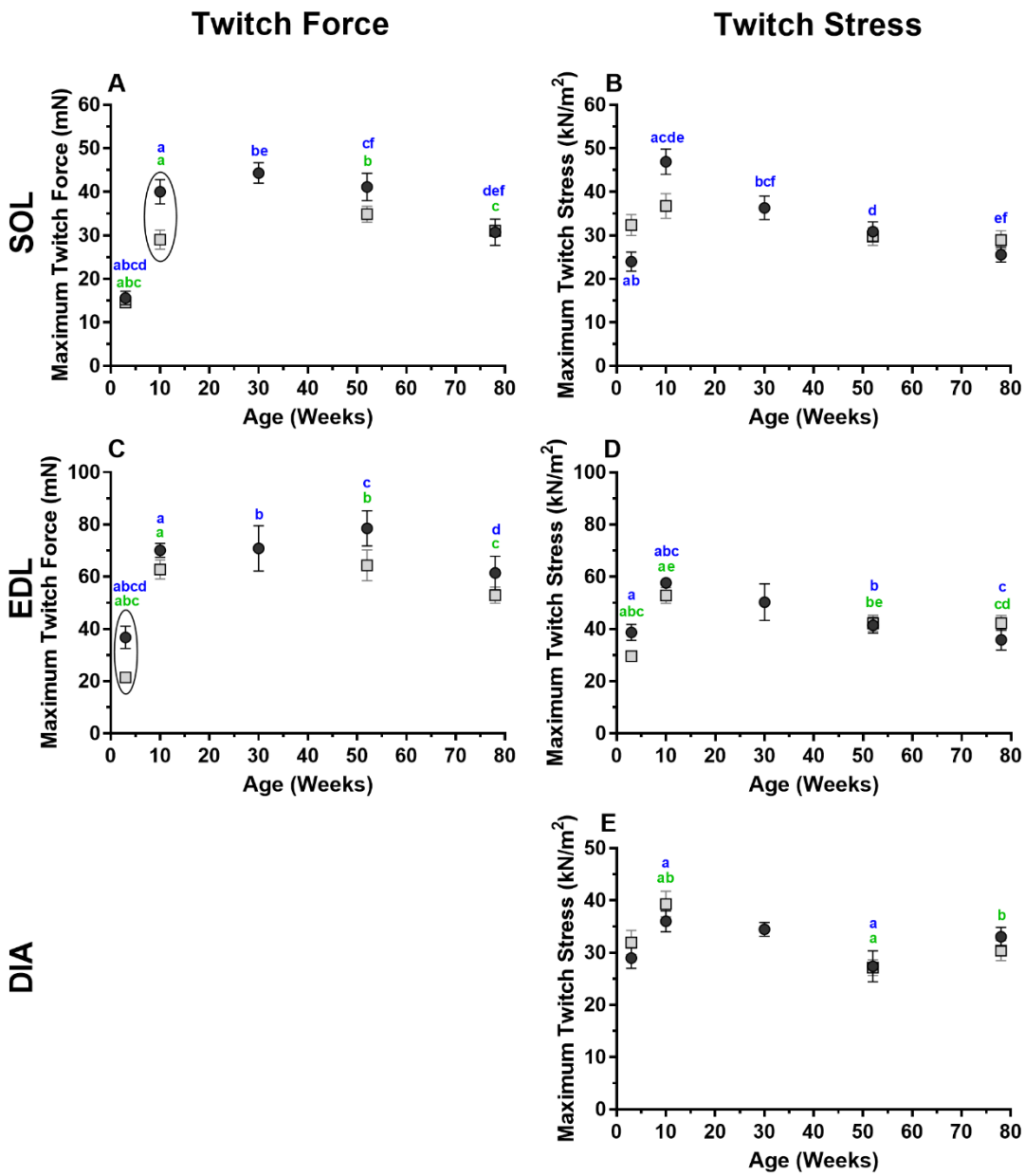


Figure 4.2 - Age-related changes in twitch force and stress for soleus (A & B), EDL, (C & D) and diaphragm (E) for males and female CD-1 mice aged 3, 10, 30 (male only), 52 and 78 weeks old. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by a common symbol; blue symbols for males and green symbols for females. Black ovals represent significant (P<0.05) sex-based differences in body mass and muscle mass at each age group.

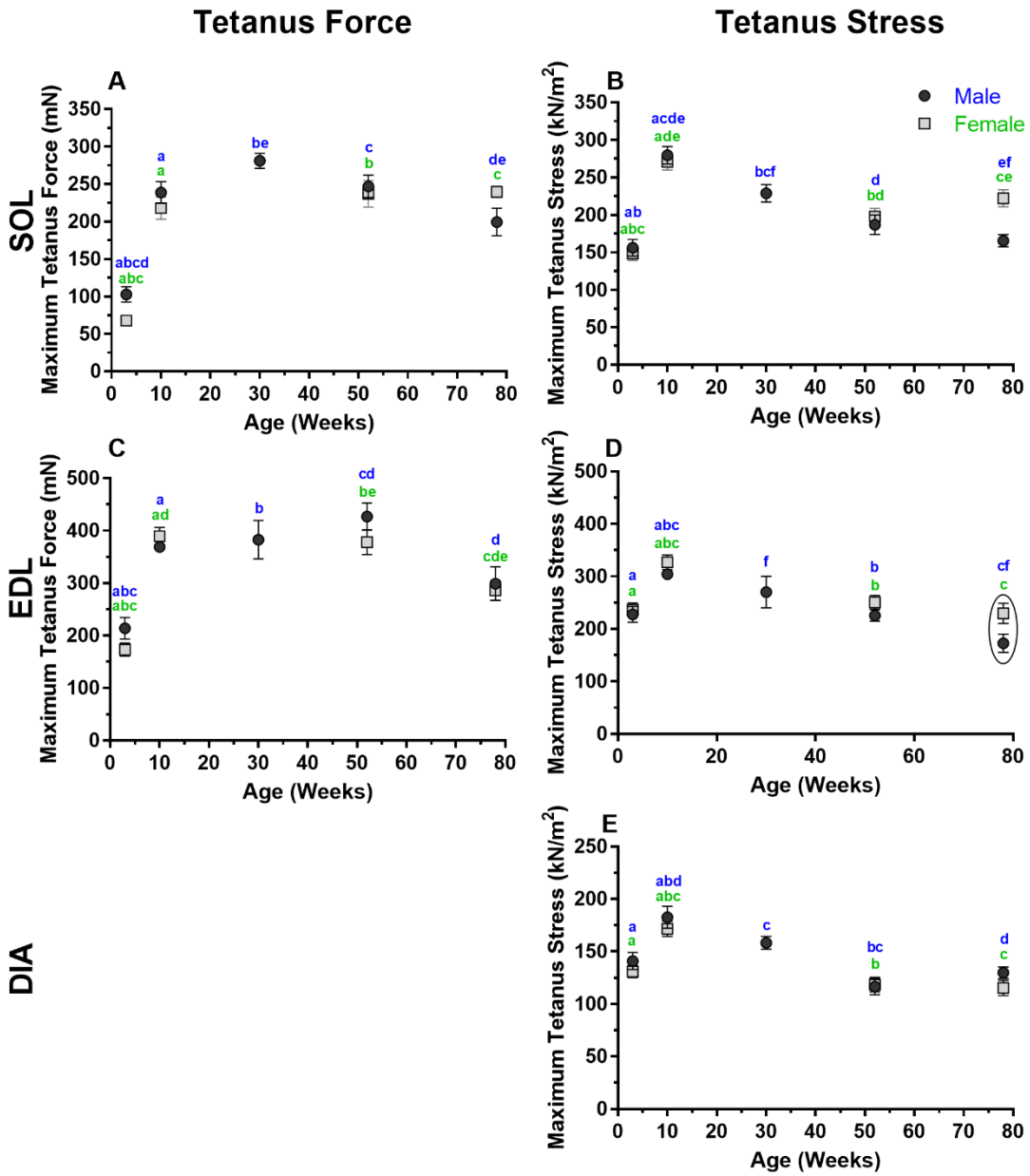


Figure 4.3 - Age-related changes in tetanus force and stress for soleus (A & B), EDL, (C & D) and diaphragm (E) for males and female CD-1 mice aged 3, 10, 30 (male only), 52 and 78 weeks old. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by a common symbol; blue symbols for males and green symbols for females. Black ovals represent significant (P<0.05) sex-based differences in body mass and muscle mass at each age group.

4.4.3 - Activation and Relaxation

Time to half-peak tetanus (THPT) was significantly affected by age for soleus (Figure 4.4A; P=0.001), Ageing did not significantly affect EDL or diaphragm activation time (Figure 4.4 C&E; P>0.06). Activation was significantly faster in male soleus and EDL (Figure 4.4 A&C; P<0.004), but no differences were observed for diaphragm (Figure 4.4E; P=0.15). A sex*age interaction was observed for EDL and diaphragm (Figure 4.4 C&E; P<0.04), but not soleus (Figure 4.4A; P=0.32). Male soleus activation was significantly faster than female soleus at 10, 52 and 78 weeks (by 17%, 14% and 12% respectively; Table 4.1; P<0.05). Male EDL activation was faster at 3 and 10 weeks than females (by 16% and 32% respectively; Table 4.1; P<0.03). For male soleus, activation time was only slower between 10 weeks and 78 weeks (by 20%) (Table 4.1; P=0.04), with no differences found between all other ages (Table 4.1; P>0.09 in all cases). Female soleus was fastest at 3 weeks and was significantly faster than at 52 and 78 weeks (by 26% and 30% respectively; Table 4.1; P<0.04).

Last stimulus to half relaxation (LSHR) was significantly affected by age for EDL and diaphragm (Figure 4.4 D&F; P<0.02 in each case) but not for soleus (Figure 4.4B; P=0.12). Relaxation time for male soleus was faster than females (Figure 4.4B; P=0.008) but was not different for EDL and diaphragm (Figure 4.4 D&F; P>0.62). No sex*age interaction was observed for all skeletal muscles (Figure 4.4 B, D&F; P>0.18). Relaxation times for the male soleus tended to be faster in 3-week-old soleus than females (Figure 4.4B; P=0.07) but not at all other ages (Figure 4.4B; P>0.23). Relaxation time of 78-week male soleus was significantly slower than 10 weeks (by 32%; Table 4.1; P=0.01) and tended to be lower between 52 weeks and 78 weeks (Table 4.1; P=0.06), though no other differences were observed at all other ages for male EDL (Table 4.1; P>0.16). Female EDL relaxation was fastest at 10 weeks and was significantly lower by 78 weeks (by 42%; Table 4.1; P=0.003), but not compared to 3 weeks and 52 weeks (Table 4.1; P>0.42). No age-related differences in diaphragm relaxation were observed for males (Table 4.1; P>0.65). Female diaphragm relaxation was fastest at 3 weeks and was significantly

slower at 10 weeks (by 26%; Table 4.1; P<0.05), 52 weeks (by 27%; Table 4.1; P=0.04) and 78 weeks (by 53%; Table 4.1; P<0.001). Moreover, relaxation was significantly slower at 78 weeks than 10 weeks (by 21%; Table 4.1; P<0.05) and tended to be slower than at 52 weeks (by 20%; Table 4.1; P=0.06).

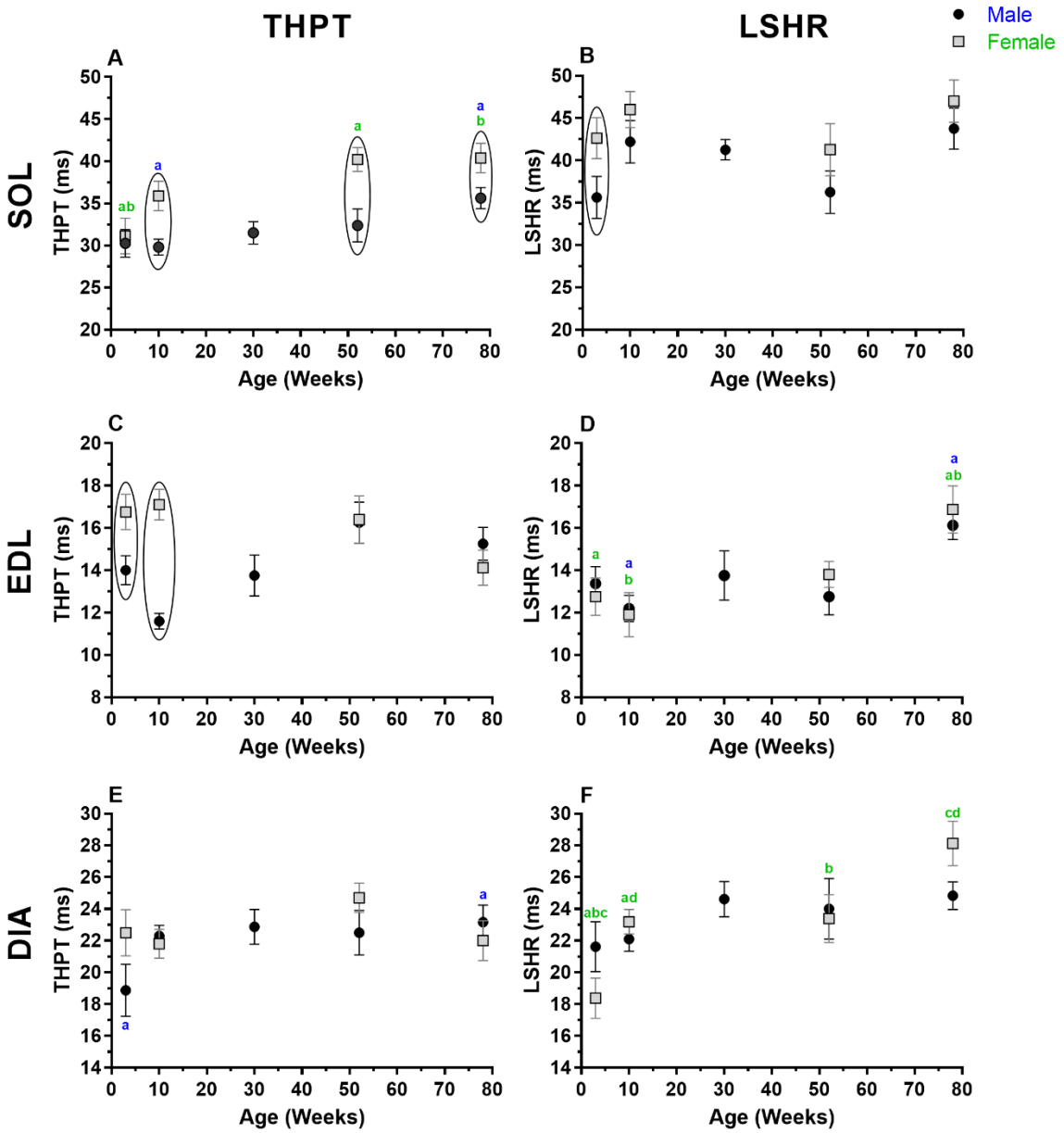


Figure 4.4 - Age-related changes in activation (THPT) and relaxation (LSHR) times for soleus (A & B), EDL, (C & D) and diaphragm (E & F) for males and female CD-1 mice aged 3, 10, 30 (male only), 52 and 78 weeks old. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by common symbols; blue symbols for males and green symbols for females. The '&' symbol indicates any statistical tendencies (P≤0.08). Black ovals represent significant (P<0.05) sex-based differences in body mass and muscle mass at each age group.

4.4.4 - Absolute Power Output and Power Output Normalised to Muscle Mass

Absolute power was significantly affected by age for soleus and EDL (Figure 4.5 A-D; P<0.001). Absolute power output increased with age and peaked at 30 weeks of age for male soleus whilst absolute power output peaked at 52 weeks for female soleus and the EDL of both sexes. By 78 weeks of age, power output declined significantly for both soleus and EDL of both sexes (P<0.001), with the greatest decline in absolute power occurring for male soleus (Table 4.1). Male soleus and EDL generated greater absolute power than females (Figure 4.5 A-D; P<0.001). For male soleus, power output was significantly greater than females at 3 weeks (by 93%), 10 weeks (by 24%) and 52 weeks (by 17%), (Table 4.1; P<0.001 in all cases) but not at 78 weeks (P=0.96). For male EDL, absolute power output was significantly greater than female EDL at 3 weeks (by 45%), 52 weeks (by 26%) and 78 weeks (by 22%), whilst mean absolute power for females was greater than males at 10 weeks (by 10%) (Table 4.1; P<0.001 in all cases). A significant sex*age interaction was observed for both muscles (Figure 4.5 A-D; P<0.001). CF significantly affected power output (Figure 4.5 A-D; P<0.001), though no sex*CF interaction was found for soleus and EDL (Figure 4.5 A-D; P>0.11) inferring sex did not influence CF required for power. An age*CF interaction was observed for soleus (Figure 4.5 A&B; P<0.001) but not for EDL (Figure 4.5 C&D; P>0.16), nor was a sex*age*CF interaction observed for both soleus and EDL (Figure 4.5 A-D; P>0.86). The age*CF effect is due to absolute power at 10Hz being similar as opposed to a change in the optimal CF for maximal power output.

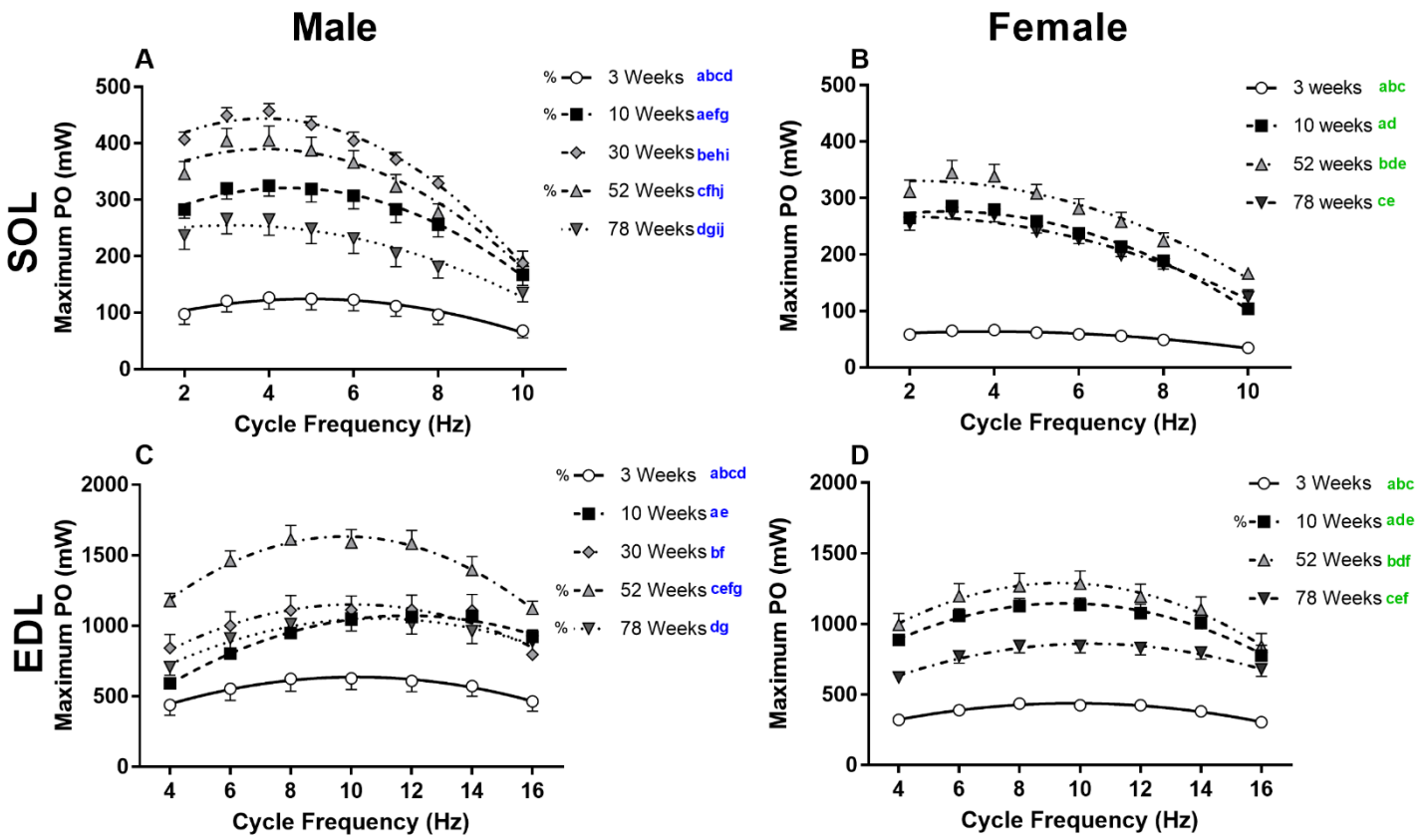


Figure 4.5 - Age-related and sex-based differences in maximal absolute WL power across a range of cycle frequencies for soleus (SOL) (A & B) and EDL (C & D) isolated from 3, 10, 30 (males only) 52 and 78-week-old male and female CD-1 mice. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by common symbols; blue symbols for males and green symbols for females. The '%' symbol next to an age group indicates significantly (P<0.05) greater WL power output compared to the opposite sex at that given age.

Power output normalised to muscle mass was affected by age for soleus, EDL and diaphragm (Figure 4.6 A-F; P<0.001). Normalised power output peaked at 10 weeks of age for all skeletal muscles, with the exception of male EDL which peaked at 52 weeks of age. From the age at which peak normalised power output occurred, further increases in age resulted in significant declines in normalised power output for all muscles of each sex (P<0.001). In addition, there were significant effects for sex on normalised power output for all skeletal muscles (Figure 4.6 A-F; P<0.02 in all cases). A sex*age interaction was observed for all skeletal muscles (Figure 4.6 A-F; P<0.001). For male soleus, normalised power was greater than females at 3 weeks (by 22%; Table 4.1; P<0.001), 10 weeks (by 11%; Table 4.1; P<0.001) and 52 weeks (by 7%; Table 4.1; P=0.02), whilst normalised soleus power for females was greater than male at 78 weeks (by 11%; Table 4.1; P=-0.001). Female EDL normalised power output was significantly greater at 10 weeks and 78 weeks than males (by 17% and 7% respectively; Table 4.1; P<0.04) but no differences were found at 3 weeks and 52 weeks (Table 4.1; P>0.59). Male diaphragm normalised power was significantly greater than females at 3 weeks only (by 13%; P=0.02), whilst female diaphragm normalised power was significantly greater at 10 weeks (by 10%; P=0.02) and 78 weeks (by 23%; P<0.001), but not at 52 weeks (P=0.06). CF significantly affected power output for all skeletal muscles (Figure 4.6 A-F; P<0.001 for all). A sex*CF interaction was not observed for all skeletal muscles (Figure 4.6 A-F; P>0.15). An age*CF interaction was found for soleus (Figure 4.6 A&B; P<0.001) but not EDL or diaphragm (Figure 4.6 C-F; P>0.46), nor was a sex*age*CF interaction observed for all muscles (Figure 4.6 A-F; P>0.43). The age*CF interaction was due to soleus normalised power at 10Hz being similar between all ages, as opposed to a leftward shift in the PO-CF curve from peak power.

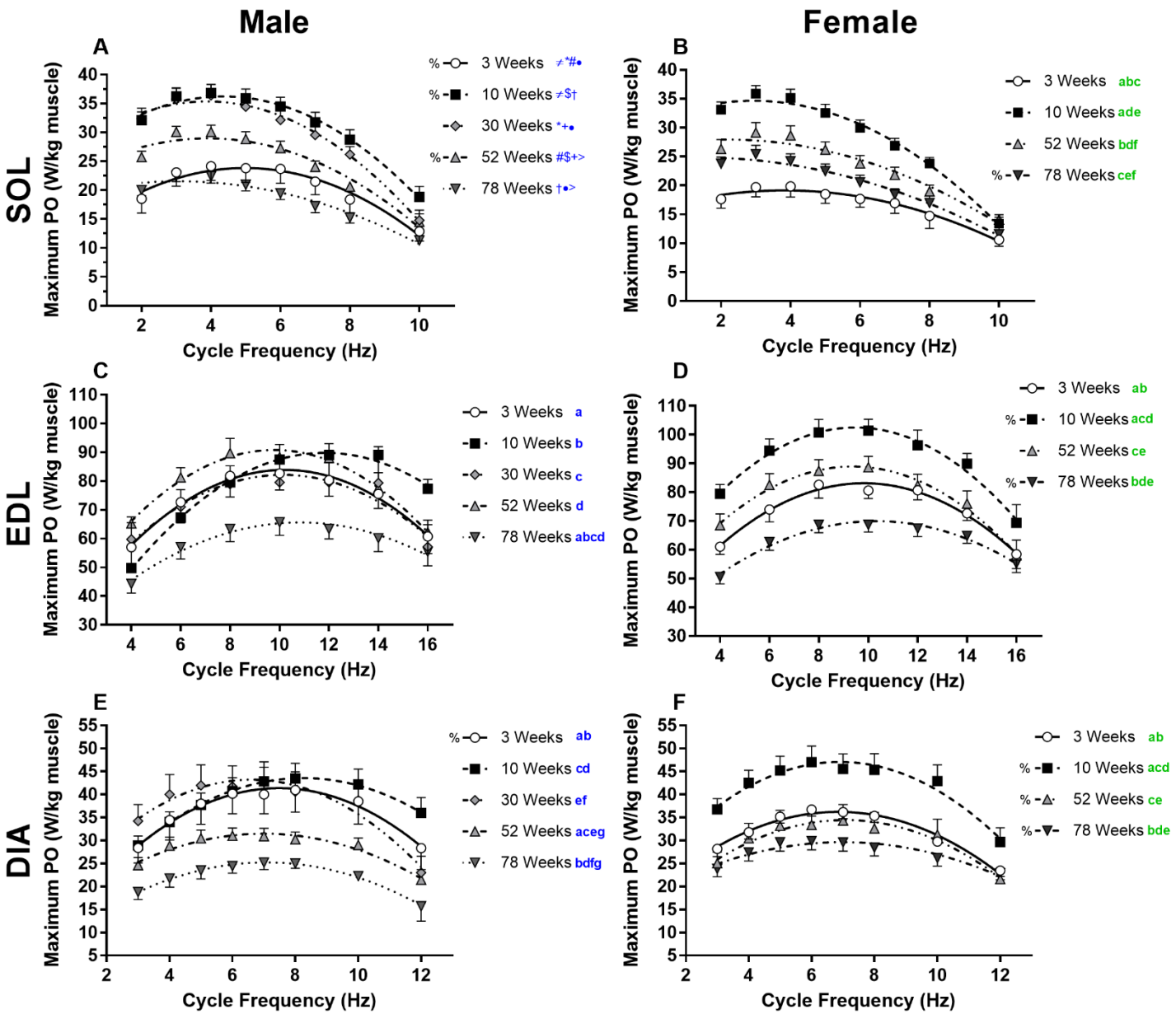


Figure 4.6 - Age-related and sex-based differences in maximal WL power normalised to muscle mass across a range of cycle frequencies for soleus (SOL) (A & B), EDL (C & D) and diaphragm (DIA) (E & F) isolated from 3, 10, 30 (males only) 52 and 78-week-old male and female CD-1 mice. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by common symbols; blue symbols for males and green symbols for females. The '%' symbol next to an age group indicates significantly (P<0.05) greater WL power output compared to the opposite sex at that given age.

	Age								
	Male					Female			
	3 weeks	10 weeks	30 weeks	52 weeks	78 weeks	3 weeks	10 weeks	52 weeks	78 weeks
					Combined				
Body Mass (g)	-56%*	-25%*	-3%	Max	-1%	-68%*	-40%*	Max	-8%†
					Soleus				
Muscle Mass (mg)	-62%*	-34%*	-6%	Max	-13%	-73%*	-33%*	Max	-10%
Muscle Length (mm)	-25%*	0%	Max	-2%	-4%	-27%	-2%	Max	-1%
Muscle CSA (m^2)	-50%*	-36%*	-7%	Max	-11%	-62%*	-33%*	Max	-9%
Twitch Force (mN)	-65%*	-10%	Max	-7%	-31%†	-58%*	-17%	Max	-11%
Twitch Stress ($kN.m^2$)	-49%*	Max	-23%*	-34%*	-46%*	-12%	Max	-19%	-21%
Tetanus Force (mN)	-63%*	-15%	Max	-12%	-29%*	-72%*	-9%	-1%	Max
Tetanus Stress ($kN.m^2$)	-44%*	Max	-18%*	-33%*	-41%*	-45%*	Max	-27%	-18%*
THPT (ms)	2%	Max	6%	13%	20%*	Max	15%	26%*	30%*
LSHR (ms)	Max	18%	16%	2%	23%	3%	11%	Max	14%
Absolute Power (mW)	-71%*	-26%*	Max	-10%*	-41%*	-80%*	-21%*	Max	-23%*
Norm. Power ($W.kg^{-1}$)	-34%*	Max	-7%	-20%*	-41%*	-39%*	Max	-15%*	-27%*
					EDL				
Muscle Mass (mg)	-59%*	-34%*	-22%*	Max	-10%	-63%*	-22%*	Max	-15%*
Muscle Length (mm)	-19%*	Max	-1%	-3%	-6%	-26%*	-4%	-2%	Max
Muscle CSA (m^2)	-50%*	-36%*	-24%*	Max	-7%	-52%*	-21%*	Max	-16%*
Twitch Force (mN)	-52%*	-11%	-10%	Max	-22%	-67%*	-2%	Max	-18%
Twitch Stress ($kN.m^2$)	-33%*	Max	-13%	-28%*	-38%*	-44%*	Max	-20%*	-20%†
Tetanus Force (mN)	-50%*	-14%	-10%	Max	-30%*	-56%*	Max	-3%	-27%*
Tetanus Stress ($kN.m^2$)	-25%*	Max	-11%	-26%	-42%	-28%*	Max	-23%*	-30%*
THPT (ms)	21%	Max	19%	40%*	31%*	19%	21%	16%	Max

LSHR (ms)	10%	Max	13%	5%	32%*	7%	Max	16%	42%*
Absolute Power (mW)	-61%*	-35%*	-29%*	Max	-34%*	-66%	-10%	Max	-31%
Norm. Power ($W.kg^{-1}$)	-7%	-2%	-8%	Max	-26%*	-19%*	Max	-14%*	-30%*
					Diaphragm				
Twitch Stress ($kN.m^2$)	-20%	Max	-4%	-24%*	-8%	-19%	Max	-31%*	-23%*
Tetanus Stress ($kN.m^2$)	-23%*	Max	-13%	-36%*	-29%*	-24%*	Max	-31%*	-33%*
THPT (ms)	Max	18%	21%	19%	23%†	3%	Max	13%	1%
LSHR (ms)	Max	2%	14%	11%	15%	Max	26%*	27%*	53%*
Norm. Power ($W.kg^{-1}$)	-5%	Max	-1%	-25%*	-41%*	-23%*	Max	-27%*	-34%*

Table 4.1 - Percentage differences in animal morphology, isometric properties and WL power output from the age at which the maximal measurement for each variable occurs. Values presented as mean. A * denotes significant ($P<0.05$) differences from the "Max" value. For time to half-peak tetanus (THPT) and last stimulus to half relaxation (LSHR), the "Max" value represents the age at which muscle activation and relaxation were fastest.

