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The Effects of Obesity on Skeletal Muscle Health
An Investigation of Contractile Performance and Nutritional Therapeutic Strategies

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The Effects of Obesity on Skeletal Muscle Health: An Investigation of Contractile Performance and Nutritional Therapeutic Strategies

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Dedication

I would like to dedicate this work to my father, John Shelley. Without your constant support and encouragement, submitting this thesis would not be possible. You are the hardest working, kindest, and most selfless parent any child could ask for. I will be forever grateful for everything you have done for me.

To my beautiful wife and best friend, Grace Shelley. Thank you for your endless love and for believing in me. I never imagined I would achieve what I have, and I would not have been able to do so without you by my side.

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Abstract

Obesity has previously shown to be detrimental to skeletal muscle contractile function, which may exacerbate the adverse health effects and comorbidities associated with obesity. Despite the damaging effects of obesity on skeletal muscle health, not all aspects of skeletal muscle contractility have been thoroughly considered. Understanding obesity effects on contractile function across a range of contractile modes and muscles is needed for a holistic understanding of how obesity effects muscle performance, which helps inform potential strategies to offset negative obesity effects. Furthermore, current non-surgical treatment strategies to reduce the impact of obesity on skeletal muscle performance have poor adherence and long-term success rates, thus alternative appealing strategies should be considered. Using a combination of human *in vivo* and rodent isolated skeletal muscle models, the present thesis examines: the contractile mode and muscle specific effects of obesity on skeletal muscle contractile function; the concomitant effects of obesity and aging on skeletal muscle contractility; potential nutritional strategies to offset the adverse effects of obesity on skeletal muscle function. Furthermore, the present work further refines the protocols used to examine isolated skeletal muscle contractile performance, appeasing some of the concerns regarding *in vivo* replicability of fatigue measures obtained from the work loop model. The current thesis is the first to report the effects of obesity on eccentric muscle performance, and uniquely identifies the simultaneous effects of obesity and aging on contractile performance, using isokinetic dynamometry, the gold standard for strength assessments *in vivo.* Isolated skeletal muscle models were used to determine the effects of high fat diet (HFD), vitamin D, and resveratrol consumption on isolated muscle contractile properties, where the focus was on the work loop (WL) model. The WL model better replicates the dynamic nature of *in vivo* skeletal muscle mechanics compared to most *in vitro* assessments of contractile function of skeletal muscle. Experimental study 1, for the first time, identifies that obesity adversely effects eccentric muscle performance in a contractile mode and muscle specific manner, which is not entirely concurrent with changes in concentric function. Furthermore, the study identifies that obesity induced changes in muscle function are not exacerbated

by aging, but are likely more substantial in older adults due to the age-related decline in muscle function. Experimental study 2 uniquely establishes that the stimulation frequency needed to evoke maximal power output in isolated mouse EDL exceeds that needed for maximal isometric force, and that submaximal stimulation frequency is needed to evoke a fatigue response representative of i*n vivo* fatigue mechanics. Using the refined WL protocol established in experimental study 2, experimental study 3 and 4 identify that HFD effects on isolated skeletal muscle function are contractile mode and muscle specific, but appear more substantial in fast twitch muscle. For the first time the present thesis identifies that HFD evokes a reduction in cumulative work production in both fast twitch EDL and slow twitch soleus during fatiguing contractions, but does not influence rate of fatigue relative to maximum power output. Experimental study 3 provides the first evidence to suggest that a high dose of vitamin D does not attenuate the reduction in contractile performance associated with HFD feeding. Experimental study 4 uniquely establishes that resveratrol reduces the impact of HFD feeding on acute and sustained power production of isolated mouse EDL. As such, this thesis provides the first direct evidence to suggest resveratrol may be an effective nutritional strategy to reduce the impact of obesity on isolated skeletal muscle function.

Presentation of Results

Data from the present thesis has been presented at international conferences and published in peer-reviewed journals.

Chapter 3 - The Effects of High Adiposity on the Concentric and Eccentric Muscle Performance of Upper and Lower Limb Musculature in Young and Old Human Males

Modified from a publication in The Journal of Applied Physiology, Nutrition, and Metabolism

Shelley, S., James, R. S., Eustace, S. J., Eyre, E., & Tallis, J. (2021). The effects of high adiposity on concentric and eccentric muscle performance of upper and lower limb musculature in young and old adults. Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme. https://doi.org/10.1139/apnm-2020-0945 [IF = 2.665; Q2]

Oral presentation of selected results was delivered at the annual British Association of Sport and Exercise Sciences (BASES) 2021 conference 'Physiology and Nutrition.'

Published abstract in The Journal of Sports Sciences

Shelley, S., James, R. S., Eustace, S. J., Eyre, E., & Tallis, J. (2021). The effects of high adiposity on concentric and eccentric muscle performance of upper and lower limb musculature in young and old adults. Journal of Sport Sciences https://doi.org/10.1080/02640414.2021.1978748 [IF = 3.337; Q1].

Chapter 4 - Effect of Stimulation Frequency on Force, Power, and Fatigue of Isolated Mouse Extensor Digitorum Longus Muscle

Modified from a publication in the Journal of Experimental Biology

Shelley, S., James, R.S., Eustace, S.J., Eyre, E., and Tallis, J. (2022) 'Effect of Stimulation Frequency on Force, Power, and Fatigue of Isolated Mouse Extensor Digitorum Longus Muscle.' Journal of Experimental Biology https://doi.org/10.1242/jeb.243285 [IF = 3.312; Q1].

Aspects of the following publication are presented in the literature review.

Modified from a publication in Biomolecules

Tallis, J., **Shelley, S.,** Degens, H., Hill, C. (2021) Age-Related Skeletal Muscle Dysfunction is Aggravated by Obesity: An Investigation of Contractile Function, Implications and Treatment. Biomolecules 11(3) 372. https://doi.org/10.3390/biom11030372 [IF = 4.569; Q2]

Abbreviations

µJ – Microjoules

µm – Micrometres

ANOVA – Analysis of variance

ADL – Activities of daily living

AMP – Adenosine monophosphate

AMPK – AMP activated protein kinase

ATP – Adenosine triphosphate

BM – Body mass

BMI – Body mass index

Ca^{2+} - Calcium ion

CF – Cycle frequency

CNS – Central nervous system

CSA – Cross-sectional area

DXA – Dual x-ray absorptiometry

EDL – Extensor digitorum longus

EE – Elbow extensor

EE – Elbow flexor

FM – Fat mass

HFD – High-fat diet

HFL - High-fat lard

HFP – High-fat palm oil

HGS – Handgrip strength

Hz – Hertz

IKD – Isokinetic dynamometer

IL-6 – Interleukin-6

IMAT – Intramuscular adipose tissue

IMCL – Intramyocellular Lipid

$kg.m^{-3}$ – 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

MF-BIA – Multi-frequency bioelectrical impedance analysis

MVC – Maximal voluntary contraction

KE – Knee extensors

KF – Knee Flexors

mN.V – Millinewtons per volt

OHa – Old high adiposity

OA – Older adults

ONa – Old normal adiposity

PO – Power output

PO-CF – Power output-cycle frequency

PTH – Pathyroid hormone

RES – Resveratrol

SD – Standard deviation

SEM – Standard error of the mean

SERCA – Sarco(endo)plasmic reticulum ATPase

SLD – Standard low-fat diet

SOL – Soleus

SR – Sarcoplasmic reticulum

STS – Sit to stand

THPT – Time to half-peak tetanus

V – Volts

V_0 – Maximum unloaded shortening velocity

V_{max} – Maximal shortening velocity

VAS – Visual analog scale

$W.g^{-1}$ – Watts per gram

$W.Kg^{-1}$ – Watts per kilogram

WL – Work loop

VitD – Vitamin D

YHa – Young high adiposity

YNa – Young normal adiposity

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Chapter 1 – Introduction and Literature Review

1.1 – Prevalence and Impact of Obesity

The prevalence of individuals who are overweight and obese has rapidly grown and has quickly become a global epidemic (Barazzoni et al. 2018). Obesity is defined as excessive or abnormal accumulation of adiposity, commonly indicated by a body mass index (BMI= Mass[kg]/height^2 [m^2]) greater than 30 (WHO 2022). Obesity exacerbates the risk and severity of infectious disease (Huttunen and Syrjänen 2010; Muscogiuri et al. 2021) and is associated with severe health risks and morbidities such as depression, type 2 diabetes, cardiovascular disease, and a variety of cancers (McGee 2005; Hruby and Hu 2014; Lu et al. 2015). Obesity is the second leading cause of premature death in Europe and the United States (NHS 2019), with 1 in 7 premature deaths in Europe associated with being overweight or obese (Di Angelantonio et al. 2016). Currently, 650 million people worldwide are categorized as obese (BMI>30), with a further 1.25 billion people classified as being overweight (BMI >25) (WHO 2022). Within the United Kingdom (UK) alone, nearly two-thirds of adults are overweight or obese (Public Health England, 2017). Since 2010 prevalence of obesity in the UK has plateaued; while this is encouraging, obesity levels remain high, with 28% of all adults in the UK considered obese, which rises to ~33-36% in older adults aged 45-74 (Baker, 2021). Obesity also has substantial financial implications for governments and health care providers, with overweight and obesity-related illnesses costing the UK upwards of £27 billion per year, including an estimated £6.1 billion burden to the National Health Service (NHS) (Public Health England, Health Matters: Obesity and The Food Environment 2017). The adverse effects of obesity on individual health and economic development may be related to and exacerbated by a reduction in skeletal muscle contractile function associated with excess adipose.

1.2 – Skeletal Muscle Contractile Function

Excessive accumulation of adiposity can reduce skeletal muscle health and function (Tallis et al. 2018; Morgan et al. 2020). Skeletal muscle contractile function represents an important link to functional independence and quality of life, as compromised muscle function reduces the capacity to be physically active and complete activities of daily living (ADL) (Bollinger 2017). The primary function of skeletal muscle is to produce force for movement, control and stabilisation (Carlson 2019). Muscle function is comprised of three elements: 1) Muscle strength; 2) Muscle power; and 3) Muscle endurance/fatigue (Beaudart et al. 2019). Muscle strength is defined, by most physiologists, as the maximal amount of force produced in a single attempt (Deschenes 2004). Skeletal muscle power is calculated as force×contraction velocity (Reid and Fielding 2012). Skeletal muscle fatigue is defined as the acute decline in the ability of skeletal muscle to generate force, that typically occurs over a bout of repeated contractions (Callahan et al. 2016). Adequate force, power and fatigue resistance are necessary for the movement and control required to complete many tasks of daily living. However, these three elements are broad categorizations and can be further subdivided into three contractile modalities: 1) Isometric (force produced at a constant length); 2) Concentric (force produced during muscle shortening); and 3) Eccentric (force produced during muscle lengthening). Isometric strength plays a pivotal role in postural control (Tallis et al. 2018). Concentric strength and power are responsible for propulsive movements such as walking, running and stair ascent (Enoka and Duchateau 2019). Eccentric contractions are responsible for producing corrective torques during postural instability and controlling braking movements such as stair descents (Choi 2016). Due to the important role adequate muscle function plays in being functionally independent and physically active, there has been an increase in literature exploring the adverse effects of excess adipose accumulation on skeletal muscle contractility.

1.3 – Obesity Effects on Skeletal Muscle Contractility

1.3.1 – The importance of Examining the Effects of Obesity on Skeletal Muscle Contractile Function

Over the last two decades an increasing amount of literature has examined the impact of obesity on skeletal muscle health and function (Maffiuletti et al. 2013; Tomlinson et al. 2016; Bollinger 2017; Tallis et al. 2017, 2021; Morgan et al. 2020; Straight et al. 2021). Findings indicate obesity effects on muscle function are not entirely uniform across anatomical location, contractile mode, age or muscle fibre type (Tallis et al. 2018). However, there is consensus that excessive accumulation of adipose can adversely affect both absolute and normalised (typically to body mass or to a lesser extent muscle size) muscle performance. Obesity induced reductions in skeletal muscle contractility can limit an individual's ability to be physically active and alter or reduce their ability to complete daily tasks, ultimately reducing quality of life (Bollinger 2017). As such, obesity related declines in skeletal muscle contractility may play a key role in exacerbating the adverse effects of obesity for two primary reasons: 1) a reduction in contractile performance may hinder the ability to be physically active, thus resulting in a reduction in energy expenditure; 2) Skeletal muscle is one of the key regulators of lipid metabolism (Morales et al. 2017). As such, skeletal muscle dysfunction evoked by obesity may contribute to, or even be a catalyst for, a negative obesity cycle, described as diminished contractile performance promoting physical inactivity, thus further adipose accumulation and exacerbation in the magnitude of obesity (Tallis et al. 2018). A variety of experimental models and protocols have been utilised throughout the literature, which has been integral for developing a detailed understanding of the effects of obesity on muscle contractility. As such, this review of the literature will summarise the effects of obesity on skeletal muscle contractile function in both *in vivo* and *in vitro* experimental models to provide a holistic overview of the current available data.

1.3.2 – Absolute Isometric and Concentric Muscle Performance (In vivo)

There has been a growing number of research studies examining the impact of obesity on the contractile performance of skeletal muscle using *in vivo* experimental models (Table 1). Whilst there are a range of techniques which have been utilised to examine contractile performance, typically isokinetic dynamometry, custom dynamometry or assessments of handgrip strength (HGS) are utilised to measure maximal force or torque (torque = Force applied x Perpendicular Distance of force from pivot: Grimshaw 2006) (Maffiuletti et al. 2013; Tomlinson et al. 2016). Absolute force or torque is then normalised to body mass, or to a lesser extent, estimated muscle mass or fat free mass (FFM), as an indication of muscle quality (Table 1).

Isometric strength is a necessity for postural control (Tallis et al. 2018) and is linked with the ability to complete functional tasks (Avlund et al. 2007). Furthermore, activities of daily living (ADL) such as walking, stair climbing and rising from a chair, require adequate mobility and concentric force and power to complete. Current literature on the effects of obesity on absolute Isometric and concentric, force and torque are somewhat ambiguous. One common suggestion is that obesity leads to greater absolute force and torque of postural muscles in young adults (<40 years) when compared to non-obese counter parts (Miyatake et al. 2000; Hulens et al. 2001; Maffiuletti et al. 2007b; Capodaglio et al. 2009; Tomlinson et al. 2014b; Erskine et al. 2017). An increase in absolute muscular performance of postural muscles in obese populations may occur as a result of an overload training stimulus from manoeuvring and controlling a larger mass (Maffiuletti et al. 2013). Furthermore, an increase in BMI and adiposity is associated with an increase in muscle volume (Tomlinson et al. 2016), which may contribute to increased absolute performance. However, an increase in absolute performance is not always observed, with many studies reporting equivalent absolute function between obese and non-obese individuals (Hulens et al. 2001; Paolillo et al. 2012; Cavuoto and Nussbaum 2013; Pajoutan et al. 2016; Erskine et al. 2017). Equivalent absolute force may relate to a reduction in activation capacity

of obese muscle (Erskine et al. 2017). Therefore, the magnitude of chronic training stimulus of supporting an elevated mass is not always great enough to overcome the reduction in activation capacity.

Studies which have assessed the effects of obesity on absolute isometric and concentric function of an elderly adult population (≥50 years) have yielded ambiguous results. Evidence exists for greater (Rolland et al. 2004; Choi et al. 2015; Erskine et al. 2017), equivalent (Miyatake et al. 2000; Zoico et al. 2004; Erskine et al. 2017) or reduced (Villareal et al. 2004; Hilton et al. 2008) capacity to produce absolute isometric force or concentric torque in postural muscles of obese individuals. Differences in the ability of musculature to adapt to elevated loading between young and old individuals may in part be attributed to an ageing-induced decline in myogenesis (Domingues-Faria et al. 2016), which may also be exacerbated by obesity (O'Leary et al. 2018).

Research examining the effects of obesity on absolute upper body muscle performance or comparing upper body and lower body musculature response to obesity is limited. The available data suggests that obesity evokes an increase in absolute performance of upper limb musculature (Rolland et al. 2004; Lafortuna et al. 2005; Pescatello et al. 2007; Cavuoto and Nussbaum 2013), irrespective of age. However, it appears the magnitude of increase in absolute performance in response to obesity is more substantial in lower limbs, attributed to mechanical loading (Lafortuna et al. 2005). It should be noted than an obesity induced increase in absolute force of upper limb musculature is not apparent when adjusting for variables such as age, height and physical activity (Rolland et al. 2004). The effects of obesity on HGS, which has been used as a proxy for overall muscle strength (Lauretani et al. 2003; Wind et al. 2010; Trosclair et al. 2011; Duchowny et al. 2017), are not well understood. In older populations there is evidence to suggest that as BMI increases so does absolute HGS (Keevil et al. 2015). However, in very similar cohorts, obesity was shown to result in a reduction (Vilaca et al. 2013)

or no change (Hulens et al. 2001; Rolland et al. 2004) in HGS. As such, the effects of obesity on the absolute force producing capacity of skeletal muscle, particularly in older adults, remains uncertain.

1.3.3 – Normalised Isometric and Concentric Muscle Performance (In vivo)

Normalising absolute performance to body mass is an important indicator for how obesity may affect an individual's ability to manoeuvre their own mass (Bollinger 2017), an important consideration for most ADL. With very few exceptions, normalised isometric and concentric muscle performance (force normalised to body mass) in obese populations is lower than that of non-obese individuals (Miyatake et al. 2000; Maffiuletti et al. 2007b; Pescatello et al. 2007; Capodaglio et al. 2009; Paolillo et al. 2012; Choi et al. 2015; Pajoutan et al. 2016; Erskine et al. 2017), irrespective of age. As such, any potential compensatory mechanisms to evoke an increase in absolute function in response to an elevated mass are still not great enough to overcome the magnitude of the increase in body mass. To achieve movement, individuals must produce force which exceeds the mass of the body part which they intend to move; thus, obese individuals have an increased force requirements to manoeuvre their own mass. Reduced normalised muscle function in obese population may have substantial effects on ADL as isometric strength in the knee extensors and plantar flexors have been shown to correlate with the ability to maintain postural control (Onambele et al. 2006; Nocera et al. 2010). Reductions in normalised force can lead to an increase in postural sway, which leads to a greater risk of loss of balance and falling (Singh et al. 2009). A decline in normalised force could be exacerbated in older adults due to the age-related decline in contractile performance (Larsson et al. 2019), and may in part explain the greater risk of falling established in an elderly obese population (Mitchell et al. 2015). As such, research has concluded that obesity induced reductions in normalised muscle function contribute to a decline in functional capacity, particularly ADL and other forms of physical activity (Maffiuletti et al. 2013).

1.3.4 – Isometric and Concentric Muscle Quality

Whilst normalising muscle performance to body mass gives an important insight into the effects of obesity on functional performance, contractile performance normalised to estimated muscle mass gives an estimation of obesity effects on the intrinsic force producing properties of the muscle i.e. muscle quality (Villareal et al. 2004). Muscle quality (force or power produced relative to muscle mass, [Hill and Tallis, 2019]) is an important consideration as obesity can result in larger muscles of poorer quality, which produce the same force and power as comparatively smaller muscles in non-obese participants, and requires a significant metabolic cost to maintain (Tallis et al. 2017). Furthermore, the larger muscles of poorer quality will contribute to an already elevated body mass, and thus, are required to produce more force to overcome bodily inertia (Tallis et al. 2017).

There are few research studies which have considered the effects of obesity on muscle quality in young adults. Those who report obesity effects on muscle quality suggest muscle quality is either comparable (Maffiuletti et al. 2007b; Pescatello et al. 2007) or reduced (Pajoutan et al. 2016; Erskine et al. 2017) in young obese adults compared to non-obese counter parts. Less ambiguity is seen in an elderly population, where typically obesity reduces the ability of muscle to produce force relative to muscle size (Villareal et al. 2004; Hilton et al. 2008; Choi et al. 2015; Rahemi et al. 2015; Erskine et al. 2017). An obesity induced decline in muscle quality has been attributed to a host of mechanisms such as a reduction in the excitation-contraction coupling process, increased intramuscular lipid, and chronic inflammation (Tallis et al. 2018). The mechanisms which contribute to an obesity induced decline in contractile performance are explored in detail in section 1.7. The ambiguity in young adults could in part be attributed to a muscle specific response to obesity (Tallis et al. 2018). Furthermore, the duration and magnitude of obesity is likely to impact muscle quality, given how long or how obese an individual is, and will influence the extent to which any mechanistic changes alter contractile performance (Hurst et al. 2019).

Methodological differences in quantification of muscle size make it difficult to make direct comparisons between studies. Magnetic resonance imaging (MRI) (Pescatello et al. 2007; Hilton et al. 2008), ultrasonography (Erskine et al. 2017), Bioelectrical Impedance Analysis (BIA) (Paolillo et al. 2012; Barbat-Artigas et al. 2012; Valenzuela et al. 2020) and dual x-ray absorptiometry (DEXA) (Rolland et al. 2004; Abdelmoula et al. 2012; Poggiogalle et al. 2019) have all being used to quantify muscle size. Studies which have reported muscle quality of a single muscle group by using total body muscle mass or FFM may not be a true representation of muscle quality (Metter et al. 1999), as obesity effects have shown to be muscle specific (Tallis et al. 2018). Furthermore, many of the methods used to quantify muscle quality give indirect estimates of muscle mass, CSA or FFM, which can make accurate readings difficult to obtain and limit the ability to make comparisons between studies (Metter et al. 1999). The combination of varying methodological approaches for quantifying tissue size, the dearth of literature examining the effects of obesity on muscle quality, and the muscle specific nature of obesity effects on contractile function, make it hard to conclude the effects of obesity on the intrinsic force producing capacity of skeletal muscle.

1.3.5 – Fatigue Resistance

The effects of obesity on muscular fatigue represents an important link to the ability to complete ADL (Maffiuletti et al. 2013), as an inability to sustain contractile performance impacts the ability to sustain locomotor performance (Maffiuletti et al. 2013; Bollinger 2017). Despite the importance fatigue resistance has in completion of daily tasks, there are few research articles examining the effects of obesity on skeletal muscle fatigue, which is why it has been highlighted as an area of interest (Maffiuletti et al. 2013). It has been proposed that irrespective of exercise intensity, obese individuals will fatigue quicker than their non-obese counterparts as muscle will have to work

at a greater intensity to overcome elevated body inertia (Tallis et al. 2017). However, it is unclear as to whether obesity evokes a reduction in the inherent fatigue resistance of muscle.

The limited available evidence examining the effects of obesity during sustained locomotor tasks suggests obesity diminishes functional capacity (Pataky et al. 2013; Manawat and Shweta 2018). During a 6 minute walk assessment, which is considered a functional assessment that incorporates an aspect of lower limb muscular endurance, obese individuals cover less distance during the walk when compared to their non-obese counterparts (Pataky et al. 2013; Manawat and Shweta 2018). However, significant obesity induced reductions in distance walked does not necessarily mean diminished ability to sustain repetitive muscular contractions. Obese participants have been shown to alter their gait pattern, resulting in lower gait speeds and shorter stride lengths, which will influence distance covered (Pataky et al. 2013). Furthermore, when compared to non-obese participants, those who are obese have an increase in metabolic demand to perform the same task, which may also contribute to poorer performance (Manawat and Shweta 2018). As such, assessing whole body locomotor tasks in obese individuals for the understanding of direct muscular effects is limited due to the other variables which contribute to a reduction in performance, including having to overcome a greater mass to produce movement.

There are few research studies directly exploring the effects of obesity on fatigue of a specific muscle group, and the responses reported are not consistent between studies. Evidence exists for unchanged voluntary fatigue resistance between obese and non-obese counter parts (Maffiuletti et al. 2008; Minetto et al. 2012; Pajoutan et al. 2017), where others report an obesity induced reduction in voluntary fatigue resistance (Maffiuletti et al. 2007b; Cavuoto and Nussbaum 2013). Studies which have considered involuntary fatigue resistance, report no differences in force production between obese and lean groups during a five-minute bout of electrically stimulated isometric contractions

(Maffiuletti et al. 2007). Furthermore, recent research reports that a reduction in central fatigue can occur without changes in peripheral fatigue (Pajoutan et al. 2017). These findings support the idea that obesity may have little effect on fatigue resistance at the muscular level, despite a reduction in whole body performance. As such, obesity induced reductions in the ability to sustain muscular contractions may relate to obese individuals having to work at a greater percent of their maximum to produce movement, as they must overcome a greater bodily inertia. Furthermore, central inhibition likely plays a role in the reduction of voluntary fatigue resistance, as obesity is associated with physical deconditioning due to prolonged periods of inactivity (Bollinger 2017). As such, obese individuals may elect to no longer sustain force or power output during bouts of fatiguing contractions due to an unfavourable and unaccustomed sensation evoked by sustained muscle activity (Bollinger 2017). The limited quantity of research and confounding variables influencing the fatigue response make it difficult to ascertain the effects of obesity on fatigue resistance at the muscular level *in vivo.*

1.3.6 – Eccentric Contractile Performance

Despite the increase in literature examining the effects of obesity on the contractile performance of skeletal muscle, this has predominately focused on isometric and concentric muscle function (Maffiuletti et al. 2013) with very few having considered the effects of obesity on eccentric muscle performance (Tallis et al. 2018). Eccentric muscle actions, defined as force produced during muscle lengthening (LaStayo et al. 2003; Nishikawa et al. 2018a), are prominent when stabilising and deaccelerating the body whilst in motion and absorbing or storing any impact forces (LaStayo et al. 2003). Given that muscles work as antagonistic pairs, eccentric muscle activity occurs during general locomotory and functional tasks (Dickinson et al. 2000; Hody et al. 2019) and to a greater extent, during activities which require dynamic balance (Lindstedt et al. 2002), such as descending stairs (McFadyen and Winter 1988; Gault and Willems 2013) and descending into a seated position (Lovering and Brooks 2014). Eccentric muscle contractions produce greater forces in comparison to concentric

contractions (Hody et al. 2019). The high force producing nature of eccentric contractions can lead to muscle fibre damage (Fridén and Lieber 1992), with subsequent reduced muscular strength and increased severity of delayed onset of muscle soreness (DOMS) (Clarkson 1992; Hody et al. 2019). An increase in eccentric demand would be expected in an obese population, given the biomechanical differences between lean and obese individuals (Tallis et al. 2018) such as an anterior shift in the centre of mass, which may increase the amount of eccentric activity needed when stabilising the larger mass. As such, an exacerbated reduction in muscular strength through sustained periods of eccentric activity could have severe consequences on functional capacity in obese individuals. Due to the importance and regularity of eccentric muscle activity during ADL, and the potential negative consequences eccentric muscle activity can have, it is important to understand how obesity affects eccentric muscle performance. However, potential effects of obesity on the physiological mechanisms resulting in eccentric force production are currently unknown. Given differences in mechanisms resulting in concentric and eccentric force production (Herzog et al. 2016), obesity effects may not be uniform across contractile modalities.

To date, there are no research studies which have directly assessed the effects of obesity on eccentric force, torque, or power. Nonetheless, there is research which has considered rate of fatigue and recovery from a bout of fatiguing eccentric muscle contractions in overweight and obese populations (Paschalis et al. 2010; Kim and So 2018; Margaritelis et al. 2019; Yoon and Kim 2020). The inflammatory response to eccentric induced muscle damage in obese populations is consistent across the literature, with inflammatory markers such as creatine kinase (Paschalis et al. 2010; Kim and So 2018; Margaritelis et al. 2019; Yoon and Kim 2020) and myoglobin (Yoon and Kim 2020) shown to be higher following eccentric contractions in high body fat or obese individuals, when compared to healthy body fat or lean controls. An increase in such inflammatory markers would suggest increased susceptibility to muscle damage following sustained eccentric activity in an obese population; this is further

supported by an increase in perceived soreness, greater reduction in isometric strength and range of motion and prolonged period of time to return to baseline perceived soreness and isometric strength in both upper (Kim and So 2018) and lower limbs (Paschalis et al. 2010; Margaritelis et al. 2019) of obese groups, compared to lean controls following bouts of eccentric fatigue. However, an increase in inflammation markers is not always associated with other markers of muscle damage. Yoon and Kim (2020) demonstrate that whilst high body fat is associated with an increase in creatine kinase and myoglobin following 2 sets of 25 eccentric contractions of the elbow flexors, rate of loss and recovery of isometric strength and perceived soreness did not differ between high and low body fat groups. There are a host of reasons as for why differences occur between studies, including muscle group assessed, eccentric protocol chosen and classification of obesity (Yoon and Kim 2020). Even with this evidence, the topic of obesity and eccentric muscle activity remain underexplored, which limits the ability to understand any potential effects, what influence this important contractile modality has in an obesity induced reduction or change in the ability to perform ADL and how feasible/sustainable eccentric training interventions could be in improving body composition. The lack of research may be a result of the perceived high risk nature of maximal eccentric contractions and the potential to cause muscle damage (LaStayo et al. 2003), particularly in an obese population where muscle function has already been shown to be diminished. Emerging evidence suggests that eccentric training may be a more favourable method of improving body composition and muscle function in an obese population when compared to other contractile modalities (Julian et al. 2018). However, if sustained eccentric activity in obese individuals evokes exacerbated muscle weakness and soreness, eccentric training may be unsustainable long term. Thus, examining the response of obese muscle during and following eccentric fatigue is an important area of investigation.