4.4.5 - Fatigue Resistance

Muscle power output during repetitive WL activation was significantly affected by age for soleus, EDL and diaphragm (Figure 4.7 A-F; P<0.001). Female soleus was more fatigue resistant than males (Figure 4.7 A&B; P=0.002) but no sex-based differences were observed for EDL and diaphragm (Figure 4.7 C-F; P>0.36 for both). A sex*age interaction was observed for EDL and diaphragm (Figure 4.7 C-F; P<0.009) but not for soleus (Figure 4.7 A&B; P=0.50).

Female soleus fatigability was not significantly affected by age (Figure 4.7B; P=0.09). For male soleus fatigue resistance at 3 weeks of age was significantly greater than all other ages (Figure 4.7A; P<0.03). No other differences were observed between the other age groups (Figure 4.7A; P=1.00). 10-week-old females were more fatigue resistant (i.e. took longer to reach 50% of pre-fatigue maximum) than 10-week male soleus (Figure 4.7 A&B; P=0.01), but no effect for 78-week female soleus to be more resistant to fatigue than males (Figure 4.7 A&B; P=0.07). No differences were observed between male and female soleus fatigue resistance at 3 weeks and 52 weeks (Figure 4.7 A&B; P>0.28).

There was no effect for age on the ability of female EDL to sustain WL power (Figure 4.7D; P=0.19). For male EDL, fatigue resistance was greatest at 3 weeks of age compared to all other ages (Figure 4.7C; P<0.05). 10-week-old male EDL was more fatigue resistant than 52-week-old EDL (Figure 4.7C; P<0.001), but not compared with 30-week and 78-week EDL (Figure 4.7C; P>0.19). 30-week-old male EDL was not more fatigue resistant than 52-week-old EDL and 78-week-old EDL (Figure 4.7C; P>0.07), with no difference between 52 weeks and 78-week-old male EDL (Figure 4.7C; P=0.44).

Male diaphragm fatigue resistance was not significantly affected by increasing age (Figure 4.7E; P=0.20). Female diaphragm was more resistant to fatigue at 3 weeks than at 52 and 78 weeks (Figure 4.7F; P<0.02), but not compared with 10-week female diaphragm (Figure 4.7F; P=0.21). At 10 weeks,

female diaphragm had greater fatigue resistance than at 52 and 78 weeks (Figure 4.7F; P<0.04), though no difference was found from 52 weeks to 78 weeks (Figure 4.7F; P=1.00).

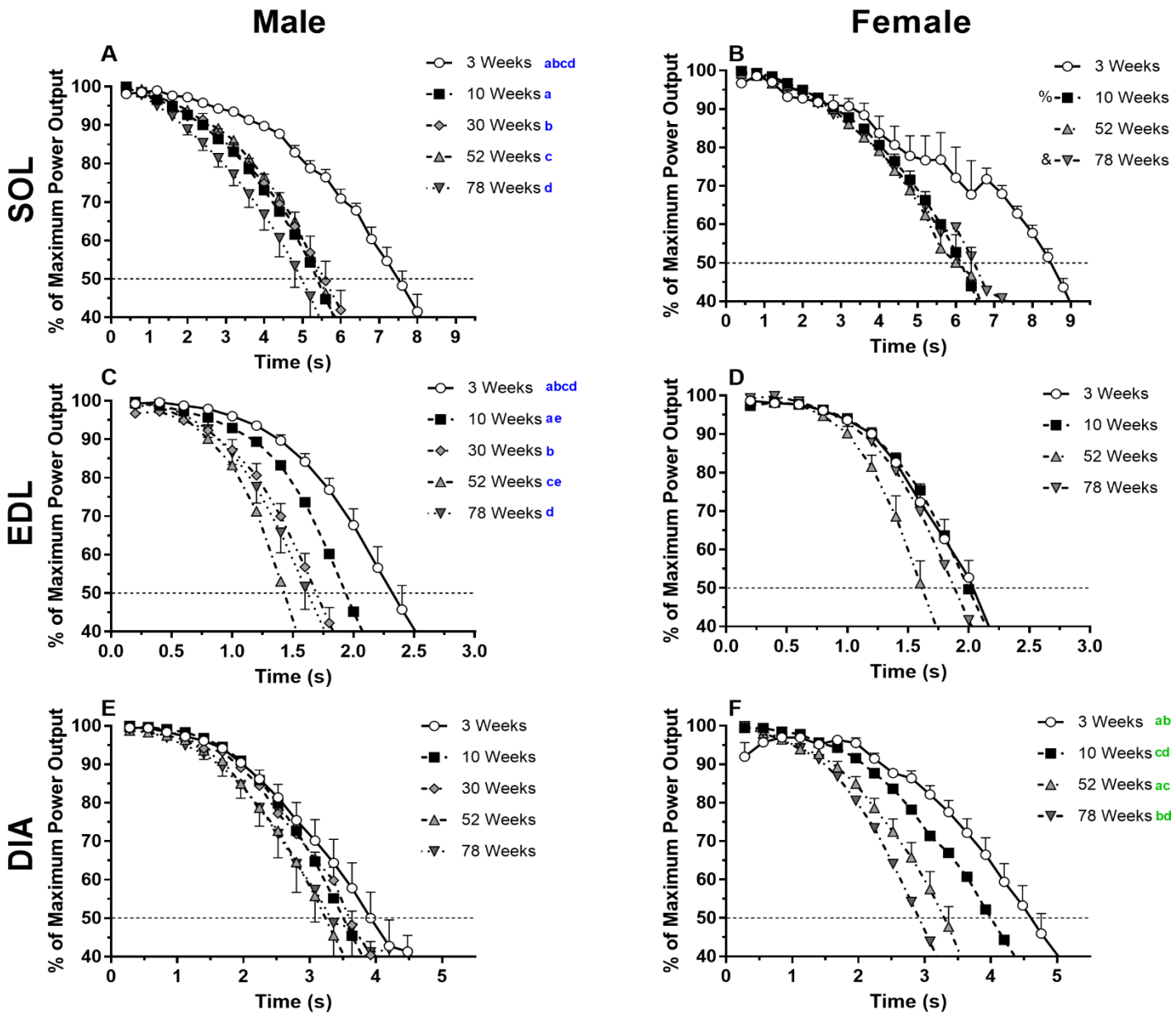


Figure 4.7 - The effect of age and sex on the ability to sustain power for male and female soleus (SOL) (A & B), EDL (C & D), and diaphragm (DIA) (E & F) at 3, 10, 30 (males only) 52 and 78 weeks of age. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by common symbols; blue symbols for males and green symbols for females. The '%' symbol next to an age group indicates significantly (P<0.05) greater fatigue resistance compared to the opposite sex at that given age.

4.4.6 - Recovery of Power

There was a significant effect of age on the recovery of power for EDL and diaphragm (Figure 4.8 A-F; P<0.03), but not soleus (P>0.09). There was a significant effect for sex on power recovery for soleus and EDL (Figure 4.8 A-D; P<0.05) but not for diaphragm (Figure 4.8 E&F; P=0.22). A significant sex*age interaction was observed for all skeletal muscles (Figure 4.8 A-F; P<0.004), but no interaction was observed for sex*time, age*time, or sex*age*time (Figure 4.8 A-F; P>0.34 for all).

The power output recovered for male and female soleus ranged from 90% to 97% of the pre-fatigue maximum by the 30th minute and was not different than that of the pre-fatigue maximum for all muscles of all ages for males and females (P>0.43), nor were there any differences between each age group for males and females (P>0.15). Additionally, power output recovery was not different than at 10 minutes and 20 minutes post-fatigue protocol for males and females of all muscles (P>0.12). Recovery of power by 30 minutes for males was greater than females at 3 weeks and 78 weeks of age (Figure 4.8 A&B; P=0.008 for both), with no difference between either sex at 10 weeks and 52 weeks (Figure 4.8 A&B; P=0.51).

For male and female EDL, power output recovery ranged from 39% to 82% of the pre-fatigue maximal power output after 30 minutes of recovery. For male EDL, peak recovery of power occurred at 3 weeks of age and was greater than at all other ages. (P<0.006). Increasing age was associated with a decline in the ability to recover power, with 30-week-old EDL exhibiting the poorest capacity to recover power after 30 minutes. There was a significant recovery in power over time for 3 week and 10-week-old male EDL (Figure 4.8C; P<0.001), but not at all other ages (Figure 4.8C; P>0.14). For female EDL, recovery of power was greatest at 78 weeks of age and was significantly greater than 10 weeks and 52-week-old EDL (Figure 4.8D; P<0.003) but was not different compared to 3-week-old EDL (P=0.10). 10-week-old EDL had the poorest capacity to recover power following fatigue but was not different compared to 52-week-old EDL (P=0.53). Significant recovery in power over time occurred at 3 weeks

and 52 weeks (Figure 4.8D; P<0.03), but not at 10 weeks and 78 weeks (Figure 4.8D; P>0.09). Males were able to recover greater power relative to the pre-fatigue maximal power than females at 3 weeks, 10 weeks and 52 weeks (Figure 4.8 C&D; P<0.05), and females to a greater extent than males at 78 weeks (Figure 4.8 C&D; P=0.001).

Recovery of male and female diaphragm power output after 30 minutes of recovery ranged from 88% to 95% of the pre-fatigue maximal power output. Recovery of power for 3-week-old male diaphragm was significantly greater than all other ages (Figure 4.8E; P<0.001), with no further differences between all other age groups (Figure 4.8E; P>0.90). Additionally, age did not affect the recovery of power for female diaphragm after 30 minutes (Figure 4.8F; P=0.76).

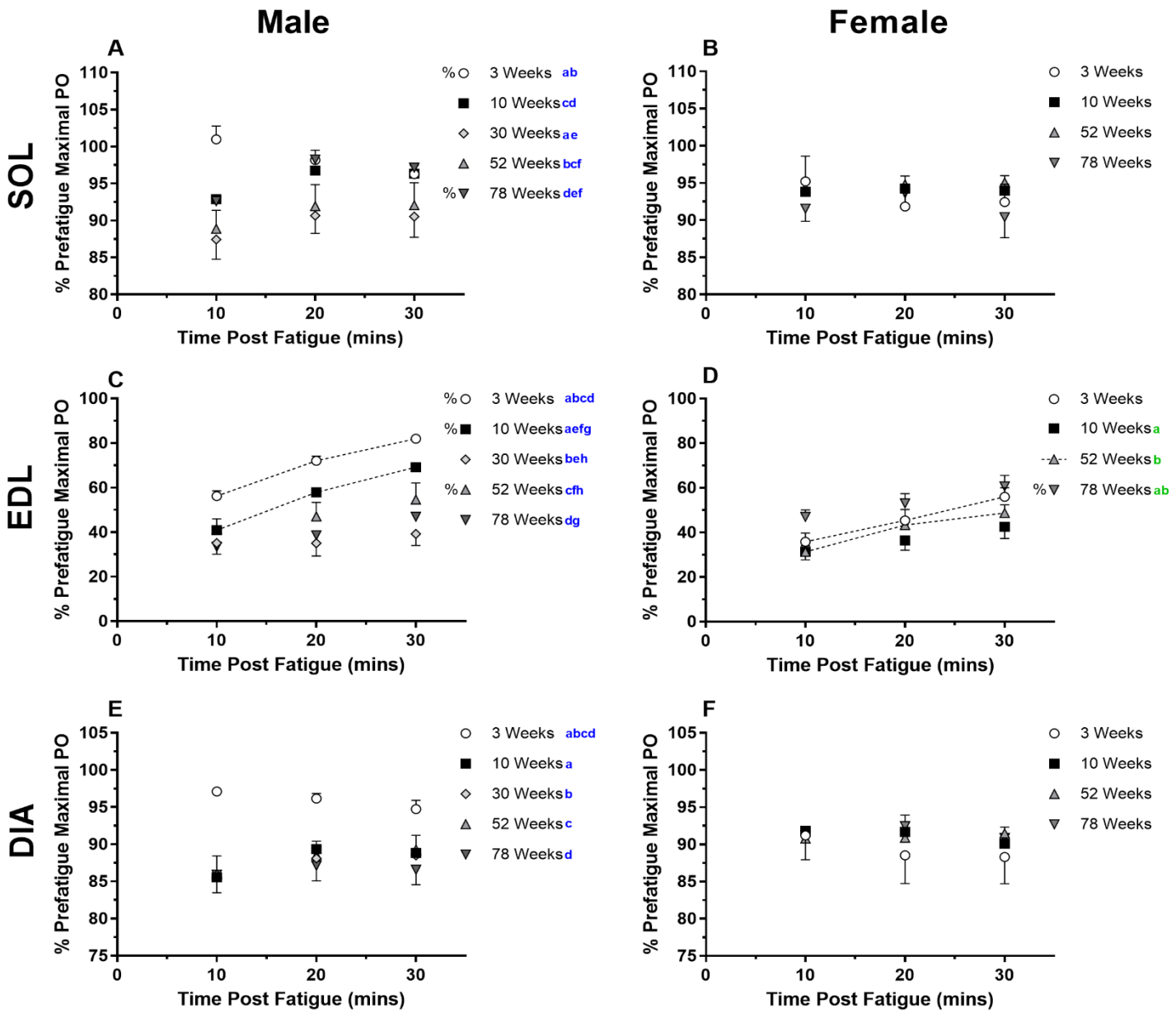


Figure 4.8 - The effect of age and sex on the ability to recover power for male and female soleus (SOL) (A & B), EDL (C & D), and diaphragm (DIA) (E & F) at 3, 10, 30 (males only) 52 and 78 weeks of age. Values presented as mean ± S.E.M. Significant differences (P<0.05) between age groups are indicated by common symbols; blue symbols for males and green symbols for females. The '%' symbol next to an age group indicates significantly (P<0.05) greater fatigue resistance compared to the opposite sex at that given age. Dashed lines between time points represent significant (P<0.05) recovery of power from 10 minutes to 30 minutes for that muscle at a specific age.

4.5 - Discussion

The present study provides a comprehensive examination of the sex-based differences in the maximal force production and WL power output of isolated locomotory and respiratory skeletal muscles, building largely upon the work conducted by Chan and Head (2010) and Tallis *et al.* (2014). In general, ageing resulted in a decline in isometric stress and normalised WL power from peak maturity whilst absolute force and absolute power improved with age up to 52 weeks, declining significantly by 78 weeks of age, without prevalent atrophy for all muscles. Between 10 weeks and 52 weeks of age, there were few sex-based differences in terms of isometric force and stress. However, absolute soleus and EDL power were greater in males than females. By 78 weeks of age, females produced greater stress and power normalised to muscle mass, indicating better quality of female skeletal muscles in old age. Irrespective of the muscle measured, the greatest decline in isometric stress occurred between 10 and 52 weeks, with the greatest loss of normalised power occurring between 52 and 78 weeks. Examinations of the power output-cycle frequency (PO-CF) curves demonstrated no leftward shift in absolute and normalised PO-CF curves. Ageing did, however, cause a significant reduction in normalised power output at slow cycle frequencies, but not fast cycle frequencies. The age-associated loss of normalised power and a general maintenance in fatigue resistance, combined with an increase and maintenance of body mass, will cause motions requiring both explosive, controlled and sustained power to be more difficult to perform *in vivo*. Examples include rising from a chair and stabilisation of the lower musculature during a fall.

4.5.1 - Comparison of Absolute and Normalised Force & Power Output

During maturation, 3-week-old male body mass (Figure 4.1A), and soleus and EDL muscle mass (Figure 4.1 B&C), was greater in males than females. When this is related to mechanical performance, male locomotor muscles generated greater absolute and normalised power than females, with no differences in absolute force or stress, whilst male diaphragm was more powerful than females. The sex-based differences in body mass, muscle mass and power output are likely to be related to

hormonal differences during maturation, where increased circulating testosterone is associated with increased muscle mass in males (Sheffield-Moore, 2000; Faigenbaum, 2008). For example, males experience a ten-fold increase in circulating testosterone levels leading to increased muscle mass and body mass, whilst increased oestrogen in females during maturation is associated with a markedly smaller increase in body mass, but greater body fat deposition in humans (Beunen and Malina, 1988; Round *et al.*, 1999).

Previous work examining age-related changes in absolute force and isometric stress reports that the decline in stress typically exceeds that of force (Phillips *et al.*, 1991; Moran *et al.*, 2005; Chan and Head, 2010; Tallis *et al.*, 2014). However, this is not always the case, where there are instances of the loss of force exceeding that of stress (Brooks and Faulkner, 1991; Brown and Hasser, 1996). In these instances, very old animals (>24 months) are usually examined, where muscle atrophy has typically occurred and does not typically occur until the final 20% of an animal's lifespan (Brown and Hasser, 1996; Chan and Head, 2010). Therefore, the loss of force in these studies is likely to be due to a reduction in muscle mass. Studies that have reported an age-related decline in isometric contractile function without prevalent atrophy demonstrates that isometric force is better maintained than isometric stress for soleus (Moran *et al.*, 2005) and EDL muscles (Chan and Head, 2010; Tallis *et al.*, 2014) for mice not within the final 20% of their lifespan. Muscle mass peaked at 52 weeks of age for male and female soleus and EDL, with a significant decline in muscle mass occurring for female EDL only. Peak absolute force and power increased with age and peaked at the age at which peak muscle mass occurred. The loss of isometric stress and normalised power output occurred from 10 weeks of age for all skeletal muscles, similar to findings of previous studies where the muscle quality exceeded that of absolute performance (Moran *et al.*, 2005; Chan and Head, 2010; Tallis *et al.*, 2017). The decline in isometric stress exceeds that of normalised power from 10 weeks to 52 weeks of age for all muscles (Table 4.1) consistent with previous isolated muscle work examining 50-week-old EDL and diaphragm (Tallis *et al.*, 2014), but contradictory to studies of human ageing where loss of absolute power

typically exceeds strength (Skelton *et al.*, 1994; Metter *et al.*, 1997; Deschenes, 2004). From 52 weeks to 78 weeks of age, however, the loss of muscular power exceeds that of isometric stress and can be attributed to the decline in absolute power output.

Tallis *et al.* (2014) examined the age-related changes in contractile performance of isolated female EDL and diaphragm, however, the effect of age on soleus power output remained unsolved. It is understood that ageing is more likely to affect skeletal muscle composed of predominantly fast-twitch muscle fibres are predisposed to a greater loss of contractile function due to a fast-to-slow shift in fibre type (Larsson, 1978; Klitgaard *et al.*, 1990; Coggan *et al.*, 1992). Therefore isometric stress of the EDL declines to a greater extent than the soleus with increasing age (Brooks and Faulkner 1988; Brown and Hasser, 1996; Lynch *et al.*, 2001; Graber *et al.*, 2015), though this is not always the case (Moran *et al.*, 2005; Rice *et al.*, 2005). By 78 weeks of age, the reduction in absolute and normalised force and power was greater for female EDL than soleus (Table 4.1). However, the decline in absolute force and isometric stress for males was similar to that of the EDL, though the decline in absolute and normalised power output was greater for male soleus than EDL (Table 4.1). In further consideration of the sex-based differences in contractile performance, Chan and Head (2010) reported a significant reduction in mouse EDL absolute force production for females but not for males, whilst isometric stress declines equally between each sex irrespective of prevalent muscle atrophy. The present work differs to the findings of Chan and Head (2010), whereby soleus and EDL absolute force and stress is not different between males and females by 78 weeks of age, but the magnitude of the decline from the age which elicited maximal force production is greater in males than females. By 78 weeks of age soleus and EDL isometric stress is significantly lower in males than females by 78 weeks of age, as is normalised power for all skeletal muscles. Isometric stress of the diaphragm was not different between each sex, but normalised power output was greater in females than males with increasing age. The magnitude of the decline in diaphragm stress and normalised power was similar between each sex, however.

As the loss of muscle quality occurs before the loss of absolute force and power and in a sex-specific manner, the reduction in normalised performance can be attributed to a reduction in hormonal factors such as testosterone and oestrogen (Lowe *et al.*, 2010). Ageing is associated with increased intramuscular adipose tissue, non-contractile elements such as collagen, and increased stiffness of non-contractile elements such as the extracellular matrix (McGregor *et al.*, 2014) where the combined effect is likely to result in greater negative work production in older skeletal muscles (Tallis *et al.*, 2014). Altered muscle architecture, such as a greater decline in fascicle length in older male skeletal muscles than females (Kubo *et al.*, 2003) could alter the length over which muscle can produce force and power in males, and could, therefore, contribute to the significantly lower muscle quality and accelerated decline in absolute force and power in males.

Maintenance of absolute force and power for soleus and EDL up to 52 weeks of age would indicate that factors intrinsic to the muscle fibre, such as inefficient actin-myosin cross-bridge kinetics (Lowe *et al.*, 2002) and excitation-contraction coupling (Renganathan *et al.*, 1997; Berchtold *et al.*, 2000) does not alter the force generating capacity of locomotor skeletal muscles. By 78 weeks of age, however, these factors may account for a reduction in absolute contractile performance and contribute further to the decline in muscle quality. Activation and relaxation times for the soleus and EDL in this muscle were not significantly different from 10 weeks to 52 weeks of age, though soleus activation and EDL relaxation times were significantly slower by 78 weeks of age (Figure 4.4 A-D). At the single fiber level, where excitation-contraction coupling and connective tissues are eliminated, ageing causes a significant reduction in isometric stress and fiber CSA for single soleus and EDL fibers (Brooks and Faulkner, 1994; Thompson and Brown, 1999), with reduced isometric stress of single fibres occurring without fibre atrophy (González *et al.*, 2000; Kim and Thompson, 2013).

At the cross-bridge level, ageing causes a 30% dissociation of myosin heads in the strong-binding state, leading to a 20% reduction in isometric force (Lowe *et al.*, 2002). During muscle shortening in a

concentric contraction, the opportunities for actin-myosin binding sites to form are lower than that during an isometric contraction (MacIntosh *et al.*, 2006). Should the level of myosin head dissociation be affected to the same extent during muscle shortening as during an isometric contraction, where force production is lower for the former compared to the latter, then poorer cross-bridge kinetics may explain the accelerated loss of power compared to force from 52 weeks to 78 weeks of age.

4.5.2 - Effects of Age and Sex on Absolute and Normalised PO-CF Curves

Whilst Tallis *et al.* (2014) reported age-related declines in maximal power outputs at a fixed cycle frequency, the relationship between contractile velocity and WL power output was not explored. The present results show that there was no leftward shift in either the absolute or normalised PO-CF curves for all muscles with increasing age. Graber *et al.* (2015) reported similar findings, in that absolute soleus and EDL power exhibited a downward, but not leftward shift, in the force-power curve. These results indicated that fibre shifting towards a more oxidative fibre composition is unlikely to have a significant effect on the contractile velocity that elicits maximal power output as with previous isolated muscle work (Lynch *et al.*, 2001).

4.5.3 - Effects of Age and Sex on Fatigue Resistance

In humans, older adults performing activities of daily living tend to be more fatigued (Mueller-Schotte *et al.*, 2016), though when examining muscular fatigue specifically findings are more ambiguous with some reporting a decline in fatigue resistance (Davies *et al.*, 1986; Sunnerhagen *et al.*, 2000; Izquierdo *et al.*, 2001) that is greater in males than females (Davies *et al.*, 1986; Hicks and McCartney, 1996) whilst others report no age-related or sex-based differences in muscular fatigue (Hicks and McCartney, 1996; Bilodeau *et al.*, 2001; Bazzucchi *et al.*, 2005). In humans, the age-related decline in fatigue resistance is largely attributable to increased central fatigability due to deterioration of the CNS (Carlson, 2004). The present work, where the CNS is removed, examines the ability for isolated muscles to sustain power, rather than sustained isometric force as in previous animal studies (Brown and Hasser, 1996; González and Delbono, 2001; Chan and Head, 2010), reveals that only male EDL and

female diaphragm were susceptible to age-related changes in fatigue resistance (Figure 4.7 C&F). For male EDL, whilst there was an age-related decline in fatigue resistance, however, there was no difference between 10-week-old EDL and 78-week-old EDL, where isometric stress (Figure 4.3C) and normalised power (Figure 4.6C) was greatest and poorest respectively. For female diaphragm the age-related decline in fatigue resistance was linear. The maintenance of fatigue resistance with increasing age for male and female soleus is due to the fibre composition of the soleus, which is primarily composed of the more fatigue resistant slow-twitch muscle fibres (Agbulut *et al.*, 2003; Tallis *et al.*, 2013). These results indicate that fatigue resistance at the muscular level is unlikely to be the main contributor to peripheral factors which govern fatigue resistance (Westerblad and Allen, 2002).

4.5.4 - The Effects of Sex and Age on the Ability to Recover from Fatigue

The recovery of power following the fatigue protocol is sex-specific and muscle-specific (Figure 4.8 A-F). In general, isolated soleus and diaphragm skeletal muscles were able to recover, and maintain, the level of power recovered over the course of the recovery protocol for all ages. For EDL, 3-week and 10-week-old male EDL, and 3-week and 52-week-old female EDL, recovered power over time. Recovery of power for male soleus and EDL was greater than that of females despite no sex-based differences in fatigue resistance for these muscles. However, the recovery of power between ages for each skeletal muscle was more variable for males than for females, where ageing had no effect on female soleus and diaphragm to recovery power. A similar pattern was found for male diaphragm, though recovery at 3 weeks of age was significantly greater than at all other ages. Female EDL and diaphragm each followed a similar pattern of recovery to the findings of Tallis *et al.* (2014), where recovery was greatest at 3 weeks, poorest at 30 weeks, and then improving with increasing age. The pattern of recovery for male EDL reciprocates that of the pattern of fatigue, though the age-related differences in soleus recovery are interesting given ageing did not affect the fatigue resistance for this muscle. By contrast, female EDL had the poorest recovery at 10 weeks and the greatest recovery at 78 weeks of age.

Little work has examined the acute ability to recover force and power following a fatiguing protocol in humans and in isolated muscle models, though of the work to have examined it, recovery of contractile function is equivocal, and could be related to the contractility mode used to induce fatigue and examine recovery. In rodents, González and Delbono (2001) reported no effect for age in the time to recover isometric stress, and the level to which stress recovered to for single soleus and EDL fibres fatigued via repeated isometric contractions. By contrast, Tallis *et al.* (2014) reported that whilst age did not affect the recovery of power for diaphragm, EDL muscles were affected by age in a fashion similar to the present findings. The recovery of power, therefore, relates to the proportion of oxidative fibres within the skeletal muscles, where those with a greater proportion of type I fibres not only has a better fatigue resistance but also better recovery, though this is not true for all skeletal muscles. Whilst the aforementioned statement can apply well to the soleus and female EDL, the same cannot be said of the male EDL and female diaphragm. Somewhat paradoxically, ageing caused a significant reduction in fatigue resistance for female diaphragm, however, ageing did not inhibit the recovery of power. As for male EDL, where fibre shifting is most associated, a reduction in the recovery of power with age is mirrored by an inability for older muscles to withstand fatigue.

4.5.5 - In Vivo Implications

Based on these results, it is expected that larger males will be less capable of completing acute tasks requiring explosive power, such as moving from a sit-to-stand position, than older females. In humans, Visser *et al.* (2002) demonstrated that lower extremity performance is associated with greater fat infiltration into skeletal muscles for both men and women. By 78 weeks of age, absolute and normalised contractile performance is significantly worse compared to all other ages. However, muscle quality is poorer in males than females. This is coupled with EDL muscle mass and animal body mass being significantly greater males than for females. The resultant effect is that older males have larger muscles that are contributing to an already elevated body mass but are of poorer quality.

Therefore, the effort of overcoming a greater limb mass and bodily inertia will be greater in males, reducing overall locomotor function and a poorer ability to complete activities of daily living.

Despite muscular fatigue of locomotor muscles being largely unaffected by age, activities of daily living requiring periods of sustained power are still more likely to give rise to poorer fatigue resistance in older adults. Fatigue resistance in this instance is represented as a relative percentage of the pre-fatigue maximal power output. As older soleus and EDL muscles produce lower isometric stress and normalised power, fatigue resistance in absolute terms is likely to be lower, and consequently reduce fatigue resistance in older adults due to weaker muscles transporting a greater bodily inertia (Pagala *et al.*, 1998). As a note, relative changes in power output over time rather than absolute changes in power output over time is reported as older muscles would start at a lower power output and therefore may appear less fatigue resistant.

4.6 - Conclusion

The present study provides a comprehensive overview of the changes in the absolute and normalised force and power and fatigue resistance of isolated mouse soleus, EDL and diaphragm for males and females at multiple time points. Males mature to a faster extent than females in terms of animal morphology, muscle mass and greater absolute and normalised WL power, and remain more powerful in absolute terms than females with increasing age. The loss of muscle quality, however, occurs equally between males and females with increasing age, though muscle quality of male skeletal muscles is significantly worse than females by 78 weeks of age. In addition to examining the force and power output of these skeletal muscles, fatigue resistance does not follow the expected pattern of fatigue as demonstrated in previous work using the WL technique. This highlights the complexities surrounding the examination of the muscle fatigue response. In lieu of an absence of skeletal muscle atrophy, the skeletal muscle ageing process may follow a two-step process, where in the first instance a decline in muscle quality could be related to increased non-contractile elements within the skeletal muscle. At the point where absolute force and power declines with age, impaired cross-bridge kinetics is likely to be a key contributor to a reduction in absolute performance and muscle quality, though a reduction in fibre CSA may also be a contributor. As there was an absence of a shift in the PO-CF curves and increase in fatigue resistance with older age, fibre type shifting may not be a key mechanism that elicits a reduction in contractile performance at the skeletal muscle level. *In vivo* contractile function is likely to be more limited in older men than women due to the need for larger muscles of poorer quality to overcome a greater bodily inertia.

Chapter 5 - The Effect of Increasing Age on the Concentric and Eccentric Contractile Properties of Isolated Mouse Soleus and Extensor Digitorum Longus Muscles

Modified from publication in The Journals of Gerontology, Series A. Biological Sciences and Medical Sciences.

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5.1 - Abstract

There is currently a limited amount of literature investigating the age-related changes in eccentric muscle function *in vitro* despite the regular occurrence during locomotion in older adults. The present study uniquely uses a strain of 0.10, which is more applicable to *in vivo* muscle length changes, in the comparison of the age and muscle-specific changes of acute and sustained concentric and eccentric power and recovery. Whole soleus or EDL muscles were isolated from 10-week and 78-week old mice, and acute and sustained concentric and eccentric WL power assessed. Despite an age-related increase in body and muscle mass, peak absolute concentric and eccentric power for both muscles was unaffected by age. Peak concentric power normalised to muscle mass declined significantly for each muscle, whilst peak normalised eccentric power declined only for soleus. Fatigue resistance and recovery for the soleus did not differ between age or contraction type. Older EDL was less resistant to concentric fatigue but was more fatigue resistant to sustained eccentric activity than young EDL. We have shown that eccentric function is better maintained for older soleus and EDL than concentric function. A greater bodily inertia is likely to further reduce *in vivo* locomotor performance in older animals, with a greater effect for concentric function *in vivo*.

5.2 - Introduction

The age-related decline in force (Lauretani *et al.*, 2003), power (Reid and Fielding, 2012) and fatigue resistance (Sunnerhagen *et al.*, 2000) have been associated with reduced mobility, quality of life and greater mortality in older adults (Rizzoli *et al.*, 2013). *In vitro* (Ballak *et al.*, 2014) and *in situ* (Warren *et al.*, 2001) studies that allow for the examination of contractile and morphological characteristics of muscle have been valuable in allowing the examination of the muscle and fibre type specific ageing response (Pagala *et al.*, 1998; Lauretani *et al.*, 2003; Ballak *et al.*, 2014). Given that ageing has been shown to affect neural recruitment (Hepple and Rice, 2016)**,** assessment of isolated muscle performance can be made, independent of the central nervous system and motivational effects, allowing true maximal force and power output to be measured. Furthermore, an isolated skeletal muscle approach allows for an accurate measurement of performance relative to muscle size (muscle quality). Assessments of muscle quality using *in vivo* methodologies may be confounded by complications in accurately measuring lean tissue mass and intramuscular adipose tissue mass (Fragala *et al.*, 2015). Finally, the effects of muscle endurance cannot be accurately determined *in vivo* given the likely elevation in body mass in older adults. Therefore, larger, older adults would have to generate greater force to overcome a greater inertia of the moving limb (Tallis *et al.*, 2017).

Previous isolated muscle work has demonstrated that increasing age is associated with a significant decline in peak absolute force and isometric stress (force relative to muscle cross-sectional area) (Moran *et al.*, 2005; Chan and Head, 2010; Graber *et al.*, 2015), and concentric power output (Tallis *et al.*, 2014). Moreover, Tallis *et al.* (2014) indicated that at the skeletal muscle level, fatigue resistance is age and muscle-specific, rationalising previous ambiguous findings examining the effects of ageing on fatigue (Pagala *et al.*, 1998; González and Delbono, 2001; Chan and Head, 2010). By contrast, there is limited evidence that examine age-related changes in eccentric power in isolated muscle models, especially given the important role of eccentric activity in older adults during locomotion and everyday

tasks such as balance, moving from a standing to seated position and stair descent (Dickinson *et al.*, 2000; LaStayo *et al.*, 2003) without undue damage or fatigue (Lovering and Brooks, 2014).

A small number of *in vitro* and *in situ* studies have assessed the effects of age on contraction-induced damage caused by eccentric muscle activity, whereby muscles are activated during substantial increases in muscle length. Such studies have demonstrated that skeletal muscles in older rodents are more susceptible to contraction-induced damage (Zerba *et al.*, 1990; Brooks and Faulkner, 1994, 1996; Chan and Head, 2010), but produce the same (Brooks and Faulkner, 1996) or greater force (Brooks and Faulkner, 1994) than younger animals. The strains used in previous animal models are substantially greater than those that would occur *in vivo* (Hoyt *et al.*, 2005; Butterfield, 2006), ranging from 20% to 50% of mean fibre length (Brooks and Faulkner, 1996; Chan and Head, 2010; Lovering and Brooks, 2014), and as such further work is needed to establish the effect of eccentric muscle activity using smaller strains that more closely approximates the *in vivo* function of skeletal muscle. Additionally, these studies fail to consider the maximal eccentric power, or eccentric power during sustained activity, which is important given that eccentric force and power is well maintained in older adults, but this hasn't been confirmed in an isolated muscle model.

The present study uses the work loop (WL) technique to better replicate *in vivo* contractile dynamics to examine the age-related changes in muscle power output (Josephson, 1985; James *et al.*, 1996; Choi and Widrick, 2009). Ageing affects isometric force production (Brooks and Faulkner, 1994; González and Delbono, 2001; Lowe *et al.*, 2002; Moran *et al.*, 2005; Chan and Head, 2010; Ballak *et al.*, 2014; Graber *et al.*, 2015) and eccentric force production during isovelocity lengthening of skeletal muscles (Zerba *et al.*, 1990; Phillips *et al.*, 1991; Brooks and Faulkner, 1994, 1996; Chan and Head, 2010), however these contraction types rarely occur *in vivo* (James *et al.*, 1996; Lynch *et al.*, 2001). Estimation of muscle power derived from isokinetic assessments of muscular strength assumes that the muscle activates and relaxes instantaneously, and fails to consider dynamic muscle length changes

(James *et al.*, 1996). As such, isometric and isovelocity methods have been shown to poorly estimate muscle power compared with the WL technique (James *et al.*, 1996; Lynch *et al.*, 2001). By using sinusoidal waveforms and stimulation parameters that more closely replicate *in vivo* conditions, the WL technique considers the muscle force production during dynamic activity, by considering simultaneous changes in force, muscle length and activation level (Josephson, 1985; James *et al.*, 1995, 1996). Previous studies that have used the WL typically use strains of 0.10 (±5% of optimal length derived from isometric contractions; section 3.5.4.1) to ascertain maximal WL power of isolated mouse soleus and EDL (James *et al.*, 1995; Askew *et al.*, 1997; Tallis *et al.*, 2013, 2014). The current work uniquely assesses the age-related changes in peak and sustained concentric and eccentric power using a strain that is more representative of the agonist-antagonist co-activation of skeletal muscles *in vivo* (Hoyt *et al.*, 2005; Butterfield, 2006; Hortobágyi and DeVita, 2006).

The aim of this study was to examine the age-related changes in concentric and eccentric muscle function of mouse soleus (predominantly slow-twitch) and EDL (predominantly fast-twitch) using parameters that better represent *in vivo* dynamic muscle activity, as these muscles are commonly examined for their contractile properties in isolated muscle models. Another aim was to determine whether utilising more common length change parameters leads to an age-related change in fatigue resistance and subsequent recovery of muscle power following a protocol of repeated maximal concentric and eccentric activation. Furthermore, the present work looked to establish differences between the absolute performance and performance normalised to muscle size derived from isometric contractions and acute measures of concentric and eccentric power, with the absolute performance providing insight into the real-world function of the muscle and the normalised measures providing valuable information with respect to changes in muscle quality.

5.3 - Methods

A more detailed overview of the methodological approach is provided in chapter 3.

5.3.1 - Animal Information

Female CD-1 mice were purchased at 8 weeks of age (Charles River, Harlow, UK) and allowed to mature in-house at Coventry University. Mice were aged to either 10 weeks (n=40) and 78 weeks (n=40) to represent young and old animals respectively. A justification for the usage of 10-week-old and 78-week-old animals is provided in section 3.1. Each age group was further split into: Young Concentric (YC), Young Eccentric (YE), Old Concentric (OC) and Old Eccentric (OE) and underwent either the repeated concentric (YC & OC) or eccentric (YE & OE) protocol (n=10 per muscle per protocol). There were no animals excluded from this study due to health complications.

Following sacrifice, animals were weighed to determine body mass, with whole soleus or EDL rapidly isolated from the right hindlimb and aluminium foil t-clips were wrapped around the distal tendons of each preparation to prevent tendon slippage during the experimental protocol. At the proximal end, a small piece of bone was left intact to allow for attachment to the mechanics' rig. Once prepared, each muscle was placed into the flow-through chamber filled with continually circulated, oxygenated (95% O_2; 5% CO_2) Krebs-Henseleit solution heated to and maintained at 37°C.

5.3.2 - Isometric Contractions

All preparations were optimised for length and stimulation parameters (14-18V for EDL, 12-16V for soleus; fixed stimulation amplitude of 160mA and pulse width of 1.2ms) through a series of isometric twitch contractions, with each parameter individually altered until peak twitch force was attained. The maximal isometric force was measured by the provision of tetanic stimulations to the preparation. The EDL received a 250ms burst of electrical stimulation and the soleus a 350ms burst of electrical stimulation. The frequency at which the stimulations were provided was altered until peak isometric

force was achieved. This was typically 200-220Hz for EDL and 120-140Hz for soleus for both ages. The duration of muscle activation and relaxation were measured as time to half-peak tetanus (THPT) and last stimulus to half tetanus relaxation (LSHR) respectively. A rest period of 5-minutes was imposed between each tetanic stimulation to allow for sufficient recovery.

5.3.3 - Assessment of Concentric Work Loop Power Output

Each muscle was held at the previously determined L_0 and the stimulation amplitude and stimulation frequency that resulted in maximal isometric force were implemented. In the first instance, maximal concentric power output for all experimental groups was determined.

A cycle frequency of 5Hz for soleus and 10Hz for EDL was used as these cycle frequencies typically elicited maximal concentric power output in young (James *et al.*, 1995; Askew *et al.*, 1997; Tallis *et al.*, 2013, 2014, 2017) and old mice (Tallis *et al.*, 2014). Phasic bursts of electrical stimulation were provided during muscle shortening for durations initially of 50ms and 65ms to the soleus and EDL respectively. The strain (typically 0.08 – 0.10 for all muscles and ages) and burst duration for each muscle was altered to ensure maximal concentric power output was achieved (Askew *et al.*, 1997). Each set of WLs was performed every 5-minutes to allow for sufficient recovery until peak power output was achieved.

5.3.4 - Assessment of Eccentric Work Loop Power Output

Eccentric power was not initially assessed for the YE and OE groups as optimisation of the muscle to achieve maximal eccentric power output may damage the muscle. Instead, the second WL of the eccentric fatigue protocol was taken to calculate eccentric PO.

5.3.5 - Repeated Concentric and Eccentric Work Loop Protocols

The ability to sustain power during repeated concentric and eccentric muscle activity for each experimental group was determined by imposing fifty consecutive WL's on each muscle. For the YC

and OC groups, the strain and stimulation parameters which elicited maximal concentric power output were maintained.

For the YE and OE groups, a cycle frequency of 5Hz and 10Hz was maintained for soleus and EDL respectively. A strain of -0.10 was used for all muscles to ensure the muscle passively shortened, followed by stimulation through lengthening. A stimulation phase shift of -10ms and -5ms for the soleus and EDL muscles respectively was maintained to ensure the stimulation was provided before the shortest muscle length. A burst duration of 72ms and 55ms was used for the soleus and EDL respectively to ensure the muscle was sufficiently stimulated throughout the lengthening phase.

Time to fatigue of the experimental groups was measured as the time taken for power to drop to 50% of the maximum concentric or eccentric PO.

5.3.6 - Recovery Protocol

The ability of each muscle to recover concentric power following the concentric and eccentric protocols was monitored for 30-minutes. The YC and OC groups were stimulated every 10-minutes as performed in previous work (Tallis *et al.*, 2013, 2014), whilst the YE and OE groups were stimulated every 5-minutes to more closely examine recovery, given no work has examined the recovery of work loop power following sustained eccentric muscle activity. The recovery of concentric power output after 30-minutes was expressed as a percentage relative to the pre-protocol maximal concentric power output for each group.

5.3.7 - Reassessment of Maximal Isometric Force and Stress

Following 5-minutes recovery after the final WL of the recovery protocol, maximal absolute force and isometric stress was reassessed in the YE and OE groups as a further means of assessing potential contraction-induced damage, with the severity of the damage determined by the magnitude of the deficit in force and stress compared to pre-fatigue measurements (Zerba *et al.*, 1990; Choi and

Widrick, 2009). This was not performed with muscles of the YC and OC groups as repeated concentric WL's do not significantly impair the recovery of power in the soleus and EDL following the same fatigue protocol used in previous studies (Tallis *et al.*, 2013, 2014).

Each experiment lasted for approximately 105 minutes from the moment of cervical dislocation through to the final stimulation. Following the experiment, the muscles were removed from the rig, tendons removed, the muscle lightly blotted on tissue paper, and then weighed to determine muscle mass to allow for normalisation of force ($kN.m^{-2}$) and power ($W.kg^{-1}$).

5.3.8 - Statistical Analyses

All data are presented as the mean ± standard error of mean (S.E.M). All data were normally distributed and showed homogeneity of variance, so parametric analyses were employed. The data for animal and muscle morphology, isometric contractile properties, maximal concentric and eccentric WL force and power were pooled for age for each muscle and analysed using independent samples T-Tests (Excel 2016, Microsoft).

A two-factor analysis of variance (ANOVA) was performed in SPSS (SPSS, IL, USA) to determine if repeated WL's caused a significant reduction in muscle power output and whether this was age-specific. Independent samples T-test were used to determine significant differences in time to fatigue between each age group for both soleus and EDL.

Recovery was assessed by a two-factor ANOVA with power and age as the main factors. An independent samples t-test was used to determine whether there was a significant difference between age groups in power output normalised to muscle mass after 30-minutes of recovery.

A repeated measures ANOVA was used to determine whether post-fatigue maximal absolute force and isometric stress of the muscles that underwent the eccentric protocol were significantly affected by age. Level of significance was set at $P<0.05$ for all analyses.

The truncated product method (Zaykin *et al.*, 2002) was used to analyse the distribution of *P*-values to provide a *P*-value for each group of multiple hypothesis tests to assess whether these values were biased via multiple hypothesis testing. The truncated product method *P*-value was <0.001, demonstrating that the results were not biased based on multiple hypothesis testing.

5.4 - Results

5.4.1 - Morphological and Isometric Contractile Properties

Ageing resulted in a significant increase in animal body mass (56%), soleus and EDL muscle mass (30% and 12% respectively) and CSA (32% and 9% respectively) with no change in muscle length (Table 5.1). Maximal isometric stress (absolute force divided by muscle cross-sectional area) for the 10-week old soleus and EDL are in line with previously reported values for the CD-1 strain (Table 5.2): 189kN.m^{-2} to 267kN.m^{-2} for soleus (Tallis *et al.*, 2012, 2013), 250kN.m^{-2} to 300kN.m^{-2} for EDL (James *et al.*, 2005; Tallis *et al.*, 2012, 2014). There was a significant age-related reduction in maximal isometric twitch and tetanus stress for both muscles, whilst absolute twitch and tetanus force declined significantly for the EDL only (Table 5.2). THPT was significantly longer for older soleus but not older EDL whilst LSHR was significantly prolonged for older EDL but not for soleus (Table 5.2).

Table 5.1 - Comparisons of pooled animal and muscle morphological measurements for each group.

Animal Morphology	**Young (10 weeks)**		**Old (78 weeks)**	
Body mass (g)	29.7 ± 0.5		46.4 ± 2.0*	
Muscle Morphology	**Soleus**		**EDL**	
	Young	**Old**	**Young**	**Old**
Muscle length (mm)	9.4 ± 0.1	9.3 ± 0.1	9.0 ± 0.1	9.2 ± 0.1
Muscle mass (mg)	7.6 ± 0.3	9.9 ± 0.3*	10.9 ± 0.3	12.0 ± 0.3*
Muscle CSA (m^2)	$7.65x10^{-7} \pm 2.76x10^{-8}$	$1.01x10^{-6} \pm 3.16x10^{-8}$*	$1.14x10^{-6} \pm 2.59x10^{-8}$	$1.24x10^{-6} \pm 2.98x10^{-8}$*

Values presented as mean ± S.E.M.

* denotes significant ($P<0.05$) difference between age groups.

Animal morphology; n=40 per age. Muscle morphology; n=20 per muscle per age.

CSA = cross-sectional area.

Table 5.2 - Pooled isometric and work loop contractile properties of young (10 weeks) and old (78 weeks) soleus and EDL muscles.

	Soleus			EDL		
Contractile Measure	**Young**	**Old**	**% change vs. young**	**Young**	**Old**	**% change vs. young**
Maximal twitch force (mN)	28 ± 1	28 ± 1	1%	64 ± 2	56 ± 3	-12%*
Maximal tetanus force (mN)	212 ± 9	224 ± 6	6%	376 ± 12	331 ± 15	-12%*
Maximal twitch stress ($kN.m^2$)	37 ± 2	29 ± 1	-23%*	57 ± 2	45 ± 2	-20%*
Maximal tetanus stress ($kN.m^2$)	280 ± 10	225 ± 7	-20%*	332 ± 9	269 ± 13	-19%*
Time to half peak tetanus (ms)	34 ± 1	39 ± 1	13%*	15 ± 1	16 ± 1	9%
Last stimulus to half relaxation (ms)	44 ± 2	48 ± 2	9%	13 ± 1	16 ± 1	29%*
Maximal concentric PO (mW)	237 ± 12	246 ± 12	4%	1066 ± 43	1007 ± 57	-6%
Maximal concentric PO ($W.kg^{-1}$ muscle mass)	31 ± 1	25 ± 1	-21%*	99 ± 5	83 ± 4	-16%*
Peak concentric force (mN)	86 ±6	80 ± 4	-7%	198 ±10	152 ±7	-23%*
Maximal eccentric PO (mW)†	-1038 ± 53	-1052 ± 48	-1%	-2682 ± 150	-2990 ± 214	-10%
Maximal eccentric PO ($W.kg^{-1}$ muscle mass)†	-146 ± 10	-115 ± 6	27%*	-260 ± 21	-253 ± 17	3%
Peak eccentric force (mN)†	294 ± 15	298 ± 9	1%	485 ± 17	484 ± 19	0.3%

Values presented as mean ± S.E.M.

* denotes significant ($P<0.05$) differences between each age group.

n=20 for all muscles and ages except for where † is placed, indicating n=10 per muscle per age.

PO = Power output. Stress = force ÷ muscle cross-sectional area.

5.4.2 - Maximal Concentric and Eccentric Work Loop Power Output and Peak Force

Power normalised to muscle mass for 10-week old animals are similar to previously reported values for the soleus, ranging from 31.7W.kg^{-1} to 33.0W.kg^{-1} for the soleus (Tallis *et al.*, 2012, 2013),: 59.8W.kg^{-1} to 99.0W.kg^{-1} for EDL (James *et al.*, 2005; Tallis *et al.*, 2012, 2014) (Table 5.2). Absolute concentric and eccentric power output generated by soleus and EDL was not significantly affected by age ($P>0.24$ for all) though when normalised to muscle mass, the maximal concentric power of both the soleus and EDL was significantly lower in the OC groups by 21% and 16% for soleus and EDL respectively (Table 5.2). Maximal eccentric power output normalised to muscle mass was not significantly altered by age for the EDL ($P>0.77$) but declined significantly for older soleus by 27% (Table 5.2).

Peak concentric force of the OC EDL was 23% lower than the peak concentric force achieved by the YC EDL muscles (Table 5.2; $P<0.002$), though there were no differences in peak concentric force for young and old soleus (Table 5.2, $P>0.41$). Likewise, there were no significant differences in the peak eccentric force achieved both muscles of each age (Table 5.2, $P>0.84$ for both).

5.4.3 - Fatigue Resistance to Repeated Concentric and Eccentric Contractions

Fifty consecutive concentric contractions resulted in significant reductions in relative power over time for both muscles (Figure 5.1 A&B; P<0.001). Age did not affect fatigue of soleus (Figure 5.1A; P=0.87). Typical WL shapes showed that the YC soleus had slightly more pronounced negative work in the early part of re-lengthening than OC soleus as demonstrated by increased passive force through re-lengthening back to L_0 with each WL, though this has a negligible effect on fatigue resistance (Figure 5.2 C&D). No differences in concentric fatigue were observed for the first 1.2 seconds for the EDL but fatigued significantly faster thereafter compared to the YC EDL group, with a 10% decrease in time to 50% of pre-protocol maximum power (Figure 5.1B; P<0.05). Negative work of the OC EDL was greater than YC EDL during the re-lengthening phase of the WL as shown by an increase in passive force through re-lengthening from the shortest muscle length with each WL, and therefore had poorer fatigue resistance (Figure 5.2 A&B).

Fifty repeated eccentric WL's did not elicit a significant reduction in relative power output over time for YE or OE soleus (Figure 5.1C; P=0.88). WL shapes indicated OE soleus have smaller WL areas at WL 2 due to absorbing less net work than YE soleus. By WL 18, YE soleus produces slightly less force during muscle lengthening, but this has no impact on muscle fatigability (Figure 5.3 C&D). By contrast, the EDL was unable to sustain eccentric power over time for both the YE and OE groups (Figure 5.1D; P<0.05). The muscles of the YE EDL lost power significantly faster than the OE EDL group (Figure 5.1D; P<0.001), with a 29% difference in the time to 50% of relative maximum PO. Positive work during the re-shortening phase of the WL is likely to be greater for the OE EDL than the YE EDL due to a downward shift in the right-hand portion of the YE EDL WL shape (Figure 5.3 A&B).

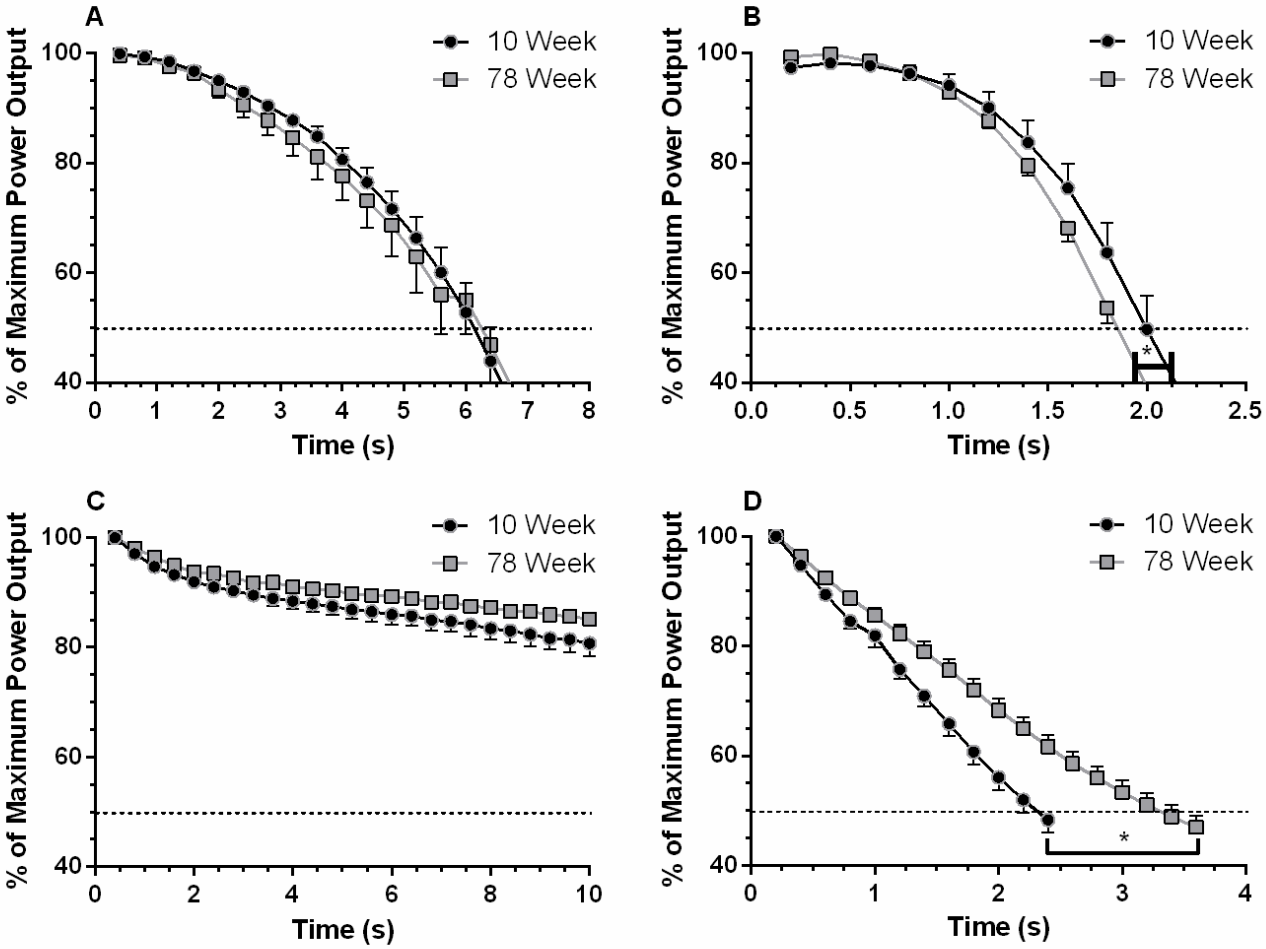

*Figure 5.1 - Time-course of sustained power production of young (●) and old (■) skeletal muscles relative to the pre-protocol maximum concentric power during fifty repeated concentric contractions (A, soleus; B, EDL), and eccentric power relative to the maximum eccentric power during fifty repeated eccentric muscle actions (C, soleus; D, EDL). Values presented as mean ± S.E.M. * significant differences between age groups for time to fatigue.*

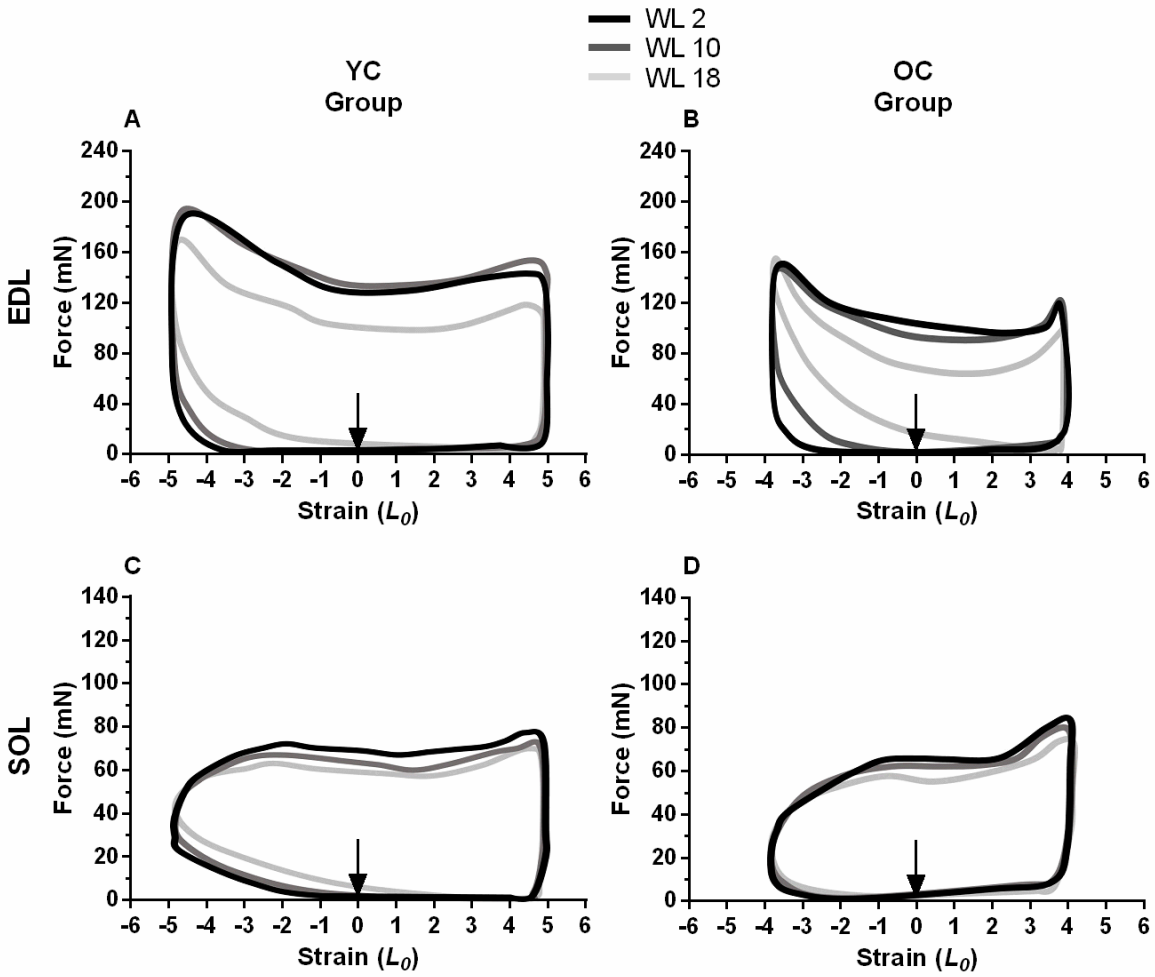

Figure 5.2 - Effect of age on typical WL shapes following concentric fatigue for the YC and OC EDL (A & B) and YC and OC soleus (C & D). Figures plotted as force against strain (%L_0). WLs 2, 10, and 18 of the fatigue protocol are shown for each group. WLs to be interpreted in the anti-clockwise (right-to-left) direction from L_0, indicated by a downwards arrow for each figure.

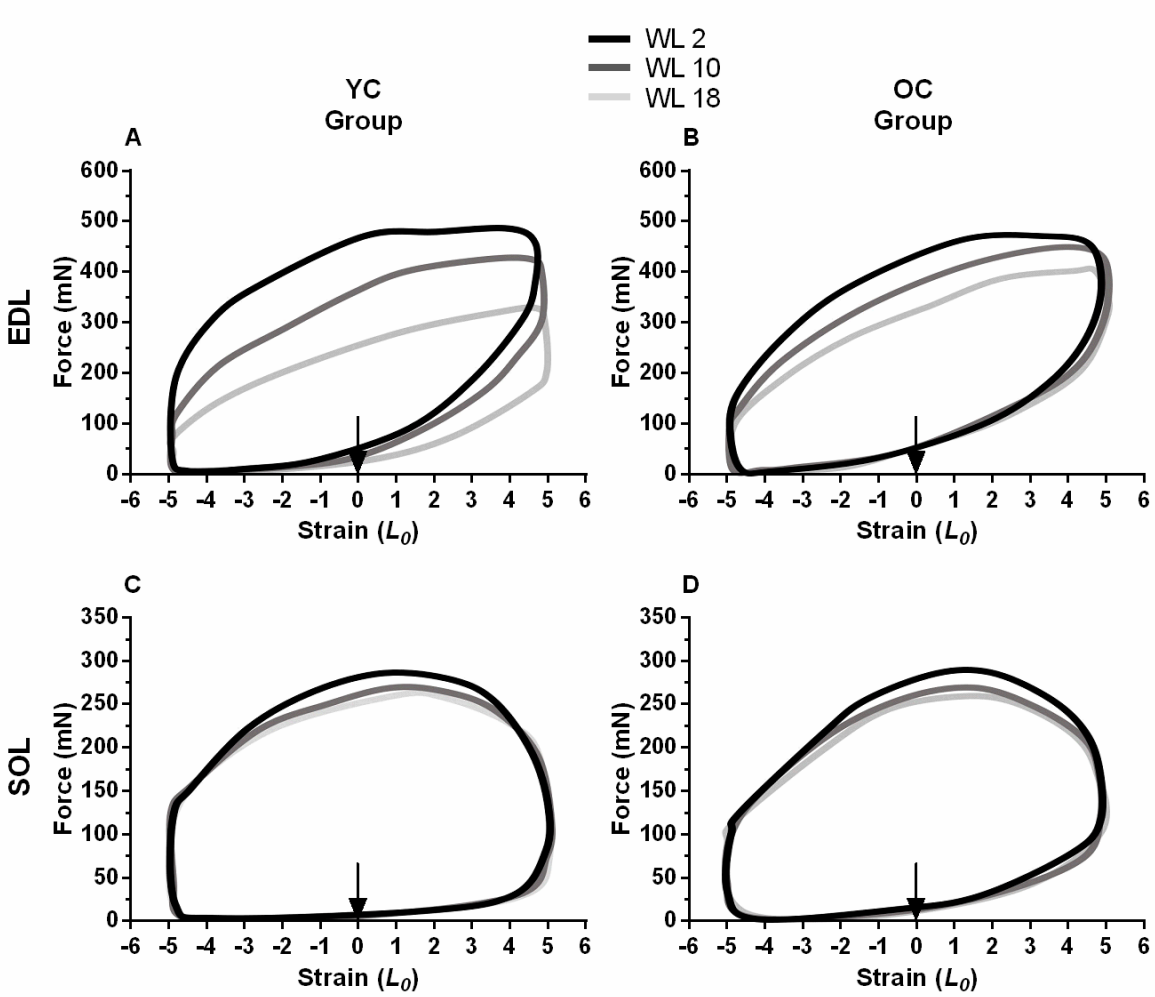

Figure 5.3 - Effect of age on typical WL shapes following eccentric fatigue at 10Hz cycle frequency for YE EDL (A) and soleus (C) and OE EDL (B) and soleus (D). Figures plotted as force against strain (%L_0). WLs 2, 10, and 18 of the fatigue protocol are shown for each group. WLs to be interpreted in the clockwise (left-to-right) direction from L_0, indicated by a downwards arrow for each figure.