Table 1. *Summary of research examining effects of obesity on the contractile performance on skeletal muscle in humans (adapted from Tallis et al., 2021)*

Author	Sex	Participants: Group & Age (Age/Age Range; yrs)	Absolute Contractile Performance	Force to Body Mass Ratio	Muscle Quality	Body Composition and Muscle Morphological Measurements
Miyatake et al., 2000	M&F	Control: Y (20-39) Middle aged (40-59) O (60-80) Obese: Y (20-39) Middle aged (40-59) O (60-80)	- O IM KE (kg) & HGS (kg) ↔ - Y & Middle-aged Obese IM KE ↑ - Middle-aged Obese Right HGS (kg) ↑ - M: Y Obese HGS (kg) ↑ - M: Middle-aged Left HGS (kg)↔ - F: Y & Middle-aged Obese Left HGS (kg) ↑	- Obese IM KE (Kg/kg^{-1}) ↓	N/A	N/A
Hulens et al., 2001	F	Lean (39.7 ± 12.2) Obese (39.9 ± 11.4)	- IK TF, TE & KE (N.m) ↑ - IK KF (N.m) & HGS (Kg) ↔	N/A	N/A	- FM & FFM (%) ↑ - FM & FFM (Kg) ↑
Rolland et al., 2004	F	Lean (80.7 ± 4.1) NW (80.2 ± 3.7) Obese (80.0 ± 3.5)	- Obese IM KE (N)↑versus lean - Obese & NW IM KE (N) ↔ - HGS (Nm^2) ↔ - Obese IM EE (N)↑versus lean & NW - Sedentary Individuals IM KE (N) ↔ Irrespective of BMI * - Active Obese IM KE (N)↑ versus Active Lean * - Active Obese and NW IM KE (N) ↔ *	N/A	N/A	- Obese FM & FFM (Kg & %) Total MM, leg MM & arm MM (Kg) ↑
Villareal et al., 2004	M&F	Non-obese non-frail (70.6 ± 0.8) Non-obese frail (77.3 ± 0.5) Obese (76.5 ± 0.9)	- Obese IK CON KE, KF ($60°s^{-1}$; N.m) ↓ versus non-obese non-fail - Obese & Non-obese frail IK CON KE, KF ($60°s^{-1}$; N.m) ↔	N/A	- Obese IK CON KE, KF torque p.u. LE LM ($60°s^{-1}$; $N.m/kg^{-1}$) ↓	- Obese total fat (Kg & %), FFM (%) ↑ - Obese FFM (Kg) ↓

Lafortuna et al., 2005	M&F	M: NW (29.2 ± 6.9) Obese (29.4 ± 7.1) F: NW (30.8 ± 3.4) Obese (30.0 ± 6.7)	- Leg & Chest Press 1RM (N) ↑ - Vertical Jump (N) ↑ - Vertical Jump (W) ↑	- Vertical Jump (N/kg^{-1}) ↓ - Vertical Jump (W/kg^{-1}) ↓	- Leg & Chest Press 1RM p.u. FFM (N/kg^{-1}) ↔ - Vertical Jump p.u. FFM (N/kg^{-1}) ↔ - Vertical Jump p.u. FFM (W/kg^{-1}) ↔	- FM & FFM (Kg) ↑
Maffiuletti et al., 2007b	M	Non-obese (27.0 ± 4.1) Obese (25.3 ± 5.2)	- IK CON KE (60°s^{-1}, 120°s^{-1} & 180°s^{-1} N.m) ↑ - IK CON KE (60°s^{-1}, 120°s^{-1} & 180°s^{-1} W) ↑ - IM KE (40°, 60° & 80° N.m) ↑	- IK CON KE (60°s^{-1}, 120°s^{-1} & 180°s^{-1} N.m/kg^{-1}) ↓ - IK CON KE (60°s^{-1}, 120°s^{-1} & 180°s^{-1} W/kg^{-1}) ↓ - IM KE (40°, 60° & 80° N.m/kg^{-1}) ↓	- IK CON KE p.u. FFM (60°s^{-1}, 120°s^{-1} & 180°s^{-1} N.m/kg^{-1}) ↓ - IK CON KE p.u. FFM (60°s^{-1}, 120°s^{-1} & 180°s^{-1} W/kg^{-1}) ↓ - IM KE p.u. FFM (40°, 60° & 80° N.m/kg^{-1}) ↓	FFM (Kg) ↑
Pescatello et al., 2007	M&F	NW (23.4 ± 0.3)** OW/Obese (25.6 ± 0.4)**	- OW & Obese IM and 1RM EF (Kg)↑	- OW & Obese IM and 1RM EF (Kg/kg^{-1}) ↔	N/A	OW & Obese Biceps Brachii CSA volume (cm^2) ↑
Hilton et al., 2008	M&F	Non-obese (58.0 ± 10.0) Obese (58.0 ± 9.2)	- IM DF, PF (N.m) ↓ - IK CON PF, DF (60°s^{-1}, 120°s^{-1}; W) ↓ - IK CON PF (60°s^{-1}, 120°s^{-1}; N.m) ↓ - IK CON DF (60°s^{-1}; N.m) ↓ - IK CON DF (120°s^{-1}; N.m) ↔	N/A	- IK CON PF (60°s^{-1}, 120°s^{-1}) p.u. MV (W/cm^3) ↓ - IK CON DF p.u. MV (120°s^{-1}; W/cm^3) ↓ - IK CON DF p.u. MV (60°s^{-1}; W/cm^3) ↔	- Distal LE IMAT volume (cm^3) ↑ - LE MV (cm^3), adipose tissue volume (cm^3) & muscle CSA (cm^2) ↔
Capodaglio et al., 2009	F	Non-obese (19-38) Obese (20-43)	- IK CON KE (60°s^{-1}, 180°s^{-1} & 240°s^{-1} N.m) ↑ - IK CON KF (60°s^{-1}, 180°s^{-1} & 240°s^{-1} N.m) ↔	- IK CON KE (60°s^{-1}, 180°s^{-1} & 240°s^{-1} N.m/kg^{-1}) ↓ - IK CON KF (60°s^{-1}, 180°s^{-1} & 240°s^{-1} N.m/kg^{-1}) ↓	N/A	N/A
Minetto et al., 2012	M&F	Non-obese (35.0 ± 12.7) Severely Obese (37.4 ± 8.8)	- IM KE (120°, N) ↑	- IM KE (120°, N/kg^{-1}) ↔	N/A	- FFM (Kg) ↔ - FM (kg), Subcutaneous tissue thickness VL & VM (mm) ↑

Paolillo et al., 2012	F	Non-obese (54.0 ± 11.0) Obese (58.0 ± 2.0)	- IK CON KE (60°s^{-1}; N.m) ↔ - IK CON KE (300°s^{-1}; W) ↑	- IK CON KE (60°s^{-1}; N.m/kg^{-1}) ↓ - IK CON KE (300°$^{-1}$; W/kg^{-1}) ↔	- IK CON KE p.u. LM (60°s^{-1}; N.m./kg^{-1}) ↓ - IK CON KE p.u. LM (300°s^{-1}; W/kg^{-1}) ↔	- BF (%), LM (kg), FM (kg) ↑
Cavuoto & Nussbaum 2013	M&F	Non-obese(22.4 ± 2.2) Obese (23.0 ± 3.8)	- IM SF & HGS (N) ↑ - IM TE (N) ↔	- IM SF, TE & HGS (N/kg^{-1}) ↓	N/A	WC, HC (cm) & W:H ↑
Tomlinson et al., 2014a	F	Y: UW (23.0 ± 6.7) NW (23.2 ± 7.9) OW (23.6 ± 8.0) Obese (30.9 ± 10.7) O: UW (63.8 ± 5.7) NW (63.5 ± 7.7) OW (68.2 ± 4.8) Obese (62.5 ± 9.0)	- Y Obese Net IM PF & IM PF (N.m) ↑ versus Y NW & UW - Y Obese & OW Net IM PF & IM PF (N.m) ↔ - O Net IM PF & IM PF (N.m) ↔ - Y IM DF (N.m) ↔ - O Obese IM DF (N.m) ↑ - Y activation & co-contraction (%) ↔ - O activation & co-contraction (%) ↔	- Y Obese IM PF (N.m/kg^{-1}) ↓ - O Obese IM PF (N.m/kg^{-1}) ↓ versus O UW - O Obese, OW & NW IM PF (N.m/kg^{-1}) ↔ - Y Obese Net IM PF (N.m/kg^{-1}) ↓ versus Y NW & UW - Y Obese & OW Net IM PF (N.m/kg^{-1}) ↔ - O Obese Net IM PF (N.m/kg^{-1}) ↓ versus O NW - O Obese, OW & UW Net IM PF (N.m/kg^{-1}) ↔	N/A	- Obese BF (%), total BF & LM (kg) leg FM (kg)↑ - O Obese Leg LM (kg) ↑versus O NW, UW & OW - Y Obese Leg LM (kg)↑versus Y NW & UW - Y Obese & OW Leg LM (kg) ↔
Tomlinson et al., 2014b	F	Y (25.5 ± 9.0): UW NW OW Obese O (64.8 ± 7.2): UW NW OW Obese	- Obese Net IM PF (N.m) ↑ versus NW & UW - Obese & OW Net IM PF (N.m) ↔ - High BF Net IM PF (N.m) ↑ versus normal BF	N/A	- Obese Net IM PF p.u. MV (N.m/cm^3)↓versus NW - Obese, UW & OW Net IM PF p.u. MV (N.m/cm^3) ↔ - Obese GM specific force (GM fascicle force/PCSA)↓versus NW & UW - Obese & OW GM specific force (GM fascicle force/PCSA) ↔ - High BF GM specific force (GM fascicle force/PCSA) & Net IM PF p.u. MV (N.m/cm^3)↓versus normal BF	- Obese MV (cm^3)↑ - High BF MV (cm^3)↑versus normal BF

Choi et al., 2015	M&F	NW (70.0 ± 2.0)** Obese (69.0 ± 2.0)**	- IK CON KE (N.m) ↑ - Type I single fibre power (μN.FLs^{-1}) ↓ - Type I fibre maximal shortening velocity (FLs^{-1}) ↓ - Type I & IIa maximal Ca^{2+} activated force (mN) ↓ - Type IIa single fibre power (μN.FLs^{-1}) ↔ - Type IIa fibre maximal shortening velocity (FLs^{-1}) ↔	- IK CON KE (N.m/kg^{-1}) ↓	- IK CON KE p.u. thigh MV (N.m/cm^3) ↓ - Type I isolated fibre power p.u. fibre size (W/litre fibre) ↓ - Type IIa isolated fibre power p.u. fibre size (W/litre fibre) ↔ - Type I & IIa maximal Ca^{2+} activated force p.u. CSA (kN/m^2) ↓	- Total thigh volume (cm^3), thigh fat volume (cm^3), thigh MV (cm^3), intramuscular fat volume (cm^3), type I & IIa fibre CSA (μm^2), type I fibre intramyocellular lipid ↑
Pajoutan et al., 2016	M&F	NW (31.5 ± 8.6) OW (31.5 ± 9.1) Obese (32.1 ± 12.3)	- IK TE (N.m) ↔	- OW & Obese IK TE (N.m/kg^{-1}) ↓	- Obese IK TE p.u. FFM (N.m/kg^{-1}) ↓	- OW & Obese BF (%), WC & HC (cm) ↑ versus NW - Obese FFM (kg) & W:H ↑ versus NW
Erskine et al., 2017	M&F	Y: Normal BF (24.0 ± 8.4) High BF (28.9 ± 9.7) O: Normal BF (65.5 ± 8.0) High BF (66.0 ± 7.3)	- High BF IM PF (N.m) ↑ - IK CON PF (60°s^{-1}; N.m) ↔ - High BF GM fascicle force (N) ↑ - High BF Activation capacity (%) ↓	- High BF IK CON (60°s^{-1}) & IM PF (N.m. kg^{-1}) ↓	- GM specific force (GM fascicle force/PCSA) ↔ - High BF IK CON (60°s^{-1}) & IM PF p.u. MV (N.m/cm^3) ↓ - Y normal BF IK CON (60°s^{-1}) & IM PF p.u. MV (N.m/cm^3) ↑ versus all other groups	- GM fascicle length ↔ - High BF GM FPA, FM (kg), LM (kg), GM volume (cm^3), GM PCSA (cm^2)↑

Abbreviations: BMI, body mass index; M, male; F, female; Y, young; O, old; NW, normal weight; UW, underweight; OW, overweight; IM, isometric; IK, isokinetic; CON, concentric; KE, knee extensors; EE, elbow extensors; EF, elbow flexors; TF, trunk flexors; TE, trunk extensors; PF, plantar flexor; DF, dorsi flexor; GM, gastrocnemius medialis; VL, vastus lateralis; VM, vastus medialis HGS, hand grip strength; FM, fat mass; FFM, fat free mass; MM, muscle mass; MV, muscle volume; BF, body fat; LM, lean mass; LE, lower extremity; 1RM, one rep maximum; IMAT, intramuscular adipose tissue; CSA, cross sectional area; PSCA, physiological cross sectional area; FPA, fascicle pennation angle; W, watts; WC, waist circumference; NC, neck circumference; W:H, waist to hip ratio; N/A, not applicable; p.u., per unit; Net, sum of maximal torque and co-contraction torque; data presented as mean ± SD; * contractile function adjusted for physical activity; ** data presented as mean ± S.E.M; ↓/↑ $P<0.05$, ↔ no change/difference.

1.4 – *In Vivo* Assessments of Skeletal Muscle Function

There are many recognised assessments of skeletal muscle performance. However, research studies which have examined the effects of obesity on skeletal muscle function typically utilise isokinetic dynamometry or hand-held dynamometers, and to a lesser extent assess sit to stand (STS) performance. Peak concentric and eccentric torque across a range of contractile velocities obtained using Isokinetic dynamometry (IKD) has previously shown to have good to excellent test-retest (Maffiuletti et al. 2007a, 2007b; Orri and Darden 2008) (intraclass correlation coefficients [ICC] ≥ 0.94 and coefficient of variation [CV] ≤ 7 for both lower and upper body assessments of peak torque) reliability. IKD is considered the gold standard in strength assessments and is often referenced as the standard to which other methods are validated. Strength assessments using IKD can measure isometric force, and concentric and eccentric force produced at a fixed angular velocity over a predefined range of motion. The IKD offers a useful approach to assessing contractile function of a specific muscle group, under controlled conditions, as parameters such as peak torque, fatigability, angle of peak force and time to peak force can all be measured (Li et al. 2006). Despite the precise assessment of strength isokinetic machines, they are not always used as they are particularly large, expensive and require training to use and maintain safety of the participants. As such, IKD cannot easily be used in clinical environments (Kannus 1994) and is largely implemented as a research tool. IKD assessments of strength during fixed angular velocities fail to represent the dynamic change in velocity during many daily tasks but do ensure that the testing regime is repeatable.

Hand-Held dynamometers (HHD) are a more user friendly, portable and relatively cost effective (Li et al. 2006) alternative to IKD. One of the most commonly used HHD is the handgrip dynamometer (Yeung et al. 2018). Handgrip strength (HGS) (kg) has been proposed to be a cost effective and valid predictor of overall muscle strength in both adult (Wind et al. 2010; Trosclair et al. 2011) and elderly populations (Lauretani et al. 2003; Duchowny et al. 2017). Furthermore, in an ageing population, low HGS is associated with an elevated risk of functional disabilities (Zamboni et al. 2008) and impairments

in completing ADL (Germain et al. 2016). However, recent work suggests that measurements of one single muscle group alone should not be considered as a proxy for overall muscle strength (Yeung et al. 2018) and that HGS has a poor association with lower extremity strength (Jenkins et al. 2014; Yeung et al. 2018). Research suggests that muscle ageing causes substantially greater decline in contractile performance of lower extremities compared to upper body performance (Samuel and Rowe 2012). In addition, there is a dearth of literature in examination of the association of HGS and eccentric muscle function; as eccentric muscle function is pivotal for the completion of ADL, when approximating overall muscle strength, eccentric force production should be considered. As such, using HGS as an approximate for overall muscle strength in an elderly population may underestimate muscle weakness (Harris-Love et al. 2018). The lack of consensus regarding the association of HGS and overall muscle strength may be attributed to the methodological inconsistencies across studies (Yeung et al. 2018). Strength values from HGS are highly variable, dependent on factors such as hand position, body position and verbal encouragement (Beaudart et al. 2019). Therefore, a standardized approach is recommended to help reduce variability between studies (Roberts et al. 2011). Despite these limitations, HGS has shown to have a good-excellent test-retest reliability (ICC ≥ 0.80) in several populations (Innes 2002; Bodilsen et al. 2015; Bohannon 2017), can be used to assess a large sample size (Beaudart et al. 2019), and has been shown to be a predictor of biomarkers such as bone mineral density, nutritional status and overall strength and function in older adults (Bohannon 2019).

Other HHD have also been designed for the assessment of lower extremity muscle function. Previous research shows good to excellent reliability and validity of HHD assessment of peak force from the hip and knee when compared to IKD assessment of peak torque (ICC ≥ 0.70) (Wang et al. 2011; Mentiplay et al. 2015). However, the use of HHD in research studies and clinical environments has been debated. It is suggested that strength assessments utilised should have <15% limit of agreement (LOA) between trials (Prentice 2015), yet a recent meta-analysis reports that the upper LOA was always >15% for HHD

assessing lower limb function (Chamorro et al. 2017). Such a wide range of LOA makes it difficult to accurately detect differences in muscle performance, given small or medium sized changes in function could be a result of instrument error. As such, IKD is still the recommended tool for strength assessments *in vivo* (Chamorro et al. 2017).

Functional assessments of lower extremity strength have also been tested through ground reaction forces (GRF) recorded by a force plate during sit to stand (STS) performance (Bohannon et al. 2010). There are number of methods employed to measure STS performance; which are typically completing a certain number of repetition's e.g. three or five-times STS (McCarthy et al. 2004; Schaubert and Bohannon 2005; Tsuji et al. 2015; Abe et al. 2016; Kera et al. 2022), or the number of repetitions completed within a certain time frame e.g. 30-second chair-stand test (Jones et al. 1999; Schaubert and Bohannon 2005). Measures of GRF obtained through STS assessments have shown good test-retest reliability (ICC ≥ 0.73) (Shen et al. 2017). Furthermore, GRF obtained through assessment of STS performance appear to predict physical function (Tsuji et al. 2011), risk of falling (Fleming et al. 1991; Abe et al. 2016), and all-cause mortality (Penninx et al. 2000), in addition to being a useful diagnostic tool for sarcopenia in a clinical setting (Kera et al. 2022). Using STS performance as an indicator for lower extremity strength and functional capacity is argued to be more functionally relevant than assessments which only measure strength of a single joint (Tsuji et al. 2015). However, there are some limitations associated with STS assessments, particularly with obese and or old participants, as significant restrictions on muscular strength and power may limit their ability to complete consecutive sit to stands (Beaudart et al. 2019).

In vivo assessments of skeletal muscle function have been fundamental for our understanding of obesity effects on *in vivo* muscle performance. However, utilising an isolated muscle model for assessing the effects of obesity on skeletal muscle contractility has some unique advantages, which

allows for the exploration of the underpinning mechanisms which mediate muscle performance *in vivo* (Tallis et al. 2018).

1.5 – Animal Isolated Muscle Model

The research studies implemented in this thesis which utilise an animal isolated skeletal muscle model use a whole muscle tendon unit, and the following review will largely focus on this approach. However, it should be noted that *in vitro* (experiments on tissues isolated from a living organism which are conducted separately from the organism itself) experiments of isolated skeletal muscle can also be conducted on a smaller muscle fibre bundles or single muscle fibres. There is a trade off when using a whole intact muscle tendon unit, whilst utilising the whole unit enables a more viable replication of *in vivo* muscle mechanics, using a whole muscle increases the chances of gradual muscle damage during experiments through increased oxygen diffusion time, resulting in the build-up of an anoxic core (Barclay 2005). Although, this is not a limiting factor in the present thesis as the magnitude of damage is limited when utilising a mouse model, due to the small size of the muscles used. Furthermore, previous work has established a linear decline in contractile output over time in isolated mouse skeletal muscle (James et al. 1995; Tallis 2013); this enables for correction once the data is obtained. An animal isolated muscle approach provides a unique assessment of contractile function, which allows for a more detailed understanding of the effects of obesity at the muscular level and the underpinning mechanisms which mediate an obesity induced change in muscle function (Tallis et al. 2018). The advantages of an animal isolated muscle approach will be discussed in detail below.

1.5.1 – Control of Diet and Feeding Duration

The duration and magnitude of obesity can be more tightly controlled using an animal model, important as these are likely two factors which play some part in how the muscular system responds

to obesity. In fact, this is highlighted in recent research showing that both magnitude of adipose accumulation, and duration of high-fat diet (HFD) feeding, influence contractile performance (Hurst et al. 2019). Another key factor which can be controlled is dietary composition. The composition of the diet used to evoke obesity can have differing effects on the morphological characteristics of skeletal muscle, which may mediate changes in contractile function, such as an earlier onset of excessive intramuscular lipids in fixed HFD compared to forage HFD (Tallis et al. 2017; Eshima et al. 2017; Messa et al. 2020). It would be difficult to control for dietary composition and duration of obesity in humans, given that the onset of obesity could occur at any time throughout the lifespan, and a detailed recollection of dietary history would not be accurate. As such, using an animal isolated muscle model allows for control of variables which may be the source of some of the ambiguity regarding the effects of obesity of muscle contractility from human research.

1.5.2 – Central Nervous System

During *in vivo* assessments of muscle contractility, the central nervous system (CNS) is an integral factor which mediates the level of activation. In consideration of *in vivo* function, accounting for the effects of obesity on the CNS is important as skeletal muscle activation will only occur once the CNS sends impulses through the spinal cord and the anterior motor neuron (McArdle et al. 2015). Obesity has been demonstrated to influence neuromuscular recruitment, with obese individuals recruiting fewer fibres to produce maximal voluntary torque of the knee extensors when compared to lean counterparts (85.1% for obese and 95.3% for lean) (Blimkie et al. 1989). However, accounting for the CNS limits our ability to understand if changes in contractile performance occur through impaired neuromuscular control or skeletal muscle dysfunction. Therefore, the removal of the CNS, via isolated muscle experiments, has been important as it has allowed researchers to establish the direct effects of obesity at the muscular level, and ensures a direct comparison between obese and lean muscle, independent of any obesity induced changes in the CNS. Furthermore, understanding the

direct effects of obesity at the muscular level has been important for targeting therapeutic strategies, as it has been established that obesity induced changes in muscle function occur, at least in part, through mechanisms altering the intrinsic force producing capacity of the muscle.

1.5.3 – Muscle Specific Response and Muscle Quality

Assessing the effects of obesity on skeletal muscle function *in vivo* involves the testing of whole muscle groups, e.g. knee extensors and knee flexors (Table 1). This approach is valuable in relation to *in vivo* function, given groups of muscles work in tandem at various levels of activation during movement (Cuesta-Vargas and González-Sánchez 2013). However, whole muscle groups differ in fibre type composition and mechanical role, highlighted in the knee extensors where the rectus femoris contains a greater proportion of fast twitch muscle fibres when compared to the predominately slow twitch vastus medialis (Kary 2010). This limits the ability of researchers to identify any potential fibre type specific responses to obesity, which have been highlighted in isolated skeletal muscle research (Tallis et al. 2018). Understanding muscle specific responses is not only integral for a detailed understanding of the effects of obesity on contractile performance but also indicates which potential therapeutic strategies may be successful in offsetting declines in contractile performance.

In whole body *in vivo* assessments, it is difficult to accurately determine muscle quality (contractile performance normalised to muscle size) as muscle groups are assessed in tandem and vary in levels of activation dependent upon the task. Therefore, it can be difficult to accurately determine which muscle experiences a reduction in contractile performance relative to muscle size. Furthermore, accurately measuring muscle or muscle group size *in vivo* is difficult, can be costly, only provides an estimation and has been reported using a variety of different techniques such as multi frequency BIA (Valenzuela et al. 2020), MRI (Hilton et al. 2008) or B-mode ultrasonography (Erskine et al. 2017). The varying approaches used to obtain muscle size likely contribute to some of the ambiguity regarding

obesity effects on muscle quality. However, assessing the effects of obesity using an isolated muscle model means the whole muscle can be directly weighed and contractile performance can be normalised to muscle mass (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019), a more accurate measure of muscle quality and an indication of any changes to the intrinsic force producing capacity of the muscle.

1.5.4 – Fatigue Resistance

Measuring fatigue resistance *in vivo* limits our ability to understand the effects of obesity directly at the muscular level, due to a variety of factors which can influence the fatigue response. Obese individuals are likely to fatigue more quickly during sustained whole-body movements, due to the need to overcome a greater bodily inertia in order to produce movement (Tallis et al. 2017). Moreover, they are likely working at a greater percentage of their maximum force or power to produce the same movement as a non-obese individual; this can be somewhat overcome by utilising a method of fatigue which involves isolation of whole muscle group without the need to manoeuvre a larger mass, such as sustained contractions using an isokinetic dynamometer (Maffiuletti et al. 2007b, 2008). However, limb mass in obese individuals will still be greater, which will contribute to the fatigue response. In isolated muscle models these factors are eradicated allowing for direct examination of how obesity affects the fatigue response at the muscular level.

1.6. High-fat Diet Effects on Isolated Skeletal Muscle Contractility

The present thesis uses a dietary induced obesity model, opposed to a genetic obesity model, as mechanistic responses differ between approaches (Matsakas et al. 2015; Tallis et al. 2018). The most notable difference between models is the decrease in muscle mass observed in genetic obesity models (Warmington et al. 2000; Kemp et al. 2009), whereas dietary obesity evokes an increase (Tallis et al. 2017; Hurst et al. 2019) or comparable muscle mass (Ciapaite et al. 2015; Eshima et al. 2017, 2020) compared to mice consuming standard chow, replicable to what has been observed in humans. Furthermore, dietary induced obesity closely replicates the onset of obesity experienced in humans, which typically occurs through a calorie rich diet. Therefore, the following narrative review will focus on experiments which have utilised a dietary induced obesity model in rodents, unless specified.

Whilst there are unique advantages to isolated skeletal muscle models, ascertaining the effects of obesity from current research utilising isolated muscle preparations can be difficult due to the variety of methodological approaches taken. Methodological differences between studies, such as species, strain, gender, and age of animals used, feeding durations, composition of diets, administration of diet and the experimental muscle test temperature are all likely to influence the contractile response to obesity (Tallis et al. 2018). This is partly shown by Hurst et al, (2019), who identify that both duration and magnitude of obesity are key determinants in the extent to which HFD feeding adversely affects contractile function. Furthermore, obesity induced alterations to the underpinning mechanisms mediating changes in muscle performance, such as intramuscular lipid (IMCL) content and oxidative capacity, are dependent on dietary composition, duration of feeding, and age of animals used (Eshima et al. 2017; Messa et al. 2020). One of the main sources of ambiguity may be the experimental temperature experiments are performed at (Tallis et al. 2022). Isometric twitch performance and power output in isolated muscle from young adult mice are greatest at a physiologically relevant temperature (~35-40°C) (James 2013; James et al. 2015). Improved contractile output at a higher

experimental temperatures are attributed to changes in calcium handling and cross bridge kinetics (Hou et al. 1992; Colombini et al. 2008). The same mechanisms are also affected by obesity (Bruton et al. 2002; Ciapaite et al. 2015). As such, studies that have conducted experiments at temperatures substantially lower than physiological relevance, as low as 20-25°C (Ciapaite et al. 2015; Bott et al. 2017), may mask some HFD effects on isolated rodent skeletal muscle function. This has been demonstrated in a recent study, which reports that HFD effects on force and power of isolated mouse soleus and diaphragm are less apparent at experimental temperatures which fall below typical physiological temperatures (Tallis et al. 2022). Despite methodological differences between studies, there are some general trends reported in the literature as to how HFD effects contractile performance.