5.4.4 - Recovery of Concentric Power

There was no significant difference in recovery of concentric power between young and old soleus following the concentric protocol (P=0.38), nor in recovery over time (Figure 5.4A; P=0.47). There was a tendency for age to affect recovery of power for YE and OE soleus (P=0.08) but there was a significant recovery over time (Figure 5.4C; P<0.001). The soleus from each age had almost fully recovered by 30-minutes (YE 98±2%; OE 98±1%).

The EDL of the OC group recovered concentric power to a greater magnitude than the YC group following the concentric protocol (Figure 5.4B; P=0.03, 59±4% vs. 43±5% respectively after 30-minutes), with significant recovery over time (P=0.03), though there was no age*time interaction (P=0.96). OE EDL had a significantly higher relative power output than YE EDL following the eccentric protocol (P<0.001), with a significant reduction in YE EDL relative power output over time (Figure 4D; P<0.001). There was a tendency for an interaction between age and time indicating a potential for older EDL to lose less power during the recovery period (P=0.08).

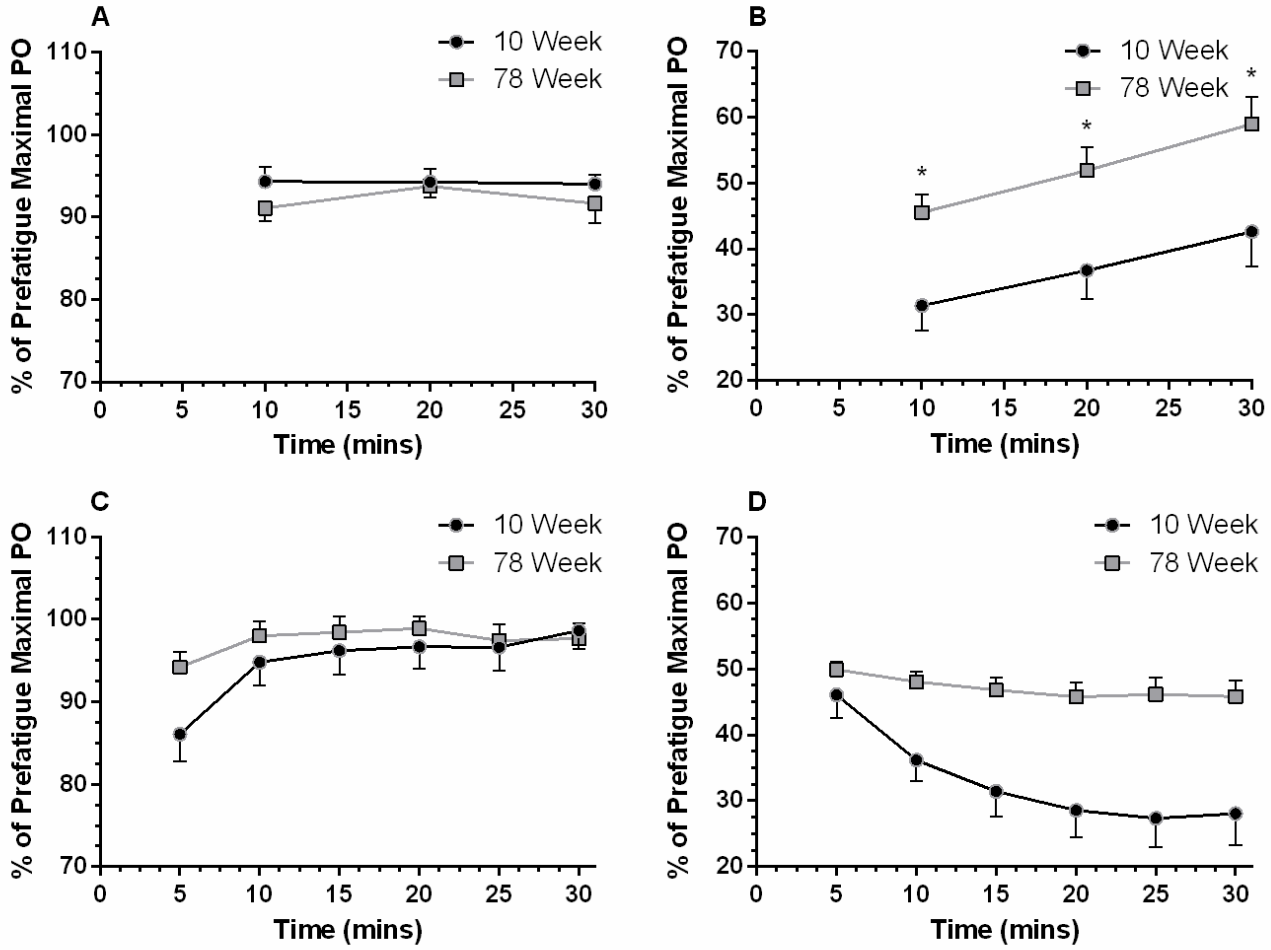

*Figure 5.4 - Time-course of recovery of concentric power output relative to the pre-protocol maximum power for young (●) and old (■) skeletal muscles every 10-minutes following repeated concentric contractions (A, soleus; B, EDL) and every 5-minutes following repeated eccentric muscle activity (C, soleus; D, EDL). Values presented as mean ± S.E.M. * significant difference between age groups at each time point.*

5.4.5 - Recovery of Absolute Force and Isometric Stress

Maximal absolute force and isometric stress of the soleus remained unchanged for both ages (Figure 5.5 A&B; P>0.36 for both). Absolute force and isometric stress declined significantly for the EDL, but to a greater magnitude in YE EDL, with force declining by 59% and 40% for YE and OE EDL respectively and stress by 57% and 38% for YE and OE EDL respectively (Figure 5.5A&B; P<0.001 for all).

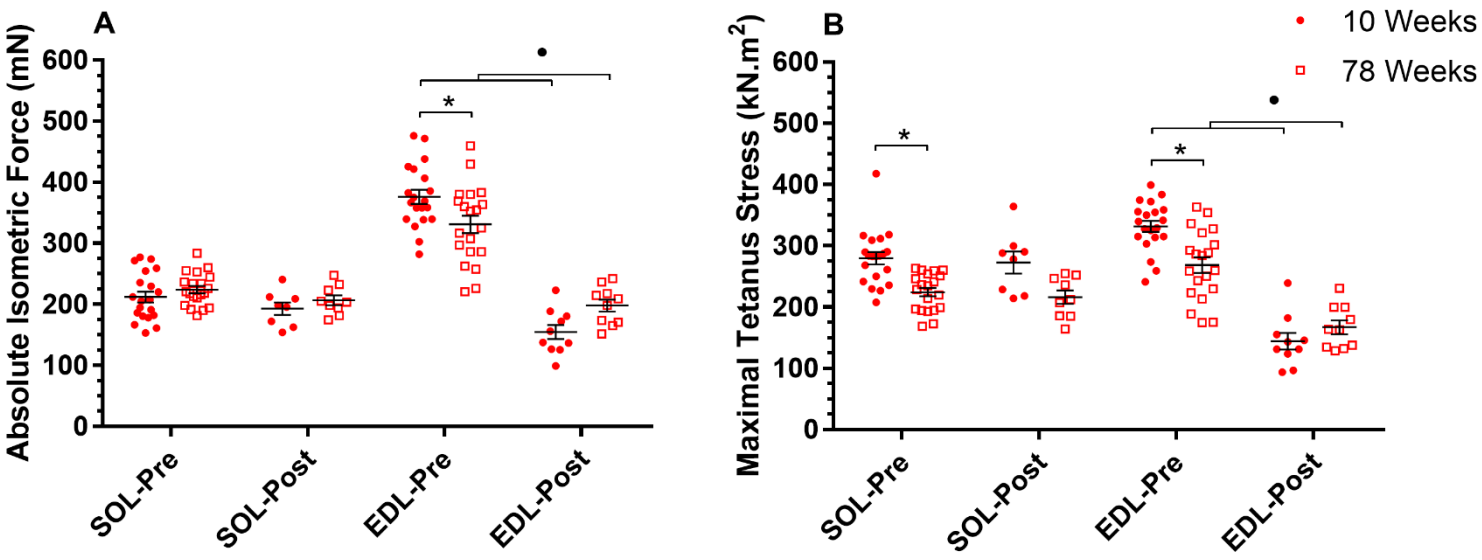

*Figure 5.5 - Age-related changes in maximal absolute force (A) and maximal isometric stress (B) prior to fatigue (SOL-Pre & EDL-Pre, n=20 for both muscles and ages). Absolute force (A) and isometric stress (B) were reassessed following the final WL of the recovery protocol in the YE & OE groups (SOL-Post & EDL-Post; n=10 for both muscles and ages). Values presented as mean ± S.E.M. * significant (P<0.05) changes in maximal absolute force or isometric stress with age. • significant (P<0.05) reductions in pre vs. post assessments of maximal absolute force or isometric stress. Stress = force ÷ muscle cross-sectional area.*

5.5 - Discussion

The present work is the first to compare the age and muscle-specific changes in acute and sustained concentric and eccentric power output by utilising the WL technique as a better representation of real-world muscle function. An age-related reduction in acute and sustained concentric power output for the EDL did not mirror those observed for eccentric muscle activity, which was largely unaffected for the older skeletal muscles. A reduction in OE soleus power output normalised to muscle mass was observed, which is likely to be detrimental to *in vivo* locomotion. Moreover, age did not affect the ability of the soleus to sustain concentric and eccentric power. The most significant finding pertains to the ability of older EDL to better withstand the damaging effects of a sustained bout of eccentric muscle activity than younger EDL, using a strain that better replicates *in vivo* muscle activity. Consequently, the reduction in eccentric contraction-induced force and power loss following sustained eccentric activity of the older EDL may indicate a reduced susceptibility to eccentric muscle damage with increasing age.

5.5.1 - Age-Related Changes in Skeletal Muscle Contractility

Similar to previous work examining the effect of ageing on isolated skeletal muscle (Phillips *et al.*, 1991; Chan and Head, 2010; Tallis *et al.*, 2014), these results infer that substantial muscle ageing can occur without the muscle wasting that defines sarcopenia. The reduction in stress and normalised concentric power indicate an age-related reduction in muscle quality. A loss in muscle quality is not consistent for all contraction types as eccentric power normalised to muscle mass was well maintained in old EDL, though normalised eccentric power for the soleus declined with age. This muscle-specific reduction in acute eccentric power may partially explain the ambiguity surrounding eccentric contractile properties in older human populations, where some have reported an age-related decline in eccentric force of lower limbs (Lindle *et al.*, 1997; Delbaere *et al.*, 2003) whilst others report no change (Poulin *et al.*, 1992; Perry *et al.*, 2007).

The age-related decline in muscle function may relate to an increase in dysfunctional Ca^{2+} handling proteins particularly given the age-related increase in soleus activation time and EDL relaxation time (Table 5.2). Previous literature has observed an age-related dysfunction in sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) activity in slow-twitch (Lamboley *et al.*, 2016) and fast-twitch (Tallis *et al.*, 2014) muscle fibres, likely causing leakage of sarcoplasmic reticulum (SR) Ca^{2+} from ryanodine receptors into the cytoplasm (Andersson *et al.*, 2011). Excitation-contraction uncoupling and reduced SERCA activity could explain why normalised concentric and eccentric power declines to a greater magnitude in older soleus than EDL (Table 5.2) given that slow-twitch fibres experience greater excitation-contraction uncoupling than fast-twitch fibres (Ryan and Ohlendieck, 2004).

Increasing age has been shown to have an effect on the interaction of the contractile proteins needed for cross-bridge formation (Lowe *et al.*, 2002). Single fibre experiments, independent of calcium kinetics and non-contractile elements, demonstrate significant reductions in isometric force production with age, resulting in fewer actin-myosin binding sites maintaining a strong-binding structure (Lowe *et al.*, 2002). By contrast, eccentric force production of single permeabilised EDL fibres of 27-month-old mice has been shown to be significantly higher than those of younger animals but was not different in whole muscles (Brooks and Faulkner, 1994). Peak eccentric force was unaffected by age but was significantly lower for older EDL during eccentric WL's (Table 5.2), indicating age-related alterations in cross-bridge kinetics are likely to be dependent on the mode of muscle activity. Additionally, intramyocellular lipid accumulation occurs with progressive ageing (Goodpaster *et al.*, 2001) which have been further associated with a reduction in muscle quality during concentric WLs (Tallis *et al.*, 2017). The relationship between obesity and eccentric muscle activity, in young and old skeletal muscles, has yet to be explored.

5.5.2 - Fatigue Response During Repeated Concentric Activity

There was no difference in concentric fatigue resistance between soleus muscles from young and old mice (Figure 5.1A) though this was not the case for older EDL (Figure 5.1C). WL shapes indicate older EDL produced more negative work during muscle re-lengthening as the fatigue protocol progressed compared with YC EDL (Figure 5.2A&B). Relaxation time increases with each WL (Askew *et al.*, 1997; Tallis *et al.*, 2013, 2014) indicating that Ca^{2+} has not been fully reabsorbed by the SR prior to the next contraction, therefore the muscle is still partially active through re-lengthening resulting in the progressive absorption of negative work leading to increased fatigability that is more pronounced in older EDL. Whilst there is a lack of evidence observing sustained concentric power using mammalian tissues, it appears 78-week old EDL muscles are able to sustain concentric power for a longer period than the 50-week old animals during the same fatigue protocol (Tallis *et al.*, 2014). Whilst absolute power is well maintained, it is expected that the older individuals will fatigue faster *in vivo* when working at the same relative intensities due to an elevated body mass (Pagala *et al.*, 1998).

5.5.3 - Fatigue Response During Repeated Eccentric Activity

The fatigue response to the eccentric protocol was age and muscle-specific. For soleus muscle, the reduction in power during sustained eccentric activity was not significantly affected by age, and as such there was very little difference in the WL shapes (Figure 5.3 C&D). As the maximal eccentric power of the older group was substantially lower, this would result in a reduced amount of relative power over the duration of the fatiguing protocol (Figure 5.1C).

Conversely, sustained power following the eccentric protocol was significantly reduced in young EDL compared to old EDL (Figure 5.1D), despite maximal eccentric power production being unaffected by age (Table 5.2). Typical WL shapes indicated that the force produced during lengthening decreased much more in young EDL than old EDL, over the series of WL's (Figure 5.3A).

Repeated eccentric activity may work to sustain locomotor performance *in vivo* given the elevated body mass. Older muscles generated the same concentric and eccentric absolute power output and peak WL force, yet body mass of older mice increased by 56%. Therefore, the older mice would have to generate greater power to overcome the bodily inertia during locomotion or braking motions at the same speed as younger mice (Tallis *et al.*, 2014).

5.5.4 - Recovery of Concentric Power

Recovery of concentric power (Figure 5.4 A&C), and recovery of absolute force and isometric stress (Figure 5.5 A&B) of the soleus following repeated concentric and eccentric activity was unaffected by age. Given that there was no age-related change in fatigue resistance of the soleus following each protocol, the consequent ability to recover concentric power indicates no undue damage or fatigue.

By contrast, the recovery of the EDL of the older age group was significantly greater following the concentric protocol when compared to the young group (Figure 5.4B). This is likely due to the ability of slower muscles to recover faster following repetitive stimulation (Tallis *et al.*, 2013), which would also explain the near full recovery of the soleus for both ages and contraction types. Tallis *et al.* (2014) observed no differences between the 10-week and 50-week old EDL in the recovery of concentric power, with the OC EDL group of this study recovering to a greater extent than the 50-week old animals.

The current study used a smaller strain as a closer representation of length changes which occur more regularly *in vivo* (Hoyt *et al.*, 2005; Butterfield, 2006), although this smaller strain may have still caused damage in the EDL for both ages as demonstrated by a consistent reduction in post eccentric protocol power (Figure 5.4D) and reduced absolute force and isometric stress (Figures 5.5 A&B). Given that high eccentric force is associated with greater muscle damage (Lovering and Brooks, 2014), this may account for the difference in recovery observed between the EDL and soleus. Interestingly, recovery

of the young EDL was impaired to a greater extent, following the eccentric protocol, than the older EDL (Figure 5.4D), which could be due to greater structural damage in the younger group. An age-related increase in structural damage of the skeletal muscles has been previously attributed to greater impairment of contractile function following eccentric muscle activity in older EDL. Zerba *et al.* (1990) found that the tetanic force deficit following 75 lengthening actions at a total mean fibre length change of 25% was significantly greater in older mouse EDL muscles compared to young and adult mice. Additionally, the relative loss of isometric force following single stretches of single permeabilised of 27-34-month-old rat EDL fibres was greater than that of 5-6-month-old rats at a strain of 10% and 20% of mean fibre length, but was not different at 5% (Brooks and Faulkner, 1996), highlighting that larger strains are required to significantly damage older muscles during acute and sustained eccentric activity.

5.6 - Conclusion

This study demonstrates that ageing is not uniform for all contraction types and muscles, which may have complex consequences for *in vivo* locomotor function in older adults. The loss of force and power relative to muscle size in the present study, as opposed to the apparent reductions in absolute force and power as observed in humans, appears to be the greatest factor in alterations of contractile function in this study, offering further support to the dynapenic mechanism of muscle ageing. The observed general reduction in muscle quality, coupled with an age-related increase in body mass observed in the present study, could be a key factor in the reduced locomotor function of older adults. However, older EDL muscles are capable of withstanding repeated eccentric muscle activity to a greater extent than younger muscles and appear to sustain less damage than younger EDL muscles. This could be important for exercise prescription, where eccentric exercises can be incorporated into training regimens to improve eccentric muscular function. Thus, locomotor capabilities and physical activity levels could be increased and overall quality of life improved.

Chapter 6 - The Effects of Dietary-Induced Obesity on the Contractile Properties of Isolated Soleus, EDL & Diaphragm Skeletal Muscles from Aged CD-1 Mice

6.1 - Abstract

Ageing and obesity independently have been shown to significantly impair isolated muscle contractile properties, though their synergistic effects are poorly understood. We uniquely examined the effects of 9 weeks of a high-fat diet (HFD) on isometric force, work loop (WL) power across a range of contractile velocities and fatigability of 79-week old soleus, extensor digitorum longus (EDL) and diaphragm compared with age-matched lean controls. The dietary intervention resulted in a significant increase in body mass and gonadal fat pad mass compared to the control group. Despite increased muscle mass for HFD soleus and EDL, absolute isometric force, isometric stress (force/CSA), WL power normalised to muscle mass and fatigability were unchanged, although absolute WL power was significantly greater. Obesity did not cause an alteration in the contractile velocity that elicited maximal power output. In the obese group, normalised diaphragm power was significantly reduced, with a tendency for reduced isometric stress and fatigability was unchanged. HFD soleus isolated from larger animals produced lower maximal power output and may impair balance in older, larger adults. The increase in absolute power is smaller than the magnitude of weight gain, meaning *in vivo* locomotor function is likely to be impaired in old obese adults. An obesity-induced reduction in the function of the diaphragm will likely impair *in vivo* respiratory function.

6.2 - Introduction

Ageing is associated with poorer muscular strength and power compounding in reduced locomotory function and quality of life (dos Santos *et al.*, 2017). It is suggested that ageing with obesity may exacerbate these effects (Tallis *et al.*, 2018). A growing body of evidence has indicated that obesity may significantly impair skeletal muscle function in young adults (Miyatake *et al.*, 2000; Garcia-Vicencio *et al.*, 2016; Tomlinson *et al.*, 2016), however, the synergistic effects of ageing and obesity on muscle function are poorly understood. The impact of obesity on the muscle function of older adults is equivocal, with evidence demonstrating either no change (Miyatake *et al.*, 2000; Zoico *et al.*, 2004) or an increase in absolute force of the lower leg musculature (Rolland *et al.*, 2004; Tomlinson *et al.*, 2014), whilst others have shown a reduction in plantar flexor and dorsiflexor absolute force and power and force relative to body mass (Tomlinson *et al.*, 2014). A recent review has indicated that assessing the effect of obesity using an isolated muscle model can help further our understanding of obesity effects on contractile performance (Tallis *et al.*, 2018). Such models have been used regularly to examine skeletal muscle ageing and more recently obesity effects, however, the present work is the first to examine the interaction between obesity and old age on isolated muscle contractile function.

Changes in muscle function may be related to an elevated body mass or contractility at the muscular level, or indeed a combination of the two, though it is difficult to ascertain which factor has the greatest influence in whole-body examinations. Evidence indicates that independently both obesity and ageing cause a reduction in muscle quality (force or power relative to muscle size), which consequently results in larger muscles of lower quality (Fragala *et al.*, 2015; Tallis *et al.*, 2017). For obese individuals particularly, such effects may contribute to an already elevated body mass for the same, or lower, mechanical work (Tallis *et al.*, 2017). Measures of muscle quality are difficult to ascertain *in vivo*, with absolute changes in strength and power commonly reported for old obese

adults (Miyatake *et al.*, 2000; Rolland *et al.*, 2004; Zoico *et al.*, 2004; Stenholm *et al.*, 2009; Tomlinson *et al.*, 2014). Whilst work has normalised contractile performance to body mass (Tomlinson *et al.*, 2014) and muscle volume (Hilton *et al.*, 2008), these approaches fail to consider whole tissue mass that can be otherwise obtained via *in vitro* examinations of whole skeletal muscles (Tallis *et al.*, 2018).

Mechanistically, the age-related decline in muscle function has been attributed to impaired calcium handling, reduced protein synthesis, reduced contractile mass, impaired cross-bridge kinetics, a shift in fibre type composition, greater lipid accumulation and chronic inflammation (Miljkovic *et al.*, 2015) where a similar mechanistic responses to ageing are shared with obesity (Akhmedov and Berdeaux, 2013; Funai *et al.*, 2013; DeNies *et al.*, 2014). As a consequence, isometric stress (force relative to muscle cross-sectional area) and power normalised to muscle mass is impaired in aged (Moran *et al.*, 2005; Chan and Head, 2010; Tallis *et al.*, 2014; Graber *et al.*, 2015; Hill *et al.*, 2017) and young obese (Ciapaite *et al.*, 2015; Matsakas *et al.*, 2015; Bott *et al.*, 2017; Tallis *et al.*, 2017) isolated skeletal muscles. Such mechanisms form the basis for an obesity-associated, muscle-specific reduction in isolated muscle contractile performance in old, obese mammalian muscles. To date, only one study has examined ageing and dietary-induced obesity on isolated skeletal muscle function, using 33-week-old C57BL/6J mice (Bott *et al.*, 2017). There were no age-related changes in peak isometric stress indicating that this study does not fully consider the effects of both old age and obesity as animals in this study were relatively young in terms of total lifespan for this strain, with a mortality rate of 50% at 28 months (121 weeks) of age (Flurkey *et al.*, 2007).

This study aims to determine whether obesity in old age impairs isolated muscle contractile performance by examining the effect of 9 weeks high-fat diet (HFD) consumption on isometric force, WL power output, and fatigue resistance of isolated mouse soleus, EDL and diaphragm muscle compared to age-matched controls. The aim was to determine whether obesity further worsened skeletal muscle contractile performance in old age. Previous isolated muscle studies examining ageing

and obesity independently typically examine isometric force (Moran *et al.*, 2005; Ciapaite *et al.*, 2015; Graber *et al.*, 2015; Matsakas *et al.*, 2015; Bott *et al.*, 2017), whereas the WL technique considers the interactions between force production during shortening, the force-velocity relationship, and the passive work required to lengthen the muscle, providing a better representation of *in vivo* muscle function (Josephson, 1985; James *et al.*, 1996). It is proposed that dietary-induced obesity will cause a muscle-specific acceleration of the deleterious effects of the muscle ageing process, such as a muscle-specific reduction in isometric stress, WL power output normalised to muscle mass and fatigue resistance, given the similar mechanistic adaptations of skeletal muscles to ageing and obesity.

6.3 - Methods

A comprehensive overview of the methodological approach for this study is provided in chapter 3.

6.3.1 - Animal Diet

A number of studies have investigated the effects of a high-fat diet (HFD) on animal and muscle morphology for a variety of durations. These have been outlined in table S1.5. In all cases, each study compared a HFD against a low-fat diet (LFD) sometimes referred to as a control. The age at the start of feeding, the feeding duration, changes in body composition and muscle morphology are reported in table S1.5 where measured. In previous studies, there is a heavy reliance on the inbred C57BL/6J mouse strain which is more susceptible to the obesity-promoting effects of a HFD (Lee *et al.*, 1995; Surwit *et al.*, 1997; Alexander *et al.*, 2006). When investigating the physiological effects of dietary-induced obesity, usage of the C57BL/6J strain lacks a full representation of a heterogeneous population further vindicating the future usage of the CD-1 strain (Gao *et al.*, 2015).

In addition to the high carbohydrate standard lab chow provided to all mice, animals in the HFD group were also provided with free *ad libitum* access to husked sunflower seeds (Advanced Protocol PicoLab, Fort Worth, USA) which formed the dietary source for the HFD. The sunflower seeds were provided to the HFD group in a self-selected, forage style diet to comply with Home Office regulations. A comparison of the nutritional value of the standard lab chow (SDS RM-1 M) diet and the sunflower seeds is provided in table 6.1.

	SDS RM-1 Maintenance	Advanced Protocol PicoLab Natural Sunflower
Calories provided by:		
Protein (%)	17.49	17.95
Fat (%)	7.42	63.66
Carbohydrates (%)	75.09	18.39
Gross energy (Kcal.g)	3.52	5.24
Digestible energy (Kcal.g)	2.57	3.80
Fatty acids content:		
Saturated (%)	0.51	2.61
Monounsaturated (%)	0.88	5.36

Table 6.1 - A nutritional comparison of the standard lab diet and sunflower seeds, which was provided in conjunction with the former to the obesity group. Both analyses assumed 10.0% moisture content of each diet (Tallis *et al.*, 2017).

Sunflower seeds provide a much greater percentage of calories through fat than carbohydrates (63.66% fat vs. 18.39% carbohydrates) whilst the majority of calories provided by the standard chow is through carbohydrates (7.42% fat vs. 75.09% carbohydrates), with calories provided by protein similar so protein availability is not a limiting factor during muscle protein synthesis for both groups (Table 6.1).

6.3.2 - Animal Information

60 female mice (strain CD-1, Charles River, UK) were purchased at 9 weeks old and matured in-house in groups of 8-10 at Coventry University without access to running wheels. Based on previous work investigating obesity in mouse models, a 9-week HFD protocol was used in this study. Feeding durations around this period of time have been shown to promote increased adipose tissue

accumulation (Lin *et al.*, 2000; de Wilde *et al.*, 2009; Tallis *et al.*, 2017) and as such ensured that there was a significant change in body mass and adipose accumulation in the present study. Access to standard lab chow and water was provided *ad libitum* until 68 weeks of age, at which mice were divided into cages of 10 and assigned to either a control (n=30) or high-fat diet (HFD; n=30) group, ensuring each group was matched for body mass (Control - 49.8±1.2g; HFD - 49.8±1.3g; mean ± SEM; P=0.99). No animals were excluded from this study due to health complications.

Animals were allowed to adapt to their new groups for 2 weeks, with each dietary protocol commencing at 70 weeks of age for a duration of 9 weeks. The control group was maintained on a diet of the standard lab chow whilst animals in the HFD group were provided with a self-selected forage diet consisting of husked sunflower seeds in addition to the standard chow. Both the HFD group and the control group had *ad libitum* access to the prescribed diet(s) and water throughout each feeding protocol. A justification for usage of a 79-week-old age group is provided in section 3.1.

6.3.3 - Assessment of Body Composition

It was important to quantify the amounts of adipose gained by the obese animals during feeding of the HFD and following completion of the dietary protocol. Mice aged 68 weeks had baseline measures of body circumference made around the lower abdominal region, with NAL and BM took in order to provide an estimate of body composition prior to feeding. Once the 9-week feeding regime had commenced, the aforementioned measures were taken every 2 weeks for each group to map the change in body composition during a HFD or control diet. Body circumference was measured in millimetres using a textiles tape measure wrapped around the mid-region beneath the thorax of the mouse. Nasoanal length (NAL) (cm) was measured from snout to anus using a set of digital callipers (Fisher Scientific™ 3417, Fisher Scientific, Loughborough, UK), whilst body mass (BM) (g) was assessed to 1 d.p. using a digital balance (PPS2102, Fisher Scientific, Loughborough, UK). These values were

then used to calculate the Body Mass Index (BMI) of the animal and Lee Index of Obesity (LIO). The BMI of the animal was calculated as follows (Equation 6.1):

$$Body\ Mass\ Index = \frac{BM[g]}{(NAL[cm]^2)}/100$$

Equation 6.1 - The equation used to calculate the Body Mass Index of a rodent (Sjögren *et al.*, 2001).

The LIO value was multiplied by 1000 as per previous research (Bernardis and Patterson, 1968; Bernardis, 1970; Bernardis *et al.*, 1978; Kanarek and Marks-Kaufman, 1979; Novelli *et al.*, 2007; Malafaia *et al.*, 2013). The equation for LIO is as follows (Equation 6.2):

$$Lee\ Index\ of\ Obesity = \frac{\sqrt[3]{BM[g]}}{NAL[cm]}\ x\ 1000$$

Equation 6.2 - The equation used to calculate the Lee Index of Obesity of a rodent (Bernardis and Patterson, 1968).

A value less than 300 for LIO is considered "normal" with anything greater than this classified as "obese" (Bernardis, 1970) for a 28-day old age group. No data exists to determine what is considered "normal" and "obese" in terms of LIO or BMI in an aged mouse population. Despite this, usage of the LIO is a useful tool as it allows for the estimation of rodent body composition *in viv*o with a high degree of accuracy when compared against total body fat (Rogers and Webb, 1980). As this project involves the sacrifice of mice, it was possible to assess body composition via *in vitro* methods, therefore cadaver analysis was also used by dissecting gonadal fat pads and weighing them.

6.3.4 - Muscle Preparation

At 79 weeks of age, animals were sacrificed and weighed to determine BM, NAL and body circumference around the abdomen. BM and NAL were used to calculate BMI (Equation 6.1) and LIO

(Equation 6.2) for each individual. Gonadal fat pad mass (FPM) was dissected and weighed to determine the differences in fat accumulation in response to each diet.

Whole EDL or soleus (n=10 per muscle per group) was dissected from the left hind limb and rapidly frozen in liquid nitrogen. The soleus and EDL of the right hind limb were isolated, and the proximal tendon secured via aluminium foil T-clips and a piece of bone left at the distal tendon. A ventral segment of the costal diaphragm (n=10 per group) was isolated from the left-hand portion of the rib cage and frozen in liquid nitrogen, with the right-hand portion prepared with an aluminium foil T-clips wrapped around the central tendon of the diaphragm segment and the two ribs anchoring the muscle left intact.

Each muscle was placed in a flow-through chamber circulated with oxygenated Krebs-Henseleit solution maintained at 37±0.2^{o}C.

6.3.5 - Assessment of Isometric Force

Muscle length and stimulation parameters (12-16V for soleus and diaphragm, 14-18V for EDL; stimulation amplitude 160mA and pulse width 1.2ms) were altered until maximal twitch force was achieved. Using these parameters, the maximal tetanic force was measured by subjecting the muscles to trains of electrical stimuli (250ms for EDL and diaphragm, 350ms for soleus) with stimulation frequency (120-140Hz for soleus and diaphragm, 200-220Hz for EDL) adjusted until peak tetanic force was achieved. Time to half peak tetanus (THPT) and last stimulus to half tetanus relaxation (LSHR) were measured as indicators of muscle activation and relaxation respectively. A 5-minutes rest period was imposed between each tetanic stimulation in order to allow for sufficient recovery. All muscles followed this process of isometric measures prior to WL protocol.

6.3.6 - Assessment of Work Loop Power

Initially, a cycle frequency (CF) of 10Hz for EDL, 7Hz for diaphragm and 5Hz for soleus was used as these CF's typically elicited maximal power output in previous research for 78-week old locomotor (Hill *et al.*, 2017) and young respiratory (Tallis *et al.*, 2014, 2017) skeletal muscles. At these CF's, phasic bursts of electrical stimulation were provided for durations of 50ms, 55ms and 65ms to the EDL, diaphragm and soleus respectively. Stimulation duration and strain was altered on an individual basis to ensure maximal WL power.

PO was determined across a range of CF's in order to produce a power output-cycle frequency curve (PO-CF) (James *et al.*, 2011). The CF denotes the rate at which a WL is performed. Production of a PO-CF curve determined if there was a shift in optimal CF to produce power following a HFD, and whether power output changed at fast and slow CF's. The CF's tested ranged from 2-10Hz for soleus, 4-18Hz for EDL, and 3-12Hz for diaphragm and were selected at random. Strain (length change amplitude) and burst duration (electrical stimulus duration) were altered at each CF for each muscle to ensure peak net work production. For the other CF's, as CF increased, strain and burst duration increased also, and vice versa.

Control sets of WLs were performed using the parameters that elicited maximal net work (soleus, 5Hz; EDL, 10Hz; diaphragm, 7Hz) every 3 to 4 sets of WLs and following examination of net work for the final CF of each muscle, to monitor changes in net work over the course of the experiment. Any variation in net work was due to an impairment in force production. Therefore, the power produced by each muscle at each CF prior to the fatigue run was corrected to the control run that yielded the greatest net work, assuming that alterations in power production were linear over time (James *et al.*, 2011).

6.3.7 - Fatigability and Recovery

Each muscle underwent 10-minutes of rest prior to the fatigue run. To determine fatigability, each muscle was subjected to fifty consecutive WLs using the optimised stimulation parameters at 5Hz, 7Hz and 10Hz for soleus, EDL & diaphragm respectively. The net work of every second WL was plotted against time until each muscle produced <50% of the pre-fatigue maximal PO. This method has been used previously to examine muscle fatigability (Tallis *et al.*, 2014, 2017; Hill *et al.*, 2017).

The ability of each muscle to recover from fatigue was monitored for 30-minutes immediately following the fatigue run. Every 10-minutes, one set of four WL cycles were performed and net work was recorded and compared to the pre-fatigue maximal power output (Tallis *et al.*, 2014; Hill *et al.*, 2017).

The experimental protocol for each muscle was ~190 minutes from the moment of cervical dislocation to the final WL performed 30-minutes after the fatigue run. Muscle performance prior to the fatigue run declined by 10±2% (S.E.M), indicating that the quality of all muscle preparations was well maintained throughout the experimental protocol as with similar studies utilising this methodological approach (James *et al.*, 1996).

Following the experiment, each muscle was removed from the mechanics' rig, with the tendons removed and the muscle blotted with tissue prior to being weighed. This allowed for force ($kN.m^{-2}$) and power ($W.kg^{-1}$) to be normalised to muscle mass.

6.3.8 - Statistical Analysis of Data

All data are presented as the mean ± S.E.M. The level of significance was set at $P<0.05$ for all analyses. Initial tests for normality and homogeneity were performed to determine the appropriate statistical analyses. Differences in animal anthropometrics and isometric properties between the control and

HFD groups were measured using an independent Student's t-test. Comparisons of the absolute and normalised PO-CF data was assessed using two-way analysis of variance (ANOVA) using SPSS (IBM SPSS, IL, USA), with diet and CF as factors.

Repeated measures two-way ANOVAs were performed to determine if the fatigue protocol induced a significant reduction in muscle power output and whether this was affected by diet. Independent samples T-test were used to determine significant differences in time taken to reach <50% of the pre-fatigue maximal power output for all muscles of each dietary group. Recovery was assessed by a two-factor ANOVA with time and diet as the factors. An independent samples t-test was used to determine whether there was a significant difference between diet groups in power output normalised to muscle mass after 30-minutes of recovery.

The truncated product method (Zaykin *et al.*, 2002) was used to analyse the distribution of *P*-values to provide a *P*-value for each group of multiple hypothesis tests to assess whether these values were biased via multiple hypothesis testing. The truncated product method *P*-value was <0.001, demonstrating that the results were not biased based on multiple hypothesis testing.

6.4 - Results

6.4.1 - Animal and Muscle Morphology

The HFD diet group had 24% greater BM, 21% greater body circumference and 119% greater FPM than the control group (Table 6.2, $P<0.0001$ in all cases). Furthermore, the HFD group nasoanal length, LIO and BMI was 2%, 5% and 13% greater respectively (Table 6.3, $P<0.04$ in all cases). The HFD group had 16% greater soleus mass and 18% greater EDL mass (Table 6.3, $P<0.04$ in both cases), though soleus and EDL muscle length was not significantly different between each group (Table 6.3, $P>0.30$ in both cases). The same comparisons of muscle morphology and contractile performance cannot be made for the diaphragm as only a section of this muscle was used in the examination of contractile performance, with weighing of whole diaphragm not viable due to the requirement of ribs being intact to the diaphragm segment required for testing. The FPM of the HFD group accounted for a greater percentage of the total BM than the control group (Table 6.2, $P<0.001$). However, when soleus and EDL muscle mass was expressed as a ratio to animal BM, there were no significant differences between the control and HFD groups for either muscle (Table 6.3, $P>0.66$).

Table 6.2 - The effects of 9-weeks of a high-fat diet on animal anthropometrics.

	Control	**High-Fat Diet**	**P-Value**
Body Mass (g)	47.2±3.0	58.6±3.6	<0.001
Nasoanal Length (cm)	11.8±0.2	12.4±0.2	<0.001
Body Circumference (cm)	8.4±0.4	10.6±0.6	<0.001
Body Mass Index ($kg.m^2$)	0.34±0.01	0.38±0.02	<0.001
Lee Index of Obesity	305±5	313±5	0.04
Fat Pad Mass (g)	3.6±0.9	7.9±1.2	<0.001
Fat Pad Mass:Body Mass (%)	7.0±0.8	12.8±0.8	<0.001

Values presented as mean ± S.E.M; n=30 control; n=30 high-fat diet.

Table 6.3 - The effects of 9-weeks of a high-fat diet on the muscle-specific morphology.

	Soleus			EDL		
	Control	**High-Fat Diet**	**P-Value**	**Control**	**High-Fat Diet**	**P-Value**
Muscle Mass (mg)	9.4±0.5	11.0±0.6	0.04	10.6±0.6	12.6±0.5	0.014
Muscle Length (mm)	9.3±0.1	9.5±0.2	0.30	9.1±0.2	9.1±0.2	0.76
Muscle CSA (m^2)	$1.0x10^{-6}$±$4.5x10^{-8}$	$1.1x10^{-6}$±$3.9x10^{-8}$	0.05	$1.1x10^{-6}$±$5.9x10^{-8}$	$1.3x10^{-6}$±$4.2x10^{-8}$	0.02
Muscle Mass:Body Mass (%)	0.52±0.04	0.55±0.03	0.67	0.45±0.04	0.45±0.02	0.98

Values presented as mean ± S.E.M; n=10 per muscle per group. Data not presented for diaphragm as morphological comparisons cannot be made due to different sections of the diaphragm isolated during each preparation.

6.4.2 - Isometric Properties

Absolute tetanus force was unaffected by diet for the soleus and EDL (Figure 6.1 A&C, P>0.21). Maximal tetanus stress was not significantly affected by diet for the soleus or EDL (Figure 6.1 B&D, P>0.63). Whilst there no significant difference between the control and HFD diaphragm in terms of maximal tetanic stress, the effect size was 0.78, indicating a moderate effect of diet on diaphragm tetanus stress (Figure 6.1E; P=0.084). Measures of absolute force and isometric stress for the control soleus and EDL were slightly lower than values reported in our previous work, where absolute force was 224mN and 331mN for the soleus and EDL respectively, and maximal isometric stress was 225kN.m^2 and 269kN.m^2 for soleus and EDL respectively (Hill *et al.*, 2017; Chapter 5). There were no significant differences between the control and HFD group in tetanus activation and relaxation times (Table 6.4, P>0.12 for all measures and muscles).

Table 6.4 - The effect of 9 weeks of HFD on isometric activation (THPT) and relaxation (LSHR) of isolated mouse soleus, EDL and diaphragm.

	THPT (ms)		**LSHR (ms)**	
	Control	**HFD**	**Control**	**HFD**
Soleus	37.6±2.3	40.1±2.2	52.3±3.2	48.0±1.9
EDL	16.0±1.1	16.3±0.9	17.4±1.2	17.6±1.1
Diaphragm	24.4±1.0	26.5±1.2	25.0±1.0	26.8±0.8

Values presented as mean ± S.E.M. n=10 for each muscle of each group. THPT, time to half-peak tetanus; LSHR, last stimulus to half-tetanus relaxation.

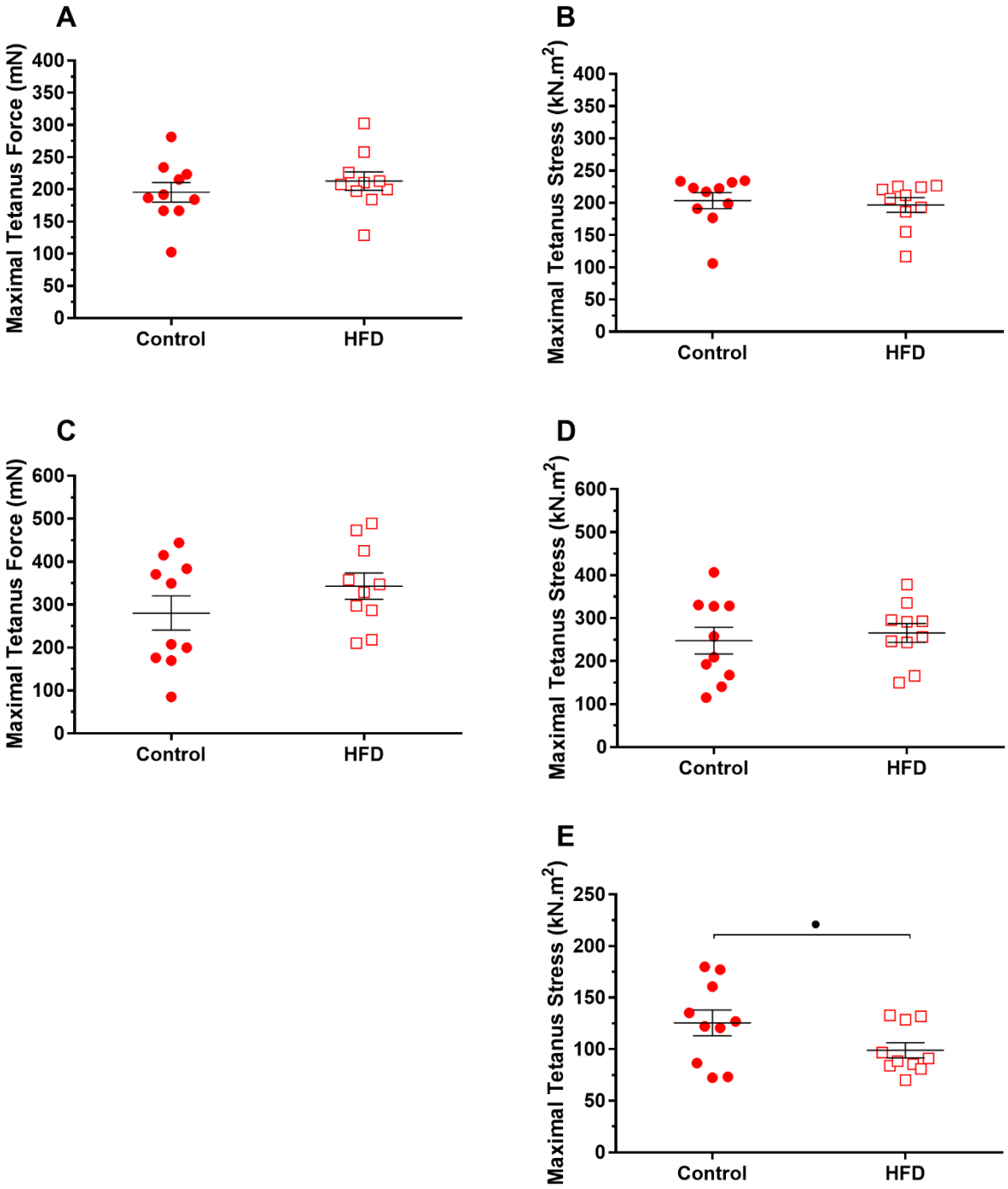

Figure 6.1 - The effect of 9-week high-fat diet (HFD) on the maximal isometric force (A and C) and maximal isometric stress (B, D and E) of isolated mouse soleus (A and B), EDL (C and D) and diaphragm (E). n=10 per muscle per group. • denotes a statistical tendency (P=0.084; ES=0.78). Values presented as mean ± S.E.M.

6.4.3 - Work Loop Power Output

The absolute power output of the soleus and EDL was significantly higher in the HFD group than the control group, increasing on average by 12.5% and 15.0% respectively (Figure 6.2 A&C, P<0.04 in each case). Whilst there was a significant effect for CF in the soleus (Figure 6.2A, P<0.001), this was not the case in the EDL (Figure 6.2C, P>0.16). The effect of CF on the soleus is due to significantly (P=0.01) lower power output at 10Hz compared to all other cycle frequencies. There was no diet*CF interaction for either muscle (P=1.00). When power was normalised to muscle mass, differences were not apparent between control and HFD soleus and EDL (Figure 6.2 B&D, P>0.62 for both muscles). Measures of absolute power and normalised power for the control soleus and EDL were slightly lower than values reported in our previous work, where absolute power CF of 5Hz and 10Hz was 246mW and 1007mW for the soleus and EDL respectively, and maximal WL power at the aforementioned CF's was 25W.kg^{-1} and 83W.kg^{-1} for soleus and EDL respectively (Hill *et al.*, 2017, Chapter 5). In contrast to the locomotory muscles, power normalised to muscle mass for the diaphragm in the HFD group was significantly lower by an average of 27% across all CF's compared to the control group (Figure 6.2E, P<0.001 in each case). CF had a significant effect on normalised and power output for all groups (Figure 6.2 B, D&E, P<0.05 in all cases). There was no interaction between diet & CF for all muscles indicating no alteration in the shape of the normalised PO-CF curves between each group (Figure 6.2 B, D&E, P=1.00 in all cases). There was no relationship between body mass and maximal normalised power output for control soleus, EDL and diaphragm (Figure 6.3A, C&E, r^2<0.39, P>0.07 in all cases). Obese soleus isolated from animals that were heavier in terms of body mass had a significantly lower power output normalised to muscle mass (Figure 6.3B, r^2=0.568, P=0.012). By contrast, there was no significant relationship between maximal normalised WL power and body mass for the HFD EDL or diaphragm (Figure 6.3D & F, r^2<32, P<0.08).

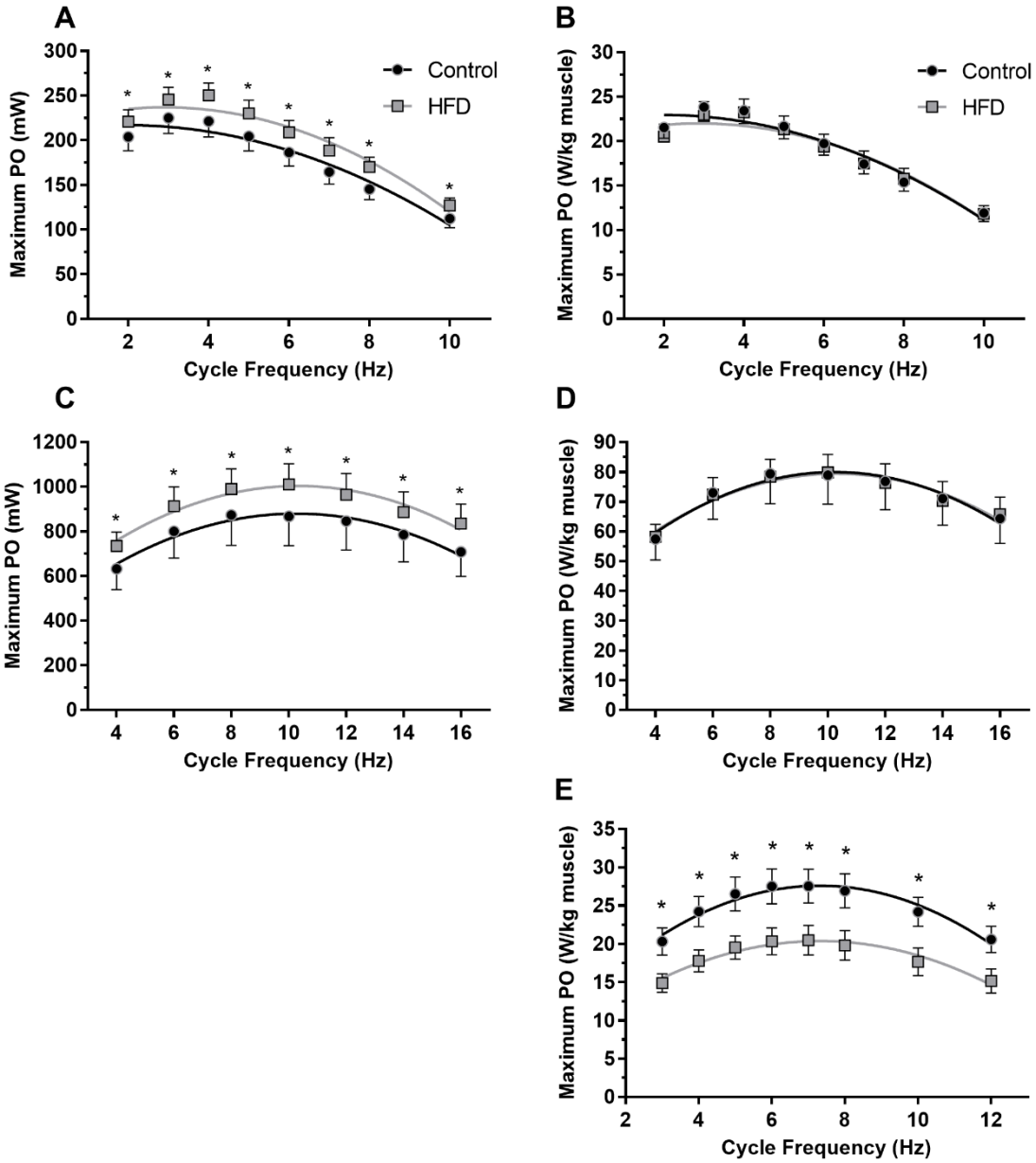

*Figure 6.2 - The effect of 9-week HFD on the maximal normalised power output (A, C and E) and absolute work loop power output (B and D) of isolated mouse soleus (A and B), EDL (C and D) and diaphragm (E) for the control (●) or HFD (■) groups. n=10 per muscle per group. Values presented as mean ± S.E.M. * indicates significant differences between each group.*

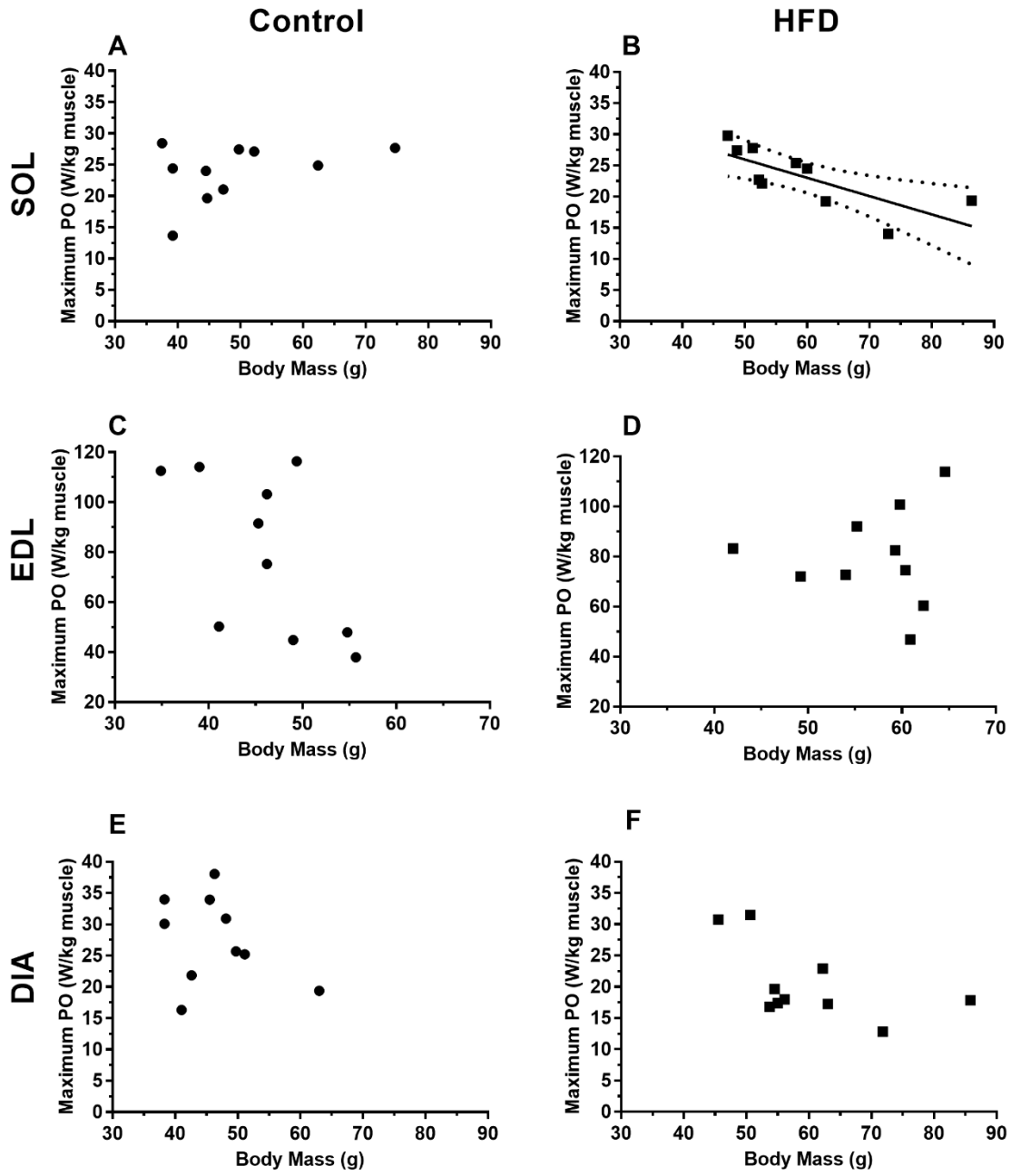

Figure 6.3 - The relationship between whole animal body mass and normalized work loop power for control (A, C, E) and HFD (B, D, F) soleus (A & B), EDL (C & D) and diaphragm (E & F) experimental groups. N=10 per muscle per group. Figure 3 B: the lines represent a first-order polynomial fitted to the data using a least squares regression and the 95% confidence limits of this line.

6.4.4 - Diaphragm Work Loop Shapes

As there were no differences in maximal WL power normalised to muscle mass between control and HFD soleus and EDL (Figure 6.2 A&B), WL shapes were not examined for these muscles.

Maximal WL power output normalised to muscle mass occurred at 7Hz for the diaphragm of the control and HFD groups (Figure 6.2C). The typical WL shapes at this cycle frequency indicate that peak force production was not significantly affected by diet (Control - 65.5±6.0mN; HFD – 58.7±5.1mN, P>0.54), however, force during muscle shortening was typically lower for the HFD diaphragm (Figure 6.4). Additionally, the passive work during lengthening and re-lengthening appears to be greater for the HFD diaphragm than the control, increasing the negative work and consequently decreasing the net work (Figure 6.4).

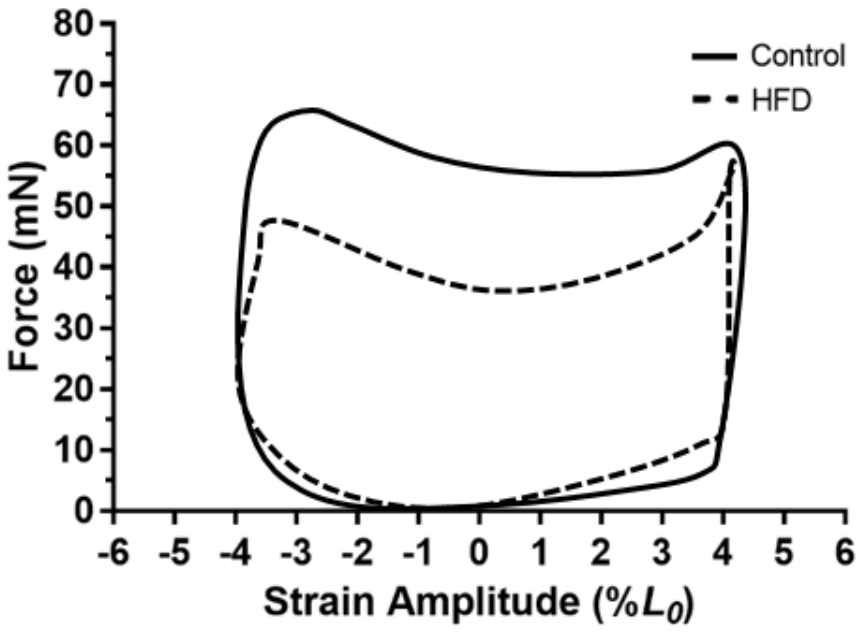

Figure 6.4 - The effect of obesity on 79-week-old diaphragm muscle net work following a control (solid) or HFD (dashed) at a cycle frequency of 7Hz, where peak power output was elicited. Figures plotted as force against strain (%L_0). The third WL of the set of four WL stimulations is shown for each group. WLs to be interpreted in the anticlockwise direction (right-to-left) from 0 of L_0.

6.4.5 - Fatigue Resistance and Recovery

Fifty consecutive WL cycles resulted in a significant reduction in PO, over time for all muscles (Figure 6.5 A, C&E; P<0.0001). However, diet did not significantly affect the time-course of fatigue for each muscle (Figure 6.5 A, C&E; P>0.29), nor time to reach 50% of the pre-fatigue maximum for all muscles (Figure 6.5 A, C&E; P>0.39).

Whilst diet did not significantly impair the ability of soleus and EDL to recover from the fatigue protocol (Figure 6.5 B&D; P>0.27), diet significantly reduced the recovery of power for the diaphragm from HFD individuals when compared to controls (Figure 6.5F; P=0.01) where power was significantly different after 30 minutes of recovery (Figure 6.5F; P=0.02). There was no time effect on the recovery of power for all three muscles (P>0.17), nor was a diet*time interaction observed (P>0.37).

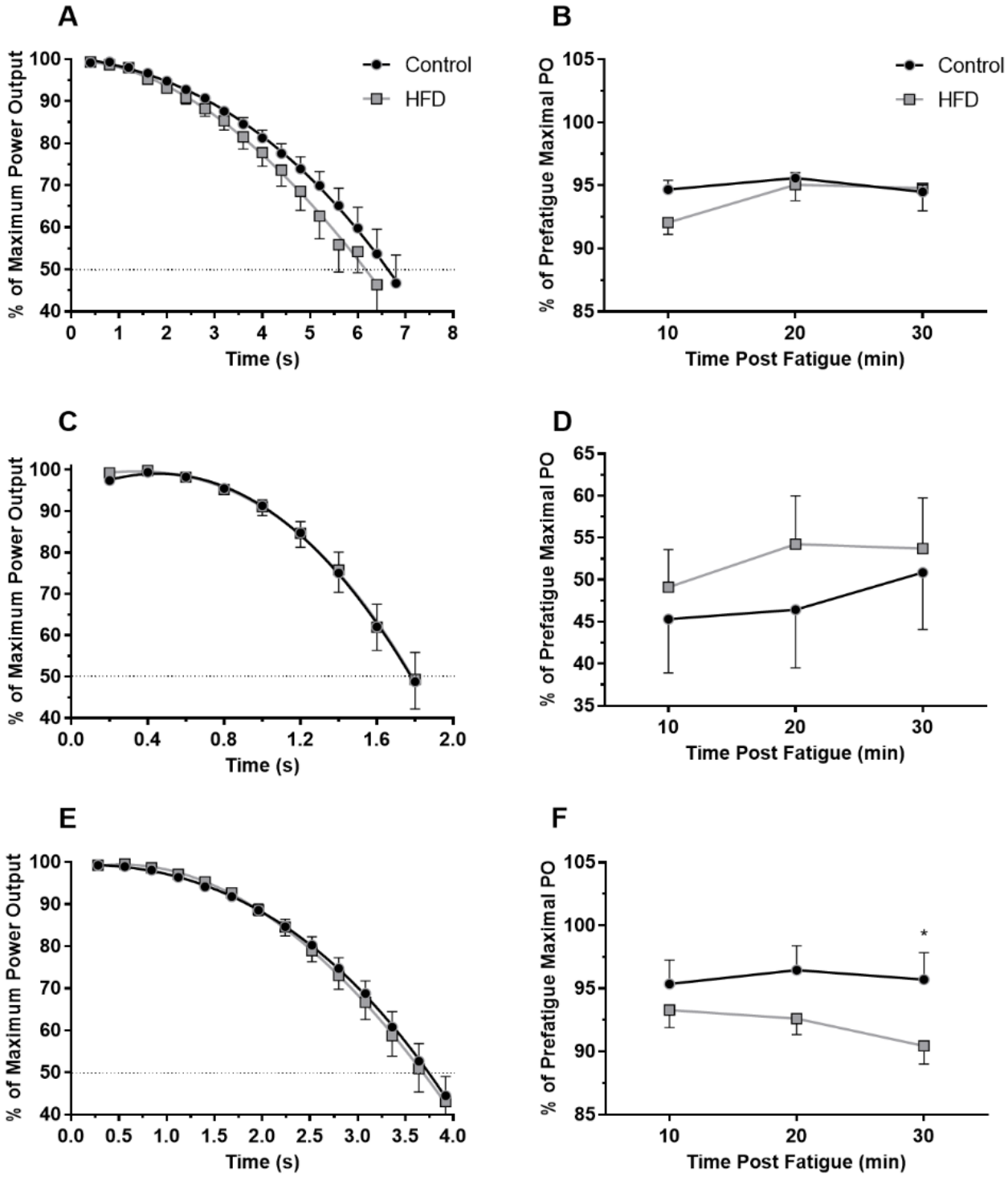

*Figure 6.5 - The effect of 9 weeks of a HFD on the fatigue resistance (A, C & E) and recovery of power (B, D & F) of maximally stimulated mouse soleus (A & B), EDL (C & D) and diaphragm (E & F)) for the control and HFD groups. Values presented as mean ± S.E.M. A * denotes a significant (P<0.05) difference in recovery of power at a given time point*

6.5 - Discussion

The present results indicate that at the muscle level obesity in old age significantly impairs respiratory, but not locomotory, isolated skeletal muscle function. Obesity significantly reduced normalised diaphragm power output of old mice following a HFD in comparison to age-matched control animals, which is likely to be related to impaired force generation during muscle shortening and increased passive work through lengthening. By contrast, absolute power output for the HFD soleus and EDL improved compared to age-matched controls, however, there was no change in muscle quality (force/power relative to muscle size). When power is correlated with body mass, however, the soleus isolated from old obese animals produced significantly lower maximal power output, with a tendency for a similar pattern in old obese diaphragm muscles. Results from younger models of obesity investigating isolated muscle performance indicate a reduction in muscle quality for soleus, EDL and diaphragm (Ciapaite *et al.*, 2015; Matsakas *et al.*, 2015; Tallis *et al.*, 2017), though only a reduction in quality was observed for old obese diaphragm. As such, there is likely to be differing consequences for old obese adults during locomotion and respiration.