1.6.1 - Absolute force and power

Experiments on the effects of obesity on isolated mouse skeletal muscle contractility are typically conducted on the soleus and EDL as they are comprised of different fibre types and play a role in locomotion. Previous *in vitro* research which has implemented an isolated muscle approach for examining dietary induced obesity effects on muscle function, typically utilised an assessment of maximal isometric force (Ciapaite et al. 2015; Seebacher et al. 2017; Tallis et al. 2017; Eshima et al. 2017, 2020; Bott et al. 2017; Hill et al. 2019; Hurst et al. 2019). Research which has reported isometric performance has yielded ambiguous results. Like that of human work, evidence indicates that obesity in rodents can result in an increase in the absolute force producing capacity of postural muscles such as the soleus (Tallis et al. 2017) which supports the suggestion that postural muscles may undergo positive adaptations to account for an elevated body mass. However, in most instances, absolute soleus force is comparable between HFD and control groups (Ciapaite et al. 2015; Hurst et al. 2019; Eshima et al. 2020). In fast twitch mouse muscle, high fat diet consumption does not elicit an increase in the isometric force producing capacity of isolated muscle (Ciapaite et al. 2015; Tallis et al. 2017;

Bott et al. 2017; Hill et al. 2019; Hurst et al. 2019) and in some cases is detrimental (Eshima et al. 2017, 2020).

There is a dearth of literature examining the capacity of isolated muscle to produce power, a notable absence given that power production is necessary for locomotion. Furthermore, the physiological mechanisms responsible for force and power production are not uniform; therefore, obesity effects on force production cannot reliably predict obesity effects on power production, as supported by previous work examining mammalian muscle (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019). Despite the potential for an increase in isometric force of the soleus, HFD consumption or increased calorie intake does not result in any significant differences in the absolute power producing capacity across a range of muscles varying in fibre type composition isolated from young adult mice (Tallis et al. 2017; Hurst et al. 2019) or zebrafish (*Danio rerio*) (Seebacher et al. 2017), when compared to control groups.

The HFD effects on absolute force and power of isolated skeletal muscle from older rodents have not been thoroughly explored. The available evidence indicates that over short feeding durations (~9weeks) a HFD does not affect mouse soleus and EDL absolute force (Hill et al. 2019). However, over longer feeding durations (~20 months) absolute soleus force is comparable, but EDL absolute force is reduced in HFD treated mice (Eshima et al. 2020). Longer HFD feeding durations are likely to evoke a more detrimental effect in fast twitch muscle, e.g. EDL. Muscle composed of a faster phenotype is unable to effectively metabolise sustained lipid overload within the muscle evoked by HFD feeding, unlike like that of muscle composed of slower fibres, e.g. soleus, which has a more favourable metabolic profile to oxidise lipids. To date, only one study has examined the impact of HFD consumption on absolute power of tissue isolated from older rodents. Hill et al, (2019) report that a

HFD evokes an increase in absolute power of isolated EDL and soleus, likely a hypertrophic response to supporting a larger mass, which can help maintain locomotor performance.

Whilst absolute function is an important marker for locomotor performance and dynamic postural control, it provides little Information on how a HFD effects the intrinsic force producing capacity of skeletal muscle. Declines in muscle quality (force or power normalised to CSA or muscle mass) has important implications for a negative obesity cycle, as larger muscles of poorer quality can be formed to perform the same mechanical tasks and contribute to an already elevated mass (Tallis et al. 2018); Effects on both muscle size and quality need to be considered to understand the likely effects *in vivo*.

1.6.2 – Isometric stress and power output normalised to muscle mass

Isometric stress, defined as force relative to muscle cross-sectional area, and power output normalised to muscle mass (Tallis et al. 2018), give a direct indication of muscle quality. Research utilising an isolated muscle model has offered the first detailed indication that a HFD reduces muscle quality in a muscle and contractile mode specific manner and is influenced by feeding duration and dietary composition. Obesity induced reductions in isometric stress of the EDL (Tallis et al. 2017; Eshima et al. 2017, 2020), diaphragm (Tallis et al. 2017; Hurst et al. 2019) and soleus (Ciapaite et al. 2015) from mice, and anterior dorsal musculature (Seebacher et al. 2017; Seebacher and James 2019) from zebrafish (danio rerio) have all been reported. Furthermore, power normalised to muscle mass has shown to be reduced in anterior dorsal musculature (Seebacher et al. 2017), EDL and diaphragm (Tallis et al. 2017; Hurst et al. 2019), following calorie surplus or HFD feeding respectively. For the soleus, power output normalised to muscle mass is reduced after 8 weeks HFD (Hurst et al. 2019), but not after feeding durations ≥ 12 weeks (Tallis et al. 2017; Hurst et al. 2019).

An obesity induced decline in muscle quality of muscle comprised of faster fibre type composition, e.g. EDL and diaphragm, is likely a result of an increase in IMAT and or IMCL due to an unfavourable metabolic profile for oxidising lipids; which increases tissue mass, with little change in quantity of contractile proteins. Furthermore, unlike the predominant weight bearing and postural muscles, there is limited hypertrophic capacity for the diaphragm, meaning an increase in adipose tissue on the diaphragm will likely increase respiratory resistance; more specifically acts as opposition to air flow during inspiration (Sharp et al. 1986; Lazarus et al. 1997). Work by Hurst et al (2019) highlights the complexity of obesity effects, as duration alone may not solely account for changes in contractile function, as individuals who accumulated adipose more rapidly may experience a decline in muscle quality earlier than 8 weeks into a HFD. As such, obesity can result in larger diaphragms and EDL's of poorer quality, which can only perform at the same absolute intensity as muscle from lean rodents, potentially limiting exercise capacity and promoting a negative obesity cycle (Tallis et al. 2018). The results reported for soleus PO normalised to muscle mass infer the soleus adapts to HFD consumption, whereby muscle quality is diminished at 8 weeks, but after 12 weeks the muscle adapts to the elevated mass; likely to ensure the maintenance of locomotor performance and dynamic postural control.

In older rodents, the effects of HFD consumption on muscle quality are equivocal. Over longer feeding durations (38 weeks or 20 months) HFD feeding evoked a reduction in isometric stress in the mixed fibre type tibialis anterior (Abrigo et al. 2016) and fast twitch EDL (Eshima et al. 2020), but no change in slow twitch soleus (Eshima et al. 2020). However, these responses are not observed for shorter HFD durations (9 or 13 weeks) where isometric stress (Bott et al. 2017; Hill et al. 2019) and power output normalised to muscle mass (Hill et al. 2019) of soleus and EDL were comparable between HFD and control treated mice, although diaphragm muscle quality was diminished (Hill et al. 2019).

Acute force and power production are important considerations for the ability to complete activities of daily living but fail to account for activities which require sustained or repetitive muscular contractions. As such, measures of fatigue resistance are an integral part of a battery of contractile assessment for a holistic understanding of how obesity effects skeletal muscle function.

1.6.3 – Fatigue Resistance

There are few studies that have assessed the effects of a HFD on the fatigue resistance of isolated skeletal muscle. Where fatigue resistance has been measured, this has been conducted via repeated isometric tetani (Shortreed et al. 2009; Thomas et al. 2014; Seebacher et al. 2017; Seebacher and James 2019) or consecutive WL contractions (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019). Generally, HFD consumption has limited effects on rate of fatigue at the muscular level, irrespective of age, muscle fibre type, mode of fatigue (repeated tetani versus consecutive WL contractions) or animal model (mouse or zebrafish) (Shortreed et al. 2009; Thomas et al. 2014; Seebacher et al. 2017; Hill et al. 2019; Seebacher and James 2019). However, there have been some observations made which create dubiety. Whilst fatigue resistance of the diaphragm is reduced irrespective of age or feeding duration (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019), reduced capacity to sustain power output in the EDL (Hurst et al. 2019) and soleus (Tallis et al. 2017) appears to be related to the duration of HFD feeding. Rate of fatigue could be diminished in the EDL over reduced feeding durations, yet maintained when subjected to prolonged HFD consumption by taking on the metabolic profile of adjacent fibres in preparation to a shift to a 'slower' fibre type expression, in accordance to the nearest neighbour theory (Pette and Staron 1997). However, such mechanistic changes appear unlikely given there have been limited HFD effects on fibre type composition in mammalian muscle (Messa et al. 2020). Where HFD induced changes in rate of fatigue do occur at the muscular level, this will likely have a profound effect *in vivo*, where a muscle which has diminished capacity to maintain power is required to work at a greater penetrance of its maximum, when compared to lean individuals,

in order to move a larger mass (Tallis et al. 2017). A notable absence from the research studies examining the effects of HFD on fatigue resistance is the absolute performance of the tissue during fatiguing contractions. The absolute amount of work achieved during fatigue, in addition to rate of fatigue, will play a key part in exercise capacity and in the ability to complete activities of daily living which require repetitive contractions.

Whilst current research has been important in establishing the effects of HFD consumption on fatigue resistance directly at the muscular level, the protocols used for measuring fatigue resistance *in vitro* are somewhat limited. The stimulation frequency used to evoke maximal force and power is typically used for fatigue protocols (Syme and Tonks 2004, Tallis et al. 2013, Hill et al. 2018, Kissane, Egginton, and Askew 2018). However, this approach often leads to a substantial amount of negative work performed during the re-lengthening phase of a contraction, which increases at each subsequent contraction (Tallis et al. 2013, Hill et al. 2018, Kissane, Egginton, and Askew 2018). It is likely that the magnitude of calcium release evoked by the stimulation frequency needed for maximal force and power overloads the muscle cytoplasm, and only a portion of the calcium can be re-sequestered back into the SR. The remaining calcium in the muscle cytoplasm results in a high degree of activation during re-lengthening. The high degree of activation during re-lengthening observed in WL traces during fatigue is unlikely to occur *in vivo* (see work loop 18 in figure 1.1, particularly in 10 and 50 week old mice), where fibre stimulation and length change waveforms can be manipulated from one length change to the next to maximise work and negate potentially damaging excessive negative work (Wakeling and Rozitis 2005).

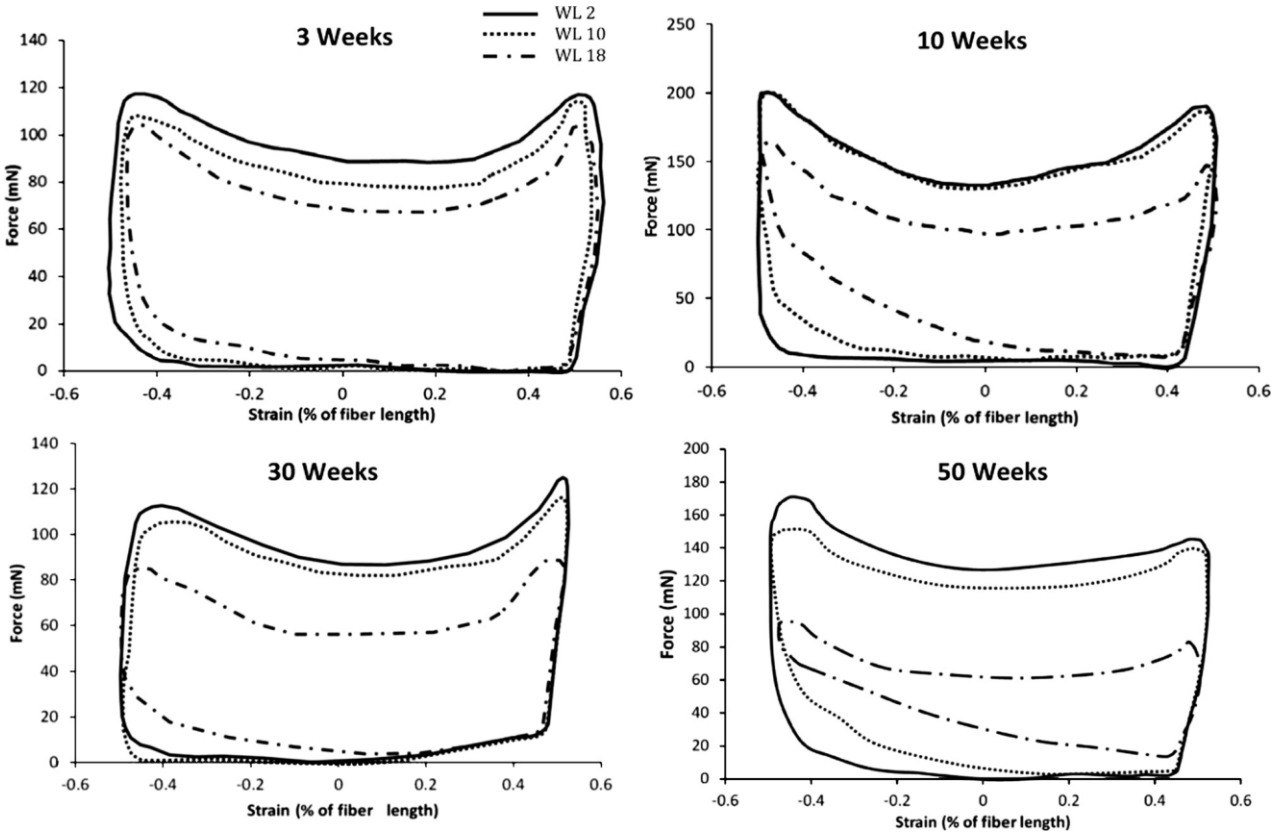

Figure 1. 1. *Effect of age on typical work-loop shapes of mouse EDL muscle during repetitive activation at 10-Hz cycle frequency for 3-, 10-, 30-, and 50-wk-old mice. Work loops (WL) 2 (at 0.2 s), 10 (at 1 s), and 18 (at 1.8 s) are shown. High degree of activation in the re-lengthening phase of the work loop as fatigue protocol progresses in isolated mouse EDL from 10- to 50-wk-old mice, using the stimulation frequency which evoked peak tetanic force (Adapted from Tallis et al., 2014).*

Table 2. *Summary of studies examining the effects of high-fat diet consumption on muscle contractile function in rodents (adapted from Tallis et al., 2021)*

Author	Animal Information	Dietary Protocol	Experimental Protocol	Absolute Contractile Performance	Muscle Quality (Contractile parameter per unit of tissue size)	Body Composition and Muscle Morphology Measurements
Shorteed et al., 2009	M C57BL6/10 mice aged 10 weeks Groups: - Control - HFD	8-week diet Control calorie (%): - fat, 20; CHO, 55; protein, 25 HFD calorie (%): - fat, 60.9; CHO, 20.1; protein, 18.3	*In situ:* IM tetanus force of whole GP complex using a range of unspecified stim. Freq.	- IM force ↔	- IM force p.u. MM ($N.g^{-1}$) ↔	- BM (g), FM (g), Type I & IIa distribution (%), IMCL (%) ↑ in HFD - CSA (μm^2) ↔
Thomas et al., 2014	M C57BL6/10 mice aged 3 weeks Groups: - Control - HFD	3-week diet Control calorie (%): - fat, 25; CHO, 55; protein, 20 HFD calorie (%): - fat, 60; CHO, 20; protein, 20	*In situ:* IM fatigue protocol of whole TS complex; 5 min of 100 Hz stimulation lasting 100 ms in 1s trains, followed by 5 min of 300 ms of 100 Hz stimulation in trains of 400 ms	- IM fatigue resistance ↔	- IM force p.u. MM ($mN.mg^{-1}$) ↔	- BM (g), FM (g), TA Type IIa & x, SOL Type IIa ↑ in HFD - TA Type IIb ↓
Ciapaite et al., 2015	M C57BL6/10 mice aged 12 weeks Groups: - Control - HFP - HFL	5-week diet Control calorie (%): - fat, 10; CHO, 70; protein, 20 HFP/HFL calorie (%): - fat, 45 (lard or palm oil); CHO, 35; protein, 20	IM twitch and tetanus force of whole SOL and EDL at 20°C	SOL: - Tetanus activation and relaxation time (ms) ↔ - Twitch activation and relaxation time (ms) ↓ in HFP versus CON EDL: - Tetanus activation and relaxation time (ms) ↔ - Twitch activation and relaxation time (ms) ↓ in HFP & HFL versus CON	SOL: - Twitch & Tetanus force p.u. MM ($N.g^{-1}$) ↓ in HFP versus CON EDL: - Twitch & Tetanus force p.u. MM ($N.g^{-1}$) ↔	BM (g) ↑ in HFP & HFL versus CON SOL: - MM, Tnnt1 ↑ in HFP & HFL versus CON - Tnnt3 ↓ in HFP & HFL versus CON - BM:MM, MHC & SERCA isoform ↔ EDL: - MM ↔ - MM:BM ↓ in HFP & HFL versus CON - PGC1α and mitochondrial oxidative phosphorylation pathway complexes III & IV, ATP synthase ↑ in HFP & HFL versus CON
Abrigo et al., 2016	M C57BL6/10 mice aged 12 weeks Groups: - Control - HFD	38-week diet. Control calorie (%): - fat, 10; CHO, 70; protein, 20	*In vivo:* - forelimb strength via weightlifting links of mass 15.5g – 54.1g *In vitro*: - IM tetanus force of whole TA	- *In vivo* strength ↓ in HFD	- *In vivo* strength p.u. body mass ↓ in HFD - Tetanic stress p.u. muscle CSA (mN/mm^2) ↓ in HFD at all stim. freq.	- Type IIa distribution (%) ↑ in HFD - Type IIb distribution (%)↓ in HFD - Percentage of fibres with a larger diameter (μm) ↓ in HFD

		HFD calorie (%): - fat, 60; CHO, 20; protein, 20	using stim. freq. 10Hz – 150 Hz at room temp.			
Bott et al., 2017	M C57BL/6 mice aged 20 weeks Groups: - Baseline - Aged-control - HFD	13-week diet Control calorie (%): - fat, 10.3; CHO, 75.9; protein, 13.7 HFD calorie (%): - fat, 45.3; CHO, 40.8; protein, 13.8	IM twitch and tetanus force of whole SOL and EDL at 25°C	SOL: - Twitch and tetanus activation time (mN/ms) ↔ - Twitch relaxation time ↔ - Tetanus relaxation time ↓ in HFD compared to baseline EDL: - Twitch activation & relaxation time ↑ in HFD compared to baseline - Tetanus activation time ↔ - Tetanus relaxation time ↓ in HFD compared to baseline	- Twitch and tetanus stress p.u. muscle CSA (mN/mm^2) ↔ across all groups	SOL: - Type I, IIa, IIx and IIb CSA (μm^2) ↑ compared to control & baseline EDL: - Type IIa and IIb ↓, type IIx ↑ compared to baseline - Type IIa ↓, IIx ↔ and IIb ↓ compared to control
Eshima et al., 2017	M C57BL/6 mice aged 8 Weeks Groups: - 4WK CON - 12WK CON - 4WK HFD - 12WK HFD	4 and 12-week diet Control calorie (%): - fat, 5.6; CHO, 53.8; protein, 22.6 HFD calorie (%): - fat, 60; CHO, 20; protein, 20	IM tetanus force of whole EDL using stim. freq. 1Hz – 150Hz	12-week: - Activation and relaxation (ms) ↔ - IM force ↓ in HFD at 75Hz - 150Hz 4-week: - Activation and relaxation (ms) ↔ - IM force ↔	12-week: - IM stress p.u. muscle CSA ↓ in HFD at 70Hz 4-week: - IM stress p.u. muscle CSA ↔	12-week: - BM (g), Abdominal visceral fat (g), IMCL levels (%), Type IIx ↑ in HFD - MM:BM, Type IIb ↓ in HFD - MM (g), CSA (m^2) ↔ 4-week: - BM (g), Abdominal visceral fat (g) ↑ in HFD - MM:BM ↓ in HFD - MM (g), CSA (m^2), IMCL levels (%), MHC composition ↔
Tallis et al., 2017	F CD-1 mice aged 4 weeks Groups: Control HFD	16-week self-selected diet Control calorie (%): fat, 7.4; CHO, 75.1, protein, 17.5 HFD calorie (%): fat, 63.7; CHO, 18.4; protein, 17.9	IM tetanus force; WL power and fatigue resistance of whole SOL, EDL and DIA at 37°C	- Activation (ms) ↔ for all muscles - Relaxation (ms) ↔ for EDL, ↓ for HFD DIA, ↑ for HFD SOL - IM force (mN) ↔ for EDL ↑ for HFD SOL - WL power ↔ for EDL & SOL	- IM stress p.u. muscle CSA ($kN.m^2$) ↔ for SOL, ↓ in HFD EDL & DIA - WL power p.u muscle mass ↔ for SOL, ↓ in HFD EDL & DIA - WL fatigue resistance ↔ for EDL & DIA, ↓ for HFD SOL	- BM (g), FM (g), BMI, Lee Index ↑ INHFD - Fast:Slow MHC ↔ for all muscles APMK ↓ for HFD SOL - MM (mg) ↔ for SOL and EDL

Hill et al., 2019	F CD-1 mice aged 70 weeks Groups: - Control - HFD	9-week self-selected diet Control calorie (%): - fat, 7.4; CHO, 75.1, protein, 17.5 HFD calorie (%): - fat, 63.7; CHO, 18.4; protein, 17.9	IM tetanus force; WL power and fatigue resistance of whole SOL, EDL and DIA at 37°C	- Activation and relaxation (ms) ↔ for all muscles - IM force (mN) ↔ for all muscles - WL power ↑ for HFD soleus & EDL	- IM stress p.u. muscle CSA (kN.m^2) ↔ for SOL & EDL, tendency for ↓ in HFD DIA - WL power p.u muscle mass ↔ for SOL & EDL, ↓ for HFD DIA - WL fatigue resistance ↔ for all muscles	- BM (g), circumference (cm), BMI, gonadal FM (g), and FM:BM ↑ in HFD - MM (mg) and CSA (m^2) ↑ in HFD SOL & EDL - MM:BM, ↔ for SOL & EDL
Hurst et al., 2019	F CD-1 mice aged 8 weeks Groups: - Control - 2WK HFD - 4WK HFD - 8WK HFD - 12WK HFD	2, 4, 8 & 12-week self-selected diet Control calorie (%): fat, 7.4; CHO, 75.1, protein, 17.5 HFD calorie (%): fat, 63.7; CHO,	IM tetanus force; WL power and fatigue resistance of whole SOL, EDL and DIA at 37°C	- Relaxation (ms) ↔ for EDL and DIA, ↑ for 8 & 12WK HFD SOL versus CON - Activation (ms) ↔ for all muscles - IM force (mN) ↔ for EDL & SOL - WL power ↔ for EDL & SOL	- IM stress p.u. muscle CSA (kN.m^2) ↔ for all muscles - WL power p.u muscle mass ↓ 8WK HFD SOL versus CON, ↓ for 8 & 12WK HFD EDL versus CON, ↓ for 12WK HFD DIA versus CON - WL fatigue resistance ↔ for EDL & DIA, ↓ for HFD SOL	- BM (g), circumference (cm), FM (g), and FM:BM ↑ in 8 & 12WK HFD versus CON - MM (mg) ↑ in 8 & 12WK HFD SOL versus CON - MM (mg) ↑ in 4, 8 & 12WK HFD EDL versus CON
Eshima et al., 2020	M C57BL/6 mice aged 2 months Groups: - Control - HFD	20-month diet Control calorie (%): - fat, 5.6; CHO, 53.8; protein, 22.6 HFD calorie (%): - fat, 60; CHO, 20; protein, 20	IM tetanus force of whole SOL and EDL using stim. freq. 1Hz – 150Hz	SOL: - IM force (mN) ↔ at all stim. freq. - Activation and relaxation time (ms) ↔ EDL: - IM force ↓ in HFD at 50Hz - 150Hz - Activation and relaxation time (ms) ↔	SOL: - IM stress p.u. muscle CSA (kN.m^2) ↔ at all stim. freq. EDL: - IM stress p.u. muscle CSA ↓ in HFD at 50Hz - 150Hz	- BM (g), Abdominal visceral fat (g), EDL IMCL droplet size (μm^2) ↑ in HFD - SOL and EDL MM (mg) ↔

Abbreviations: M, male; F, female; HFD, high-fat diet; HFL, high-fat lard diet; HFP, high-fat palm oil; CHO, carbohydrates; TA, tibialis anterior; EDL, extensor digitorum longus; SOL, soleus; DIA, diaphragm; TS, triceps surae; GP, gastrocnemius plantaris; stim. freq., stimulation frequency; IM, isometric; WL, work loop; CSA, cross-sectional area; BMI, body mass index; BM, body mass; MM, muscle mass; FM, fat mass; FM:BM, fat mass to body mass ratio; MM:BM; muscle mass to body mass ratio; IMCL, intramyocellular lipid; p.u., per unit. ↓/↑ $P < 0.05$, ↔ no change/difference.

1.7 – Contractile Assessments of Isolated Muscle

1.7.1 – Isometric activations

The most commonly employed contractile assessment of isolated muscle is the measurement of isometric force (James et al. 1996; Medler 2002; Syme 2005; Nishikawa et al. 2018b). Such assessments involve the measurement of fixed length force production when subjected to a single stimulus (twitch) or burst (tetanus) of electrical stimulation (see Figure 1.2) (Tallis et al. 2015). Typically, muscle length, stimulation amplitude and electrical stimulation frequency are manipulated to determine the maximal force producing capacity of the muscle, which is then normalised to muscle cross sectional area to determine isometric stress. Furthermore, measuring the speed of activation and relaxation during this assessment provides important insight into muscle Ca^{2+} kinetics (Ebashi and Endo 1968).

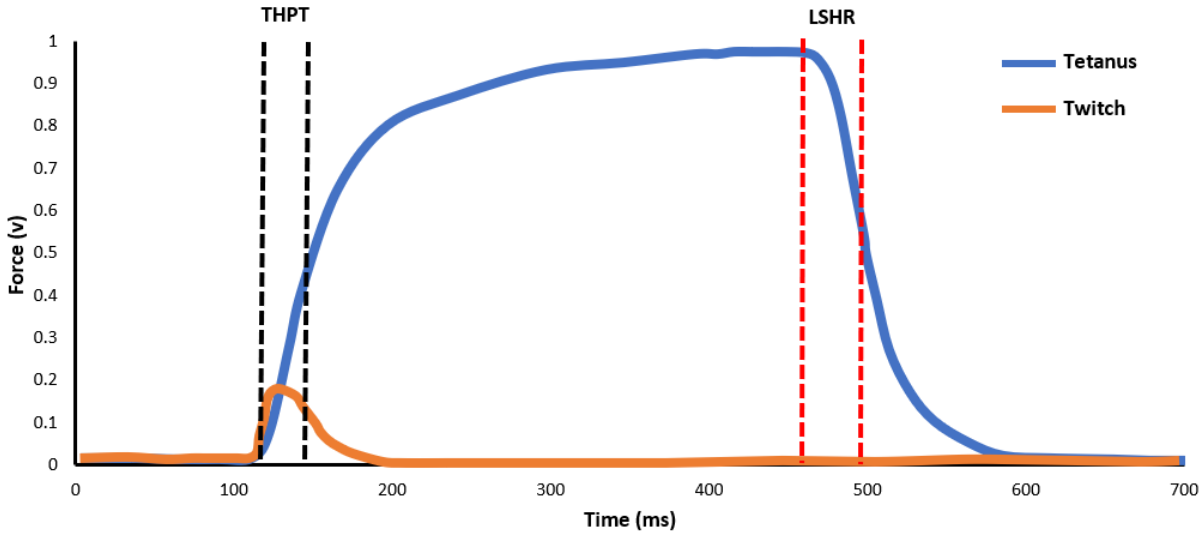

Figure 1. 2. *Example of twitch (orange) and tetanus (blue) activation produced by isolated mouse soleus (Chapter 5). THPT: time to half peak tetanus; LSHR: last stimuli half relaxation time.*

1.7.2 – Isometric Twitch Activation

When isolated muscle is subjected to a single electrical stimulus it will initiate a twitch response (MacIntosh et al. 2006). Twitch activations are not instantaneous upon electrical stimulation.

There is a small electromechanical delay (lag phase) whilst the action potential released reaches the fibres membranes and for calcium (Ca^{2+}) to be released from the sarcoplasmic reticulum (SR), although the speed of delay is muscle specific (Eberstein and Goodgold 1968). Force production from a twitch activation is usually only small due to limited Ca^{2+} release, meaning there are few actin binding sites and cross bridge formations, thus, small number of rotating myosin heads to evoke force production.

The studies in this thesis which utilised an isolated skeletal muscle model, used twitch activations to identify the optimal length (L_0) and stimulation amplitude for the muscle to produce force. The force length relationship has been well established for over a century, indicating force production will increase as length is increased, until a certain threshold whereby force begins to plateau and then reduce (Blix 1892). When muscle length is too short, there a smaller number of actin binding sites for myosin to interact with due to limited overlapping actin filaments; when muscle length is too long, the overlap between thick and thin filaments decreases, both of which reduce cross bridge formation and force production (Matthews 2003).