6.5.1 - Effects of Ageing & Obesity on Animal and Muscle Morphology

Provision of a calorie-rich diet resulted in the excessive accumulation of gonadal fat and elevated skeletal muscle mass, where an elevated FPM contributed to the greatest difference in body mass between the two groups rather than skeletal muscle mass (Table 6.2 & Table 6.3). The increase in soleus and EDL muscle mass is likely, in part, due to the ectopic accumulation of fat within the muscle (Addison *et al.*, 2014), although the added load upon the locomotor skeletal muscles in the HFD group may stimulate a hypertrophic effect (Maffiuletti *et al.*, 2013). Bott *et al.* (2017) reported a hypertrophic effect of 33-week-old C57BL/6J soleus type I, type IIa, type IIx and type IIb fibre CSA in line with an elevated body mass for their HFD group compared to baseline measures and the age-matched control group, which is unsurprising given the postural position of the soleus, though it is interesting to note

the comparative fibre atrophy of the non-weight bearing EDL in their study following an obesogenic diet.

6.5.2 - Effect of Obesity on Isometric Force and Work Loop Power

The increased absolute power output of the soleus (Figure 6.2A) aligns with previous *in vivo* work demonstrating an increased maximal force of "antigravity", weight-bearing muscles in older obese adults (Miyatake *et al.*, 2000; Rolland *et al.*, 2004; Zoico *et al.*, 2004; Stenholm *et al.*, 2009; Tomlinson *et al.*, 2016), which is unsurprising given the added load of the fat free mass acting as a training stimulus on postural muscles (Garcia-Vicencio *et al.*, 2016). However, the increase in absolute power for the EDL of the HFD group (Figure 6.2C) is surprising given *in vivo* work demonstrates little change in absolute force for non-weight-bearing skeletal muscles of old obese adults, and may consequently be an effect of the CNS (Rolland *et al.*, 2004; Tomlinson *et al.*, 2016). The increased absolute power may seem to be a positive response to obesity, however, *in vivo* locomotor performance is likely to be impaired due to the elevated muscle mass and fat-free mass contributing further to an already elevated body mass. Furthermore, the magnitude of the increase in body mass (24%) is not reciprocated by a similarly proportioned increase in absolute power for the soleus (13%) and EDL (15%), meaning the ability to overcome a greater bodily inertia will require greater muscular effort. It has been shown that, for the soleus, despite unchanged isometric stress and an increase in absolute power output and maintenance of normalised WL power in the HFD group, there is a negative association between animal body mass and maximal normalised WL power which is not evident for the EDL (Figure 6.3 A&B). This muscle-specific effect for the soleus would mean locomotor capabilities, active stabilisation at the ankle (James *et al.*, 1995) and postural control in older obese adults may be significantly impacted, leading to a functional incapacity to perform activities of daily living that are compounded by a poorer gait, slower speed of performing activities, and lower fatigue resistance (Hirani *et al.*, 2017). For example, older adults with a BMI greater than 30 are at greater risk of

experiencing a fall (Mitchell *et al.*, 2014) whilst those with a BMI greater than 35 are more frail than leaner, but not underweight (i.e. BMI<20), older adults (Hubbard *et al.,* 2010).

By contrast, a HFD in older animals caused a significant reduction in diaphragm power output relative to muscle mass compared to age-matched controls (Figure 6.2E). It is likely that, as with locomotor muscle, fat is likely to be stored ectopically within the diaphragm, increasing the non-contractile mass and work required to lengthen the muscle. However, unlike locomotor muscles, adipose tissue loading on the diaphragm is unlikely to induce a hypertrophic effect given that adipose tissue accumulates in the thoracic cavity of obese adults and as such increases respiratory resistance (Sharp *et al.*, 1986; Lazarus *et al.*, 1997). In terms of the WL, a greater non-contractile mass and lower tissue compliance would amplify the work required (negative work) to lengthen the muscle, and would, therefore, decrease maximal net work and power output (Josephson, 1985). The impairment in power output does not appear to be limited by the ability for old, obese diaphragm to produce peak force during cyclical work but instead, maintenance of force during shortening is lower, with a tendency for greater eccentric (i.e. negative) work during re-lengthening compared to control animals (Figure 6.4). Consequently, it is plausible that a reduced capacity for old obese diaphragm to generate power to be a contributor to the increased metabolic and cardiovascular disease risk in old obese adults (Chuang *et al.*, 2016).

6.5.3 - Fatigability and Recovery

Obesity did not cause a significant reduction in the ability to sustain power output over repeated WLs for old obese soleus, EDL or diaphragm (Figure 6.5 A, C&E), nor for locomotor muscles to recover from the fatigue protocol (Figure 6.5 B&D) as found with young obese female mice (DeNies *et al.*, 2014). It should be noted that whilst the pattern of fatigue appears the same for both the control and HFD diaphragm, each data set is plotted as a percentage of the pre-fatigue maximal power. Power normalised to muscle mass is significantly lower in the HFD group, so it should be considered that

power output at 100% for the HFD group to be significantly lower due to a lower starting normalised power, and therefore would be likely to fatigue faster *in vivo* when working against a comparable load to the control diaphragm. Recovery of old obese diaphragm is significantly impaired (Figure 6.5F) despite no change in the fatigue response, and as such requires further investigation. The added load of an increased bodily inertia in older adults could be a significant contributor towards reduced muscular endurance in older adults (Izquierdo *et al.*, 2001). It is expected that the added muscle mass and fat-free mass in old obese adults is likely to further contribute to a reduction in fatigue resistance when working at the same relative intensities due to isolated skeletal muscles fatiguing at the same rate with no change in muscle quality (Tallis *et al.*, 2017). As such, it is more likely the increased demand placed on the muscle due to an elevated body mass, rather than the ability of the skeletal muscle to withstand fatigue, may potentially explain the reduction in whole animal exercise tolerance following a HFD (Matsakas *et al.*, 2015) and slower gait velocity in old obese adults (Huo *et al.*, 2016).

6.5.4 - Comparisons of Contractile Performance between Young and Old Models of Obesity

Whilst obesity studies in young rodents share similar characteristics with the present study, such as inducing obesity via diet, and comparing the soleus and EDL to represent phenotypic differences, comparisons are difficult due to the different methodological approaches including feeding duration and diet composition, a lack of classification of what is considered obese for rodent models, and different test temperature for isolated skeletal muscles (Tallis *et al.*, 2018).

For soleus, absolute force, isometric stress, and absolute and normalised power output remain unchanged or even improve following an obesogenic diet in young mice (Bott *et al.*, 2017; Tallis *et al.*, 2017) which does not differ to the current findings. The EDL response, however, is more ambiguous with previous studies reporting a reduction in force and stress in young obese EDL (Matsakas *et al.*, 2015), whereas others report no change in isometric stress (Ciapaite *et al.*, 2015; Bott *et al.*, 2017). The diaphragm appears to be affected by obesity irrespective of age (Tallis *et al.*, 2017). It is possible

that obesity has a phenotypic effect on skeletal muscle fibres, where the contractile function of type II and IIa/x fibres are affected to a greater extent than type I fibres, which may explain the muscle-specific differences in young and old obese contractile performance. With age, muscle composed of predominantly fast-twitch muscle fibres experience shifting towards a slow-twitch composition, which consequently may be less affected by obesity than fast-twitch fibres, hence no change in muscle quality for old EDL and soleus. To date, no study has examined the effects of obesity on single fibre contractile function to determine whether obesity has a phenotypic response.

6.6 - Conclusion

Absolute force and power, muscle quality, and fatigability of locomotor muscles are well maintained following a HFD in old animals. By contrast muscle quality of old obese diaphragm is significantly lower compared to lean, age-matched counterparts. These findings differ to that of young obese skeletal muscles, where both force and power normalised to muscle mass generally declines for EDL and diaphragm, though soleus muscle quality is well maintained irrespective of age. An elevated body mass in old obese adults is likely to act as a training stimulus on the soleus and EDL, as demonstrated by an increase in the absolute power for the locomotor muscles. Particularly for the soleus, where a larger body mass correlated with poorer normalised power output, an increased bodily inertia will mean acute and sustained *in vivo* locomotor performance is likely to be substantially affected due to a larger limb mass and body mass creating a greater work demand on the skeletal muscle. The elevated fat mass loaded on the diaphragm *in vivo* could be a plausible contributor to a reduction in muscle quality as found *in vitro*. This may potentially elevate respiratory disease risk and further contribute to the negative cycle of obesity.

Chapter 7 - General Conclusions

The current findings from the thesis have provided a detailed account of the age-related changes in animal and muscle morphology, and the contractile properties of isolated locomotor and respiratory skeletal muscles, considering the confounding effects of sex, mode of muscle activity (isometric, concentric and eccentric), and dietary-induced obesity.

A summary of the key findings from the thesis are detailed below:

1. 3-week males are morphologically larger in terms of body mass and skeletal muscle size and have more powerful muscles than age-matched female counterparts. The sex-based differences in skeletal muscle size and function at this age are likely to be related to hormonal differences (**Chapter 4**).

2. In the first instance, the loss of muscle isometric stress exceeds that of the loss of normalised power up to the age of 52 weeks of age. By 78 weeks of age, however, the loss of normalised power exceeds the loss of isometric stress. The loss of normalised contractile performance occurs to a greater extent for males than for females, with the loss of performance occurring to a greater extent for soleus and diaphragm (**Chapter 4**).

3. Absolute tetanic force and absolute power output are well maintained until 30 and 52 weeks of age for soleus and EDL, and in some cases increased, with absolute power greater in males than females but not differently so in terms of absolute force. A significant reduction in absolute force and power occurs from 52 weeks to 78 weeks of age for all muscles. The reduction in absolute force and power from the maximal occurs to a similar magnitude to that of normalised contractile performance. **(Chapter 4).**

4. Unlike Tallis *et al.* (2014) a clear pattern of fatigue is not present in this study. Whilst the greatest fatigue resistance occurred at 3 weeks of age for all skeletal muscles, only male EDL and female diaphragm underwent any further reductions the ability to sustain concentric power. Moreover, there are few instances of any sex-based differences in the fatigue response (**Chapter 4**).

5. Absolute eccentric power output and eccentric power output normalised to muscle mass of isolated soleus and EDL is relatively well maintained in 78-week-old animals versus 10-week-old animals compared to the age-related decline in concentric power when utilising a strain amplitude that more closely aligns to *in vivo* muscle strains. As muscles produce the same, or lower, eccentric power output, but are larger, fall risk in older adults may in part be related to a decreased ability to overcome an increased bodily inertia (**Chapter 5**).

6. Changes in eccentric power during sustained eccentric muscle activity and the ability to recover concentric power was muscle specific. There were no age-related changes in eccentric power over time for the soleus and was highly resistant to the fatiguing effects of sustained eccentric activity. By contrast, 78-week-old EDL was able to sustain eccentric power for a longer time period than 10-week-old EDL, and consequently recover greater concentric power. Eccentric activity could be a useful integration into an exercise regimen for older adults (**Chapter 5**).

7. Consumption of a HFD in old age may cause an ectopic accumulation of fat, though this does not translate to reduced contractile performance of locomotor muscles. The added load of an elevated body mass, due to an increased fat mass, may instead act as a training stimulus on the load on the muscles and as such a hypertrophic effect may be present for old obese soleus and EDL (**Chapter 6**).

8. Dietary-induced obesity in old age causes a significant increase in absolute power of isolated soleus and EDL, but this did not translate to improved absolute force, performance normalised to muscle mass or altered fatigue resistance, despite a significantly greater body mass. This is likely to transpose to poorer *in vivo* locomotor performance given that a HFD leads to greater adiposity leads to greater body mass for the same power output (**Chapter 6**).

9. As with younger models of obesity, obesity in old mice has a significantly detrimental effect on diaphragm muscle quality that is linked to reduced force during muscle shortening. Therefore, poorer diaphragmatic power could be a limitation of fat oxidation and therefore contribute further to the negative cycle of obesity (**Chapter 6**).

The results from this thesis demonstrate that skeletal muscle ageing is not a uniform, single factor process, where the sex of an animal, the skeletal muscle examined, and the mode of muscle activity has a significant impact on the age-related changes in isolated skeletal muscle contractile performance. Additionally, changes in contractile function are dependent on whether force or power is expressed in absolute terms or normalised to muscle mass. The comprehensive analysis of the morphological and contractile properties of isolated skeletal muscles allows for targeting specific muscle at a specific age for both males and females in the exploration of therapeutic strategies that can sustain or improve muscle function with increasing age.

In general, ageing results in a greater loss of muscle quality (i.e. isometric stress and normalised concentric power output) compared to a relative maintenance, and even improvement, of absolute force and power. The loss of muscle quality occurs without a significant decline in muscle mass with increasing age, indicating that dynapenia rather than sarcopenia remains a key determinant of poorer skeletal muscle function in CD-1 mice at 50% of their expected lifespan (Navarro *et al.*, 2002; Clark and Manini, 2008). Whilst previous work has confirmed that ageing causes a decline in muscle quality

without prevalent atrophy (Chan and Head, 2010; Tallis *et al.*, 2014) the present work is the first to demonstrate the sex-based differences, muscle-specific responses, contraction mode-specific, and the influence of obesity, on skeletal muscle power output across a range of cycle frequencies using the WL technique (Josephson, 1985).

In humans, comparing contractile performance, specifically muscle quality, between sexes is methodologically difficult, and ultimately fails to capture the muscle-specific nature of skeletal muscle ageing (Edwén *et al.*, 2014; Fragala *et al.*, 2015). When considering all contractile parameters in tandem, males are more prone to a decline in contractile function than females, with the decline in contractile performance for male soleus most greatly affected compared with all other muscles. When examining isometric contractions, ageing causes a decline in isometric force and stress, though there are relatively few differences between males and females for all skeletal muscles, that is, until 78 weeks of age where male soleus and EDL produces lower isometric stress than females. When considering power output, however, males are more powerful than females in absolute terms, therefore there is a greater capacity for a loss of absolute power (Doherty, 2003). The loss of absolute power output occurs to a greater extent for the soleus than the EDL, though the decline in power tends to occur between 52 weeks and 78 weeks of age for both muscles of each sex, whilst normalised power declines progressively from 10 weeks of age for all muscles. Whilst males are more powerful in absolute terms than females, as soleus and EDL muscle mass is generally greater than females in old age, there are relatively few sex-based differences in normalised power output between 10 weeks and 52 weeks of age for all muscles. Only by 78 weeks of age do female skeletal muscles produce significantly greater normalised power than males. As for the diaphragm, ageing caused a progressive decline in normalised power output for males and females, though female diaphragm is more powerful.

The current results demonstrate that the loss of isometric stress occurs before the loss of normalised concentric power output from 10 weeks to 52 weeks of age, atypical of *in vivo* conditions where the loss of power exceeds the loss of strength with increasing age (Skelton *et al.*, 1994; Metter *et al.*, 1997; Deschenes, 2004). Only between 52 weeks and 78 weeks of age does the loss of normalised power exceed that of isometric stress, primarily due to a greater decline absolute concentric power compared to isometric force. *In vivo* locomotor function is not solely dependent on isometric and concentric contractile function, with eccentric muscle activity a fundamental requirement for successful completion of activities of daily living. Unlike previous studies examining eccentric activity through isovelocity lengthening protocols, where skeletal muscles are deliberately damaged (Call and Lowe, 2016), the present work demonstrates that ageing does not diminish EDL normalised eccentric power output when activated eccentrically via the WL technique. As with chapter 4, ageing caused a significant decline in isometric stress and normalised concentric power for the soleus and EDL, though there was a significant decline in normalised eccentric power output for the soleus. It is worth noting that the differences in absolute force and absolute power of the female soleus, and EDL absolute force, is similar to that in chapter 5. However, female EDL absolute power was approximately 24% lower in study 4 compared with chapter 5 and may be related to the genetic heterogeneity of the CD-1 mouse strain.

One hypothesis for an age-related reduction in contractile function is that elevated adiposity in old age leads to a decline in muscle quality. By inducing obesity via 9 weeks of a HFD, the present thesis shows that obesity in old age does not further reduce isometric stress or normalised power output of isolated locomotor muscles. When body mass and normalised power output was correlated, soleus power output isolated from heavier animals were significantly less powerful than leaner counterparts. Old obese diaphragm, however, had a tendency to generate lower isometric stress and generated significantly lower power output at all cycle frequencies. The greater decline in diaphragm contractile

function with ageing and obesity may, therefore, be a significant contributor to greater respiratory disease risk on old obese adults (Chuang *et al.*, 2016).

Ageing resulted in a significant increase in body mass, with an obesogenic diet in old age causing significant adiposity as demonstrated by an elevated body mass and gonadal fat mass compared to the control animals. Concentric and eccentric absolute power was well preserved with increasing age for the soleus and EDL, with absolute power increasing with age up to 52 weeks of age. In absolute terms, older adults would generate greater absolute power to overcome the increased bodily inertia to maintain locomotor function. However, in the oldest group (i.e. 78 weeks old), absolute concentric power output declines significantly with age with no reduction in body mass. As a consequence, it is expected that locomotor performance would be most inhibited by this age. However, normalised concentric power, and normalised eccentric power declines with increasing age. In the case of the former, larger muscles of poorer quality adds to an already elevated body mass and will consequently limit acute and sustained concentric power *in vivo*, limiting tasks such as stair ascent and moving from a stand-to-sit position (Tallis *et al.*, 2018). As for eccentric muscle activity, the generation of power is limited for soleus, but not EDL, and coupled with an elevated body mass, may contribute to an inability to stabilise the lower musculature and maintain balance. Activities requiring sustained eccentric muscle activity, however, may result in better fatigue resistance *in vivo*. Whilst obesity had little impact on the quality of isolated soleus and EDL, an obesogenic diet exacerbated the decline in muscle quality of the diaphragm, which can further inhibit respiratory function and further contribute to the negative cycle of obesity.

Ageing is associated with greater fall risk and all-cause mortality in older adults (McPhee *et al.*, 2016), largely due to muscle weakness and reduced muscular endurance in older adults (Schwendner *et al.*, 1997; Wang *et al.*, 2016). In addition to a greater number of falls in older adults, females over the age of 60 are more likely to experience a fall compared to males (Gale *et al.*, 2016). In the present study,

the quality of locomotor skeletal muscle in terms of normalised WL power is lost to a much greater extent for males than females, with mean normalised soleus and EDL power output significantly lower in males than females at 78 weeks of age. However, absolute power is significantly greater in 78-week-old male EDL muscle compared to females. As a broad application for both sexes, the loss of tissue quality occurs before the loss of muscle mass and is compounded by an increase in body mass. As the quality of soleus and EDL reduced with age, with either a maintenance or decline in fatigue resistance, a combination of an elevated bodily inertia and muscle mass and a reduction in contractile function means locomotor function is likely to be further inhibited. The magnitude of the effect may be greater in males than females, where males have a greater muscle mass but lower muscle quality than females so would have to generate greater power to overcome a greater bodily inertia. The maintenance in absolute power is likely to be a key in maintaining locomotor performance in light of a reduction in muscle quality.

Fatigue resistance in each study was determined by reporting the change in power output normalised to muscle mass as a percentage of the pre-fatigue maximal power output over time, with the time taken to reach 50% of the pre-fatigue maximal power output as a measure of fatigue resistance (Tallis *et al.*, 2014, 2017). One point of note was that as muscle mass increases with age (Figure 4.1 B&C; Table 5.1) and following the consumption of a high-fat diet (Table 6.2), the increased muscle mass is likely to contribute further to an increased bodily inertia, and therefore decrease *in vivo* fatigue resistance at the muscular level, even with no alteration at the muscular level in terms of fatigue resistance. Little is known, however, to what extent an increase in whole muscle mass relates to the fatigue resistance of a muscle at the muscular level, as it is expected that a greater bodily inertia can contribute to poorer fatigue resistance in older and obese adults (Tallis *et al.*, 2018). Therefore, for each study, muscle mass was correlated against time to reach 50% of the pre-fatigue maximal power output during concentric fatigue to determine whether there was a relationship between increased muscle mass and poorer fatigue resistance. The diaphragm was not examined for each study due to

different segments of the diaphragm taken for each preparation so comparisons between preparations are difficult to make. The correlation analyses are presented in table 7.1. To increase statistical power, common age groups were pooled. Therefore, 10-week-old and 78-week-old animals from chapter five, and 78-week-old animals from chapter six were pooled with the muscles isolated from 10 week and 78-week-old female animals in chapter four.

Table 7.1 - The relationship between muscle mass and time to 50% fatigue.

	Male			
	Soleus		EDL	
Group	r^2	P-Value	r^2	P-Value
3 weeks	0.04	0.65	0.75	0.005
10 weeks	0.20	0.19	<0.01	0.84
30 weeks	0.02	0.72	0.24	0.22
52 weeks	0.08	0.49	0.02	0.75
78 weeks	0.40	0.09	0.13	0.39
	Female			
	Soleus		EDL	
Group	r^2	P-Value	r^2	P-Value
3 weeks	<0.01	0.98	<0.01	0.83
10 weeks•	0.10	0.39	0.28	0.13
52 weeks	0.15	0.27	0.03	0.66
78 weeks*	0.03	0.36	0.04	0.31
	Obese Females			
	Soleus		EDL	
Group	r^2	P-Value	r^2	P-Value
High-fat Diet	0.04	0.60	0.08	0.42

N = 8-10 per muscle per group.

A * indicates n = 28-30 per muscle per group.

A • indicates n = 20 per muscle per group.

The correlation analyses revealed that, generally, that there was no relationship between muscle size and the ability to withstand the fatiguing effects of sustained concentric activity at the muscular level (Table 7.1). Even with an increase in muscle mass following a HFD in 78-week-old soleus and EDL, no relationship was observed between muscle mass and fatigue resistance (Table 7.1). Only one instance was observed where there was a significant relationship between muscle mass and fatigue resistance (Table 7.1). 3-week-old male EDL exhibited a significant relationship between muscle mass and fatigue resistance, where larger muscles exhibited poorer resistance to fatigue (Table 7.1). Despite this, no significant differences in fatigue resistance were observed between 3-week-old male and female EDL fatigue resistance, with 3-week old male EDL more fatigue resistant than all other ages despite this association.

An increased muscle mass is therefore unlikely to alter fatigue resistance at the muscular level, with increased appendicular limb fat mass and body mass, contributing to a greater bodily inertia, more likely to contribute to the poorer fatigue resistance exhibited in larger and older adults more so than the contractile properties at the muscular level (Izquierdo *et al.*, 2001; Huo *et al.*, 2016). This is particularly pertinent where gonadal fat pad mass accounted for a significantly greater proportion of animal body mass following a HFD compared with controls (Table 6.2), though no differences between muscle mass to body mass ratio was observed for either muscle following a HFD (Table 6.3).

The quality of the diaphragm declines linearly with age to equal magnitudes between males and females, though female diaphragm is more powerful than male diaphragm between 10 weeks and 78 weeks of age (chapter 4). This progressive decline in power occurs faster in the early stages and later stages of ageing compared to soleus and EDL. As a consequence, cardiorespiratory function is more likely to be impaired earlier than the reduction in locomotor muscle quality. This may ultimately contribute significantly to the increased cardiorespiratory disease risk that is associated with a reduction in diaphragm contractile function in older adults (Polkey *et al.*, 1997; Criswell *et al.*, 2003).

Chapter 8 - Limitations and Future Work

There are a number of considerations to make given the context of the findings of this thesis. Interpretation of these results is limited by several factors, which in turn provide unique opportunities for further studies. The limitations of the thesis are outlined below.

This thesis provides a simplified approach to assessing dynamic muscle activity that occurs *in vivo*. A sinusoidal waveform was used as an approximation of the otherwise complex cyclical length change patterns that occur during *in vivo* locomotion (Dickinson *et al.*, 2000). In reality, fibre recruitment and length change waveform are likely to be manipulated throughout the activity *in vivo* (Wakeling and Rozitis, 2005). During locomotion, the pattern of activation is likely to frequently change to maximise positive work and minimise negative work during concentric activity, and vice versa during eccentric activity, to maximise the muscle's ability to sustain power output for longer durations.

Whilst reporting isometric stress and normalised WL power provides a measure of muscle quality, the reduction in stress with ageing is likely to be primarily caused by a reduction in the performance of contractile tissues rather than the increase in non-contractile mass (Tallis *et al.*, 2017). In older muscles, it is expected that a smaller proportion of the muscle mass is contractile proteins due to a greater non-contractile mass, such as increased intramuscular fat deposition, meaning the density of the muscle is likely to decrease. As we assumed a constant muscle density with age, the muscle CSA may be underestimated in the older skeletal muscles due to an overestimated muscle density (Tallis *et al.*, 2014). This may mean that the isometric stress is likely to be an overestimate in older muscles, meaning the magnitude of the decline in normalised force may be greater than reported. It would be useful in future studies to analyse skeletal muscle protein and contractile protein across a similar age range and normalise contractile function to lean tissue mass. Doing so shall provide a valuable insight into the underpinning mechanisms for observed changes in contractile function relative to the increase in muscle mass in older animals.

A 9-week HFD was used to induce a significant gain in weight and adipose tissue in mice. It is recognised that during normal feeding habits in humans, it is not common for older adults to become obese in a short duration via a HFD. Obesity in older adults is generally a transient process over many years of poor dietary choices and leading a sedentary lifestyle. Examining the impact obesity has on skeletal muscle function during feeding over a larger proportion of an animal's lifespan, and provided at a younger age, would provide a more relatable scenario to human dietary habits.

In light of the findings from the thesis, a number of future potential studies can be performed to further our understanding of the effects of age and obesity on skeletal muscle, and potential strategies that can be used to negate their negative effects on muscle contractility.

- What are the actual *in vivo* implications related to a greater bodily inertia and poorer muscle quality? Biomechanical analyses and a battery of functional tests, such as treadmill running, gait analysis and grip strength, comparing leaner and larger humans or rodents can provide a useful insight into the impact body size, or appendicular limb fat mass has on biomechanical factors and the ability to complete functional tasks.

- How does ageing and obesity in old age affect the cross-bridge kinetics of single muscle fibres? Ageing and obesity is associated with an increase in intramuscular adipose tissue and may increase muscle stiffness. By removing the muscle belly and non-contractile elements, an examination of the contractile properties of single fibres of isolated skeletal muscles can be made. Many studies have examined single fibre contractile function with increasing age, but generally, use isometric contractions to examine isometric force and stress (Brooks and Faulkner, 1994; Thompson and Brown, 1999; González *et al.*, 2000; Kim and Thompson, 2012, 2013; Kung *et al.*, 2014). Moreover, no study to date has examined the impact obesity has on force or power production of single fibres in either young or old obesity models. Usage of the

WL technique to examine changes in absolute and normalised power during ageing and obesity can provide a unique insight into the consequential effects on contractile performance at the cross-bridge level, independent of the muscle belly and Ca^{2+} kinetics.

- What impact will a prolonged HFD have on *in vivo* locomotor performance and *in vitro* skeletal muscle contractile function? Many studies examining the effects of obesity on skeletal muscle contractile performance use relatively short HFD protocols which rarely exceed 10 weeks (Shortreed *et al.*, 2009; Thomas *et al.*, 2014; Ciapaite *et al.*, 2015; Matsakas *et al.*, 2015; Bott *et al.*, 2017; Eshima *et al.*, 2017). Work by DeNies *et al.* (2014) shows that a prolonged feeding duration of 52 weeks significantly alters animal morphology and skeletal muscle morphology and composition in a muscle-specific and sex-specific manner. However, the corresponding alterations in skeletal muscle contractile function in response to the chronic provision of a HFD has yet to be explored.

- Can calorie restriction prolong skeletal muscle function? Emerging evidence in primates (Colman *et al.*, 2014; Pifferi *et al.*, 2018) and rodents (Heilbronn and Ravussin, 2003) have demonstrated that following a regimen of a reduced calorific intake can prolong animal and human lifespan. The impact of calorie restriction, in both a control and obesity group, on muscle morphology and skeletal muscle contractile function have yet to be explored.

- Acceleration of muscular atrophy during limb disuse. Human studies report significant muscle atrophy and consequent reductions in contractile performance during periods of bed rest, immobilisation, hindlimb unloading and spaceflight (Gao *et al.*, 2018). In older adults, physical inactivity due to poorer locomotory capabilities, and hospitalisation as a result of illness and debilitation, can accelerate the ageing process due to a lack of skeletal muscle usage. These open a number of interesting areas of study; firstly, quantifying the acceleration of the muscle-

specific loss of muscle quality following limb immobilisation in a young and old rodent model, and determining to what extent training and other therapeutic strategies following limb immobilisation can restore skeletal muscle function.

- Serum 25-hydroxyvitamin D [25 (OH) D], more commonly known as vitamin D, has been shown to be a deficient vitamin in US and UK adults, with the vitamin crucial for normal physiological process, including regulation of muscle mass and function (Forrest and Stuhldreher, 2011; Girgis *et al.*, 2013). Evidence in humans has demonstrated vitamin D deficiency is associated with reduced skeletal muscle force production, power output and balance and fall risk (Girgis *et al.*, 2013), with muscle strength, but not muscle mass or power, affected by vitamin D supplementation in humans (Beaudart *et al.*, 2014). However, measures of force and power of isolated skeletal muscle have rarely been performed (Girgis *et al.*, 2013). Those which have performed such work report reduced tricep surae force in vitamin D deficient chicks (Pleasure *et al.*, 1979), significantly lower diaphragm isometric force, but not EDL force, in dietary-induced vitamin D deficient mice (Ray *et al.*, 2013), whilst Schubert and DeLuca (2010) report no effect of vitamin D deficiency on isolated rat soleus force production. No work to date has examined the effect of vitamin D deficiency on the contractile properties of isolated skeletal muscles of older animals, or whether vitamin D provision can have an impact on isolated muscle contractile function in both young and old skeletal muscles, whether this is via a diet rich in vitamin D or through UV ray exposure.

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Appendix

Author	Participant Information	Muscle Assessed	Protocol	Change in Performance?	Comments
			Muscular Strength		
Larsson *et al.* (1979)	114 M (11 – 70 years)	Quadriceps	MI & MD strength tested through knee-extensor activity Muscle biopsies (n=51, 22 – 65 years)	**Yes**	Isometric and dynamic strength increased to 3rd decade, sustained through to the 5th and continued to decline with increasing age.
Murray *et al.* (1980)	72 M (20 – 86 years) Three age groups of 24 (20 – 35 years, 50 – 65 years, 70 – 86 years)	KF & KE	MI and MD strength measured at three joint positions (35°, 45° and 60°) at a speed of 36°s^{-1}. Isometric contractions sustained for 5 seconds.	**Yes**	MI strength 55-65% ↓in oldest vs. youngest. MD strength 45-65% ↓ in oldest vs youngest
Young *et al.* (1984)	50 healthy females in their 20's (n=25) and 70's (n=25)	Quadriceps	MMV isometric strength; CSA measured via ultrasound imaging at mid-thigh.	**Yes**	Strength 35% and CSA 33% ↓in old than young, ($P<0.001$ for both).

Young *et al.* (1985)	24 healthy males in their 20's (n=12) and 70's (n=12)	Quadriceps	MMV isometric strength; CSA measured via ultrasound imaging at mid-thigh.	**Yes**	Strength 39% and CSA 29% ↓ in old than young (P<0.001 for both).
Murray *et al.* (1985)	72 F (20-86 years) Three age groups of 24 (20-35 years, 42 – 61 years, 70 – 86 years)	KF & KE	MI and MD strength measured at three joint positions (35°, 45° and 60°) at a speed of $36°s^{-1}$. Isometric contractions sustained for 5 seconds.	**Yes**	Strength 63% ↓ in oldest vs youngest
Viitasalo *et al.* (1985)	338 M (131 aged 32.9±1.4 yrs.; 138 aged 53.1±1.5 yrs.; 119 aged 72.7±1.4 yrs.)	HG, EF, KE, TF, TE	MI strength for all muscles	**Yes**	KE ↓ 47% from youngest to oldest. HG ↓ 42% youngest to oldest TE ↓ 42% youngest to oldest TF ↓ 35% youngest to oldest EF ↓ 35% youngest to oldest.
Kallman *et al.* (1990)	Cross sectional:847 participants in 7 age groups, 20's (20-29) to 80's (80-89) Longitudinal: 342 participants	HG	HG strength with hand held dynamometer	**Yes**	Cross-sectional: Peak in 30's, but not different vs 20's and 40's Strength ↓ 37% in 80's vs. 30's, accelerates

	from 30's to 70's assessed on average 9 years after initial assessment.				with age from 40's Longitudinal: 30's strength ↑ 0.33±0.23 kg.yr after 8.5 years. Strength ↓ 40's to 80's (-0.31±0.12 kg.yr vs. 1.27±0.21 kg.yr) HG strength signif. correlates with MM (r^2 = 0.16) but correlates stronger with age (r^2 = 0.38)
Reed *et al.* (1991)	296 M & F: Middle aged – (33 M & 45 F, 55-64 yrs) Young-old – (74 M & 87 F, 65-	KF, KE, EF, EE, ankle dorsiflexor	MI strength for all muscles in right and left limb	**Yes**	Signif. ↓ strength for all muscles with age for M & F. F strength ↓ more than M strength for all muscles. Arm muscle circumference & area ↓ with

	74 yrs) Old-old – 26 M & 31 F, 75+ yrs)				age in M but not F MQ ↓with age for all muscles but no effect for gender
Overend *et al.* (1992)	13 young M (24.4 yrs) 12 elderly M (70.7 yrs)	KF, KE	KE MI at 60° and KF MI at 30° with MD strength at $0°s^{-1}$ & $120°s^{-1}$, thus creating four test groups	**Yes**	KE MD at $120°s^{-1}$ and $0°s^{-1}$↓ 32.4% and 24.0% in young to old respectively KF MD at $120°s^{-1}$ and $0°s^{-1}$↓ 32.0% and 24.3% in young to old respectively KE MI at $120°s^{-1}$ and $0°s^{-1}$↓ 31.5% and 23.6% in young to old respectively KF MI at $120°s^{-1}$ and $0°s^{-1}$↓ 30.6% and 22.3% in young to old respectively Rate of strength loss ↑ than rate of CSA loss in elderly Strength/CSA ↔ at $0°s^{-1}$ for KE & KF, ↓

					significantly in elderly at 120°s^{-1} in KE & KF in elderly
Skelton *et al.* (1994)	50 M & 50 F n=5 per gender per group every ½ decade (65-69, 70-74, 75-79, 80-84 & 85-89 years of age)	KE, EF, HG	MI strength for all muscles	**Yes**	BM – M heavier than F, though significant negative correlation with age for both (m-r=-0.39; F – r=-0.47). No effect for gender on changes in strength with M strength ↑ than F for all ages. Loss of strength by 1-2% per year.
Bäckman *et al.* (1995)	128 M & F 63 F aged 17-70 years 65 M aged 17- 70 years	EF, KE, KF, DF, HF, HA,	MI strength via dynamometry	**Yes**	F absolute strength 65-70% of strength achieved by men. ↔ when strength normalised to BM Strength peaked at 17-18 years, generally ↔ to 40 years, ↓ by 60 years.

Lindle *et al*. (1997)	346 M & 308 F aged 20-92 years	KE	MI & MD strength at 0, 30 and 180°s^{-1}	**Yes**	MI and MD strength ↑in M vs. F at all ages and velocities. (P<0.001) MI & MD strength ↓ with inc. age in M & F Onset of decline typical occurs in 40's for M & F
Metter *et al.* (1997)	Longitudinal analysis of M (n=837) & F (n=106) at every decade between 20-80 years Cross-sectional analysis of M (n=993) and F (n=184) at every decade between 20-80 years	HG	Longitudinal - HG strength using a handheld dynamometer measured every 1-2 years for average of 9.6 years for M and 3.9 years for F. Cross-sectional – As above but single examination at each decade	**Yes**	Longitudinal - ↓ from 20-80 years in M (P<0.001) but not for F Cross-sectional - HG strength ↓ significantly after 39.8 years for M and 44 years for F (p<0.001). Significant 34% and 32% ↓ in strength for M and F from 20-80 years (P<0.01).
Lynch *et al.* (1999)	364 M & 339 F aged 19-93 years Muscle mass in 224 M & 278 F	KF, KE, EF & EE	MD force measured in KF & KE at 45°s^{-1} & KF & KE at 30°S^{-1} Corrected to MM	**Yes**	30% ↓ in EF & EE strength vs. KF & KE for all ages and genders. M arm and leg MQ ↓ at similar rate with age

					F arm MQ ↓ 20% faster than leg MQ with age Arm MQ ↓ 28% and 20% with age in M & F respectively Leg MQ ↓ 40% for both M & F with age
Frontera *et al.* (2000)	12 M (65.4 ± 4.2 years) assessed, 9 of which assessed after 12 years	KF, KE, EF & EE	MD force measured in 1985-86; reassessed in 1997-98 CSA of thigh, all thigh muscles, quadriceps femoris and flexor muscles taken via tomography Muscle biopsies taken from vastus lateralis	**Yes**	Strength 20-30% ↓ KE & EF ($P<0.05$) CSA ↓ in thigh (12.5%), thigh muscles (14.7%) quadriceps femoris (16.1%) and flexor muscles (14.9%) ($P<0.05$ in all cases)
Klein *et al.* (2001)	22 young M (22.6±2.7 yrs) 13 old M (81.0±6.5 yrs)	EE, EF	MVC during isometric contraction	**Yes**	EF & EE ↓ 27% & 33% in old vs. young respectively Magnitude of decline in each muscle ↔

					EF & EE PCSA ↓ 19% and 28% in old vs. young respectively EF & EE strength/PCSA ↓ in old vs. young
Lauretani *et al.* (2003)	469 M & 561 F (20-85+ years) (20-29 youngest, 85+ oldest)	KE, HG	Maximal torque from knee extension HG strength with hand held dynamometer	**Yes**	M torque - ↓ 60% in oldest vs. youngest group F torque - ↓ 57% in oldest vs. youngest group M HG – ↓ 55% in oldest vs. youngest group F HG - ↓ 59% in oldest vs. youngest group
Bazzucchi *et al.* (2005)	12 M 6 young (28.3±4.8 yrs) 6 old (71.3±0.8 yrs) All participants had similar absolute force.	EF	MI and MD EF strength during a MVC. Torque-velocity measures at 13, 30 60 90 120 and 150°s^{-1} Muscle CSA measured via MRI scan	**Yes and No**	↔ in isometric MVC absolute force, MVC force relative to BMI, or MCV relative to muscle CSA ↔ in muscle CSA with age Torque-velocity ↓ from 60-150°s^{-1} with age

Delmonico *et al.* (2009)	813 M (73.6±2.8 yrs) & 865 F (73.2±2.89 yrs)	KE	MD strength measured at baseline and in a 5-year follow up	**Yes**	M & F ↓ 16.1±20.6% and 13.4±23.0% respectively. Thigh CSA for M & F ↓ 4.9±7.4% and 3.2±7.9% respectively. MQ for M & F ↓ 13.1±20.4% and 11.1±23.8% respectively. Loss of strength greater than loss of muscle CSA
Dey *et al.* (2009)	38 M & 49 F (75 years for both)	HG, KE, EF	MI HG strength, EF strength and KE strength measured at baseline (75 years) and after 5 years (80 years) Body composition determined via bioelectrical impedance	**Yes**	↓ in M & F for all muscle groups (P<0.001). Rate of decline faster in M than F. Fat-free mass ↓in M & F, but more so in M (P<0.001) Body fat percentage ↑in M (P<0.05), F ↔.
			Muscular Power		

(Bassey *et al.*, 1992)					
Skelton *et al*. (1994)	50 M & 50 F n=5 per gender per group every ½ decade (65-69, 70-74, 75-79, 80-84 & 85-89 years of age)	Lower muscle groups	Absolute PO (W) and relative PO ($W.Kg^{-1}$ BM) calculated from leg extension	**Yes**	PO M vs F - greater in M than F (P<0.01) though rate of loss in PO (W) ↑ in M (P=0.002) Loss of PO greater than loss of strength in M (P=0.0001) but not F (P=0.08), with PO declining by 3.5% per year.
Metter *et al.* (1997)	As above	Upper body	Longitudinal - HG strength using a handheld dynamometer measured every 1-2 years for average of 9.6 years for M and 3.9 years for F. Cross-sectional – As above but single examination at each decade	**Yes**	Power peaked in the 30's and 50's for males and females respectively and significantly (P<0.001) declined with age. When expressed as a percentage of power for 20 year olds, power significantly (P<0.001) declined by 42% from 20's to 80's

			Upper body arm crank; maximal effort for 10-15 seconds at 4 separate loads with 30 seconds rest between each load		years of age in men and by 46% in women, though not significantly (P=0.33)
De Vito *et al.* (1998)	52 F (62.2 ± 6.6 years)	Lower leg muscle groups	PPO calculated from CMJ and SJ on force platform	**N/A**	When correlated against age, negative correlations between absolute (W) (P<0.001 for CMJ & SJ) and relative (W.Kg BM) (P<0.05 for CMF & SJ) PO.
Lauretani *et al.* (2003)	As above	Lower body muscle groups	PPO calculated from leg extension Calf CSA	**Yes**	M PO – 74% ↓ in oldest vs. youngest group F PO – 76% ↓ in oldest vs. youngest group M CSA – 31% ↓ in oldest vs. youngest group F CSA – 15% ↓ in oldest vs. youngest group
Macaluso and De Vito (2003)	20 M 10 young (22.8±5.7 years) 10 old (69.5±2.4 years)	Lower body muscle groups	PPO (Watts) calculated from isometric force and velocity during leg press at different loads, with optimal load used to calculated power	**Yes**	PPO ↓ by 61% for old vs. young* Peak force ↓ 52% lower for old vs. young* Optimal velocity ↓21% for old vs. young*

					Loss of power ↑ than loss of strength, with 22.1% difference when expressed as a ratio*
Pojednic *et al.* (2012)	79 M & F 25 middle-aged adults (MH; 47.2±4.5 years) 28 older healthy adults (OH; 73.6±3.5 years) 26 older mobility limited adults (OML; 77.9±4.3 years)	KE and lower leg muscles	MVC during leg extension to produced torque Maximal contraction velocity at 40% of 1RM during unilateral leg extension Muscle CSA measured via CT scan PPO measured at 180°s^{-1} Torque and PO normalised to CSA	**Yes**	MH – absolute and normalised torque, but not velocity, associated with absolute and normalised PO. OH – both force and power significantly associated with PO OML – velocity, but not absolute and normalised torque, associated with absolute and normalised power. Mobility limitations likely due to contraction velocity and not power.
Edwén *et al.* (2014)	127 M & 188 F 107 young (18-34 years)	Lower leg muscle groups	PPO (W. Kg BM) calculated from bilateral CMJ on a force platform	**Yes**	M PPO – ↓ 0.44 $W.Kg^{-1}$ per year F PPO - ↓ 0.29 $W.Kg^{-1}$ per year PPO M vs. F - greater in M than F ($P<0.001$)

	54 middle-aged (35-55 years) 154 old (65-81 years)		Force at PPO (FPpeak) and velocity at PPO measured (VPpeak)		M FPpeak - ↓ 0.07 N.Kg per year F FPpeak - ↓ 0.04 N.Kg per year. FPpeak M vs. F - greater in M than women (P<0.001) M VPpeak - ↓ 0.02 $m.s^{-1}$ per year F VPpeak - ↓ 0.01 $m.s^{-1}$ per year VPpeak M vs. F - greater in M than F (P=0.002)
			Fatigue Resistance		
Davies *et al.* (1986)	32 M and 19 F 12 young M (21.5±2.4 yrs) 8 young F (21.9±2.2 yrs) 20 old M (69.7±2.8 yrs) 11 old F (68.5±2.9 yrs)	Triceps surae	20Hz train of stimulation for 300ms at every second for 2 minutes. Force plotted over the time course	**Yes**	Signif. Age-related ↓ in fatigue for M & F (P<0.01 for both) Old M had signif. ↓ fatigue resistance than F (P<0.01). ↔ for young M and F.

Klein *et al.* (1988)	17 M 10 young (19-32 years) 7 old (64-69 years)	Triceps surae	20Hz train of stimulation for 300ms every second for 10 minutes. Force plotted over the time course	**No**	Signif. decline in force for both groups (P<0.01). ↔ in fatigue resistance for age.
Bäckman *et al.* (1995)	As above	As above	Sustained isometric contractions until RPE reached 17.	**No**	Endurance peaked at 20-29 years for M and 40-49 years for F but did not decline with age
Hicks and McCartney (1996)	56 control M & F 14 old F (65±2.5 yrs) 21 older F (73±3.5 yrs) 13 old M (64±2.04 yrs) 6 older M (72±2.89 yrs)	EF, DF	Intermittent isometric contractions (5s active, 2s rest) for 3 mins. Fatigue defined as loss of torque vs pre-fatigue maximum	**Yes**	M signif ↓ fatigue resistance than F for both EF and DF. Older group signif. ↑ fatigue resistance than old group for both EF and DF.
Lindström *et al.* (1997)	38 M & F 22 young: - 14 M (28±5 years) - 8 F (28±7 years)	KE	100 repeated KE contractions at $90°s^{-1}$ of dominant limb Split into two phases for analyses: - first 40 contractions = fatigue phase - last 30 contractions = endurance level	**Yes and No**	↓ in peak torque and endurance level with age. ↔ for fatigue relative to peak torque or fatigue rate during the fatigue phase.

	16 old: - 8 M (73±2 years) - 8 F (73±4 years)				
Bilodeau *et al.* (2001)	10 young (5 M, 5 F; 25.5±2.7 yrs) 11 old (7 M, 4 F; 76.3±5.8 yrs)	EF	Maximal isometric EF until torque ↓ 50% of prefatigued MVC for 5s.	**Yes**	Time to 50% of prefatigued MVC signif. ↑ in old than young (P<0.05). F signif ↑ time to fatigue than M (P<0.05)
Bazzucchi *et al.* (2005)	As above	EF	Continual, maximal isometric contraction at 30%, 50% & 80% MVC until torque ↓ by 10% for 3 s Time to maintain contraction measured.	**Yes**	Endurance time signif. ↑ in old than young at 50% and 80% MVC (P<0.05 in both cases). ↔ at 30% MVC

Table S1.1 - In vivo assessments of muscle strength, power and fatigue resistance in cross-sectional and longitudinal ageing studies.

Abbreviations as follows: males =M, females = F; knee extensors = KE; knee flexors = KF; elbow flexors = EF; elbow extensors = EE; dorsiflexors = DF; trunk extension = TE; trunk flexors = TF; hand grip = HG; mean maximal voluntary = MMV; maximal voluntary contraction = MVC; maximal isometric = MI; maximal dynamic = MD; muscle quality = MQ; peak power output = PPO; cross-sectional area = CSA; physiological cross-sectional area = PCSA; one rep maximum = 1RM; countermovement jump = CMJ; squat jump = SJ; rating of perceived exertion = RPE; .

Author	Animal Information	Muscle Tested	Protocol	Change in Performance	Comments
			Isometric Testing		
Gutmann and Carlson (1976)	Male rats (Wistar): Young (3 months; n=7) and aged (24 months; n=7).	Whole EDL.	Maximal isometric stimulations *in vitro*. $TwtP_O$, $TetP_O$, TPT, $1/2_{RT}$. Tested at 36°C.	$TwtP_O$ - ↔ with age. $TetP_O$ - ↓ in aged vs. young TPT - ↔ with age. $1/2_{RT}$ - ↑ in aged vs. young.	MM - ↓ in aged vs. young.
Brooks and Faulkner (1988)	Male mice (C57BL/6): Young (2-3 months; n=11), adult (9-10 months; n=14) and aged (26-27 months; n=14).	Whole Sol, EDL.	Maximal isometric and isotonic stimulations *in vitro*. $TetP_O$, $TetSP_O$, activation and relaxation, V_{max}. Tested at 25°C.	$TetP_o$ – EDL ↓ 73% adult to aged.* - Sol ↓78% adult to aged.* $TetSP_O$ – EDL ↓ 78% adult to aged.* - ↔ for Sol with age. V_{max} - ↔ for EDL or Sol with age. THPT & LSHR - ↔ EDL with age. - Sol ↑ in adult and aged for both*	BM - ↑ by 25% from young to adult* and then ↓ by 13% in aged.* MM – EDL ↓ by 13% from adult to aged* - Sol ↓ by 20% from adult to aged*

Zhang and Kelsen (1990)	42 golden hamsters: Young adult (4.9±0.4 months; n=15), adult (12.8±0.2 months; n=13) and aged (18.8±0.3 months; n=14).	DIA strips.	Maximal isometric stimulations *in vitro*. $TetSP_O$, F-Freq THT, $1/2_{RT}$, F-V relationship	$TetSP_O$ – ↓ in maximum and at all muscle fibre lengths with age* - ↔ in fibre length for max $TetSP_O$ F-Freq - signif. leftward shift from 10-40Hz with age (P<0.003). - ↔ with age 50-150Hz (P>0.50). THT & $1/2_{RT}$ - ↑ in aged vs. young and adult* - ↔ in young vs. adult. F-V - ↓ in velocity at given load with age*	BM - ↔ with age Lung volume - ↑ in adult and aged vs. young adult*
Brooks and Faulkner (1991)	Male mice (C57BL/6): Young (2-3 months), adult (12-13 months)	Whole EDL.	Maximal isometric stimulations *in situ.* $TetP_O$, $TetSP_O$. Mouse placed on heated platform at 35°C.	$TetP_O$ - ↓ in young and aged mice vs adult* $TetSP_O$ - ↔ with age.	BM - ↓ in young vs adult and aged*. ↔ from adult to aged. MM - ↓ in young and aged vs adult*

	and aged (26-27 months)				
Phillips *et al.* (1991)	Mice (C57BL/10 or tan coat mutation of the C57 black animal): Young (2.5-8 months; n=12) or aged (28-31 months; n=8).	Whole Sol from left and right hind limb.	Maximal isometric stimulations *in vitro*. $TetP_O$, $TetSP_O$, Activation, Relaxation. Tested at 25°C.	$TetP_O$ - ↓ in aged vs. young $TetSP_O$ - ↓13.3% in aged vs. young Activ. - ↔ with age Relax. - ↑ in aged vs. young	↔ in dry muscle mass/unit of fibre length
Brooks and Faulkner (1994)	Male mice (C57BL/6): Adult (12 months) and aged (27 months)	Single intact EDL fibres	Maximal isometric stimulations *in vitro*. $TetSP_O$, V_{max}, a/P_O. Tested at 25°C.	$TetSP_O$ - ↔ with age V_{max} - ↔ with age a/P_O - ↔ with age.	↔ in fibre CSA or sarcomere length with age.

Brown and Hasser (1996)	Male rats (Fischer 334 x Brown Norway hybrids): 6, 12, 28 and 36 months old	Whole Sol, EDL	Maximal isometric stimulations *in situ*. Stimulated via nerve and direct muscle. $TwtP_O$, $TetP_O$, $TetSP_O$, TPT, $1/2_{RT}$. Tested at 37°C.	$TwtP_O$ – EDL ↔ with age. - Sol ↓ 31% 6-28 mo., signif diff. by 38 mo.** $TetP_O$ – EDL ↔ 6-28 mo., ↓ 48% by 36 mo. ‡ - Sol ↔ 6-28 mo., ↓ 62% by 36 mo. ** $TetSP_O$ – EDL ↔ 6-28 mo., ↓ 30% 6-36 mo.* - Sol ↔ 6-28 mo., ↓ 31% 6-36 mo.* TPT – EDL ↑ at 28 mo. vs. all ages* - Sol ↓ at 6 mo. vs. all ages* $1/2_{RT}$ – EDL ↓ at 28 mo. vs all ages* - Sol ↑ at 6 mo. vs. all ages*	BM - ↑ 6-12 mo.*, 28 mo. ↑ vs. 6 & 36 mo.*, 36 mo. ↑ than 6 mo.* MM – EDL ↔ with age. - Sol ↓18% 6-36 mo.* ↔ between direct muscle stimulation and nerve stimulation.

Kadhiresan *et al*. (1996)	Rats (Fisher 344): Adult (10-12 months; n=10) and aged (24-26 months; n=8).	Whole medial Gas	Maximal isometric stimulations *in vitro*. $TetP_O$, $TetSP_O$, F-Freq, TPT. Tested at 37°C.	$TetP_O$ - ↓ 29% in aged vs. adult* $TetSP_O$ - ↓ 85% in aged vs. adult* F-Freq - ↔ with age. TPT - ↔ with age.	BM - ↔ with age. MM - ↓15% in aged vs. adult* CSA - ↓ 15% in aged vs. adult* L_0 - ↔ with age.
Criswell *et al.* (1997)	Male rats (Fischer 344): young adult (9 months; n=12) and senescent adult (26 months; n=13)	DIA strip	Maximal isometric stimulations *in vitro*. $TetSP_O$. Tested at 24±0.5°C	$TetSP_O$ - ↓ 16.4% in young vs. adult*	↔ in BM, MM, BM:MM, total MM and L_0 with age.
Lynch *et al.* (1997)	Mice: (C57BL/10ScSn) Young (4-6 months, n=15)	DIA strips	$TetSP_o$, TPT, $1/2_{RT}$, F-Freq. Tested at 25°C	↔ for $TetSP_o$, TPT, $1/2_{RT}$, F-Freq from young to aged	N/A

	Aged (24 months, n=7)				
Pagala *et al.* (1998)	Male mice (C57BL/6J): Young (3-6 months) and old (34-37 months)	Whole Sol, EDL	Maximal and submaximal isometric stimulations *in vitro*. Max and submax tetP_o, TetSP_O at 100Hz (max), TetSP_O at 30Hz (submax). Tested at 20°C	TetP_o - EDL ↓ from young to old‡ - Sol ↓ from young to old* Submax tension - EDL ↓ from young to old‡ - Sol ↓ from young to old* Max tetSP_O – EDL & Sol ↔ with age Submax tetSP_O – EDL & Sol ↔ with age	Muscle Length - EDL & Sol ↔ with age MM – EDL ↓16.4% with age* - Sol ↓ 19.5% with age*
Thompson and Brown (1999)	Male rats (Fischer 344 x Brown Norway F1 Hybrid): Young adult (12 months; n=31), adult (24 months; n=27)	Single intact Sol fibers	Maximal isometric stimulations *in vitro*. TetP_O, TetSP_O, V_{max} Tested at 15°C	TetP_O – greatest at 12 mo., ↓ 31% by 24 mo.* and ↓ 50% by 36 mo. vs 12 mo., 24 mo. vs 37 mo. signif. lower* TetSP_O – greatest at 12 mo., ↓20% by 24 mo.* & maintained to 36 mo. with ↓ 32% - 37mo. signif. ↓ vs 12, 24 & 30mo.*	BM - ↑32% from 12-30 mo.* - ↓by 36 mo vs 30 mo.* MM - ↔12-30 mo. - ↓26% by 36-37mo.* MM:BM - ↔12-24 mo. - ↓33% 12-37 mo.*

	older adult (30 months), 36 months (n=19) and 37 months (n=27).			V_{max} - ↔ 12-24 mo., then ↓ 50% between 24-30mo. and maintained*	
González *et al.* (2000)	Mice (DBA and FVB): Young (2-6 months; n=14), adult (12-14 months; n=7) and aged (20-24 months; n=15).	Single intact EDL, Sol fibers	Maximal isometric stimulations *in vitro*. $TwtSP_O$, $TetSP_O$, TPT, $1/2_{RT}$. Tested at 20-21°C.	$TwtSP_O$ – EDL ↓ from adult to aged* - Sol ↔ with age. $TetSP_O$ – EDL ↓ in aged vs young and adult* - Sol ↓ in aged vs young and adult* TPT – EDL & Sol ↔ with age $1/2_{RT}$ – EDL & Sol ↔ with age	Fiber diameter – EDL ↔ with age - Sol ↓ in aged vs young and adult CSA – EDL ↔ with age - Sol ↓ in aged vs young and adult.
Lynch *et al.* (2001)	Male mice (C57BL): Aged ~6, ~17, ~24 & ~ 28 months.	Whole Sol, EDL	Maximal isometric stimulations *in vitro*. $TetP_O$, $TetSP_O$. Tested at 25°C.	$TetP_O$ – EDL ↓ 36% from 6-28 mo. ‡ - Sol ↓ 15% from 6-28 mo. ‡ $TetSP_O$ – EDL ↔ 6-24 mo., ↓ 26% 24-28	BM - ↑ 16% 6-17 mo., ↓ 10% 17-24 mo. and 32% by 28 mo. ‡ MM – EDL ↓ 22% 6-28 mo.‡ - Sol ↓ 16% 6-28 mo.‡

				mo.* - Sol ↓ 15% 6-28 mo.	
Criswell *et al.* (2003)	Male rats (Fischer 344/Brown Norway F1 hybrid): Aged 4 and 30 months	DIA strips	Maximal isometric stimulations *in vitro*. $TwtSP_O$, $TetSP_O$ 1/2TPT, $1/2_{RT}$. Tested at 24±0.5°C.	$TwtSP_O$ - ↔ with age. $TetSP_O$ - ↓ 13% 4-30mo.* 1/2TPT - ↑ with age* $1/2_{RT}$ - ↔ with age.	BM - ↑ with age* MM - ↑ with age* MM:BM - ↔ with age
McArdle *et al.* (2004)	Male and female mice (wild type B6XSJL): Adult (10-12 months) and aged (26-28 months)	Whole EDL	Maximal isometric stimulations *in situ*. $TwtP_O$, $TetP_O$, $TetSP_O$, TPT, $1/2_{RT}$.	$TwtP_O$ - ↓ from adult to aged*. $TwtSP_O$ - ↔ with age. $TetP_O$ - ↓ 28% from adult to aged* $TetSP_O$ - ↓ 26% from adult to aged* TPT - ↔ with age. $1/2_{RT}$ - ↔ with age.	BM – 13% smaller but ↔ with age. MM - ↓ 15% from adult to aged* BM/MM - ↓ with age*
Moran *et al.* (2005)	Female mice (C57BL/6J):	Whole Sol from one	Maximal isometric stimulations *in vitro*. $TwtP_O$, $TwtSP_O$, $TetP_O$,	$TwtP_O$ - ↔ for EDL & Sol with age $TetP_O$ – EDL ↔ with age	BM – Affected by age‡ -16, 24 & 28 mo.>4 & 8 mo.

~4 mo. (n=7), 8 mo. (n=7), 16 mo. (n=8), 24 mo. (n=5) and 28 mo. (n=10)	hindlimb, EDL from contralateral hindlimb	$TetSP_O$, active stiffness, passive stiffness, TPT, $1/2_{RT}$. Tested at 25°C.	- Sol ↓ 23% 4-28 mo. - 28 mo. Sol signif. ↓ vs. 4 & 8 mo.* - Sol $tetP_O$ vs. age (r=-0.63; P<0.001) but no correlation for EDL (r=-.014, P=0.44) $TetSP_O$ – EDL ↔ with age - Sol ↓ 26% 16 & 28 mo. vs 8 mo. * Act. stiffness – EDL ↔ with age - Sol ↓ 23% 8-28 mo.* Pass. stiffness – EDL ↑ 20% 4 & 8-28 mo. * - Sol ↔ with age TPT – EDL & Sol ↔ with age $1/2_{RT}$ – EDL ↑ 30% 4-28 mo.* - Sol ↔ with age	↔ EDL & Sol MM or L_0

Rice *et al.* (2005)	Male rats (Fischer 344/NNiaHSd; F344/N): Adult (6 months) and aged (24 months) vs. male rats (Fischer 344/NNiaHSd x Brown Norway/BiNia; F344/NXBN): Adult (6 months) and aged (38 months).	Whole Sol, EDL	Maximal isometric stimulations *in vitro*. $TwtP_O$, $TetP_O$, $TetSP_O$, TPT, $1/2_{RT}$. Tested at 24°C.	F334/N: - No changes in contractile function for EDL & SOL F334/NXBN: $TwtP_O$ – EDL ↓ ~44% adult to aged* - Sol ~67% adult to aged* $TetP_O$ – EDL ↓ ~65% adult to aged* - Sol ↓ ~77% adult to aged* $TetSP_O$ – EDL ↓ ~42% adult to aged* - Sol ↓ ~ 65% adult to aged* TPT ↔ EDL and Sol with age $1/2_{RT}$ – EDL ↑ ~24% with age - Sol ↔ with age	F334/N: BM - ↑ 14% in aged vs. adult* MM – ↔ EDL & Sol with age CSA - ↔ EDL & Sol with age MM:BM - ↔ with age F334/NXBN: BM - ↔ with age. MM – EDL ↓ ~38% adult to aged* - Sol ↓ 37% adult to aged* CSA - ↓ adult to aged* MM:BM - ↓ adult to aged*

Carter *et al.* (2010)	Male rats (F344BN): Young adults (7-10 months; n=9) and aged (35 months; n=9)	Sol, Gastrocnemius -plantaris complex (G-P)	Maximal twitch and tetanus isometric force *in situ*. $TwtP_O$, $TetP_O$, $TetSP_O$, TPT, $1/2_{RT}$. Rat placed on heated platform at 37°C.	$TwtP_O$ – Sol ↓ 38% with age. * - G-P ↓ 50% with age* TetPO – Sol ↓~67% with age* - G-P ↓ ~61% with age* $TetSP_O$ – Sol ↓ ~58% with age* - G-P ↓ ~36% with age* TPT - Sol ↔ with age. - G-P ↑ with age* $1/2_{RT}$ – Sol ↑ 70% with age* - G-P ↑ 51% with age*	BM - ↔ with age MM – Sol ↓35% with age* - GAS ↓ 55% with age*
Chan and Head (2010)	Male & female mice (129/ReJ): Adult (2-6 months; n=39 M, n=23 F) and	Whole EDL	Maximal isometric stimulations *in vitro*. $TetP_O$, $TetSP_O$, TPT, $1/2_{RT}$. Tested at ~22-24°C	TetPo – M ↔ with age. - ♀F↓ 7.2% with age (P=0.0069) $TetSP_O$ - M ↓ 13% with age• - F ↓ 13% with age (P=0.0016) TPT - M ↑ 1.9ms in aged (P=0.0003)	MM - M ↑ 27% in aged• - F ↔ with age CSA - M ↑ 21% in aged• - F ↔ with age

	aged (20-22 months; n=20 M, n=12 F)			- F ↔ with age $1/2_{RT}$ - ↔ M or F with age	
Kayani *et al.* (2010)	Mice (wild type): Adult (10-12 months; n=8) and aged (26-28 months, n=8)	Whole EDL	Maximal isometric stimulations *in situ*. $TwtP_O$, $TetP_O$, TPT, $1/2_{RT}$.	$TwtP_O$ - ↔ with age. $TetP_O$ - ↓ in aged vs. adult* TPT - ↔ with age $1/2_{RT}$ - ↔ with age	BM - ↔ with age MM - ↔ with age CSA - ↓ in aged vs. adult*
Kim and Thompson (2012)	Male rats (Fischer 344 Brown Norway F1 hybrid): Young (5-12 moths) adult (24-31 months) and aged (32-40 months)	Single Sol fibers	Maximal isometric stimulations *in vitro*. $TetP_O$, $TetSP_O$, V_{max}	$TetP_O$ - ↓ young to adult*, ↔ adult to aged, ↔ adult to aged $TetSP_O$ - ↔ with age V_{max} - ↔ with age	Diameter - ↓young adult to adult*, ↑ adult to aged*, ↔ young adult to aged.