1.7.3 – Isometric Tetanus Activation

Prolonged bursts of electrical stimulation provided to a muscle at a constant length, at a stimulation frequency above a certain threshold, will evoke a fused tetanus activation. Fused tetanus activations determine the amount of force the muscle can produce, in the case of this thesis, at the length for optimal twitch force. Fused tetanus can occur utilising submaximal or maximal stimulation frequencies (Vassilakos et al. 2009; Tallis et al. 2013). Increasing stimulation frequency evokes greater force production until a certain threshold is met (Vassilakos et al. 2009). Once maximal Ca^{2+} release is achieved, the number of cross bridge formations will not change and therefore force generating capacity will not increase. The stimulation frequency needed to elicit maximal isometric tetanus force is higher in fast twitch muscle, when compared to relatively slow or mixed fibre type muscle (Segal et

al. 1986; James et al. 1995), given a need to evoke greater and sustained SR Ca^{2+} release (Baylor and Hollingworth 2003). When stimulation frequency falls below a certain threshold, a small period of relaxation occurs between stimuli, allowing for some reuptake of Ca^{2+} back into the SR, ultimately reducing force production. This response is referred to as an unfused tetanus activation (see figure 1.3)

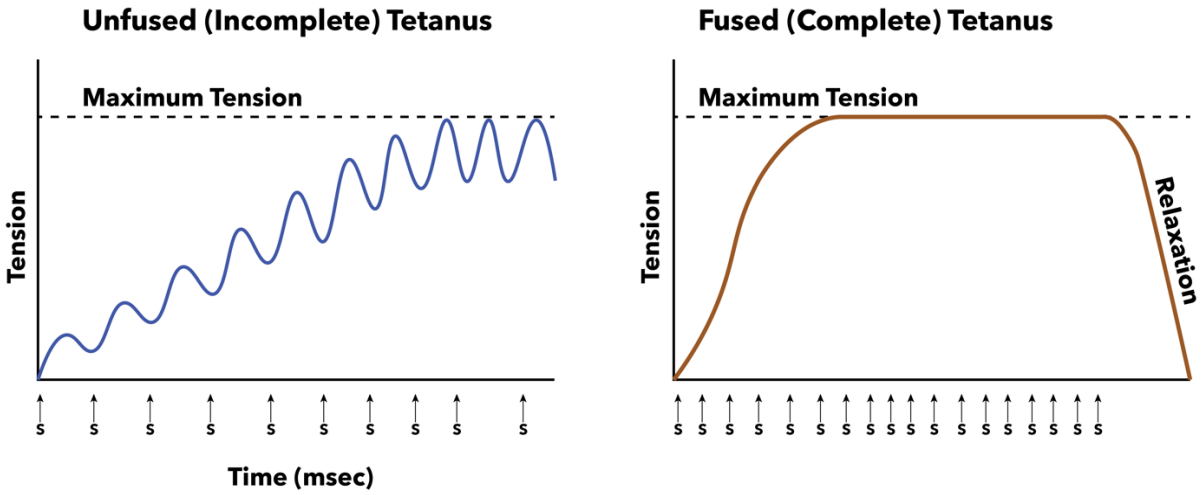

Figure 1. 3. *Example of unfused v fused tetanic activation (adapted from Walsh and Sved, 2019)*

Activation and relaxation time of tetanus force can be measured to provide an insight into the release and subsequent reuptake of Ca^{2+} back into the SR (Ebashi and Endo 1968). In the present thesis time to half peak tetanus (THPT) was measured to indicate activation time, which is the time from first point of electrical stimulation to reach half maximal force. Last stimulus to half tetanus relaxation (LSHR) time i.e. time elapsed between final electrical stimulation to relaxation to half of maximal force, was used as a measure of relaxation time (see figure 1.2). An insight into Ca^{2+} kinetics is important for the present thesis, given that obesity induced changes in acute force and power, and fatigue resistance, have in part been linked to alterations in Ca^{2+} handling and cross bridge kinetics (Tallis et al. 2018). Activation and relaxation kinetics are muscle specific, whereby fast twitch muscle will generate force and relax at much quicker rates than muscle comprised of slow twitch fibres (Scott et al. 2001; Tallis et al. 2017; Hill et al. 2020).

1.7.4 – Isotonic and Isovelocity Contractions

Whilst isometric muscle activity has some important *in vivo* applications, particularly for postural control (Loram et al. 2004), it fails to represent dynamic power producing muscle activity (i.e. the action of muscle that produce work to initiate movement; work done ÷ time) that is essential for locomotion (Josephson 1985; James et al. 1996). Measures of skeletal muscle power provide important insight into the *in vivo* application of findings derived from isolated skeletal muscle assessments, and should be considered as an integral part of an assessment battery given that evidence demonstrates contractile mode specific responses to environmental and experimental stimuli (Syme 2005; James et al. 2011; Tallis et al. 2018, 2021; Stoehr et al. 2020). Thus, studies that only assess a single contractile mode may be limited. Therefore, research of muscle function should consider both force production and assessments of dynamic muscle activity. Traditionally, assessments of isolated skeletal muscle power have been derived from force-velocity experiments which utilise a constant force or fixed shortening velocity (Josephson 1993; Caiozzo 2002).

To undertake isotonic experiments, isometric parameters must be optimised (length and, stimulation amplitude and frequency) for maximal force. Once maximal force is achieved, the muscle will undergo shortening contractions against a variety of predetermined loads i.e. set amount of force, to create a force-velocity relationship (Hill 1938; Nishikawa et al. 2018b). The shortening velocities achieved at the systematically established predetermined loads are plotted against each other (force against velocity). The force velocity relationship identifies that higher forces are produced at slower shortening velocities, and low amounts of force at quicker shortening velocities (Fenwick et al. 2017). This occurs due to cross bridge properties at the sarcomere, as producing high amounts of force means there are greater number of cross bridge formations, which results in a slow shortening velocity, but quicker shortening velocities occur at low forces, as few cross bridges are needed to meet the force

requirements, highlighted in Figure 1.4 (Stehle and Brenner 2000). Each shortening velocity recorded at each predefined load can be multiplied by one another (velocity×force) to determine power output (PO), which also means the relationship between contractile velocity and PO can also be established.

Another technique for determining PO, again through establishing a force-velocity relationship, is measuring performance via isovelocity contractions. However, instead of implementing a predetermined force based on maximum tetanus force, isovelocity experiments utilise a fixed shortening velocity. Whilst there a variety of methods for performing isovelocity contractions, most common is the step-release protocol (Cecchi et al. 1978). During the step-release protocol maximum tetanus force is achieved before rapidly shortening, evoking a reduction in both muscle length and force production. Once shortened, the muscle will undergo further shortening at a constant known velocity, which is able to sustain the force production achieved after rapid shortening (Lou et al. 2002).

Figure 1. 4. *The isotonic force-velocity relationship of a typical mouse soleus muscle (Askew & Marsh, 1998)*

The ‘iso’ experiments described have been an integral part of developing the understanding of isolated skeletal muscle mechanics. However, deriving maximal instantaneous power via these methods is limited in its application to *in vivo* skeletal mechanics, as it fails to consider the net energy cost of a skeletal muscle length change cycle (Abbott et al. 1952; Josephson 1985; James et al. 1995) and the calculation infers that maximal force production and relaxation is instantaneous (James et al. 1996; Caiozzo 2002). For this reason, ‘iso’ assessments overestimate power production, with the isotonic technique producing twice the power output achieved via the work loop (WL) method (James et al. 1996).

1.7.5 – The Work Loop Technique

The WL technique (Machin and Pringle 1959; Josephson 1985) assesses the ability of a muscle to produce work during cyclical length changes, as per *in vivo* power producing muscles (James et al. 1996). More specifically, power ($J.s^{-1}$) is derived from the net work (Joules) of a WL length change cycle, multiplied by the number of length change cycles per second (s^{-1}) (James et al. 1996). The WL technique considers not only the work produced during active muscle shortening, but also work required to lengthen the muscle, and the influence of activation and relaxation time on force production which are not considered in instantaneous power assessments (Josephson 1985; James et al. 1996; Caiozzo 2002). Thus, determining net work over the duration of a length change cycle is more indicative of *in vivo* muscle function (Josephson 1985; James et al. 1995, 1996; Caiozzo 2002). As such, there has been a growth in the use of the WL model across published literature (Josephson 1993; James et al. 1995; Young and Rome 2001; Caiozzo 2002; Syme 2005; Choi and Widrick 2009; Tallis et al. 2018; Kissane et al. 2018; Nishikawa et al. 2018b; Padilla et al. 2020; Hessel et al. 2021). Initial implementation of the WL technique was for the assessment of asynchronous flight muscle of insects (Machin and Pringle 1959). The addition of phasic stimulation (Josephson 1985) further enhanced the

technique as it allows for synchronous muscular contractions i.e. one phase of stimulation producing one full WL cycle.

Like 'iso' experiments, isometric parameters are optimised for maximal force prior to WL assessments. The WL technique involves muscle being subjected to length changes, typically shortening and lengthening around the muscle length established to produce maximal twitch or tetanus force. Previous research examining mouse isolated skeletal muscle has implemented symmetrical (sinusoidal) (James et al. 1995) or non-symmetrical (sawtooth) (Askew and Marsh 1997) waveform length changes. However, the present thesis utilised sinusoidal length changes, to allow for direct comparisons between previous work examining the impact of HFD consumption on PO and fatigue resistance of isolated mouse soleus and extensor digitorum longus (EDL) muscle (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019). It should be noted that a sinusoidal length change is a simplistic approximation of the complex length changes likely to occur during *in vivo* locomotion (Dickinson et al., 2000), with estimates of the muscle length changes used by mouse EDL and soleus during running deviating from a sine wave (James et al. 1995). Despite this, sinusoidal length change waveforms are commonly used for WL assessments (James et al. 2004; Choi and Widrick 2009; Tallis et al. 2014; Hessel and Nishikawa 2017; Stoehr et al. 2020; Padilla et al. 2020) as sine waves provide a general approximation of muscle length changes found *in vivo* (James et al. 1995, 1996). The experiments in this thesis were conducted to achieve positive net work i.e. concentric work. To do this, external electrical stimuli are provided to the muscle during the shortening phase of length change cycle (see figure 1.5) i.e. muscle is stimulated at its greatest length and stimuli are provided for the most of shortening. External electrical stimulation does not occur during initial lengthening or re-lengthening, to ensure the muscle remains predominately passive during these phases. Whilst not utilised in the present thesis, others have instead provided electrical stimulation during muscle lengthening, but not muscle shortening, to quantify negative or eccentric work (Hessel and Nishikawa 2017; Hill et al. 2018).

The magnitude of length change is commonly referred to as strain to indicate the length as a percentage or proportion of its resting length e.g. a strain of 0.10 will evoke a total length change of 10% (5% lengthening, 10% shortening and 5% re-lengthening back to starting length). Strain is manipulated to achieve maximal work, which varies dependent on muscle fibre type and length change velocity (manipulated via change in cycle frequency). WL traces are formed by plotting strain amplitude (as a proportion of L_0) against force (Josephson 1985; James et al. 1995). The area within a plotted WL is indicative of net work (total work minus negative work), described in detail in Figure 1.6.

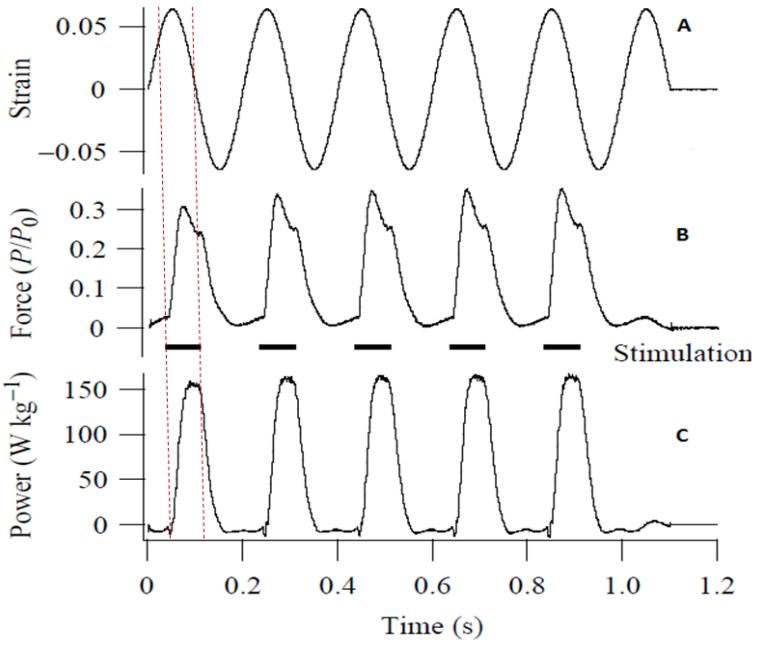

Figure 1. 5. *Example of the sinusoidal length trajectory at a cycle frequency of 5 Hz. Strain changes with sine waves (A); the central panel shows force produced by a mouse soleus. The black horizontal bars show the time for which the muscle was stimulated, where P is muscle force whilst shortening and P_0 is isometric stress (B). Lower panel shows instantaneous net power output, calculated as force × velocity (C) (Adapted from Askew, Young, and Altringham 1997)*

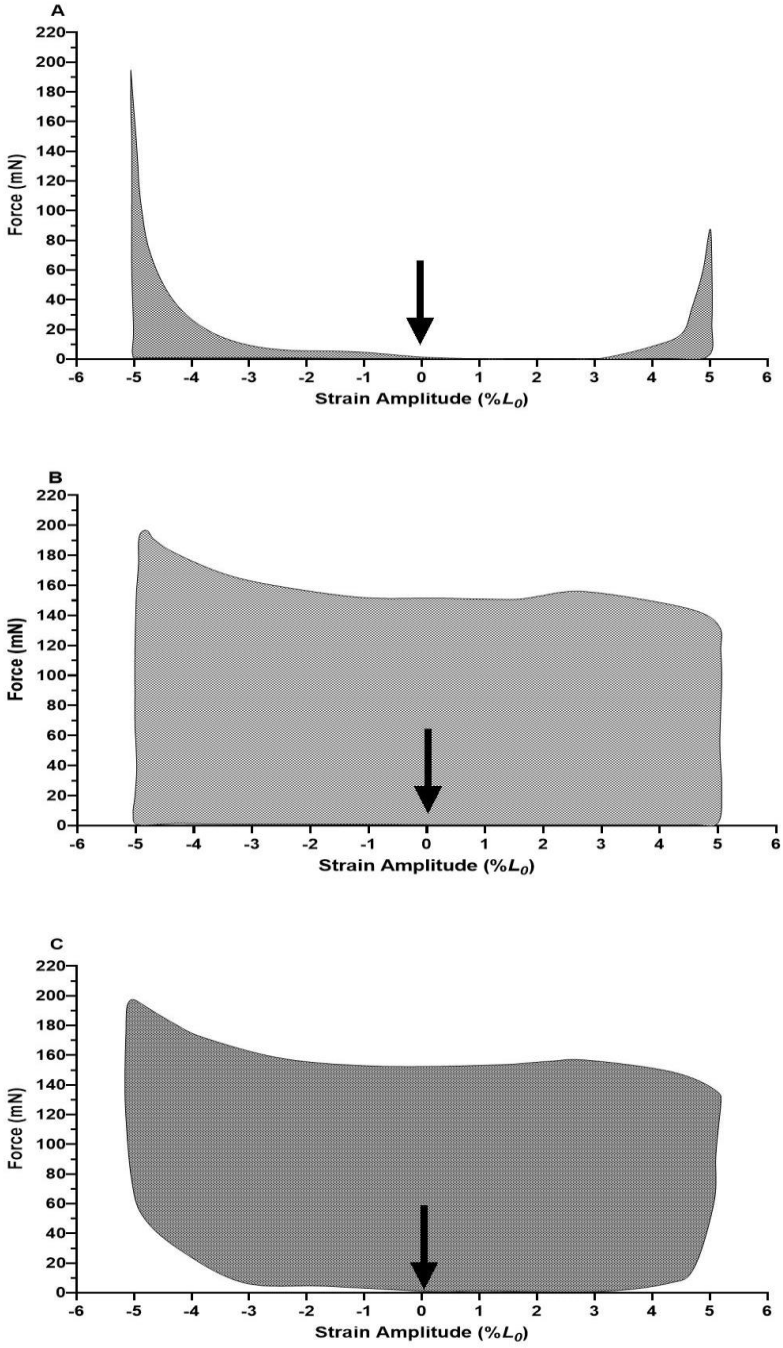

Figure 1. 6. *Components of a mouse extensor digitorum longus work loop cycle optimised for maximal work at 10Hz cycle frequency and 260Hz stimulation frequency. A: Work performed on the muscle during lengthening (termed as negative work); B: Work performed by the muscle during the shortening phase of the length change cycle (positive work); C: Net work produced during the entirety of the length change cycle, calculated as total work minus negative work (C = B – A). Work loops are performed in the anti-clockwise direction, with the initiation of the work loop starting at* L_0 *as indicated via the arrow. (Adapted from Shelley et al. 2022)*

1.8 – Mechanisms Associated with Obesity Induced Changes in Contractile Performance

There are emerging trends from the current literature examining the effects of obesity on contractile function and there has been an increasing quantity of literature examining potential mechanisms which may contribute to obesity induced changes in muscle performance. However, few research studies have directly explored and linked the obesity induced changes in underpinning mechanisms to contractile data (Tallis et al. 2018). The mechanisms which result in obesity induced changes in skeletal muscle function are unlikely to be uniform across muscles or contractile modes, given the complexity of obesity effects on contractile function. Although there is limited direct evidence, there are several important mechanisms hypothesised to contribute to poor skeletal muscle health in obese individuals.

1.8.1 – Fibre Type Composition

Skeletal muscle is composed of different fibre types which determine contractile performance and metabolic properties (McArdle et al. 2015). The fibre types of muscles are often classified in respect to their myosin heavy chain (MHC) isoform composition. Mammalian skeletal muscle is separated into slow type I and fast type IIa, IIx and IIb fibres, although type IIb fibres are not expressed in human skeletal muscle (Talbot and Maves 2016). An overview of the characteristic difference between fibre types is provided in Table 3.

Table 3. *Characteristics of different fibre type compositions (McArdle et al., 2015)*

Muscle composed of slow fibres relies on oxidative phosphorylation for ATP production and is highly resistant to fatigue (Ciapaite et al. 2015). However, slow fibres have a low shortening velocity, with relatively low force and power production (Bassel-Duby and Olson 2006). Conversely, fast fibre types are associated with glycolysis to produce energy and higher shortening velocities, resulting in greater force and power production; they are however more susceptible to fatigue (Bassel-Duby and Olson 2006). Even within muscle classified as the same fibre type there can be structural and functional differences observed between muscle (Plotkin et al. 2021), such as differences in the isoforms of proteins which are involved in excitation contraction coupling and relaxation (Brotto et al. 2006). It has been well documented that environmental stimuli can determine and remodel the fibre type expression of skeletal muscle (Plotkin et al. 2021). A common scenario where remodelling of fibre type expression occurs, is through sustained high load-low reps resistance training, where oxidative type II fibres (x or a/x) will shift to a more pure type IIa phenotype (Adams et al. 1993; Tobias and Galpin 2020). These adaptations occur as skeletal muscle composition altered, by up to 45%, in order to meet prolonged external/environmental demands (Simoneau and Bouchard 1995).

It has been proposed that many of the obesity induced changes to contractile function of skeletal muscle occur though changes in fibre type composition (Tallis et al. 2018). One predominant suggestion is that obesity promotes a slow to fast shift in fibre type expression (Kriketos et al. 1997; Tanner et al. 2002; de Wilde et al. 2008; Stuart et al. 2013; DeNies et al. 2014; Seebacher et al. 2017), proposed to occur through a reduction in 5'-adenosine monophosphate-activated protein kinase (AMPK) activity (Tallis et al. 2018). Reduced AMPK activity promotes the class II histone deacetylase (HDAC), which suppresses myocyte enhancer factor 2 (MEF2), a mechanism, which when activated, is in part responsible for formation of the type I fibres (Tallis et al. 2018). However, literature reporting the effects of obesity on fibre type composition is equivocal as there is also evidence for a shift to slower (Wade et al. 1990; Warmington et al. 2000; Kemp et al. 2009; Trajcevski et al. 2013), or no change (Turpin et al. 2009; Messa et al. 2020), in muscle phenotype. Differences in obesity related fibre type shifts have been attributed to variances in muscles testing, durations of feeding/obesity and methods used to quantify fibre type (Tallis et al. 2017).

Due to equivocal data, it is difficult to determine the extent to which potential alterations in fibre type composition has on an obesity induced change in contractile performance. It is difficult to suggest shift in fibre type is a driving mechanisms for a reduction in contractile performance, given reductions in EDL muscle quality have been observed without any alterations to fibre type in obese muscle (Tallis et al. 2017) or changes in power output- cycle frequency curves (Hurst et al. 2019) . Although, a shift in fibre type cannot be ruled out as a contributing to factor to a change in contractile performance in some instances.

1.8.2 - Calcium Handling

Obesity has been shown to impair calcium handling (Funai et al. 2013; Eshima et al. 2020), which may contribute to an obesity induced decline in the force producing capacity of skeletal muscle

(Tallis et al. 2017, 2018). Calcium handling is vital in force production as the quantity of Ca^{2+}, and ability to release and reuptake Ca^{2+} from and to the SR directly impacts the rate and magnitude of force production (Maffiuletti et al. 2016). Previous research indicates a HFD induced reduction in peak Ca^{2+} levels, indicative of diminished ability to release calcium, corresponds with a reduction in isometric stress of mouse EDL (Eshima et al. 2020). Furthermore, Funai et al., (2013) demonstrated an obesity related reduction in Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA), which is responsible for Ca^{2+} reuptake from the cytoplasm back to the SR (Periasamy et al. 2017). The reduction in SERCA will increase muscle relaxation time, supported by previous evidence demonstrating increased LSHR in time in mouse soleus (Tallis et al. 2017; Hurst et al. 2019) and EDL (Eshima et al. 2020) isolated from obese individuals. During dynamic contractions, reduction in SERCA may result in the muscle remaining active during the re-lengthening phase; thus, requiring a greater amount of work to lengthen the muscle (negative work), which will result in a decline in net work and force production (Tallis et al. 2017).

Troponin T (TNNT) is a component of the regulatory complex troponin which is key in regulation of calcium sensitivity during muscle relaxion (Leavis et al. 1984). Variations in TNNT isoforms, which are encoded by two separate genes (slow TNNT1 and fast TNNT3), have been shown to influence the contractile performance of skeletal muscle (Ogut et al. 1999). Furthermore, obesity induced changes in TNNT isoform composition have also been proposed to inhibit contractile performance, irrespective of a change in MHC isoform composition (Ciapaite et al. 2015). Reduction in peak force of mouse soleus subject to a 5 week HFD, has been attributed to a reduction in the fast TNNT3 isoform and increase in the slow TNNT1 isoform (Ciapaite et al. 2015). However, no such changes in TNNT composition were found in the comparatively faster twitch EDL or diaphragm, leading the authors to suggest obesity induced changes in TNNT isoforms and subsequent changes in contractile performance, are possibly muscle fibre type I (i.e. soleus specific (Ciapaite et al. 2015). This is unlikely

the case as more recent evidence indicates that a reduction in fast troponin isoforms in the EDL (Eshima et al. 2017), of mice fed a 60% HFD for 12 weeks, contributed to a decline in EDL isometric stress. Therefore, the duration of HFD feeding needed to evoke obesity induced changes in TNNT isoforms and subsequent reductions in contractile performance are likely muscle specific.

1.8.3 - AMPK Activity

5'-adenosine monophosphate-activated protein kinase (AMPK) plays an important role in homeostasis as it is a key regulator of energy balance, particularly in skeletal muscle (Tallis et al. 2018). Depletion of high energy ATP and a greater ratio of low energy adenosine monophosphate (AMP) to ATP, which can be brought about by bouts of muscular contractions or calorie restriction, result in the activation of AMPK (Hardie et al. 2006; Canto et al. 2009). AMPK activity can aid with regeneration of ATP through glycolysis and or fatty acid oxidation (Moffat and Ellen Harper 2010). Obesity has been shown to have suppressive effects upon AMPK activity (Steinberg et al. 2006; Tallis et al. 2017) and reduces levels of adiponectin, which is a protein hormone that triggers AMPK activity (Yamauchi et al. 2002). Suppressed AMPK activity could possibly explain an obesity related reduction in fatigue resistance in the soleus, where AMPK was also reduced, through inhibition of the regeneration of ATP, subsequently, reducing contractile performance (Tallis et al. 2017). Furthermore, obesity may not only reduce contractile performance but when exercise stimulated activation of AMPK is inhibited in obese individuals, exercise tolerance can also be diminished (Lee-Young et al. 2010), which would further exacerbate an increase in time to voluntarily fatigue and contribute to a negative obesity cycle. Obesity induced reductions in AMPK activity can also promote fat deposition through impaired lipid metabolism and increased chronic low-grade inflammation and oxidative stress (Steinberg et al. 2006; Fu et al. 2013; Tallis et al. 2018; Lyons and Roche 2018), factors which independently could affect contractile performance.

1.8.4 – Intramuscular Lipids and Inflammation

Obesity is associated with an increase in lipid accumulation in non-adipose tissues. Once the magnitude of circulating lipids reaches a certain threshold and the adipocytes in adipose tissue reach their limit for lipid capacity, fat is stored in other tissue organs, referred to as ectopic fat accumulation (van Herpen and Schrauwen-Hinderling 2008). More specifically, obesity can result in significant lipid infiltrations within skeletal muscle fibres (Messa et al. 2020; Eshima et al. 2020), a process known as myosteatosis (Corley et al. 2020). The increase in intramuscular lipids associated with obesity corresponds with reduction in both isometric stress (Eshima et al. 2020) and muscle quality (Hurst et al. 2019; Messa et al. 2020). In part because a greater portion of the contractile tissues will be made up of lipids in obese muscle when compared to lean (Rahemi et al. 2015), with previous evidence showing intramuscular lipids account for 5% of mass in obese muscle, but only 1.5% in non-obese muscle (Malenfant et al. 2001).

An increase in intramuscular lipids can also have implications for chronic inflammation. Intramuscular adipose tissue acts as chemoattractant to macrophages (Kewalramani et al. 2010), evoking increased circulation of interleukin – 6 (IL-6) (Park et al. 2005). High quantities of circulating proinflammatory cytokines could inhibit the processes involved in myogenesis, such as breakdown of muscle protein (Johnson et al. 1973; Erskine et al. 2017). Where this occurs, this is likely to have profound effects on skeletal muscle contractility, given a reduction in myogenesis is integral for repair and replacement of contractile proteins during recovery from damage, which might be substantial due to chronic-low grade intensity overloading of obese skeletal muscle due to supporting and moving a larger mass (Erskine et al. 2017); this is supported by the fact the increased circulating levels of proinflammatory cytokines in obese individuals were associated with a reduction in muscle quality of the plantar and dorsi flexors, in young and older adults (Erskine et al. 2017).

The responses to, and alterations of mechanisms associated with, obesity are not uniform across age groups, muscle, and contractile modes (Tallis et al. 2018); whilst literature has begun to establish some muscle specific responses, work on the specific mechanistic changes to contractile function due to obesity is still incomplete (Tallis et al. 2018). However, general trends have been observed for both obesity induced changes in contractility, and in the underpinning mechanisms which contribute to a reduction in contractile performance. As such, it is important to consider potential therapeutic strategies which could help alleviate the decline in skeletal muscle health associated with obesity (Tallis et al. 2021)

1.9 – Strategies to Reduce the Impact of Obesity on Skeletal Muscle Contractility

1.9.1 Current Strategies

Skeletal muscle health represents an important link to physical function and quality of life, and obesity induced changes to contractile performance of skeletal muscle have been proposed to exacerbate the adverse effects of obesity and be the catalyst for a negative obesity cycle (Tallis et al. 2018). As such, targeting strategies to improve skeletal muscle health in obese populations could be an initial step in reducing the impact of obesity on overall health.

Lifestyle interventions such as dietary interventions and increased physical activity are the commonly promoted obesity treatment strategies. A wealth of evidence indicates that well managed lifestyle interventions are successful in reducing overall body mass and adiposity, improving the metabolic profile, blood pressure and glucose control, and reducing the risk of cardiovascular disease in obese populations (Ross 2000; Ryan et al. 2003; Goodpaster et al. 2010; Touati et al. 2011; Foster-Schubert et al. 2012; Julian et al. 2018). The positive effects of lifestyle interventions on obesity outcomes are likely, in part, through lifestyle interventions alleviating the decline in contractile performance

associated with obesity, such as improving absolute force and muscle quality (Daly et al. 2005; Wang et al. 2007; Frimel et al. 2008; Villareal et al. 2011; Straight et al. 2012).

Whilst calorific restriction is the most effective lifestyle intervention to evoke clinically meaningful weight loss, such interventions are limited in that they can cause a concurrent loss of lean mass (Willoughby et al. 2018) resulting in no change or detrimental effects on skeletal muscle performance (Weiss et al. 2007; Beavers et al. 2015; Seebacher et al. 2017). Furthermore, despite well-established benefits of lifestyle interventions, non-surgical treatment strategies have not appeared to gain widespread compliance, and have poor adherence and success rates (Turk et al. 2009; Fildes et al. 2015; Hall and Kahan 2018) partly due to the recidivism associated with lifestyle interventions. In fact, only <1% of obese (body mass index [BMI] >30) and ~ 0.1% of morbidly obese individuals (BMI >40) attain a normal weight status (BMI 18-25) over a 9 year period (Fildes et al. 2015). As such, any potential benefits of non-surgical interventions are substantially influenced by poor adherence. The most invasive therapeutic method to reduce obesity, and the most effective in promoting weight loss, is bariatric surgery (Wolfe et al. 2016). Although research examining its effectiveness for improving contractile performance and physical function has yielded ambiguous results (Stegen et al. 2011; Lyytinen et al. 2013; Alba et al. 2019). Irrespective of the success of bariatric surgery, it is not a global solution as there is significant risk and costing involved (Chang et al. 2014; Arterburn and Courcoulas 2014). As such, there is a need to explore other strategies which require minimal lifestyle modification to promote long term adherence, but have minimal risk and cost involved.

1.9.2 Nutritional Supplementation

Nutritional supplementation holds potential as an alternative to current treatment strategies for reducing the impact of obesity on skeletal muscle health, as it is relatively time and cost effective, and requires minimal lifestyle modification (Tallis et al. 2021). There are host of nutritional

supplements which would mechanistically appear promising in offsetting a reduction in contractile performance associated with obesity, including, but not limited to, curcumin (Alappat and Awad 2010), quercetin (Nabavi et al. 2015), omega-3 fatty acids (Albracht-Schulte et al. 2018), sufficient protein intake (Tallis et al. 2021), resveratrol (Aguirre et al. 2014) and vitamin D (Marcotorchino et al. 2014). However, the studies in the present thesis utilise vitamin D and resveratrol, as there is evidence to support both elicit changes in some of the key mechanisms contributing to poor skeletal muscle health in obese individuals, and individually these have been shown to improve contractile function of skeletal muscle in healthy and disease models.

1.9.3 – Vitamin D

Supplementation of Vitamin D (or in its biologically active form *1α,25(OH)2D3, 24R,25-dihydroxyvitamin D3 [24R,25(OH)2D3]*) may be a viable treatment strategy to alleviate the detrimental effects of obesity on skeletal muscle health (Tallis et al. 2021). Obesity and vitamin D deficiency (VDD) or insufficiency (typically defined as serum 25-hydroxyvitamin D [25(OH)D] concentrations <50 nmol/L and 50-75 nmol/L respectively (Liu et al. 2018) often coincide, with some suggestion that obesity may exacerbate the risk of VDD (Duan et al. 2020). This is likely a result of a combination of factors, such as, reduced sunlight exposure, greater distribution of vitamin D to compartments (e.g. fat mass) other than serum when exposed to sunlight, reduced dietary intake, a reduction in synthetic capacity, reduced intestinal absorption, altered metabolism and elevated accumulation in fat mass (Vanlint 2013; Golzarand et al. 2018; Vranić et al. 2019). VDD is both associated with declines in contractile performance of skeletal muscle (Toffanello et al., 2012, Visser et al., 2003, Gerdhem et al., 2005) and may also promote further accumulation of adiposity, as low serum 25(OH)D has been shown to contribute to elevated lipogenesis and suppressed lipolysis through elevated parathyroid hormone (PTH) and intracellular Ca^{2+} (Golzarand et al. 2018). As such, VDD itself may be a contributing factor to a HFD induced decline in muscle contractility. Thus, chronic

supplementation of vitamin D appears a suitable strategy to moderate the adverse effects of obesity on skeletal muscle health (Tallis et al. 2021).

The evidence to support that vitamin D could improve contractile function of obese muscle is twofold. Firstly evidence indicates chronic supplementation of vitamin D, particularly in individuals who are VDD, improves skeletal muscle contractility in a number of populations (Bischoff et al. 2003; Rejnmark 2011; Tomlinson et al. 2015; Zhang et al. 2019), including older adults; this is noteworthy given changes in skeletal muscle function associated with aging and obesity have been attributed to similar mechanistic responses (Pérez et al. 2016; Tallis et al. 2021). Secondly, chronic supplementation of vitamin D ameliorates mechanisms reported to contribute to obesity induced declines in muscle function, such as modulating inflammation (Farhangi et al. 2017; Szymczak-Pajor and Śliwińska 2019) and reducing/attenuating adiposity in both obese individuals (Vanlint 2013; Golzarand et al. 2018; Vranić et al. 2019) and mice consuming a HFD (Marcotorchino et al. 2014; Fan et al. 2016; Benetti et al. 2018).

Data from healthy rodent models show that chronic vitamin D supplementation (20,000IU/kg feed for 4 weeks) resulted in greater tetanic stress of isolated mouse soleus when compared to healthy weight, vitamin D sufficient mice (Debruin et al. 2019; Hayes et al. 2019). Improvement in muscle contractility in non-disease models may be attributed to vitamin D modulating mitochondrial function, muscle insulin signalling, contractile protein synthesis, calcium and phosphate homeostasis, and inflammation (Girgis et al., 2013, Vanlint, 2013, Montenegro et al., 2019, Szymczak-Pajor and Śliwińska, 2019), mechanisms which contribute to high functioning skeletal muscle. As such, chronic supplementation of vitamin D would appear to be a suitable supplement to target improved muscle contractility in HFD treated muscle. However, despite theoretical evidence for improved contractility in HFD treated

muscle, the effects of chronic supplementation of vitamin D on isolated skeletal muscle contractility in an obese model have not been directly investigated.

1.9.4 – Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene), a natural polyphenolic compound, sourced in trace amounts from grapes, wine, and peanuts, has received considerable attention for its numerous anti-obesogenic properties (Fernández-Quintela et al. 2016; Carpéné et al. 2019). Whilst evidence is limited, in obese humans, short term (30 days of 150mg) supplementation of RES has been shown to increase AMPK activity and reduce IMCL levels and inflammatory markers (Timmers et al. 2011), and reduce adipocyte size (Konings et al. 2014). Longer term supplementation (90 days of 1,500mg) is associated with positive changes in body composition including a reduction in overall adiposity (Méndez-del Villar et al. 2014).

The therapeutic effects of RES in obese rodent models has also shown promise. A review which considered the anti-obesity mechanisms of RES has proposed that supplementation improves body composition by targeting adipose tissue and lipid metabolism of the liver and skeletal muscle; results in a reduction in the accumulation of adiposity through decreased adipogenesis, increased apoptosis, and stimulation of the lipolytic and oxidative pathways (Aguirre et al. 2014). There is also evidence indicating that supplementation of 100mg-4000mg of RES per kg of high-fat diet feed, for anywhere between 4-15 weeks, reduces overall body mass and adiposity when compared to HFD alone (Lagouge et al. 2006, Kim et al. 2011, Wang et al. 2015). RES not only targets adipose tissue but also abates some of the mechanisms which have been proposed to contribute to an obesity-induced decline in contractile performance by increasing AMPK activity and mitochondrial function, and reducing chronic inflammation and IMAT (Baur et al. 2006; Lagouge et al. 2006; Kim et al. 2011; Wang et al. 2015; Shabani et al. 2020).

Despite RES mechanistically showing the potential to alleviate an obesity-induced decline in muscle function, there is limited evidence of its effects on muscle function in obese humans or HFD treated rodents. However, in healthy young and old rodents, *in vivo* absolute and relative forelimb grip strength (measured via electronic grip testers) and time to exhaustion (measured by either a swimming test or motor-driven treadmill test) was improved in 9-10 week old male ICR mice and 25-month-old male SD rats, who were fed a standard laboratory diet (SLD) and 25-150mg of RES per kg of body mass per day, via oral gavage for 3-6 weeks, when compared to mice fed a SLD only (Wu et al. 2013; Kan et al. 2018; Zhou et al. 2019). In 4-5 week old male MDX mice (muscular dystrophy model), *in situ* assessments identify that 100mg of RES per kg of body mass diluted into the animals drinking water every second day for 8 weeks, resulted in a significant increase in stress and stress normalised for body mass of the triceps surae complex, when compared to a control group (Gordon et al. 2014). Given that RES can alter adipose morphology, mediate mechanisms which promote obesity-induced muscle dysfunction (Baur et al. 2006; Lagouge et al. 2006; Kim et al. 2011; Wang et al. 2015; Shabani et al. 2020), and has shown potential to improve muscle function in healthy and disease rodent models (Wu et al. 2013; Kan et al. 2018; Zhou et al. 2019), there is rationale to believe that RES could be an effective nutritional strategy to alleviate the decline in contractile performance associated with dietary-induced obesity.

1.10 – Gaps in the Literature

The following experimental chapters will aim to explore the current gaps in the literature:

- There a few research studies which have considered the concomitant effects of obesity and ageing on muscle function and those that have typically focus on a single muscle group, a single mode of contractility, with many failing to compare against a young control group, leading to ambiguity in findings.

- To date there have been no research studies directly examining the effect of obesity on eccentric muscle performance. Eccentric muscle performance plays an integral role in daily function as it is responsible for absorbing impact forces and deacceleration, which occur during stair descents, descending into a seated position and during activities which require dynamic balance i.e. some key acts of daily living. Given that obesity effects are contractile mode specific and the physiological mechanisms for concentric and eccentric force production are different, it is unclear how obesity will affect eccentric muscle performance.
- There is also a lack of literature which has examined how obesity affects upper limb musculature that is not subject to the same increase or magnitude in mechanical loading as postural muscles. Detrimental effects of obesity on upper body musculature could have consequences on functional impairment and quality of life, given that upper extremity functional strength is necessary for completing many ADL, including, moving objects, and washing.
- There are several limitations with current work loop protocols used to measure contractile performance of isolated skeletal muscle. The stimulation frequency which evokes maximal isometric force is typically utilised for all contractile assessments. However, Vassilakos et al, (2009) report that the stimulation frequency needed to evoke maximal power output exceeded that needed to evoke maximal isometric force in slow twitch soleus, thus previous studies may have underestimated true power output. Although, how stimulation frequency affects the force and power producing capacity of predominantly fast twitch muscle (i.e. EDL) remains to be explored.
- Similarly, utilising the stimulation frequency for maximal isometric force for assessment of fatigue often evokes substantial activation during re-lengthening, which is unlikely to occur *in vivo.* It is currently unclear if differing stimulation frequencies are needed to achieve maximal force and power, and to evoke a fatigue response typical of *in vivo* fatigue mechanics, in isolated fast twitch muscle.

- Currently, only Seebacher et al, (2017 and 2018) have examined potential therapeutic strategies to offset the decline in isolated skeletal muscle mechanics associated with obesity. However, the strategies used were calorific restriction and increased physical activity (resistance-based swimming) in obese zebrafish. Whilst lifestyle interventions such as increased physical activity or calorie restriction are successful in the short term for evoking clinically meaningful weight loss, and in some cases improved contractile function, they are not well maintained long term in humans. Nutritional strategies hold potential as an appealing and sustainable alternative to current therapeutic strategies. Evidence indicates that chronic supplementation of vitamin D and resveratrol mediate many of the underpinning mechanisms contributing to obesity induced decline in muscle function. However, to date, there is no direct evidence exploring the effect of vitamin D or resveratrol supplementation on isolated skeletal muscle mechanics following dietary induced obesity.

Chapter 2 – Research Questions

Thesis Research Questions –

How does obesity affect skeletal muscle function, both in vivo (human), and in isolated skeletal muscle (rodent), across a range of contractile modes and muscles?

Can the adverse effects of obesity on muscle contractility be reduced through targeted nutritional supplementation in the form of vitamin D or resveratrol?

These research questions have been divided into 4 experimental studies, each with distinct aims to contribute to the overarching questions of this thesis. Provided below are the research questions and hypotheses for each experimental chapter. More detailed introductions and rationale can be found in each experimental chapter.

2.1. Experimental Studies: Aims, Objectives and Hypotheses

The research questions and hypotheses for each study are provided below. However, the rationale for these studies are provided in the experimental chapters to demonstrate the linkage and progression between experimental chapters of this thesis.

Experimental Study 1 - ***The Effects of High Adiposity on the Concentric and Eccentric Muscle Performance of Upper and Lower Limb Musculature in Young and Old Human Males***

Study Aim:

- Using IKD, the aim of study 1 was to uniquely examine the effects of high adiposity and older age on concentric and eccentric maximal voluntary torque and fatigue resistance of the elbow flexors and extensors and knee flexors and extensors.

This study sought to address the following questions:

1. Does high adiposity affect eccentric muscle performance?
2. Are high adiposity induced changes in eccentric performance consistent with changes in concentric function?
3. Are the effects of high adiposity on muscle performance uniform between young and older adults?
4. Are the effects of high adiposity similar between upper and lower limb musculature?

Hypotheses and rationale:

- The effects of high adiposity will be more substantial in older adults due to an age-related decline in muscle function and the cumulative alterations in mechanisms responsible for optimal contractile performance.
- The magnitude of high adiposity effects on eccentric function will be reduced when compared to concentric function. An increase in eccentric demand in high adipose individuals may act as a favourable training adaptation, preserving eccentric contractile performance. Furthermore, previous evidence indicates maintained eccentric performance in populations which share similar mechanistic and contractile changes in skeletal muscle evoked via high adiposity. As such, we hypothesise the magnitude of high adiposity effects on eccentric function will be reduced when compared to concentric function.
- The effects of high adiposity on contractile performance will vary between upper and lower limb musculature, due to differences in mechanical loading.

Experimental Study 2 **-** ***Effect of Stimulation Frequency on Force, Power, and Fatigue of Isolated Mouse Extensor Digitorum Longus Muscle***

Study Aim:

- The aim of study 2 was to refine the work loop protocol to be used in later chapters by examining the optimal stimulation frequencies needed for maximal force and power, and to evoke a fatigue response more replicable of *in vivo* muscle mechanics.

This study sought to address the following questions:

1. Does the optimal stimulation frequency for isometric force evoke maximal work loop power output?
2. Do the stimulation frequencies which produce maximal isometric force and work loop power output evoke a fatigue response typical of *in vivo* fatigue mechanics?

Hypotheses and Rationale:

- The stimulation frequency needed to evoke maximal work loop power output will exceed that needed for maximal isometric force. Previous research demonstrates that the stimulation frequency-force relationship for isometric conditions cannot reliability predict stimulation-force relationship for dynamic muscle activity in rodent gastrocnemius (de Haan 1998), and in slow twitch mouse soleus muscle, the stimulation frequency for maximal isometric force is less than that needed for maximal power output (Vassilakos et al. 2009).
- Stimulation frequencies which evoke maximal force and power will elicit quicker rates of fatigue, reduced or equal cumulative work, and increased force requirements to relengthen (negative work) the muscle due to prolonged relaxation. As such, sub maximal stimulation frequencies will produce a more physiologically relevant fatigue response.

Experimental Study 3 **- *The Effects of a High-Fat Diet and vitamin D on Maximal Force, Power and Fatigue Resistance of Isolated Female CD-1 Mouse Soleus and EDL Muscles***

Thesis Progression and Study Aim:

- The main aims of experimental study 3 (chapter 5) was to examine the impact of high-fat diet on isolated muscle contractility and, to establish if vitamin D could both improve muscle contractility in standard low-fat diet treated mice and reduce the magnitude of high-fat induced muscle contractile dysfunction.

This study sought to address the following questions:

1. The effects of 12 weeks high-fat diet (45%) on the contractile performance of isolated skeletal muscle.
2. Can a high dose of dietary vitamin D (20,000IU/kg^{-1}) enhance isometric force and work loop power output and fatigue resistance of isolated fast or slow twitch locomotor muscles from control mice?
3. Does a high dose of dietary vitamin D offset the increase in body mass and adiposity evoked by a high-fat diet?
4. Can a high dose of dietary vitamin D alleviate the adverse effects of high-fat diet consumption on the contractile performance of isolated skeletal muscle?

Hypotheses and Rationale:

- A 12 weeks fixed high-fat diet will adversely affect the contractile performance of isolated mouse skeletal muscle; particularly in predominately fast twitch EDL, probably due to increased rate of overall and intramuscular adipose accumulation.
- High dose vitamin D will improve contractile performance in standard low-fat diet treated mice through promoting mechanisms involved in optimal contractile performance, such as, improved mitochondrial function, contractile protein synthesis and calcium handling (Girgis et al. 2013; Dahlquist et al. 2015).

- Vitamin D will reduce the impact of a high-fat diet on the accumulation of adipose, probably through inhibition of adipogenesis and increase in fatty acid oxidation (Marcotorchino et al. 2014; Fan et al. 2016).
- Supplementation of vitamin D will reduce the adverse effects of high-fat diet consumption on contractile performance, suspected to occur because of two primary reasons. Firstly, vitamin D will modulate mechanisms responsible for optimal muscle function. Secondly, dietary enrichment of vitamin D in high-fat treated mice will likely attenuate changes in mechanisms attributed to an obesity induced decline in muscle performance, such as, reducing myosteatosis through inhibition in lipogenic pathways (Benetti et al. 2018) and increase in fatty acid oxidation (Marcotorchino et al. 2014), and modulating mitochondrial function, in part through an increase in UCP3 (Fan et al. 2016).

Experimental Study 4 **-** ***The Effects of a High-Fat Diet and Resveratrol on Maximal Force, Power and Fatigue Resistance of Isolated Female CD-1 Mouse Soleus and EDL Muscles***

Study Aim:

- Using the work loop protocol refined in experimental study 2 (chapter 4), the aims of experimental study 4 (chapter 5) was to examine if RES could be used to reduce adverse effects of high-fat diet consumption on the contractile performance of isolated skeletal muscle; particularly in fast twitch EDL, where high-fat diet effects appear most substantial. Furthermore, this study aimed to examine the impact of RES on whole body and muscle morphology of standard and high-fat diet treated mice.

This study sought to address the following questions:

1. Does resveratrol offset the increase in body mass and adiposity evoked by a high-fat diet?

2. Can resveratrol be used as an effective nutritional strategy to alleviate the adverse effects of high-fat diet consumption on the contractile performance of isolated skeletal muscle?

Hypotheses and Rationale:

- Dietary enrichment of RES when consumed with a high-fat diet will reduce the accumulation of adipose as RES directly targets adipose tissue and enhances lipid metabolism (Kim et al. 2011).
- Declines in contractile function associated with HFD consumption will be at least partially mitigated in RES treated muscle through modulation of mechanisms responsible for obesity muscular dysfunction such as attenuating: the increase in intramuscular adipose and inflammation; and reduction in mitochondrial function and AMPK activity (Lagouge et al. 2006)

Chapter 3 - The Effects of High Adiposity on the Concentric and Eccentric Muscle Performance of Upper and Lower Limb Musculature in Young and Old Human Males

Modified from a publication in The Journal of Applied Physiology, Nutrition and Metabolism

Shelley, S., James, R. S., Eustace, S. J., Eyre, E., & Tallis, J. (2021). The effects of high adiposity on concentric and eccentric muscle performance of upper and lower limb musculature in young and old adults. Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme. https://doi.org/10.1139/apnm-2020-0945

Oral presentation of selected results was delivered at the annual British Association of Sport and Exercise Sciences (BASES) 2021 conference 'Physiology and Nutrition'

Published abstract in The Journal of Sports Sciences

Shelley, S., James, R. S., Eustace, S. J., Eyre, E., & Tallis, J. (2021). The effects of high adiposity on concentric and eccentric muscle performance of upper and lower limb musculature in young and old adults. Journal of Sport Sciences https://doi.org/10.1080/02640414.2021.19787

3.1 – Abstract

The present study uniquely examined the influence of old age and adiposity on maximal concentric and eccentric torque and fatigue of the elbow (EF, EE) and knee (KF, KE) flexors and extensors. 40 males were recruited and categorised into young (*n*=21, 23.7±3.4 years) and old (*n*=19, 68.3±6.1 years) and then further into normal (young = 16.9±2.5%, old = 20.6±3.1%) and high adiposity (young = 28.9±5.0%, old = 31.3±4.2%) groups. Handgrip strength, sit-to-stand performance, and isokinetic assessments of peak torque at 60°, 120° and 180° s^{-1} were measured. Older men produced significantly less concentric and eccentric peak torque ($P < 0.016$) but this was not influenced by adiposity ($P > 0.055$). For KE and KF, high adiposity groups demonstrated reduced peak torque normalised to body mass ($P < 0.021$), and muscle and contractile mode specific reduction in torque normalised to segmental lean mass. Eccentric fatigue resistance was unaffected by both age and adiposity ($P > 0.30$) and perceived muscle soreness, measured up to 72 hours post, was only enhanced in the upper body of the young group following eccentric fatigue ($P = 0.009$). Despite the impact of adiposity on skeletal muscle function being comparable between ages, these results suggest high adiposity will have greater impact on functional performance of older adults.

3.2 – Introduction

Recent research indicates that obesity may be detrimental to skeletal muscle function (Maffiuletti et al. 2013; Bollinger 2017; Tallis et al. 2018). However, current work has focused on isometric (force produced whilst the muscle remains at a constant length) and concentric (force produced during shortening) muscle actions of the lower extremities (Miyatake et al. 2000; Hulens et al. 2001; Villareal et al. 2004; Maffiuletti et al. 2007b; Capodaglio et al. 2009; Paolillo et al. 2012; Tomlinson et al. 2014b). Effects of obesity on eccentric (force produced during lengthening) muscle actions have not been thoroughly considered. Adequate eccentric force is important for maintaining balance, deacceleration and absorbing impact (Delbaere et al. 2003; Nishikawa et al. 2018a). As such, eccentric muscle function is important for physical activity and the completion of activities of daily living (ADLs) including stair descents, descending into a seated position and dynamic balance (Choi 2016; Nishikawa et al. 2018a). Obesity effects on isometric and concentric muscle function may not directly translate to eccentric performance. Potential effects of adiposity on the physiological mechanisms resulting in eccentric force production are currently unknown and given differences in mechanisms resulting in concentric and eccentric force production (Herzog et al. 2016), adiposity effects may not be uniform across contractile modalities. Given that changes in concentric and isometric muscle performance are similar in muscle ageing and adiposity it may be that eccentric function is better preserved compared to concentric and isometric contractile function in obesity as in aging (Roig et al. 2010). Furthermore, excessive adiposity is likely to increase eccentric demand (Tallis et al. 2018), particularly during stabilisation and deaccelerating phase of movements, which may result in a favourable eccentric training adaptation.

There is a growing suggestion that obesity may exacerbate the muscle ageing response (Tomlinson et al. 2014a; Hill and Tallis 2019; Eshima et al. 2020). Ageing and obesity induced declines in muscle function share similar mechanistic responses, such as impaired excitation contraction coupling, shifts in fibre type composition and a reduction in myogenesis (Tallis et al. 2018; Larsson et al. 2019). Unlike

in a young population, obesity has been shown to have little effect on the absolute force producing capacity of elderly muscle (Maffiuletti et al. 2013). However, obesity can result in a decline in force to body mass ratio (Miyatake et al. 2000; Villareal et al. 2004; Paolillo et al. 2012; Tomlinson et al. 2014b) and force normalised to muscle size (muscle quality), irrespective of age (Villareal et al. 2004; Tomlinson et al. 2014c; Valenzuela et al. 2020). Such diminished muscle quality reflects reduced contractile function per unit of muscle size, and in the case of obesity, results in larger, heavier muscles and in turn an increased force requirement for movement (Tallis et al. 2018). Despite this important insight, understanding of obesity induced changes in skeletal muscle function is limited. Previous research typically focuses on a single muscle group and a single mode of contractility (isometric or concentric), with limited assessment of muscular fatigue or direct comparisons between young and older groups (Miyatake et al. 2000; Villareal et al. 2004; Capodaglio et al. 2009; Paolillo et al. 2012; Erskine et al. 2017). Work examining the effect of obesity on eccentric function and the concomitant effects of obesity and increasing age on muscle function has been identified as an area of priority (Tallis et al. 2018)

As such, the present study used isokinetic dynamometry (IKD) to uniquely examine the effects of high adiposity and older age on concentric and eccentric maximal voluntary torque and fatigue resistance of the elbow flexors (EF) and extensors (EE) and knee flexors (KF) and extensors (KE). Torque values were also normalised for body mass and segmental muscle mass, the latter as an indication of muscle quality. Measures of hand grip strength (HGS) and sit to stand (STS) kinetics were used to determine whether differences in concentric and eccentric function were detectable in assessments frequently used as muscle health screening tools (Beaudart et al. 2019). STS performance also served to provide insight into the functional consequences of changes in peak torque.

3.3 – Methodology

All procedures were conducted following ethics approval from Coventry University (P76153). Participants visited the human performance laboratory at Coventry University on three separate occasions. Participants provided written consent and upon each visit completed a departmental health screening questionnaire. Visits were separated by a minimum of seven days and assessments were performed at the same time of day on each occasion. Participants were required to be healthy, non-resistance trained, have no contraindications to exercise, nor suffer from uncontrolled hypertension. Participants were asked to abstain from vigorous physical activity 48 hours prior to testing and to empty their bladder upon arrival. Participants (*N*=40) were categorised into young (18-30) or old (60-80), then sub categorised by age specific body fat percentage (%) into young normal adiposity (YNa; 8-19.9%; N=10), young high adiposity (YHa; >20%; N=11), old normal adiposity (ONa; 13-24.9%; N=11) and old high adiposity (OHa; >25%; N=8), derived using hand to foot multi frequency bioelectrical impedance analysis (MF-BIA; following instructions from the manufacturer, which utilised boundaries from Gallagher et al. 2000) Participant information and anthropometric data for groups are presented in Table 4.

3.3.1 – Experimental Procedures

Familiarisation

The intention of the first visit was to familiarise participants to the experimental procedures to be used in the study.

Body Composition

Shoes, heavy clothing, and jewellery were removed prior to measures being taken. Height was measured using a stadiometer (SECA Instruments Ltd., Germany). Body composition was then determined using hand to foot MF-BIA (TANITA MC-780, TANITA, Japan; impedance frequencies 5, 50 and 250kHz). MF-BIA has previously been shown to have acceptable accuracy for overall and

segmental adiposity and lean mass, when compared to dual-energy X-ray absorptiometry (DEXA) (ICC ≥ 0.832 and $R^2 \geq 0.85$) (Faria et al. 2014; Yamada et al. 2017; Ramírez-Vélez et al. 2018), and is a reliable method of obtaining fat mass in the populations being used (ICC = 0.925) (Ballesteros-Pomar et al. 2022). MF-BIA devices have been developed to allow for measurements at any time of the day, without the need to impose nutritional constraints (Verney et al. 2015). From BIA assessment, body mass (kg), overall and segmental adiposity (%) and muscle mass (kg) of the limbs and trunk, and body mass index (BMI) were all recorded. All values recorded were measured to the nearest 0.1 kilogram or percentage.

Sit to Stand

Measures of lower extremity function were obtained through three consecutive STS on a force plate (AMTI, AccuGait, MA, USA) sampling at 100Hz (Regterschot et al. 2016). During the STS, arms were folded across the chest (Janssen et al. 2002), and participants were instructed to stand up as quickly as possible from the chair (seat height 0.45m; width 0.41m; depth 0.38.5m; floor to center of back support 0.68m) and remain standing for a minimum of ten seconds before sitting down. Participants were given verbal queues on when to rise and sit down. Peak propulsive force normalised to body mass ($N.kg^{-1}$), rate of force development (RFD; $N.kg^{-1}.s^{-1}$) and time to stabilisation (TTS; s) were recorded from each trial. RFD was calculated by dividing the peak force in the standing phase of the movement by time to peak force. Time to stabilisation (s) was calculated by subtracting the time to which the participant was within 5% of body weight (Wikstrom et al. 2005) for the first of 50 data points by the time at which peak propulsive force occurs. The average results of the three consecutive STS were taken for each trial. All calculations for STS were completed in Microsoft Excel (Windows v. 2016).