Kim and Thompson (2013)	Male rats (Fischer 344 Brown Norway F1 hybrid): Young adult (5-12 months) adult (24-31 months) and aged (32-37 months). N=21	Single MHC-I and MHC-II fibers from Gas	Maximal isometric stimulations *in vitro*. $TetP_O$, $TetSP_O$, V_{max}. Tested at 15°C	$TetP_O$ – MHC-I ↓ 29% young adult to adult*, ↓32% young adult to elderly ‡ - MHC-II ↓ 29% and 25% in aged vs. young adult‡ and adult‡ respectively. $TetSP_O$ – MHC-I ↓ 15% young adult to aged* - MHC-II ↓ 14% young adult to aged* V_{max} – MHC-I ↔ with age - MHC-II ↓ 32% young adult to aged ‡	Diameter – MHC-I ↔ with age - MHC-II ↓ aged vs. young adult* and adult*
Graber *et al.* (2013)	Male mice (C57BL/6): Adult (6-7 months; n=20), old (24-26 months; n=12) and	Whole EDL	Maximal isometric stimulations *in vitro*. $TetP_O$, Tested at 25°C	$TetP_O$ - ↓ 28% from adult to aged ‡ - ↔ adult to old despite 18% ↓ - ↔ old to aged - age and $TetP_O$ correlate (r=-0.569; P<0.001)	BM - ↔ with age

	aged (>28 months; n=23)				
Greising *et al.* (2013)	Mice (Wild type – C57BL6 x 129): Young (5 months; 100% survival) and aged (23 months; ~75% survival)	DIA strips	Maximal isometric stimulations *in vitro*. $TwtP_O$, $TetSP_O$, Tested at 26°C.	$TwtP_o$ - ↓ in aged vs. young* $TetSP_O$ - ↓ 34% young vs. aged‡	BM - ↑23% in old vs. young*
Kung *et al.* (2014)	Male rats (Brown-Norway): Adult (11-13 months; n=19) and aged (36-37 months; n=12)	Whole EDL and single EDL fibers via motor unit stimulation	Maximal isometric stimulations *in situ*. $TetP_O$, $TetSP_O$ for whole EDL. $TetP_O$. Tested at 36°C	Whole EDL: $TetP_O$ - ↓ 65% with age* $TetSP_O$ - ↓ 46% with age* Single fiber: $TetP_O$ - ↔ with age	BM - ↔ with age Whole EDL: MM - ↓ 40% with age* L_0 - ↓ with age* CSA - ↓ with age*
Tallis *et al.* (2014)	Female mice (CD1): 3, 10, 30 and 50	Whole EDL, DIA strips	Maximal isometric stimulations *in vitro*. $TwtP_O$, $TwtSP_O$, $TetP_O$,	$TwtP_O$ – EDL ↑3-10 wks. and maintained - DIA ↑3-50 wks. and peaks	BM - ↑ with age & peak at 50 wks.**

	weeks old (n=8 per muscle per age)	$TetSP_O$, THPT, LSHR. Tested at 37±0.5°C.	$TwtSP_O$ – EDL peak at 10 wks., ↓ 39%, 20% and 27% at 3, 30 & 50 wks. respectively**. 3<30wk* - DIA peak at 10 wks., ↓34% and 27% at 30 & 50 wks. *. Tendency for 3>30wk* $TetP_O$ – EDL ↑ 3-10 wks. and maintained - DIA ↑ 3-10 wks. and maintained $TetSP_O$ – EDL peak at 10 wks., ↓ 17%, 18% and 22% at 3, 30 & 50 wks. respectively* 3>50wks† - DIA peak at 10 wks., ↓ 10%, 28% and 33% at 3, 30 & 50 wks. respectively* 3>50wks* THPT – EDL ↑ 46% at 3 wks. than all ages $	MM – EDL peak at 50 weeks and smallest at 3 wks. ‡. ↓ 19% and 13% at 10 & 30wks vs. 50 wks. ‡.

				- DIA ↑ 19% at 30 wks. than 10 wks.* LSHR – EDL ↑ 32% at 50 wks. than all ages* - DIA ↑ at 50 weeks than 3 & 10 wks.*	
Graber *et al.* (2015)	Male mice (C57BL/6) Adult (5-7 months; n=17), old (22-26 months; n=14) and elderly (28-32 months; n=24).	Whole EDL, Sol	Maximal isometric stimulations *in vitro*. $TetP_O$, $TetSP_O$, force normalised to BM (TetBM), Activation. Tested at 25°C.	$TetP_O$ – EDL ↓ 22% and 28% in old ‡ and elderly* vs. adult - Sol ↔ with age. $TetSP_O$ – EDL ↔ with age - Sol ↔ with age. TetBM – EDL ↓ 20% and 29% in old ‡ and elderly ‡ vs. adult - Sol ↓22% old vs. adults*, ↔ elderly vs. adult Activ. – EDL ↔ adult to old, ↓ 16% and	BM – EDL group; peak in old, ↓17% and 13% in adult** and elderly * - Sol group; peak in old, ↓33% and 27% in adult ‡ and elderly ‡ MM - EDL ↓ 25% adult to aged‡ - Sol ↔ with age. CSA – EDL ↓27% adult to elderly ‡, ↓17 % adult to old* - Sol ↔ with age.

				33% elderly vs old* and adult ‡ respectively - Sol ↔ with age.	MM:BM – EDL ↓ 25% and 27% in old ‡ and elderly ‡ vs. adult - Sol ↓ 30% old vs. adult†
Elliott *et al.* (2016)	Male rats (Fisher 344 and Brown Norway) Fisher 344: - Young (6 months) - Old (24 months) Brown Norway: - Young (6 months) - Old (32 months)	DIA strips	Maximal isometric stimulations *in vitro*. TwtSP$_o$, TetSP$_o$, 1/2$_{RT}$, F-freq from 5Hz-100Hz Tested at 26°C.	TwtSPo - ↓ with age* - Fisher 344 ↓ 17% and BN ↓ 14% TetSP$_o$ - ↓ with age* - Fisher 344 ↓ 24% and BN ↓ 13% 1/2$_{RT}$ - ↔ with age F-freq - ↓ with age* - Fisher 344 ↓ >30Hz	BM - ↔ with age

Author	***Animal Info***	***Muscle tested***	***Protocol***	***Change in Performance***	***Comments***

Power Output					
Brooks and Faulkner (1991)	Male mice (C57BL/6): Young (2-3 months), adult (12-13 months) and aged (26-27 months)	Whole EDL	Maximal isovelocity shortening contractions from 105% L_0 to 95% L_0 *in situ*. Force development measured during muscle relengthening. PO(Watts), PO(w.kg) Mouse placed on heated platform at 35°C	PO (Watts) - ↓30% in young & aged vs adult* PO (W.kg) - ↓ 20% adult to aged*	BM - ↓ in young vs adult and aged*. ↔ from adult to aged. MM - ↓ in young and aged vs adult*
Lynch *et al.* (1997)	Mice: (C57BL/10ScSn) Young (4-6 months, n=15) Aged (24 months, n=7)	DIA strips	Normalised power derived from isovelocity shortening contractions by 10% from 105% of mean fiber length.	↔ in normalised power with age	N/A

Lynch *et al.* (2001)	Male mice (C57BL): Aged ~6, ~17, ~24 & ~ 28 months.	Whole Sol, EDL	Maximal isovelocity shortening contractions from 105% L_0 - 95% L_0. *in vitro*. PO(mwatts), PO(w.kg) Tested at 25°C.	PO (mwatts) – EDL ↓31% and 21% 17-24 mo. & 17-28 mo. respectively ‡ - Sol ↓ 46% 17-24 mo. ‡ PO (w.kg) – EDL ↓24% 17-24 mo. ‡ - Sol ↓36% 17-24 mo. ‡	N/A
Kim and Thompson (2012)	Male rats (Fischer 344 Brown Norway F1 hybrid): Young (5-12 moths) adult (24-31 months) and aged (32-40 months)	Single Sol fibers	Absolute PO (µN·fiber length [FL]·s^{-1}) derived from $TetP_O$, V_{max} and a/P_O. Normalised power ($kN·m^{-2}·FL·s^{-1}$) derived from product of $tetSP_O$ and shortening velocity ($FL.s^{-1}$)	Abs. PO - ↓ young adult to aged*, ↓ young adult to adult*, ↑ adult to aged* Norm. PO - ↔ with age.	N/A
Kim and Thompson (2013)	Male rats (Fischer 344 Brown Norway F1 hybrid):	Single MHC-I and MHC-II	Absolute PO (µN·fiber length [FL]·s^{-1}) derived from $tetP_O$, V_{max} and a/P_O.	Abs. PO – MHC-I ↓ 32% and 33% in aged vs. young adult ‡ and adult * respectively - MHC-II ↓ 47% and 38% in aged	N/A

	Young adult (5-12 months) adult (24-31 months) and aged (32-37 months). N=21	fibers from Gas	Normalised power ($kN \cdot m^{-2} \cdot FL \cdot s^{-1}$) derived from product of $TetSP_O$ and shortening velocity ($FL.s^{-1}$)	vs. young adult ‡ and adult** respectively Norm. PO – MHC-I ↔ with age - MHC-II ↓ aged vs. young adult and adult*	
Tallis *et al.* (2014)	Female mice (CD-1): 3, 10, 30 and 50 weeks old (n=8 per muscle per age)	Whole EDL, DIA strips	WL maximal normalised PO to muscle mass (w.kg) and body mass, PO (w.g)	PO(w.kg) – EDL peak at 10 wks., ↓ 20% and 13% at 3 and 50 wks.* - DIA peak at 10 wks., ↓ 23% at 50 wks.** 3wks>50wk * PO (w.g) – EDL peak at 10 wks., ↓ 20%, 19% and 22% at 3, 30 and 50 wks. *. - DIA whole muscle not measured	N/A
Graber *et al.* (2015)	Male mice (C57BL/6) Adult (5-7 months; n=17), old (22-26	Whole EDL, Sol	Maximal PO derived as force at % $TetP_O$ x maximum velocity at the % $TetP_O$.	MaxPO – EDL ↓ 40% adult to elderly ‡, ↓ 21% adult to old‡, ↓ 24% old to elderly *	N/A

	months; n=14) and elderly (28-32 months; n=24).			- SOL ↓ 28% adult to elderly**, ↔ adult to old.	
Author	**Animal Info**	**Muscle tested**	**Protocol**	**Change in Performance**	**Comments**
			Fatigue Resistance		
Brooks and Faulkner (1991)	Male mice (C57BL/6): Young (2-3 months), adult (12-13 months) and aged (26-27 months)	Whole EDL.	Repeated isovelocity contractions to measure power reduction *in situ* at a stim. freq. of 150Hz,	Fatigue of adult and old EDL better maintained at low train rates (speed of contractions) than young EDL.	N/A
Brown and Hasser (1996)	Male rats (Fischer 334 x Brown Norway hybrids):	Whole Sol, EDL	5-minutes of repeated tetani: SOL - 250ms trains at 100Hz at 75 trains/min *in situ*	Sol - ↔ with age EDL - ↑ by 36 months, with force ↓ 42±3%	N/A

	6, 12, 28 and 36 months old		EDL – 250ms trains at 150Hz at 50 trains/min	vs. 60±4%, 58±4% and 65±4% for 6, 12 and 28 months.	
Pagala *et al.* (1998)	Male mice (C57BL/6J): Young (3-6 months) and old (34-37 months)	Whole Sol and EDL	Repeated tetanic stimulation at 30Hz for 500ms every 2.5s for each muscle and age. Fatigue defined as drop below 50% of pre-fatigue maximal force, or until 10-minutes of fatigue	Sol – ↑ with age* - Time to 50% fatigue young vs. old was 482.2±68.9s vs. 1134.0±233.4s. - Less loss of force for old than young during fatigue* EDL - ↔ with age	N/A
González and Delbono (2001)	Mice (DBA or FVB) Young (2-6 months) and old (20-24 months)	Single, intact Sol and EDL fibers	Two protocols of short intervals (SI - 1s) and long intervals (LI, 3.65s) of repeated tetanic stimulations 350ms trains of stimulation at frequency which produced maximal $TetP_o$.	↔ in Sol or EDL fatigue resistance with age for both SI and LI protocols	N/A

Brotto *et al.* (2002)	Mice (B6C3F1J) Adult (15 months) and old (30 months)	Whole Sol and EDL	Repeated tetanic stimulations at high and low frequency SOL – High freq. = 100Hz Low freq. = ~44Hz. EDL – High freq. = 140Hz Low freq. = ~21Hz 300ms trains every 0.8ms for 5 minutes, alternating between high and low every minute	Sol ↑ fatigue resistance than EDL* ↔ for Sol or EDL post-fatigue $TetP_o$ at high freq. ↓ at low freq. for SOL with age, but ↔ for EDL	Leftward shift in F-Freq. relationship for young Sol and EDL. Potential protective mechanism from damaging effects of fatigue.
Criswell *et al.* (2003)	Male rats (Fischer 344/Brown Norway F1 hybrid): Aged 4 and 30 months	DIA strips	Repeated tetanic stimulations at 30Hz for 250ms every 2s for 30-minutes	↔ for DIA fatigue resistance with age	N/A

Chan and Head (2010)	Male & female mice (129/ReJ): Adult (2-6 months; n=39 M, n=23 F) and aged (20-22 months; n=20 M, n=12 F)	Whole EDL	Repeated tetanic stimulations at 100Hz for 1 second every 2s for 30s. F-Freq, as described above, reassessed after fatigue	↑ in fatigue resistance with age for male and female EDL Greater recovery of $TetP_O$ in older EDL - Male – Old, 54.2±1.3%; adult, 43.8±1.1%* - Female – Old, 49.2±1.2%; adult, 43.5±0.7%*	N/A
Tallis *et al.* (2014)	Female mice (CD-1): 3, 10, 30 and 50 weeks old (n=8 per muscle per age)	Whole EDL, DIA strips	Fifty repeated WL stimulations lasting 5s for EDL and 7s for DIA at parameters that elicited maximal WL PO	EDL – greatest fatigue resistance at 3 weeks - ↓ at 50 weeks vs. all other ages‡ - 10 weeks ↓ vs. 3 and 30 weeks* DIA – 10 weeks ↓ vs 3 weeks** and tendency vs 30 weeks - 50 weeks ↓ than weeks*	N/A

Table S1.2 - Age-related changes in contractile performance of isolated locomotory and respiratory muscle tested under isometric, isovelocity and dynamic conditions.

Abbreviations are as follows: ***Muscle:*** *soleus = SOL; extensor digitorum longus = EDL; diaphragm = DIA; gastrocnemius = GAS; myosin heavy chain = MHC.* ***Isometric measures:*** *twitch contraction = twt; tetanus contraction = tet; absolute force = P_O; specific force = SP_O; maximal velocity of unloaded shortening = V_{max}; time to peak twitch force = TPT; half-twitch relaxation time = $1/2_{RT}$; time to half-peak tetanus = THPT; last stimulus to half relaxation = LSHR; force-frequency relationship – F-Freq.* ***Power measures:*** *absolute power output = PO(mwatts); power output normalised to muscle mass = PO(w.kg); power output normalised to body mass = PO($W.g^{-1}$); mechanical constant to describe force-velocity relationship curvature = a/P_O.* ***Comments:*** *body mass = BM; muscle mass = MM; cross-sectional area = CSA; muscle mass to body mass ratio = MM:BM.* ***Change in Performance:*** *no significant change = ↔; significantly decreased/shorter = ↓; significantly increased/longer = ↑.*

*P values where significant differences are observed and reported are denoted by the following symbols - *≤0.05, ** - ≤0.01, $ - ≤0.003, ‡ - ≤0.001, • - ≤0.0001.*

Author	Participant Information	Protocol	Change in Performance	Comments
Vandervoort *et al.* (1990)	26 young F (20-29 years) and elderly F (66-89 years)	KE and KF eccentric torque tested at 45°.s^{-1} and 90°.s^{-1}	**Yes**	Age caused KE and KF eccentric strength ↓ by 35% (P<0.001) but torque was greater in eccentric than concentric measures for both ages.
Poulin *et al.* (1992)	12 young M (23-32 years) and old M (60—75 years)	KE and EE eccentric torque	**No**	2% decline
Hortobágyi *et al.* (1995)	60 M (18-80 years) and 30 F (20-74 years) sedentary but healthy	Quadricep eccentric strength at 1.05, 2.09 & 3.14rad.$^{\circ-1}$	**No**	Eccentric force ↓ 9 N per decade. Regression analysis revealed no correlation between eccentric strength and fat-free mass.
Porter *et al.* (1995)	28 young M (26±3 years) and 25 old M (71±7 years) 27 young F (25±3 years) and 26 old F (73±6 years)	KE eccentric torque measured at 90°.s between 100° to 0° knee flexion.	**Yes**	Eccentric torque ↓ by 24.9% and 39.3% for M and F respectively. Loss of concentric torque ↑ than eccentric torque (P<0.01).

Lindle *et al.* (1997)	654 M & F aged 20-93 years	KE eccentric torque measured at 0.52 rad/s and 3.14 rad/s.	**Yes**	Absolute torque ↓ at both angular velocities (P<0.001), with M producing ↑ torque than F across all ages (P<0.001). M lost 19% eccentric torque whilst F lost only 11%.
Porter *et al.* (1997)	16 young F (27±4 years) and 16 old F (67±4 years)	PF and DF eccentric torque measured at 30°/s through a range of 20° for PF and 10° for DF.	**No**	Whilst concentric torque ↓ significantly, eccentric torque for both PF and DF ↔.
Phillips *et al.* (1998)	18 healthy older F (84.7±1.2 years)	Adductor pollicis (hand) eccentric strength measured during MVC	**Yes**	Eccentric strength ↑ with age (P<0.002)
Horstmann *et al.* (1999)	60 sedentary M (20 – 60 years) Four groups with mean age 24, 34, 45 and 55 years of age	Peak torque of knee and ankle measured at 60°.s^{-1} and 120°.s^{-1}.	**No**	↔ with age at all angular velocities.

Pousson *et al.* (2001)	6 young M (21±2 years) and 6 young F (20±2 years) 4 elderly M (73±6 years) and 6 elderly F (67±2 years)	EF eccentric torque measured at 60°.s^{-1} and 120°.s^{-1}.	**Yes**	↓in eccentric torque with age for both genders at both angular velocities (P<0.001), with ↑ loss in concentric than eccentric torque. Young M ↑ eccentric torque than F (P=0.0001) ↔ for gender in older group.
Christou and Carlton (2002)	12 young M (26.0±2.9 years) and 12 young F (24.7±2.6 years) 12 old M (72.6±4.8) and 12 old F (74.0±6.3)	KE eccentric torque measured at 25°.s^{-1} across a range of 10° for 200ms.	**Yes**	Eccentric torque ↓with age for both genders (P<0.01). Young M ↑force than F (P<0.01) ↔in the old group (P=0.213).
Delbaere *et al.* (2003)	15 young M (27.5±4.1 years) and 19 young F (28.1±5.4 years) 20 adult M (50.5±4.6 years) and 20 adult F (47.5±4.6 years) 10 old M (75.9±8.3 years) and 17 old F (72.1±8.3 years)	KE, KF, EF and EE eccentric strength measured at 60°.s^{-1} and 120°.s^{-1} from 25° to 110° for KE and KF and from 15° to 110° for EF and EE.	**Yes**	Eccentric strength for all muscles ↓ in old group vs. young and adult (P<0.01), ↔ for gender. EF & EE eccentric strength is better maintained than KF and KE strength (P<0.01). The decline in eccentric, concentric and isometric strength was similar with age.

Klass *et al.* (2005)	10 young M (25.4±1.0 years) and 10 young F (25.9±1.4 years) 10 old M (78.7±1.8 years) and 9 old F (74.6±1.9 years)	DF eccentric torque measured at 5, 25, 50, 75 and 100°.s^{-1} through a 30° range of motion.	**Yes & No**	Absolute eccentric torque ↓ significantly for the M only (10.5%, P<0.05) ↔ for F (P>0.05). Loss of isometric and concentric force greater than eccentric torque
Perry *et al.* (2007)	44 young (29.3±0.6 years) 44 older fallers (75.9±0.6 years 34 older non-fallers (76.4±0.8 years)	Quadricep, Hamstring, DF and PF bilateral eccentric torque measured at 50°s^{-1} and 150°.s^{-1}.	**Yes**	Both fallers and non-fallers produced ↓ eccentric torque for all muscles and angular velocities (P= 0.003-<0.0001) with fallers generally weaker than non-fallers (P<0.0001).

Table S1.3 - An overview of the in vivo studies to have assessed the age-related changes in acute eccentric muscle activity.

Author	Participant Information	Muscle/Motion	Protocol	Change in Performance	Comments
			Changes in Strength		
Miyatake *et al.* (2000)	M & F obese & control (no Information of age-matched n values) - obese = BMI ≥26.4 Adult (≥40 years<60 years) Old (≥60 years<80 years	HG, KE	HG strength via hand held dynamometer for right and left hand Torque via leg extension for right and left leg	**Yes & No**	Right & left HG strength – adult ↑ for obese vs control for male* and female**, ↔ for old. Right & left KE strength – adult ↑ for obese vs control for male** and female**, ↔ for old. ↑ fat % for M & F in old vs adult
Rolland *et al.* (2004)	1443 F 598 Lean (80.7±4.1 years) 630 Normal (80.2±3.7 years) 215 Obese (80.0±3.5 years)	EE, KE, HG	MI MVC for knees and elbow HG strength via hand held dynamometer	**Yes**	Obese EE ↑ vs. normal and lean* Obese KE ↑ vs. lean, ↔ vs normal* ↔ in HG strength between groups.

					BM, BMI, fat mass, fat free mass, leg and arm skeletal muscle mass ↑ for obese vs. normal and lean
Zoico *et al.* (2004)	167 F (71.7±2.4 years) Split into normal, overweight and obese based on BMI	KE	MI MVC for knees	**No**	↔ in strength between all groups
Hilton *et al.* (2008)	12 M & F 6 overweight (4 M, 2F; 58.0±9.2 years) 6 obese (4M, 2 F; 58.0±10.0 years)	PF, DF	MI MVC strength at 0° MD torque at $60°s^{-1}$ and $120°s^{-1}$	**Yes**	PF – obese 41% ↓ MI vs. overweight - obese 70% and 74% ↓ MD at 60 and $120°s^{-1}$ vs. overweight. DF - obese 77% ↓ MI vs. overweight - obese 79% ↓ MD at $60°s^{-1}$ vs. overweight, ↔ at $120°s^{-1}$ vs. overweight.

					IMAT – Obese 55% ↑ vs. overweight Muscle and adipose tissue volume - ↔ between groups
Stenholm *et al.* (2009)	930 adults aged ≥65 years split into 4 groups: Obesity (n=162 71.9±50.2 years) Low strength (n=239; 77.7±7.4 years) "Both" (n=71; 77.3±6.6 years) "Neither" (n=458; 74.1±6.8 years)	KE	MI MVC strength of knees	**Yes**	Obesity and "neither" groups KE strength ↑ vs. low strength and "both" group for M & F at baseline*

Huo *et al.* (2016)	680 adults (238 M, 442 F) aged ≥75 years split into 4 groups: Sarcopenic (n=284; 81±7 years) Obese (n=109; 85±7 years) "Both" (n=96; 78±6 years) "Neither" (n=191; 79±7 years)	HG	HG strength measured by hand held dynamometer	**Yes & No**	"Both" group ↓ HG strength vs. obese and "neither" group, ↔ vs. sarcopenic
			Changes in Power		
Hilton *et al.* (2008)	As above	PF, DF	Peak absolute PO and PO relative to muscle volume ($W.cm^3$) at $60°s^{-1}$ and $120°s^{-1}$	**Yes**	Absolute DF PO – Obese 86% and 83% ↓ at 60 and $120°s^{-1}$ vs. overweight. Absolute PF PO – Obese 73% and 77% ↓ at 60 and $120°s^{-1}$ vs. overweight.

		Relative DF PO – Obese 45% ↓at 120°s^{-1} vs. overweight; ↔ at 60°s^{-1} vs. overweight. Relative PF PO – Obese 77% and 80% ↓ at 60 and 120°s^{-1} vs. overweight.

Table S1.4 - The effects of obesity on the contractile performance of lower limb skeletal muscle groups in sarcopenic obese adults.

Author	Animal Information	Age at Start of Diet	Feeding Duration	Change in Body Composition	Muscle Morphology
Lin *et al.* (2000)	Male mice (C57BL/6J): n=48 (n=6 per feeding group)	3 weeks	1, 8, 15 & 19 weeks	Body mass: - Signif. diff. after 2 weeks* - 8 weeks – 11.4% greater than LFD ‡ - 15 weeks – 23.1% greater than LFD ‡ - 19 weeks – 30.5% greater than LFD ‡ Epididymal and perirenal fat mass: - Signif. diff. after 8, 15 and 19 weeks* - Epididymal fat mass inc. by 144% - Perirenal fat mass inc. by 130%	Not measured
Winzell and Ahrén (2004)	Female mice (C57BL/6J): n=~500 (LFD n=240; HFD n=259)	4 weeks	52 weeks	First 12 weeks of diet: - LFD – ↑ 0.40 ±0.03 g/week - HFD – ↑ 0.68 ± 0.04 g/week ‡ Week 13 onwards: - LFD – ↑ 0.10 ± 0.01 g/week - HFD – ↑ 0.18 ± 0.03 g/week ‡	Not measured

				HFD vs. LFD signif. Diff. after 4 weeks	
de Wilde *et al.* (2008)	Male mice (C57BL/6J): n=12 (n=6 per feeding group)	9 weeks	3 weeks	Not reported, ↔ vs. LFD post-feeding	Not measured
Shortreed *et al.* (2009)	Male mice (C57Bl6/J): N=40 (n=20 per feeding group)	10 weeks	8 weeks	Body mass: - LFD - ↑ first 4 weeks before stabilising - HFD – signif ↑ by 4 weeks and continued to grow. ~ 40% ↑ in BM by 8 weeks vs pre-diet Epidydimal fat mass: - Signif two-fold ↑ in fat mass for HFD group	↔ in SOL or tibialis anterior muscle mass.
de Wilde *et al.* (2009)	Male mice (C57BL/6J): n=20 (n=10 per feeding group)	9 weeks – LFD 12 weeks - HFD	LFD 3 weeks HFD 8 weeks	BM – HFD ↑ after 4** and 8 weeks ‡ vs. LFD Fat mass – Both ↑ 0-8 weeks (LFD ‡; HFD ‡); but HFD ↑ after 4** and 8 weeks ‡ vs. LFD Lean mass – Both ↑ after 4 weeks (LFD ‡; HFD*) with only LFD increasing 0-8 weeks*	Not measured

Anderson *et al.* (2008)	Male mice (C57BL/6J): n=32 (n=16 per feeding group)	BM ~14g	9 weeks	BM of HFD was signif. diff. after 4 weeks of feeding and each week after vs. fasting group	Not measured
Breslin *et al.* (2010) [Study 2]	Male mice (CD-1): n=24 (n=12 per feeding group)	Young – 8-10 weeks Adult – 19-22 weeks	12 weeks	Young – ↑ 82% from baseline ‡ Adult – ↑ 63% from baseline ‡ BM was signif. diff. from baseline after 4 weeks in both group. *	Not measured
DeNies *et al.* (2014)	Male and female mice (C57BL/6J): N vale for each condition not reported.	3 weeks	~ 52 weeks	LFD 39.2g ± 2.7 vs. 56.9g ± 3.4 in HFD ‡ (Weights were pooled)	Signif. ↑ in soleus, plantaris and gastrocnemius mass between LFD and HFD. No difference in soleus mass for males and females.

Thomas *et al.* (2014)	Male mice (C57BL6/J): n=24 (12 per feeding group)	4 weeks	3 weeks	HFD significant ↑ in body mas vs. LFD* Significant ↑ in HFD epididymal fat vs. LFD*	↔ for SOL, triceps surae and tibialis anterior mass between HFD and LFD group
Ciapaite *et al.* (2015)	Male mice (C57BL/J6): n=48 (n=16 per feeding group)	12 weeks	5 weeks	Low fat diet (LFD) - ↔ High-fat lard (HFL) - ↑ 25.8g ± 0.3 to 30.2g ± 0.4‡ High-fat palm oil (HFP) - ↑ 25.1g ± 0.4 to 31.1g ± 0.6‡	EDL – LFD – 10.4 ± 0.4mg HFL - 10.2 ± 0.2mg HFP – 10.8 ± 0.2mg SOL – LFD – 7.0 ± 0.2mg - HFL – 7.7 ± 0.2mg* - HFP – 7.8 ± 0.2 mg*
Gao *et al.* (2015)	Female mice (CD-1): n=20 (n=10 per feeding group)	6 weeks	12 weeks	LFD – ↑ ~0.4g/week HFD - ↑ ~1.4g/week. Sig. diff. at week 3*.	Not measured

Table S1.5 - An overview of the effects of different durations of HFD provision to mice on animal body composition and muscle morphology.

*Levels of significance are denoted by the following symbols: * = P<0.05 vs. LFD; ** = P<0.01 vs. LFD; ‡ = p<0.001 vs. LFD.*