Handgrip Strength

Handgrip strength (HGS; kg) was measured using an isometric hand dynamometer (Takei Physical Fitness Test, GRIP-D, Takei Scientific instrument Co. LTD, Japan). Participants were instructed to stand upright, with their arms by their side, whilst gripping the dynamometer maximally (Yeung et al. 2018). Assessment of handgrip was performed three times with the dominant hand for approximately 2-3 seconds, with peak HGS being recorded. Each attempt was separated by 60s rest. Participants were given verbal queues on when to grip the device. Previous literature has proposed that assessing HGS can be a cost effective and valid predictor of overall muscle strength in young and older adults (OAs) (Wind et al. 2010; Bohannon et al. 2012)

Isokinetic Dynamometry

Skeletal muscle contractile performance was further assessed using IKD, in accordance to previously published protocols (Impellizzeri et al. 2008; Tallis et al. 2016). Initially, participants completed a standardised upper body warm-up, consisting of five minutes of arm crank ergometry (Monark 857E Ergomedic, Monark, Varberg, Sweden) using an unloaded cradle and a fixed cadence of 70 revs min^{-1}. This was immediately followed by 5 minutes of dynamic stretches, focusing on the elbow flexors and extensors. Maximal voluntary isokinetic concentric and eccentric torque (N.m) of the elbow flexors and extensors for the dominant side were measured using IKD (Cybex NORM, Humac2009, v10.000.0082, CA, USA) set up in accordance with the manufacturer's instructions. Participants were strapped to the dynamometer chair in a supine position. The rotational axis of the dynamometer head was aligned with the lateral epicondyle of the humerus. A hand grip bar at the opposing end of the lever arm was adjusted to the length of the hand and forearm to allow the participant a comfortable grip. Participant settings were retained and used for subsequent visits. During concentric measures, participants were instructed to pull upwards towards their shoulder (flexion) and extend their arm outward (extension) as hard a possible through a fixed range of 40° - 120° (relative to anatomical zero for the elbow). This range of motion falls within ranges used during ADL (Morrey et al. 1981; Malagelada et al. 2014). During eccentric measures, participants were asked

to resist the movement of the lever arm moving from 120° - 40°. Maximal concentric and eccentric force were measured at angular velocities of 60°, 120° and 180° s^{-1}. Participants performed several submaximal attempts at each angular velocity to become familiarised with the movements and test speeds (Feiring et al. 1990). During the assessment of maximal voluntary torque, participants performed a series of tests at each angular velocity, with peak torque occurring within 3 repetitions, similar to previous work (Sole et al. 2007; Impellizzeri et al. 2008; Tallis and Yavuz 2018). A two-minute rest period was implemented between each set to minimise fatigue. Participants were then familiarised to part of the fatigue protocol to be used in the experimental trials. Participants completed 10 consecutive concentric contractions at 180° s^{-1} of the elbow flexors and extensors. Following a 2-minute rest period, participants then completed 10 eccentric contractions at 180° s^{-1} of the elbow flexors and extensors. All torque values collected were corrected for gravity effects by estimation of limb weight, prior to the assessment of maximal voluntary torque.

Participants then completed a standardised warm up of the lower body, consisting of 5 minutes of cycling (Monark 824E, Ergomedic, Monark, Varberg, Sweden) using an unloaded cradle and a fixed cadence of 70 revs min^{-1}, immediately followed by 5 minutes of dynamic stretches, focusing on the knee extensors and flexors. The IKD was then set up for the assessment of maximal voluntary concentric and eccentric isokinetic torque (N.m) of knee flexors and extensors. Participants were strapped to the dynamometer chair in a seated position, and the lever arm axis of rotation was aligned with the lateral femoral epicondyle of the dominant limb. The distal end of the lever arm was fitted with a shin pad approximately 3cm above the lateral malleolus. A strap was placed across the midpoint of the upper limb of the test leg. Throughout the duration of the test, participants were instructed to hold the handles provided on the chair. The range of motion was fixed at 20°-80° (relative to anatomical zero for the knee) to ensure all participants were able to generate torque throughout the whole movement. The testing protocol was then carried out in the manner previously described.

Experimental Trial One: Concentric Muscle Function

Participants completed the STS, HGS and the concentric IKD protocols, following the procedures previously described. Immediately following both the upper body and lower body assessments of maximal voluntary force, fatigability was assessed via 5 sets of 10 concentric contractions at 180° s^{-1}, with 10 seconds separating each set. Immediately following the session and at 24-hour intervals for the following three days, perceived soreness was assessed using a visual analogue scale (VAS) (Mattacola et al. 1997). Perceived soreness was recorded individually for the upper body and lower body to establish if high adiposity or old age exacerbated the delayed onset of muscle soreness following bouts of consecutive contractions. To assess perceived soreness of the lower body participants were instructed to assume an unweighted squat of approximately 90 degrees for 3-5 seconds before noting soreness. For the upper body, participants were required to extend their arms to near anatomical zero in front of them, whilst holding a weighted object and then marked their perceived soreness. All absolute torque values were saved and later normalised for body mass (torque/body mass; $N.m.kg^{-1}$) and segmental muscle mass of the limb used (muscle quality; torque/segmental muscle mass; $N.m.kg^{-1}$).

Experimental Trial Two: Eccentric Muscle Function

Participants were asked to complete only the eccentric IKD protocol. Immediately following both the upper body and lower body assessments of maximal voluntary eccentric torque, fatigability was assessed via 5 sets of 10 eccentric contractions at 180° s^{-1}, with 10 seconds of rest separating each set. Immediately following the session and at 24-hour intervals for the following three days, perceived soreness was assessed using a visual analogue scale (VAS) in the manner previously described. Trials were always performed in the order described as recovery from eccentric induced muscle damage is not well known in these populations and participants may experience a decline in concentric peak torque more than 7 days post eccentric fatigue (Paulsen et al. 2010).

3.3.2 – Data Analysis

Statistical analysis was performed using SPSS v.25 (IBM SPSS Statistics for Windows, IBM Corp, Armonk, NY, USA). All data are presented as mean ± SD. Tests of normality (Shapiro-Wilk) and homogeneity (Levenes) were utilised to ensure appropriate analysis of data. When parametric assumptions were not met, non-parametric alternatives were used where appropriate. As such, Mann-Whitney test was utilised to assess significant differences in age, body mass and BMI. When non-parametric equivalents were not possible, data were transformed using either log10 or SQRT transformations dependent on if the data was left or right skewed. Once transformed, data were reanalysed for normality and homogeneity. Comparisons of anthropometric data, absolute torque, and torque normalised to body mass and segmental muscle mass, HGS and STS performance of experimental groups were measured using a two-way analysis of variance (ANOVA), with age and adiposity as the factors. Comparisons of percentage torque loss during fatigue protocols were measured using a three factor (age, adiposity and set) ANOVA, following arcsine transformation of fatigue data (Winer 1971). Significant interactions were explored using Bonferroni post hoc for multiple comparisons. Partial eta squared (ηp^2) was calculated to estimate effect sizes for all significant main effects. Thresholds for Partial eta squared effect size were classified as small (<0.05), moderate (0.06-0.137) or large (>0.138) (Cohen 1988). Cohen's *d* was calculated to measure effect size of any interactions observed. Cohen's *d* was then corrected for bias using Hedges' g due to the appropriate sample size of each experimental group (Hedges 1981). Cohen's *d* effect size was interpreted as trivial (<0.2), small (0.2-0.6), moderate (0.6-1.2) or large (>1.2) (Hopkins et al. 2009). The truncated product method (Zaykin et al. 2002) was utilised to combine all P values obtained from statistical analysis in this study to determine whether there was a bias from multiple hypothesis testing. The P value of < 0.001 obtained from the truncated product method suggests that our results were not biased. The level of significance was set at $P \leq 0.05$.

3.4 – Results

3.4.1 – Participant Characteristics

Table 4 displays age and anthropometric measures of participants. Individuals with high adiposity had higher body mass, BMI, body fat percentage and muscle mass ($P < 0.021$, $\eta p^2 > 0.140$) compared to normal adipose individuals. A main effect of age was observed for all characteristics ($P < 0.001$, $\eta p^2 > 0.301$), other than body mass, BMI, trunk fat (%) and left arm fat (%) ($P > 0.116$; $\eta p^2 < 0.040$ in each case, except for left arm fat (%) where $P = 0.062$, $\eta p^2 = 0.940$). There were no age*adiposity interactions ($P > 0.119$; $\eta p^2 < 0.074$).

Table 4. *Characteristics of Participants Grouped by Age and Adiposity*

	Young Normal Adiposity	Young High Adiposity	Old Normal Adiposity	Old High Adiposity	Age Effect		Adiposity Effect		Interaction	
	N=10	*N*=11	*N*=11	*N*=8	*P* value	ηp^2	*P* value	ηp^2	*P* value	ηp^2
Age	23.0 ± 2.7	23.9 ± 4.2	63.4 ± 5.6	66.8 ± 7.3	**< 0.001**	**0.566**	0.349	0.005	*n/a*	*n/a*
Height (cm)	180.4 ± 5.2	177.9 ± 6.3	173.9 ± 4.4	172.1 ± 5.3	**< 0.001**	**0.269**	0.214	0.043	0.829	0.001
Mass (kg)	76.8 ± 7.5	105.0 ± 17.0	74.3 ± 5.4	96.5 ± 18.5	0.116	0.001	**< 0.001**	**0.539**	*n/a*	*n/a*
BMI	23.5 ± 1.4	33.1 ± 3.4	24.5 ± 1.2	32.4 ± 3.8	0.674	0.040	**< 0.001**	**0.400**	*n/a*	*n/a*
Bodyfat (%)	16.9 ± 2.5	28.9 ± 5.0	20.6 ± 3.1	31.3 ± 4.2	**0.021**	**0.140**	**< 0.001**	**0.705**	0.598	0.008
Muscle Mass (kg)	60.2 ± 5.9	70.4 ± 7.8	56.0 ± 3.8	62.5 ± 8.2	**0.006**	**0.191**	**< 0.001**	**0.310**	0.442	0.016
Right Leg Fat (%)	13.7 ± 2.5	23.8 ± 5.1	21.0 ± 2.9	29.8 ± 8.5	**< 0.001**	**0.379**	**< 0.001**	**0.524**	0.119	0.066
Right Leg Muscle Mass (kg)	10.7 ± 1.0	12.8 ± 1.5	8.6 ± 0.7	10.2 ± 1.8	**< 0.001**	**0.524**	**< 0.001**	**0.372**	0.827	0.001
Left Leg Fat (%)	14.6 ± 2.5	23.6 ± 2.5	20.3 ± 2.7	29.5 ± 7.3	**< 0.001**	**0.354**	**< 0.001**	**0.557**	0.321	0.027
Left Leg Muscle Mass (kg)	10.3 ± 0.9	12.7 ± 1.5	8.6 ± 0.7	10.2 ± 1.7	**< 0.001**	**0.465**	**< 0.001**	**0.402**	0.657	0.006
Right Arm Fat (%)	14.9 ± 1.5	26.0 ± 5.4	20.0 ± 2.4	28 ± 3.6	**0.013**	**0.159**	**< 0.001**	**0.610**	0.099	0.074
Right Arm Muscle Mass (kg)	3.6 ± 0.4	4.3 ± 0.5	2.9 ± 0.3	3.5 ± 0.8	**< 0.001**	**0.524**	**< 0.001**	**0.319**	0.836	0.001
Left Arm Fat (%)	15.8 ± 2.7	27.2 ± 6.0	19.9 ± 2.6	29.0 ± 4.0	0.062	0.940	**< 0.001**	**0.603**	0.224	0.041
Left Arm Muscle Mass (kg)	3.6 ± 0.5	4.3 ± 0.6	3.0 ± 0.3	3.6 ± 0.7	**< 0.001**	**0.306**	**< 0.001**	**0.301**	0.880	0.001
Trunk Fat (%)	19.9 ± 3.9	32.5 ± 5.4	20.6 ± 3.8	32.5 ± 4.8	0.844	0.001	**< 0.001**	**0.681**	0.800	0.002

3.4.2 – Adiposity and Age Effects on Maximal Concentric Muscle Function

For absolute concentric peak torque of the EF, EE, KF and KE, young individuals produced greater absolute torque than OAs (Fig. 3.1A & D, 3.2A & D. $P < 0.002$, $\eta p^2 > 0.263$). There was no main effect of adiposity ($P > 0.185$, $\eta p^2 < 0.048$ in each case, except for peak torque of the EE at angular velocity of 180° s^{-1} where $P = 0.055$, $\eta p^2 = 0.099$).

Young (Fig 3.1B & E, 3.2B & E. $P < 0.009$, $\eta p^2 > 0.174$) and normal adiposity (Fig 3.1B & E, 3.2B & E. $P < 0.021$, $\eta p^2 > 0.144$) groups produced greater concentric torque relative to body mass in all muscles, when compared to their old or high adiposity counterparts, except at an angular velocity of 180° s^{-1} at the EE, where no significant effect of adiposity was observed ($P = 0.086$, $\eta p^2 = 0.080$).

The young (Fig 3.1F, 3.2C & F. $P < 0.013$, $\eta p^2 > 0.159$) and normal adiposity (Fig 3.1F, 3.2C & F. $P < 0.041$, $\eta p^2 > 0.111$) groups KE, KF and EF produced greater concentric torque normalised to segmental muscle mass compared to that of the older and high adiposity groups respectively; with the exception of the EF at 120° s^{-1} where no effect of adiposity was observed ($P = 0.086$, $\eta p^2 = 0.080$). There was no effect of age or adiposity observed on concentric muscle quality of the EE (Fig 3.1C. $P > 0.233$, $\eta p^2 < 0.041$).

There was no age*adiposity interaction observed for absolute peak concentric torque, peak concentric torque normalised to body mass or peak concentric muscle quality in any of the muscle groups ($P > 0.085$, $\eta p^2 < 0.065$).

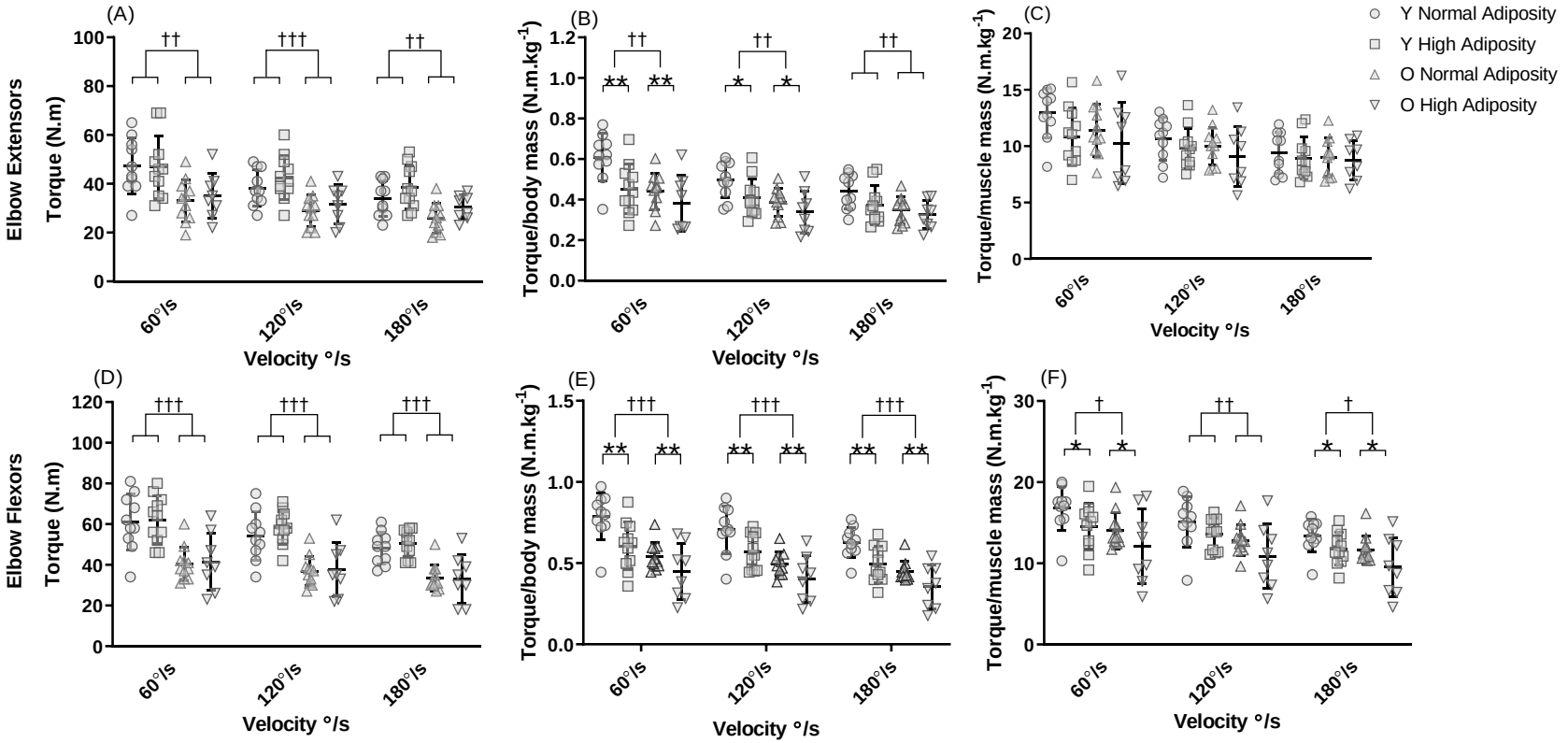

Figure 3. 1. *The effect of age and adiposity on absolute concentric torque (A, D), torque normalised to body mass (B, E) and torque normalised to segmental muscle mass of the dominant arm (C, F) of the elbow extensors and flexors. Values are presented as means ± SD; †, ††, ††† and *, **, *** indicate a significant difference between young and old and normal and high adiposity respectively at P < 0.05, P < 0.01 and P < 0.001.*

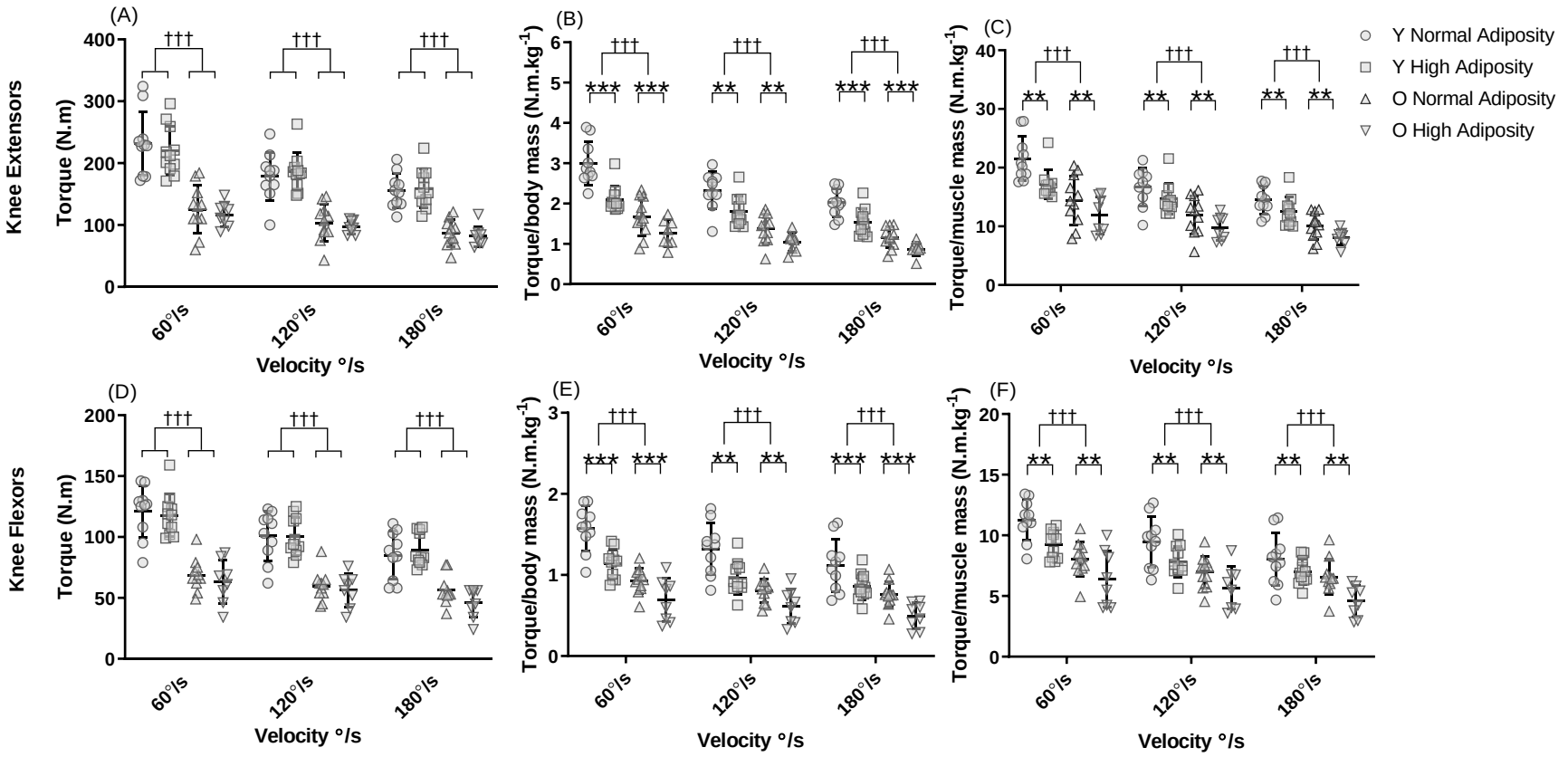

Figure 3. 2. *The effect of age and adiposity on absolute concentric torque (A, D), torque normalised to body mass (B, E) and torque normalised to segmental muscle mass of the dominant leg (C, F) of the knee extensors and flexors. Values are presented as means ± SD; †, ††, ††† and *, **, *** indicate a significant difference between young and old and normal and high adiposity respectively at P < 0.05, P < 0.01 and P < 0.001.*

3.4.3 – Adiposity and Age Effects on Maximal Eccentric Muscle Function

Young individuals produced greater absolute eccentric torque when compared to OAs (Fig. 3.3A & D, 3.4A & D. $P < 0.016$, $\eta p^2 > 0.152$). There was no main effect of adiposity ($P > 0.209$, $\eta p^2 < 0.043$) and no age*adiposity interaction observed in any muscle group ($P > 0.126$, $\eta p^2 < 0.064$).

The young (Fig 3.4B & E. $P < 0.012$, $\eta p^2 > 0.162$) and normal adiposity (Fig 3.4B & E. $P < 0.010$, $\eta p^2 > 0.172$) groups KE and KF produced greater eccentric torque normalised to body mass compared to older and high adiposity groups respectively. At the EE, younger individuals produced greater eccentric peak torque relative to body mass than OAs (Fig 3.3B $P < 0.015$, $\eta p^2 > 0.155$), except at an angular velocity of 120° s^{-1} ($P = 0.115$ $\eta p^2 = 0.068$). At the EE, normal adipose participants produced greater eccentric peak torque normalised to body mass at an angular velocity of 120° s^{-1} (Fig 3.3B $P = 0.013$ $\eta p^2 = 0.160$), but not at 60° or 180° s^{-1} ($P > 0.580$ $\eta p^2 < 0.096$). There was no age*adiposity interaction observed for the EE, KE and KF ($P > 0.234$, $\eta p^2 < 0.039$). However, for the EF, there was an age*adiposity interaction (Fig 3.3E. $P < 0.026$, $\eta p^2 > 0.142$) for torque normalised to body mass. Bonferroni multiple comparisons indicated that YNa produced significantly greater peak eccentric torque normalised to body mass than all other experimental groups ($P < 0.001$, $d > 1.85$); the other experimental groups were not different to each other ($P > 0.999$, $d < 0.46$).

For the EE and EF, there was no main effect of age (Fig 3.3C & F. $P > 0.071$, $\eta p^2 < 0.088$) observed for eccentric muscle quality. Similarly, for the KE and KF, there was no main effect of age on eccentric muscle quality (Fig 3.4C & F $P > 0.103$, $\eta p^2 < 0.072$) except at an angular velocity of 60° s^{-1}, where younger individuals produced greater eccentric torque normalised to segmental muscle mass compared to OAs ($P < 0.025$, $\eta p^2 > 0.132$). For the EE and KE, there was no main effect of adiposity (Fig 3.3C & 3.4C $P > 0.224$, $\eta p^2 < 0.040$) observed for eccentric muscle quality. However, for the EF and KF, those with normal adiposity produced greater eccentric torque normalised to segmental muscle mass when compared to those with a high adiposity (Fig 3.3F $P < 0.034$, $\eta p^2 > 0.120$, Fig 3.4F $P < 0.010$, $\eta p^2 > 0.170$).

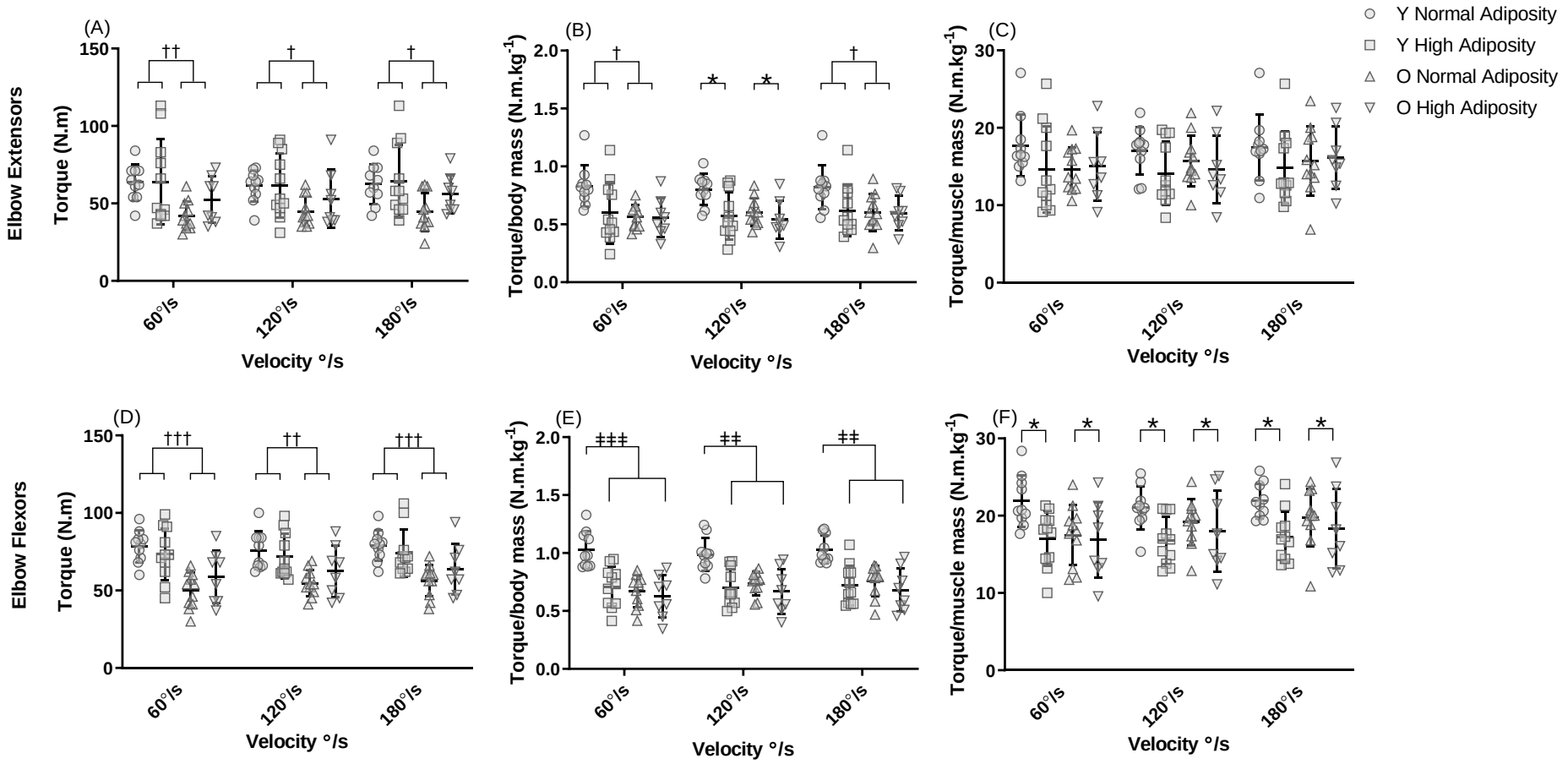

Figure 3. 3. *The effect of age and adiposity on absolute eccentric torque (A, D), torque normalised to body mass (B, E) and torque normalised to segmental muscle mass of the dominant arm (C, F) of the elbow extensors and flexors. Values are presented as means ± SD; †, ††, †††, *, **, *** and ǂ, ǂǂ, ǂǂǂ indicate a significant difference between young and old, normal and high adiposity, and YNa and all other groups respectively at P < 0.05, P < 0.01 and P < 0.001.*

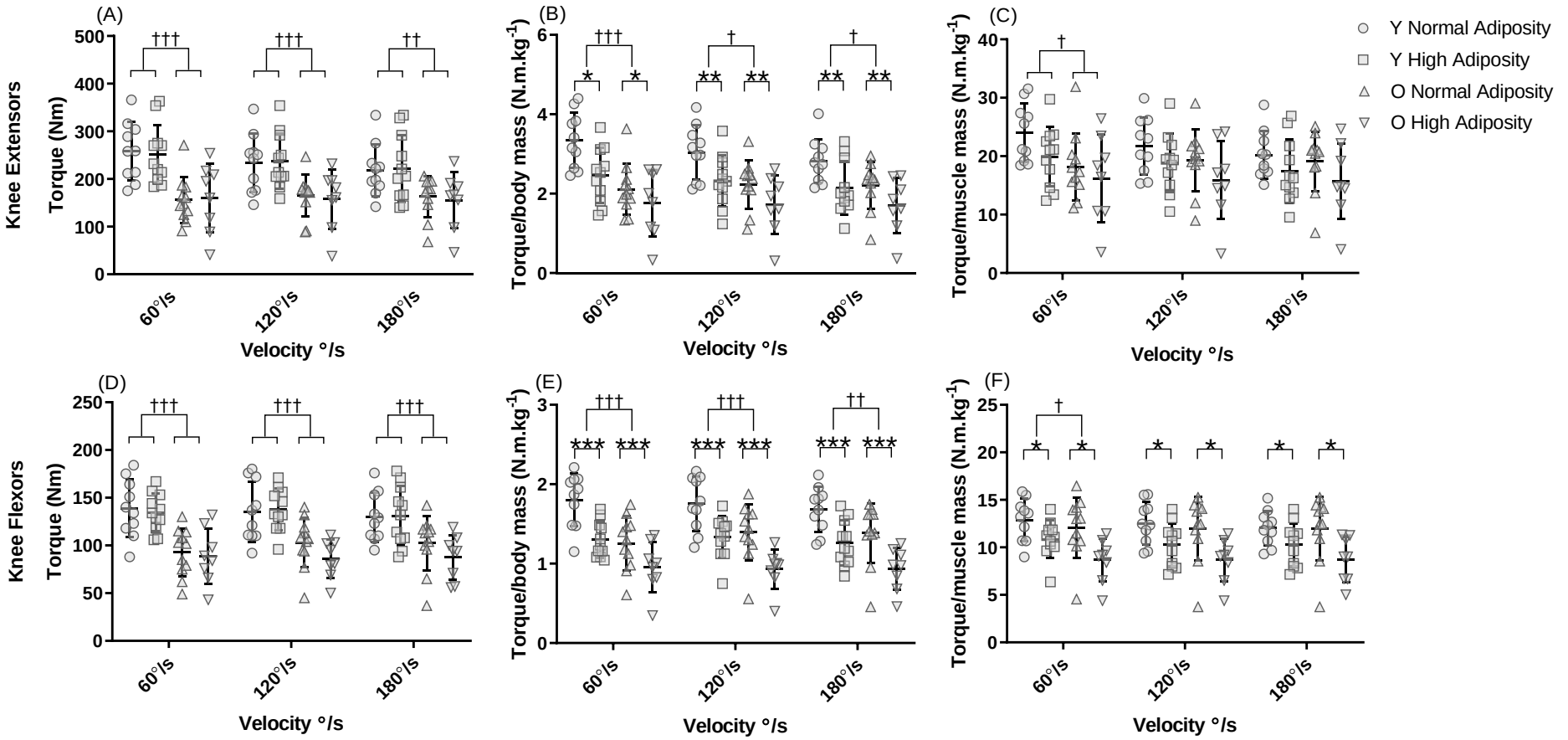

Figure 3. 4. *The effect of age and adiposity on absolute eccentric torque (A, D), torque normalised to body mass (B, E) and torque normalised to segmental muscle mass of the dominant leg (C, F) of the knee extensors and flexors. Values are presented as means ± SD; †, ††, ††† and *, **, *** indicate a significant difference between young and old and normal and high adiposity respectively at P < 0.05, P < 0.01 and P < 0.001.*

3.4.4 – Adiposity and Age Effects on Concentric Muscle Fatigue

There was a decline in concentric torque over time in all experimental groups and muscles assessed (Fig 3.5A, C, E & G. $P < 0.001$, $\eta p^2 > 0.652$). There was no main effect of adiposity observed in any muscle group (Fig 3.5A, C & G. $P > 0.145$, $\eta p^2 < 0.120$). For the KE, there was a main effect of age (Fig 3.5G $P = 0.013$, $\eta p^2 = 0.034$) on percentage torque loss during concentric muscle actions. For the EE and EF, there was an age*set interaction (Fig 3.5A & C. $P < 0.001$, $\eta p^2 > 0.122$). Bonferroni comparisons indicated a significant difference ($P < 0.001$, $d > 1.24$) in percentage of maximum torque between young and old individuals at sets 3, 4 and 5. The main effect of age and age*set interaction indicated that the younger groups experience a greater percentage decline in concentric torque relative to their maximum, compared to older groups. For the KF, an age*adiposity interaction (Fig 3.5E. $P = 0.044$, $\eta p^2 = 0.022$) was observed. However, Bonferroni comparisons indicated no difference between groups ($P > 0.459$, $d < 0.47$, except for comparison of fatigue in YHa and OHa, where $P = 0.459$, $d = 0.76$). There were no other interactions ($P > 0.144$, $\eta p^2 < 0.012$).

3.4.5 – Adiposity and Age Effects on Eccentric Muscle Fatigue

There was a decline in eccentric torque over time in all experimental groups and muscles assessed (Fig 3.5B, D & H. $P < 0.001$, $\eta p^2 > 0.232$), except at the KE (Fig 3.5H. $P = 0.052$, $\eta p^2 = 0.051$). For the EF, KF, and KE, there were no main effects of age (Fig 3.5B, F & H. $P > 0.302$, $\eta p^2 < 0.006$) or adiposity (Fig 3.5B, F & H. $P > 0.695$, $\eta p^2 < 0.001$) observed for percentage eccentric torque loss. For the EE, there was an age*adiposity*set interaction (Fig 3.5D, $P = 0.017$, $\eta p^2 = 0.064$). Bonferroni comparisons indicated a significant difference between YNa and YHa at set 1 and 4 ($P < 0.024$, set 1 Cohen's d = 0.88, set 4 Cohen's d = 1.26) and YNa and ONa at set 4 and 5 ($P = 0.038$, $d = 1.32$). The interaction indicated the YNa group started at a greater percentage of their maximum but experienced greater fatigue at set 4 when compared to the YHa group. Furthermore, the YNa group experienced

greater torque loss at sets 4 and 5 compared to ONa. No other interactions were observed ($P > 0.063$, $\eta p^2 < 0.043$).

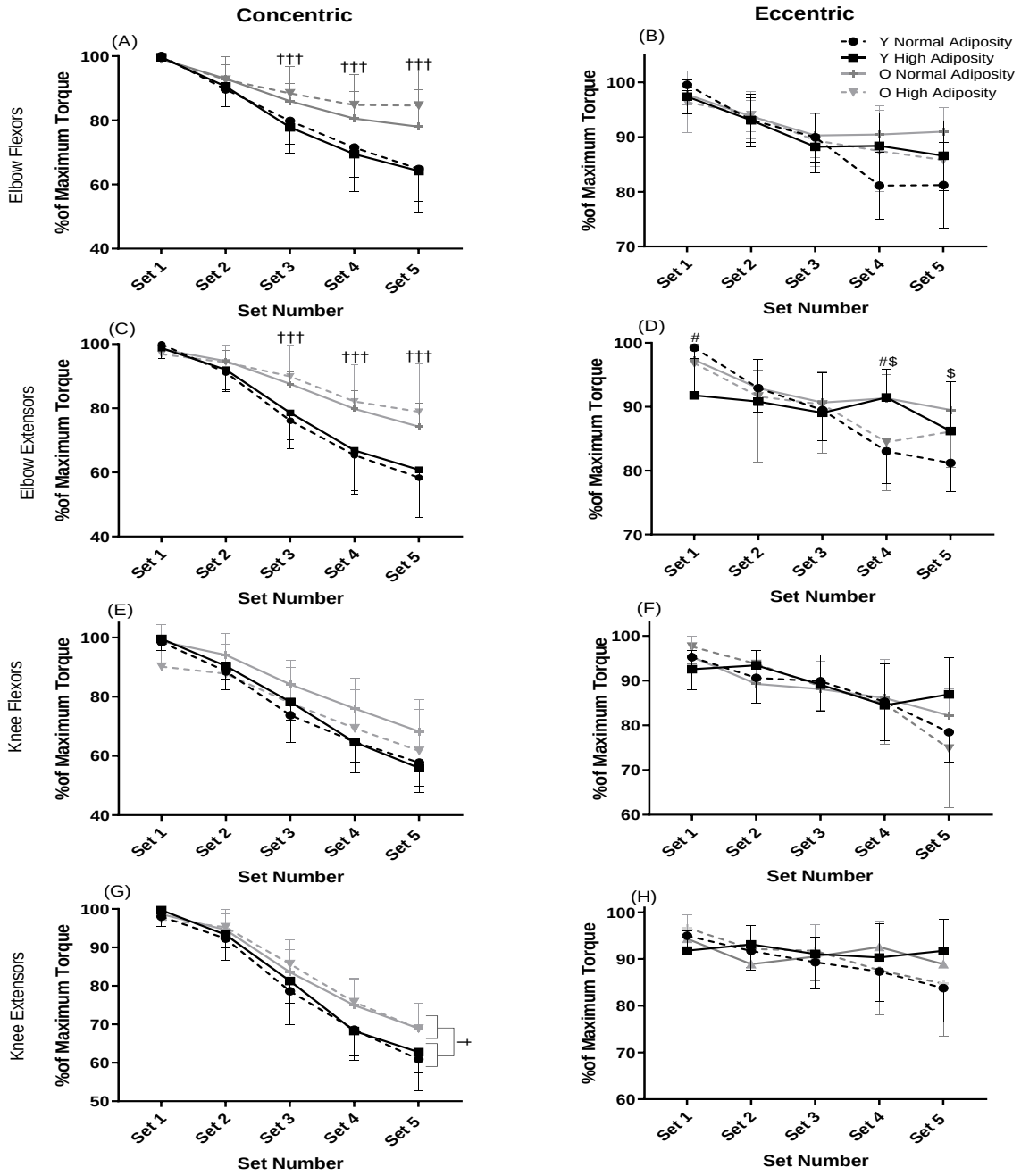

Figure 3. 5. *The effect of age and adiposity on the fatigue resistance of the elbow flexors (A, B), elbow extensors (C, D), knee flexors (E, F) and knee extensors (G, H) following five sets of 10 concentric or eccentric isokinetic contractions. Values are presented as means ± SD; †, †† and ††† indicate a significant difference between young and old at P < 0.05, P < 0.01 and P < 0.001; # and $ indicate a significant difference between YNa and YHa, and YNa and ONa respectively at P < 0.05.*

3.4.6 – Adiposity and Age Effects on Perceived Soreness Following Fatigue

Table 5 displays perceived soreness immediately following, and every 24-hours for 3 days after, the fatigue protocols. Following eccentric fatigue of the dominant arm, younger individuals experienced greater soreness compared to OAs ($P = 0.009$, $\eta p^2 = 0.047$). There were no main effects or interactions observed following concentric fatigue protocols ($P > 0.263$, $\eta p^2 < 0.009$). There was however an age*adiposity interaction ($P = 0.034$, $\eta p^2 = 0.030$) in perceived soreness of the dominant leg following the eccentric fatigue protocol. However, Bonferroni comparisons identified no significant difference in perceived soreness between groups ($P > 0.470$, $d < 0.55$).

3.4.7 – Adiposity and Age Effects on STS performance and HGS

Table 6 displays descriptive values of HGS and STS performance. All outcome variables for STS and HGS revealed a main effect of age ($P < 0.037$, $\eta p^2 > 0.115$*)*, whereby young individuals outperformed OAs. A main effect of adiposity was revealed for peak force during STS ($P < 0.001$, $\eta p^2 > 0.316$), indicating those with normal adiposity produced greater force normalised to body mass when compared to those with high body adiposity. There were no age*adiposity interactions observed ($p > 0.188$, $np^2 < 0.048$).

Table 5. *The Effects of Age and Adiposity on the Perceived Soreness Following Fatigue*

	Young Normal Adiposity	Young High Adiposity	Old Normal Adiposity	Old High Adiposity	Age Effect		Adiposity Effect		Interaction	
	N=10	*N*=11	*N*=11	*N*=8	*P* value	ηp^2	*P* value	ηp^2	*P* value	ηp^2
Perceived Soreness 0-10										
Concentric Arm					0.748	0.001	0.782	0.001	0.559	0.002
Immediate	7.8 ± 0.8	6.0 ± 2.1	6.7 ± 0.9	6.6 ± 1.1						
24 Hours	1.0± 1.3	1.1 ± 2.0	1.8 ± 1.2	1.7 ± 2.0						
48 Hours	0.0 ± 0.2	0.6 ± 1.6	0.5 ± 0.7	0.7 ± 0.8						
72 Hours	0.0 ± 0.0	0.1 ± 0.5	0.1 ± 0.3	0.4 ± 0.6						
Concentric Leg					0.263	0.009	0.680	0.001	0.429	0.004
Immediate	7.4 ± 1.1	6.6 ± 2.1	6.2 ± 1.1	6.3 ± 1.7						
24 Hours	0.6 ± 0.7	0.7 ± 0.9	1.1 ± 0.9	1.0 ± 0.5						
48 Hours	0.0 ± 0.2	0.2 ± 0.7	0.4 ± 0.6	0.4 ± 0.5						
72 Hours	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	0.3 ± 0.4						
Eccentric Arm					**0.009**	**0.470**	0.318	0.007	0.715	0.001
Immediate	7.6 ± 0.1	7.5 ± 1.4	6.2 ± 1.4	6.5 ± 1.8						
24 Hours	4.8 ± 3.0	3.8 ± 2.2	3.4 ± 2.6	4.2 ± 2.5						
48 Hours	4.5 ± 3.2	3.8 ± 2.2	2.7 ± 3.0	3.6 ± 2.9						
72 Hours	3.4 ± 2.3	2.2 ± 1.9	1.8 ± 2.2	3.0 ± 2.6						
Eccentric Leg					**0.034**	**0.310**	0.885	0.006	**0.034**	**0.030**
Immediate	7.1 ± 1.1	7.6 ± 1.3	6.0 ± 1.8	6.8 ± 1.7						
24 Hours	4.3 ± 2.9	4.4 ± 2.5	3.0 ± 2.8	3.5 ± 2.8						
48 Hours	3.4 ± 3.0	3.8 ± 2.5	2.3 ± 3.0	2.6 ± 2.5						
72 Hours	2.2 ± 2.2	2.2 ± 2.2	1.3 ± 2.1	1.7 ± 2.2						

Table 6. *The Effects of Age and Adiposity on Handgrip Strength and Sit to Stand Performance*

	Young Normal Adiposity	Young High Adiposity	Old Normal Adiposity	Old High Adiposity	Age Effect		Adiposity Effect		Interaction	
	N=10	*N*=11	*N*=11	*N*=8	*P* value	ηp^2	*P* value	ηp^2	*P* value	ηp^2
Handgrip										
HGS (kg)	47.9 ± 5.1	50.6 ± 9.4	36.6 ± 6.6	38.6 ± 10.4	**< 0.001**	**0.455**	0.863	0.001	0.21	0.043
Sit to Stand										
Peak Force (N.kg^{-1})	1.60 ± 0.10	1.49 ± 0.06	1.40 ± 0.06	1.29 ± 0.12	**< 0.001**	**0.615**	**< 0.001**	**0.316**	0.82	0.001
RFD (N.kg^{-1}.s^{-1})	4.31 ± 1.18	4.05 ± 0.84	3.80 ± 0.63	3.10 ± 0.97	**0.018**	**0.145**	0.950	0.075	0.336	0.026
TTS (s)	0.64 ± 0.11	0.67 ± 0.16	0.66 ± 0.10	0.83 ± 0.19	**0.037**	**0.115**	0.062	0.093	0.188	0.048

3.5 – Discussion

The present study uniquely examined the influence of both old age and high adiposity on the concentric and eccentric performance of upper and lower limb musculature in men. These data indicate an age-related decline in peak eccentric and concentric maximal voluntary torque but no change with adiposity. Whilst concentric muscle quality (torque normalised to segmental muscle mass) was reduced with increasing age, eccentric muscle quality was generally unaffected. In comparison to absolute function and force to body mass ratio, the present findings suggest that the effects of high adiposity on muscle quality are more complex. Concentric muscle quality was reduced with high adiposity, except for the EE. Whereas eccentric muscle quality was only reduced in the KF and EF. Across the contractile modalities and muscles groups assessed, older age and adiposity typically resulted in reduced maximal voluntary torque normalised to body mass. Whilst these results suggest that the severity of high adiposity effects on muscle performance are not greater in older males, implications of adiposity induced losses in muscle quality and torque relative to body mass will have much greater consequences for OAs given pronounced age-related declines in contractile function.

3.5.1 – Adiposity and Age Effects on Peak Torque

An age-related decline in muscular strength, including both concentric and eccentric torque production, has been well established (Proctor et al. 1998; Pousson et al. 2001; Lauretani et al. 2003; Larsson et al. 2019). The data in this chapter supports these findings, with young individuals producing greater absolute peak torque across all muscle groups and modes of contractility, when compared to OAs. An age-related decline in absolute function is characterised by muscular atrophy (Newman et al. 2003), and as seen in Table 4, whole body and segmental muscle mass was lower in the OAs. Mechanistic and architectural changes to skeletal muscle such as a reduction in sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) (Tallis et al. 2014), impaired calcium handling (Tallis et al. 2014)

reduction in fascicle pennation angle (Morse et al. 2005) and atrophy of high force producing type II fibres (Lexell and Taylor 1991), likely account for the demonstrated difference in absolute torque between young and old adults. Due to the age range used to categorise older adults (60-80 years old), it is possible the age-induced reduction in absolute muscle performance may be more pronounced in the oldest participants (70-80 years old) when compared to those aged 60-69 years old (Milanović et al., 2013). Given the magnitude of differences in absolute muscular strength between young and older participants, any differences in absolute muscular performance between older adults are unlikely to effect the trends in the current data.

Irrespective of age, high adiposity had no effects on concentric or eccentric peak torque of either the upper or lower limb musculature. Previous work has indicated that maximal isometric and concentric force of anti-gravitational musculature may be increased in high adiposity groups, due to the greater mechanical loading required to support the elevated body mass (Rolland et al. 2004; Villareal et al. 2004; Maffiuletti et al. 2007b; Capodaglio et al. 2009; Tomlinson et al. 2014a; Erskine et al. 2017). However, as with the present findings, an increase in absolute force production is not always shown in young (Hulens et al. 2001; Lafortuna et al. 2005; Paolillo et al. 2012; Cavuoto and Nussbaum 2013; Pajoutan et al. 2016) or old obese individuals (Miyatake et al. 2000; Erskine et al. 2017). Such findings may in part be attributed to a reduction in muscle activation capacity, which has previously been observed in both young and old obese groups (Tomlinson et al. 2014a). Furthermore, as obesity impairs myogenesis, particularly in older muscle (O'Leary et al. 2018), this may limit the ability of muscle to adapt to an elevated mass. However, variability in strength assessments, physical activity, age, contractile modality assessed, muscle or muscle groups tested and duration and magnitude of adiposity, may account for disparity in findings between studies (Tallis et al. 2018). Data from the present study are the first to indicate a consistent trend for changes in absolute maximal voluntary

torque between concentric and eccentric modes of activity and refute the idea that elevated eccentric loading in high adiposity individuals will provoke a stimulus to improve maximal eccentric torque.

Results from HGS also identified an age-related reduction in strength, with no significant effect of adiposity. The results from HGS reflect the IKD assessment of strength and therefore may be an effective measurement of absolute strength, particularly in the upper body. However, given the ambiguity of absolute function of lower limbs and other postural muscles, HGS should be used with caution when attempting to relate results to whole body strength.

3.5.2 – Adiposity and Age Effects on Normalised Peak Torque

Normalising absolute performance to body mass is an important indicator for how high adiposity and old age may affect an individual's ability to manoeuvre their own mass (Bollinger 2017). The decline in relative concentric torque of lower limb musculature in high adiposity and OAs are comparable to previous literature (Maffiuletti et al. 2007b; Capodaglio et al. 2009; Paolillo et al. 2012). However, the present study is the first to identify that relative eccentric function of lower limb musculature is impaired by adiposity. A decline in relative eccentric function is likely to have profound effects in individuals with high adiposity, as an increased eccentric demand is expected during many ADL (Tallis et al. 2018). The declines in relative performance will in part be because of muscles having to overcome greater inertia to move, carry and stabilise the elevated mass. Whilst the magnitude of adiposity effects does not differ across the age groups assessed, OHa individuals are likely to experience greater functional impairment given a significant age-related decline in muscle function. Furthermore, the present data generally infer that the reduction in relative concentric torque is uniform between both upper and lower body musculature. Adiposity effects on relative eccentric function of upper limb musculature appear age and muscle specific, with YNa producing significantly

greater torque than all other groups in the EF and adiposity and old age generally resulting in poorer relative eccentric performance of the EE.

Normalising absolute performance for segmental muscle mass gives an indication of muscle quality i.e. intrinsic force producing capacity of muscle. Adiposity induced changes in muscle quality have been debated (Tallis et al. 2018), however results from the present study add weight to the growing pool of evidence indicating that adiposity will reduce concentric muscle quality (Villareal et al. 2004; Morse et al. 2005; Delmonico et al. 2009; Paolillo et al. 2012; Erskine et al. 2017). Larger muscles of poorer quality will contribute to an already elevated mass thus increasing bodily inertia, have a greater metabolic cost to maintain and could promote a negative obesity cycle (Tallis et al. 2018).

The present study uniquely examined eccentric muscle quality. Previous literature in humans demonstrates limited ageing effects on eccentric muscle quality (Pasco et al. 2020), which is supported by our results. However, current evidence from animal models indicates that age-induced changes in eccentric muscle quality are likely muscle/fibre type specific (Hill et al. 2018). The present study indicates eccentric muscle quality was reduced in high adipose individuals in a manner that was not concurrent with changes in concentric muscle quality. This lack of consistency supports the idea of contractile mode and muscle specific effects of high adiposity (Ciapaite et al. 2015; Tallis et al. 2018). The present work is the first to indicate differences between concentric and eccentric muscle quality and force to body mass ratio, in high adipose individuals, which may be attributed to mechanistic differences between concentric and eccentric contractions.

3.5.3 – Adiposity and Age Effects on Fatigue Resistance

Similar to previous findings, the present study indicated improved capacity to maintain maximal voluntary concentric contractions in an ageing population (Chung et al. 2007; Russ et al. 2008), although no differences were observed for eccentric fatigue resistance between young and older individuals. The effects of ageing on fatigue remain ambiguous, as other work indicates a reduction in both concentric and eccentric fatigue resistance of the ankle dorsiflexor (Baudry et al. 2007). Disparity in findings may be due to muscle specific responses, as work utilising isolated mouse muscle indicates an age-induced decline in concentric and eccentric fatigue resistance of extensor digitorum longus, but no change in soleus (Hill et al. 2018). Whilst fatigability was improved or unchanged in the OAs, data are plotted from 100% of an individual's MVC reflecting the rate of fatigue. Given the age-related decline in contractile function, OAs will be required to produce force at a higher percentage of MVC during ADL's, and thus, likely fatigue more quickly.

Results from the present study demonstrate that high adiposity does not influence concentric or eccentric fatigue resistance. Effects of high adiposity remain unresolved - whilst there is evidence indicating that time to task failure or percentage torque loss remain unaffected irrespective of BMI (Maffiuletti et al. 2008; Minetto et al. 2012), other findings show greater percentage torque loss in obese individuals when compared to lean counterparts (Maffiuletti et al. 2007b). Despite this, the data in this chapter support the idea that faster fatigue seen during functional tasks is likely due to elevated body inertia (Tallis et al. 2017).

In line with previous research, these results indicate no differences in perceived soreness between high and low adipose groups following bouts of eccentric fatigue (Yoon and Kim 2020). Similarly, there was no difference in perceived soreness following concentric contractions between young and old groups, although soreness was significantly lower in OAs following eccentric contractions, albeit with

a small effect size. Such results provide promise with respect to the ability to sustain chronic eccentric interventions to improve muscle function.

3.5.4 – Future Directions and Limitations

The extent and magnitude of both obesity and ageing are likely to impact how mechanistic responses alter contractile performance. Therefore, future work should consider how duration of obesity, and magnitude and distribution of adiposity, impact contractile performance and if they exacerbate an age-related decline in muscle function.

The mechanisms underpinning obesity-induced changes in eccentric function are speculative and require further investigation. A particular focus on the function of the giant protein titin in response to ageing and elevated adiposity may be an important starting point, given its important role in the development of eccentric force (Enoka and Duchateau 2019). Furthermore, future work should focus specifically on defining the impact of intramuscular fat on contractile performance, given it is proposed to contribute to poor muscle quality (Rahemi et al. 2015). However, previous research on this topic typically considers only concentric muscle quality such that the impact of intramuscular fat on the mechanisms responsible for eccentric force production remain unknown.

Given that high adiposity adversely affects muscle performance in both young and older adults, across multiple modes of contractility, there is a need to consider therapeutic strategies to alleviate high adiposity induced declines in contractile performance. Targeted nutritional supplementation, such as vitamin D or resveratrol, is proposed to be an appealing alternative to current conventional non-surgical treatment strategies e.g. increased physical activity or calorific restriction, which are not well-maintained long term (Tallis et al, 2021). Despite nutritional strategies displaying numerous anti-obesogenic properties, to date, their efficacy on reducing the impact of obesity on skeletal muscle function has not been directly explored.

Despite using a previously established method for determining muscle quality (Maffiuletti et al. 2007b; Valenzuela et al. 2020), we recognise that these results may overestimate the age-related decline in muscle quality given that MF-BIA was developed and validated against DEXA (Yamada et al. 2017). Previous work suggests that despite having a strong and significant relationship between assessments, DEXA may overestimate muscle thigh volume of older males by ~6.1% when compared to an MRI assessment (Maden-Wilkinson et al. 2013). Although, given that differences in measurements between assessments in males is small, this is unlikely to affect the trends demonstrated in the current data.

A potential limitation of the study is that physical activity (PA) was not objectively measured. However, 80% of YNa, 64% of YHa, 100% of ONa and 50% of OHa self-reported that they met the minimum guidelines for PA of at least 150 minutes of moderate or 75 minutes of vigorous intensity activity per week. These findings would appear representative given that high adiposity is associated with lower levels of PA (Zhu et al. 2020). As such, it is possible that increased PA levels may negate some of the detrimental effects of high adiposity on contractile performance (Rolland et al. 2004)

3.5.5 – Conclusion

The present findings demonstrate that the age-related decline in maximal voluntary concentric and eccentric torque is not exacerbated by adiposity in men. However, torque normalised to body mass and muscle quality were reduced, albeit in a muscle and contractile mode specific manner in the case of the latter. Although the severity of these effects was uniform across both young and older age groups, the consequences for OAs with respect to the safe completion of ADLs is likely to be more substantial given that both torque relative to body mass and muscle quality are already compromised by increasing age. These data suggest a need to focus on novel and appealing therapeutic strategies to alleviate the detrimental effect of high adiposity on skeletal muscle quality and improve performance in functional tasks

3.5.6 – Thesis Progression

This experimental chapter provides a detailed account of the effects of high adiposity on skeletal muscle performance in human males. More specifically, this study is the first to identify that high adiposity adversely effects normalised eccentric muscle function in a manner which is not concurrent with changes in concentric function. One future direction from the present study, and highlighted as an area of priority in a recent review (Tallis et al., 2021), is to consider cost effective, sustainable strategies to offset the decline in muscle function associated with obesity. When examining therapeutic strategies, an animal isolated muscle approach offers some distinct advantages over human models, including control of confounding variables, such as duration of obesity, and allows for direct examination of muscle and fibre type specific responses to treatments. Furthermore, much of the detailed understanding surrounding the muscle, contractile mode and fibre type specific effects of obesity on contractile function have been developed using an isolated skeletal muscle model. However, isolated muscle models have previously been criticised as they may lack *in vivo* relevance. There has been an increase in research studies utilising the work loop model to assess skeletal muscle contractility as it addresses many of the criticisms of previously utilised isolated muscle models. However, there is opportunity to refine work loop protocols, essential for applying findings from isolated skeletal muscle mechanics to *in vivo* muscle performance. As such, the following experimental chapter (Chapter 4) aims to refine the work loop protocol, which will be used in later chapters, by examining the optimal stimulation frequencies needed for maximal force and power, and to evoke a fatigue response more replicable of *in vivo* muscle mechanics.

Chapter 4 - Effect of Stimulation Frequency on Force, Power, and Fatigue of Isolated Mouse Extensor Digitorum Longus Muscle

Modified from a publication in the Journal of Experimental Biology

Shelley, S., James, R.S., Eustace, S.J., Eyre, E., and Tallis, J. (2022) 'Effect of Stimulation Frequency on Force, Power, and Fatigue of Isolated Mouse Extensor Digitorum Longus Muscle'. Journal of Experimental Biology https://doi.org/10.1242/jeb.243285

4.1 – Abstract

The present study examined the effect of stimulation frequency (140, 200, 230 and 260Hz) on isometric force, work loop (WL) power, and the fatigue resistance of extensor digitorum longus (EDL) muscle (n=32), isolated from 8–10-week-old CD-1 female mice. Stimulation frequency had significant effects on the isometric properties of isolated mouse EDL, whereby increasing stimulation frequency evoked increased isometric force, quicker activation, and prolonged relaxation ($P < 0.047$), until 230Hz thereafter force and activation did not differ ($P > 0.137$). Increasing stimulation frequency increased maximal WL power output ($P < 0.001$; 140Hz, 71.3 ± 3.5; 200Hz, 105.4 ± 4.1; 230Hz, 115.5 ± 4.1; 260Hz, 121.1 ± 4.1 $W.kg^{-1}$), but resulted in significantly quicker rates of fatigue during consecutive WL's ($P < 0.004$). WL shapes indicate impaired muscle relaxation at the end of shortening and subsequent increased negative work was an important contributor to fatigue at 230Hz and 260Hz, but not at lower stimulation frequencies. Although cumulative work production did not differ between each stimulation frequency ($P > 0.054$). These findings demonstrate that stimulation frequency affects force, power, and fatigue in mouse EDL, but effects are not uniform between different methods of assessment of contractile performance. Therefore, future work examining contractile properties of isolated skeletal muscle should consider increasing stimulation frequency beyond that needed for maximal force when examining maximal power, but utilise a sub-maximal stimulation frequency for fatigue assessments to avoid a high degree of negative work atypical of *in vivo* function.

4.2 – Introduction

The assessment of isolated skeletal muscle function has been integral to the current understanding of skeletal muscle mechanics (Brooks and Faulkner 1991; James et al. 1995; Askew et al. 1997b; Hessel et al. 2021). Isolated muscle models have also been necessary for developing understanding of the ageing process (Brooks and Faulkner 1988; Brown and Hasser 1996; Tallis et al. 2014), metabolic diseases (Tallis et al. 2017; Eshima et al. 2017, 2020; Hurst et al. 2019), nutrition (James et al. 2004; Tallis et al. 2012), and species diversity and evolution (Altringham and Johnston 1986; Stoehr et al. 2020; Padilla et al. 2020). Except for measures of fatigue, the methodological approaches for the small number of techniques employed to assess the contractility of isolated skeletal muscle are typically standardised (James et al. 1995; Askew et al. 1997b; Park et al. 2012; Moorwood et al. 2013). The work loop (WL) technique is an increasingly common assessment to measure power due to its applicability to *in vivo* muscle mechanics (James et al. 1996; Nishikawa et al. 2018b); the stimulation frequency that evokes peak isometric force is typically utilised in work loop experiments (Ahn et al. 2003; James et al. 2004; Choi and Widrick 2009; Tallis et al. 2017; Padilla et al. 2020; Hessel et al. 2021). However, this may limit understanding of true physiological contractile performance as the stimulation frequency force relationship for isometric conditions cannot reliably predict force output during dynamic contractions (de Haan 1998; Caiozzo 2002). Therefore, further research is needed to establish the optimal stimulation frequency for peak force and power, and to evoke a fatigue response that is more representative of *in vivo* fatigue skeletal muscle mechanics, which will improve the quality of data obtained.

The benefits of *in vitro* assessments of isolated skeletal muscle function have previously been discussed in detail (Caiozzo, 2002; Josephson, 1993; Nishikawa et al., 2018; Syme, 2005; James et al. 1996; Tallis et al., 2021). In brief, *In vitro* methodological approaches remove the confounding influence of the neural system, and so allow for direct assessments of muscle performance (Askew et

al. 1997b; James et al. 2004). The most commonly employed contractile assessment is the measurement of isometric force (Fulton 1925; James et al. 1996; Medler 2002; Syme 2005; Nishikawa et al. 2018b), which provides information on absolute force and stress (force normalised to cross sectional area), and through measuring the rate of activation and relaxation, provide insight into muscle calcium kinetics (Ebashi and Endo 1968). Whilst isometric muscle activity has important *in vivo* applications, particularly for postural control (Loram et al. 2004), it fails to represent dynamic power producing muscle activity (i.e. work done ÷ time) that is essential for locomotion (Josephson 1985; James et al. 1996).

Traditionally, assessments of isolated muscle power have been derived from force velocity experiments, measured via isotonic or isovelocity assessments, which utilise a constant force or fixed shortening velocity, respectively (Josephson 1993; Caiozzo 2002). However, isotonic assessments overestimate power production, and have been shown to produce about twice the power output achieved via the WL method (James et al. 1996). The WL technique (Josephson 1985) assesses the ability of a muscle to produce work during cyclical length changes, as per *in vivo* power producing muscle (James et al. 1996). More specifically, power is derived from the net work of a WL length change cycle multiplied by the number of length change cycles per second (referred to as the cycle frequency) (James et al. 1996). As such, the WL technique considers not only the work produced during active muscle shortening but also work required to lengthen the muscle and the influence of activation and relaxation time (Josephson 1985; James et al. 1996). One limitation of current WL protocols is that standard practice is to apply the stimulation frequency used to elicit maximal isometric tetanus force to the assessment of power (Ahn et al. 2003; Choi and Widrick 2009; Hessel and Nishikawa 2017; Hill et al. 2018; Padilla et al. 2020). This approach may be limited, given that the stimulation frequency-force relationship for isometric activity may not be applicable to dynamic conditions (de Haan 1998; Caiozzo 2002). In slow twitch mouse soleus muscle, the stimulation frequency needed to evoke

maximal power exceeds that needed for maximal isometric force (Vassilakos et al. 2009). However, this relationship may not be directly applicable to fast twitch muscle, such as the extensor digitorum longus (EDL). The EDL has high type IIx-IIb fibre composition (Brooks and Faulkner 1991; Bobinac et al. 2000) and as such, the stimulation frequency required to achieve maximal isometric force is much greater than in soleus (Scott et al. 2001; Tallis et al. 2017; Hill et al. 2020). This is likely due to the need to evoke greater and sustained sarcoplasmic reticulum calcium release, and increased relaxation rates (Baylor and Hollingworth 2003). Thus, the optimal stimulation frequency to elicit maximal WL power in type II fibres remains unknown.

Isolated muscle models are also used to assess fatigue resistance, typically utilising repeated isometric or WL activations (Askew et al. 1997b; Syme and Tonks 2004; Russ and Lovering 2006; Vassilakos et al. 2009; Choi and Widrick 2009; Kissane et al. 2018; Padilla et al. 2020). The ability to sustain force or power during repetitive muscular contractions has important *in vivo* applications, particularly for effective locomotion and health (Karatzaferi and Chase 2013). Whilst the parameters used in fatigue protocols are more wide ranging than assessments of muscular force and power and are often not well justified or lack *in vivo* relevance, generally the stimulation parameters used to evoke peak force and power is used to elicit fatigue (Syme and Tonks 2004; Tallis et al. 2013; Kissane et al. 2018; Hill et al. 2018). However, in some instances, this approach can result in a high degree of activation during re-lengthening and a subsequent increase in negative work (Tallis et al. 2013; Kissane et al. 2018; Hill et al. 2018). This is atypical of *in vivo* fatigue mechanics, where fibre stimulation and length change waveforms can be manipulated from one length change to the next to optimise work and negate potentially damaging excessive negative work (Wakeling and Rozitis 2005). To counteract this, some studies have made arbitrary adjustments in stimulation frequency, burst duration and cycle frequency (James et al. 2011; Seebacher et al. 2014), where the impact of these manipulations have not been systematically considered. Given that stimulation frequency influences the magnitude of calcium

release into the muscle cytoplasm, a reduction in stimulation frequency may play an important role in negating excessive negative work and may evoke equal or greater cumulative work during the WL's assessment of fatigue, though this has yet to be systematically explored in fast twitch muscle.

Current evidence suggests that the standardised practice of utilising fixed stimulation parameters for all contractile assessments may underestimate true WL PO and produce a pattern of fatigue atypical of *in vivo* response. As such, the present chapter aimed to determine the effects of stimulation frequency on maximal isometric force, activation and relaxation kinetics, WL PO and fatigue resistance of whole isolated fast twitch mouse EDL muscle. Such work provides the first comprehensive insight into the effect of stimulation frequency on fast muscle mechanics, important for furthering the understanding of muscle physiology and for the future utility of isolated skeletal muscle assessments. It was hypothesised that the stimulation frequency needed to evoke maximal PO would exceed that needed for maximal isometric force and that higher stimulation frequencies during fatiguing contractions will result in prolonged relaxation and greater negative work, a faster reduction in peak power and reduced cumulative work.

4.3 – Materials and methods

The procedures outlined in this study and the use of animals was approved by the ethics committee of Coventry University (P108131). Female CD-1 mice (Charles River, Kent, UK) aged between 8 to 10 weeks (body mass 29.6 ± 0.7 g. N = 19) were used in the experimental procedures.

4.3.1 – Experimental setup

Animals were culled via cervical dislocation (in accordance with the British Home Office Animals Scientific Procedures Act 1986, Schedule 1). Whole extensor digitorum longus (EDL) was rapidly isolated from either one (N = 6) or both hindlimbs (N = 13) in refrigerated (1-3°C) oxygenated (95% O_2:5% CO_2) Krebs Henseleit solution (in mM: NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; $CaCl_2$ 2.54 in each case; pH 7.55 at room temperature). For each EDL, the tendon and proximal bone were left intact and an aluminium foil T-clip was wrapped around the distal tendon, as close to the muscle as possible to avoid slippage when the muscle was producing force (Ford et al., 1977; Goldman and Simmons, 1984; James et al., 1995; Tallis et al., 2012). Each EDL muscle (N = 32) was placed in a perspex flow through chamber filled with circulating oxygenated Krebs Henseleit solution. The temperature within the bath was continuously monitored using a digital thermometer (Traceable, Fisherbrand, Fisher Scientific, Loughborough, UK) and adjusted accordingly to maintain a physiologically relevant 37°C (± 0.2°C range) via an external heater/cooler (Grant LTD6G, Grant Instruments, Shepreth, UK). Using the bone at the proximal end, the muscle was attached to a crocodile clip connected to a force transducer (UF1, Pioden Controls Ltd, Henwood Ashford, UK) and the T-foil clip at the distal end was attached to a crocodile clip, connected to a motor arm (V201, Ling Dynamic Systems, Royston, UK). The muscle was electrically stimulated to produce force via parallel platinum electrodes submerged in the Krebs solution inside the muscle chamber. The electrical currents were provided by a tabletop power supply (PL320 Thurlby Instruments, Huntington, UK). Initially, muscle length and stimulation amplitude (typically 14-18V) were optimised to produce

maximal isometric twitch force, as determined via digital storage oscilloscope (2211 or 1002, Tektronix, Marlow, UK). The rig used for the experimental testing was custom built and the set up can be seen in schematic 1. The muscle optimal length for isometric twitch performance was measured, using an eyepiece graticule and microscope, and defined as L_0. Estimated fibre length for the EDL (8.2 ± 0.3mm) was calculated as 75% of L_0 (James et al. 1995). During isometric and WL protocols stimulation and length change parameters were controlled using custom-written software (Testpoint, CEC) via a D/A board (KPCI3108, Keithley Instruments) on a standard desktop personal computer or laptop.

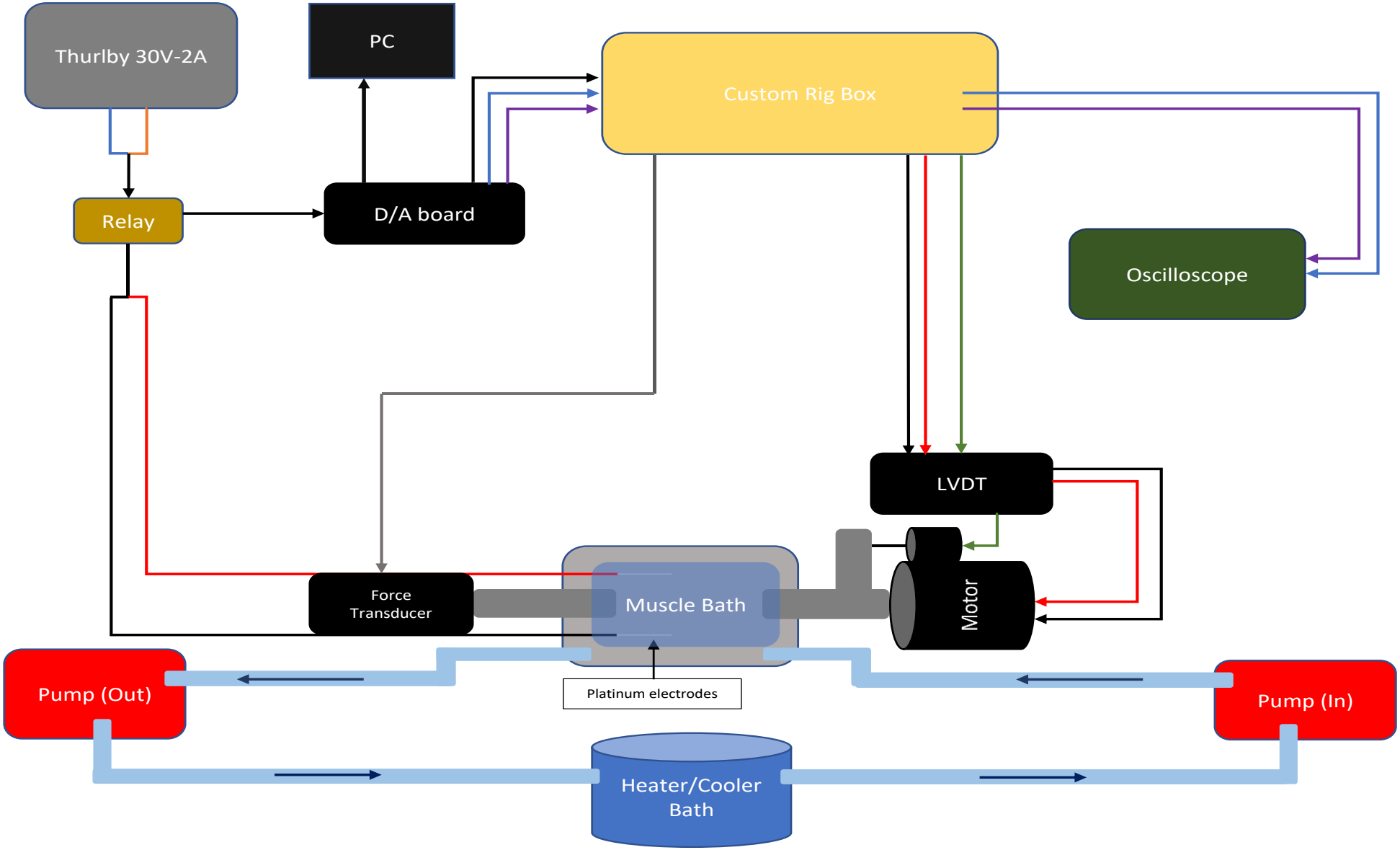

Figure 4. 1. *Experimental set up for contractile assessment of isolated skeletal muscle*

4.3.2 – Calibration of the force transducer

To obtain true values of force, the force transducer was calibrated at the start of every week prior to experiments. Calibration was performed by hanging known weights in a randomised order and plotting the magnitude of force obtained via the oscilloscope. Calculating the slope and the intercept of this data allowed for the calculation of the force transducer calibration (in $mN.V^{-1}$).

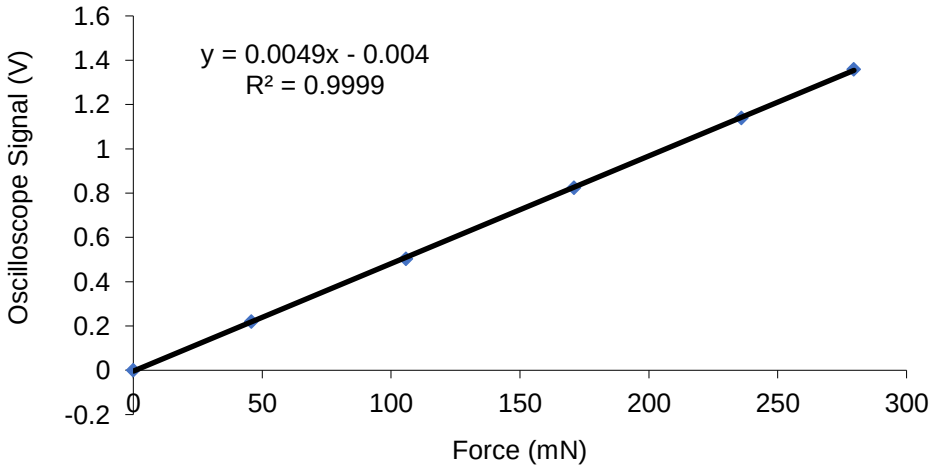

Figure 4. 2. *Example calibration of the force transducer; y axis = force production (dependant variable); x axis = voltage recorded on the oscilloscope (independent variable)*

4.3.3 – Calibration of length change from L_0

A small screw was placed into the crocodile clip which was anchored to the length change motor arm. A strain was inputted into the Testpoint software, and continual sinusoidal wavelength changes were performed. Using an eye piece graticule, fitted to a microscope, the units of length change from the initial starting position was recorded, using the screw fitted within the crocodile clip as the reference point (a). The magnitude of sinusoidal wavelength change was measured on the oscilloscope in Volts (b). The voltage recorded is equivalent to the physical length change from L_0. The physical length of the screw was recorded in millimetres (c) and measured in units under the eye piece graticule (d). The length change calibration equation is: [(c/d) * a]/b = length change (mm/V); a = units

moved on graticule during sinusoidal waveforms; b = delta voltage of sinusoidal wavelength changes; c = physical length of the screw measured; d = length of the screw derived from the graticule.

4.3.4 – Contractility measures

Isometric force

The procedures utilised to measure the contractile properties of isolated mouse EDL in this study are based on well-established protocols which have been utilised within the field for several decades, many of which have been adopted in work published from our laboratory. Using the established optimal parameters for isometric twitch performance, the muscle was then subjected to a train of electrical stimuli (250ms) across several stimulation frequencies (140, 200, 230 and 260Hz). Except for 200Hz, which was implemented first to be used as the control stimulation frequency in all muscles, isometric tetanus force was recorded across the range of stimulation frequencies in a randomised order. A recovery period of 5 minutes was implemented between each tetanus (Askew and Marsh, 1997; James et al. 1996). Once a tetanus had been performed at each experimental stimulation frequency, a control tetanus (200Hz) was performed to monitor change in performance over time. Time to half peak tetanus (THPT) and last stimulus to half tetanus relaxation time (LSHR) were measured at each stimulation frequency as indicative measures of activation and relaxation time, respectively (Ebashi and Endo 1968).

Work loop power output

Following measurements of isometric performance, PO was measured using the WL technique (Machin and Pringle 1959; Josephson 1985, 1993). The WL technique involved each muscle being subjected to four sinusoidal length changes around L_0 and stimulated to produce force during shortening. Length changes were driven via a motor (V201, Ling Dynamic Systems, Royston, UK), the

position of which was measured using a Linear Variable Displacement Transformer (DFG5.0, Solartron Metrology, Bognor Regis, UK). During the length change cycle, instantaneous force and velocity were sampled at 10kHz and plotted against each other, forming a WL, to give a visual representation of performance. Instantaneous PO was calculated for every data point in each work loop cycle by multiplying instantaneous velocity by instantaneous force. Instantaneous PO values were averaged to generate an average PO for each length change cycle (Van Wassenbergh et al. 2007; Vanhooydonck et al. 2014)

The previously determined L_0 and stimulation amplitude which evoked maximal isometric tetanic force were implemented for the WL assessment of PO, as has been utilised in previous research examining WL PO of the EDL (James et al. 1995). The following parameters were standardised and utilised for each muscle preparation, based on previous work indicating that these parameters elicit maximal WL PO of the EDL of young female mice of a similar or same strain (James et al. 1995; Tallis et al. 2014, 2017; Hill et al. 2018). The cycle frequency of length change was set at 10Hz. A strain of 0.10 of L_0 was implemented, resulting in the muscle lengthening by 5%, shortening by 10% before being re-lengthened by 5% back to L_0. Burst duration, 50ms, was used to elicit maximal net work during shortening. The phase, i.e. time stimulation with respect to reaching maximal length, was set at -2ms as activation is not instantaneous, thus ensuring the muscle is active upon shortening. After each set of four WL cycles, a 5-minute rest period was implemented to allow for sufficient recovery (Altringham and Young, 1991; James et al., 1995; Young and Rome, 2001). To examine the effect of stimulation frequency on WL PO, a range of stimulation frequencies (140, 200, 230 and 260Hz) were used. The order in which stimulation frequencies were performed was randomised, except for 200Hz, which was used as the control stimulation frequency in all trials to measure change in performance over time. Utilising a control stimulation frequency allows for the correction of WL power, which declines slowly over time due to the build of a small anoxic core (Barclay 2005). A linear decline in the contractile

function of isolated mouse skeletal muscle over time has been previously demonstrated (James et al. 1995; Tallis, 2013) and to account for this all WL PO's were corrected assuming a linear decline in performance as per previous work (Vassilakos et al. 2009, Hurst et al. 2019, Hill et al. 2020).

Fatigue resistance

The effect of stimulation frequency (140, 200, 230 and 260Hz) on fatigue resistance was measured by subjecting the EDL to 50 consecutive WL cycles, using the same parameters implemented for assessment of maximal WL PO (cycle frequency 10Hz; strain 0.10; burst duration 50ms; phase -2ms). Only one fatigue run was performed per muscle (n=8 for each stimulation frequency). The change in WL PO was plotted as percentage decline relative to maximum power produced during the fatigue run (Tallis et al. 2014; Hurst et al. 2019; Hill et al. 2020). Assessing the relative decline in WL PO over the time course of the fatiguing contractions provides information regarding the impact of stimulation frequency on the rate of fatigue. However, cumulative work, calculated as the sum of the net work performed in each cycle (Askew et al. 1997b) was also determined to infer absolute differences in the fatigue response, accounting for higher work initially expected at greater stimulation frequencies but associated with a faster rate of fatigue. WL shapes were plotted as force against strain ($\%L_0$) over the individual force and length data points for each work loop cycle. The components of a typical WL cycle of mouse EDL optimised for maximal work at 10Hz cycle frequency using a 260Hz stimulation frequency are provided in Figure 1.6 as an example of how a work loop cycle is plotted. Following the fatigue protocol, the ability of each muscle to recover concentric power production was measured every 10-minutes for 30-minutes, using a fixed stimulation frequency of 200Hz. The recovery of power was expressed as a percentage relative to the pre-fatigue control WL PO.

4.3.5 – Muscle size calculations

Upon completion of contractile assessments, the muscle was detached from the crocodile clips and removed from the muscle chamber. The tendons, T-foil clip and bone were removed leaving only muscle tissue, which was then blotted on absorbent paper to remove the excess Krebs solution and weighed to determine wet muscle mass (muscle mass = 8.9 ± 0.3 mg; TL-64, Denver Instrument Company, Arvada, CO). Mean muscle cross-sectional area was calculated from L_0, muscle mass, and an assumed density of 1060 kg m^{-3} (Méndez and Keys 1960). Maximal isometric stress (kN.m^{-2}) at each stimulation frequency was calculated by dividing peak tetanic force by mean muscle cross sectional area (CSA). Absolute PO (Watts) was calculated as net work (work output during shortening minus work input during lengthening) multiplied by cycle frequency. WL PO normalised to muscle mass (W.kg^{-1} muscle mass) was calculated by dividing absolute PO by muscle mass.

4.3.6 – *Statistical analysis*

Statistical analysis was performed using RStudio (R Foundation for Statistical Computing, Version 4.0.4, Boston, US). All data were normally distributed (checked via histogram using descriptive statistics) with acceptable limits of skewedness of ≤±2 observed (Gravetter and Wallnau 2014) and showed homogeneity of variance (checked via Levene's test), thus parametric analysis was performed. A repeated measures mixed effects model using the lmerTest package in RStudio assessed the effect of stimulation frequency on isometric stress, THPT, LSHR and WL PO normalised to muscle mass. A mixed effects model was utilised to assess the effect of stimulation frequency on rate of fatigue and cumulative work production after 50 consecutive loops. A mixed effects model, with stimulation frequency and time as factors, was used to assess effect of the stimulation frequency used during the fatigue protocol on the ability of the EDL to recover maximal WL PO. The random effect (allowed to vary using random intercepts and slopes) of specimen accounted for when both EDL (n=13) were isolated from an individual (Hurlbert 1984). Significant main effects and interactions observed were

explored using Bonferonni pairwise comparisons. Cohen's *d* was calculated to measure effect size and was then corrected for bias using Hedges' g according to the appropriate sample size (Hedges 1981). Cohen's *d* effect size was interpreted as trivial (<0.2), small (0.2-0.6), moderate (0.6-1.2) or large (>1.2) (Hopkins et al. 2009). Cohen's *d* effect size was interpreted as trivial (<0.2), small (0.2-0.6), moderate (0.6-1.2) or large (>1.2) (Hopkins et al. 2009). The level of significance was set at $P \leq 0.05$.

4.4 – Results

4.4.1 – Isometric tetanic stress

There was a significant effect of stimulation frequency on maximal isometric tetanic stress with an overall rise in stress as stimulation frequency increased (Fig 4.3A $P < 0.001$; 140Hz, 244.5 ± 9.3; 200Hz, 293.1 ± 8.5; 230Hz, 315 ± 10.9; 260Hz, 326.3 ± 8.8 $kN.m^{-2}$). Bonferroni multiple comparisons indicated significant differences in maximal isometric tetanic stress between most stimulation frequencies (140 vs. 200Hz: $P < 0.001$, $d = 0.96$; 140 vs. 230 & 260Hz: $P < 0.001$, $d > 1.23$; 200 vs. 230Hz: $P = 0.009$, $d = 0.4$; 200 vs. 260Hz: $P < 0.001$, $d = 0.68$) except between 230Hz and 260Hz ($P = 0.377$, $d = 0.19$).

There was a significant effect of stimulation frequency on time to half peak tetanus (THPT) with an overall decrease in time with increased stimulation frequency (Fig 4.3B $P < 0.001$; 140Hz, 18.4 ± 0.9; 200Hz, 15.2 ± 0.9; 230Hz, 13.0 ± 0.7; 260Hz, 12.5 ± 0.7 ms). Bonferroni multiple comparisons indicated significant differences in THPT (140 vs. 200Hz: $P < 0.001$, $d = 0.81$; 140 vs. 230 & 260Hz: $P < 0.001$, $d > 1.54$; 200 vs. 230 & 260Hz: $P < 0.002$, $d > 0.63$) between all stimulation frequencies except between 230 and 260Hz (THPT: $P > 0.999$, $d = 0.15$). Furthermore, we identified a significant effect of stimulation frequency on last stimulus to half relaxation time (Fig 4.3C $P < 0.001$; 140Hz, 7.6 ± 0.3; 200Hz, 8.8 ± 0.3; 230Hz, 12.1 ± 0.5; 260Hz, 13.4 ± 0.5 ms). Bonferroni multiple comparisons indicated significant differences in LSHR between all stimulation frequencies, with relaxation time increasing with stimulation frequency LSHR (140 vs. 200Hz: $P < 0.001$, $d = 1.01$; 140 vs. 230 & 260Hz: $P < 0.001$, $d > 2.39$; 200 vs. 230 & 260Hz: $P < 0.001$, $d > 1.79$; 230 vs. 260Hz: $P = 0.004$, $d = 0.59$).

4.4.2 – Work loop power output

There was a significant effect of stimulation frequency on WL PO normalised to muscle mass (Fig 4.3D $P < 0.001$; 140Hz, 71.3 ± 3.5; 200Hz, 105.4 ± 4.1; 230Hz, 115.5 ± 4.1; 260Hz, 121.1 ± 4.1 $W.kg^{-1}$). Bonferroni multiple comparisons indicated significant differences in WL PO between all stimulation frequencies, with higher stimulation frequency resulting in greater WL PO (140 vs. 200, 230 & 260Hz: $P < 0.001$, $d > 1.58$; 200 vs. 230Hz: $P < 0.001$, $d = 0.44$: 200 vs. 260Hz: $P < 0.001$, $d = 0.68$; 230 vs. 260Hz: $P < 0.001$, $d = 0.24$).

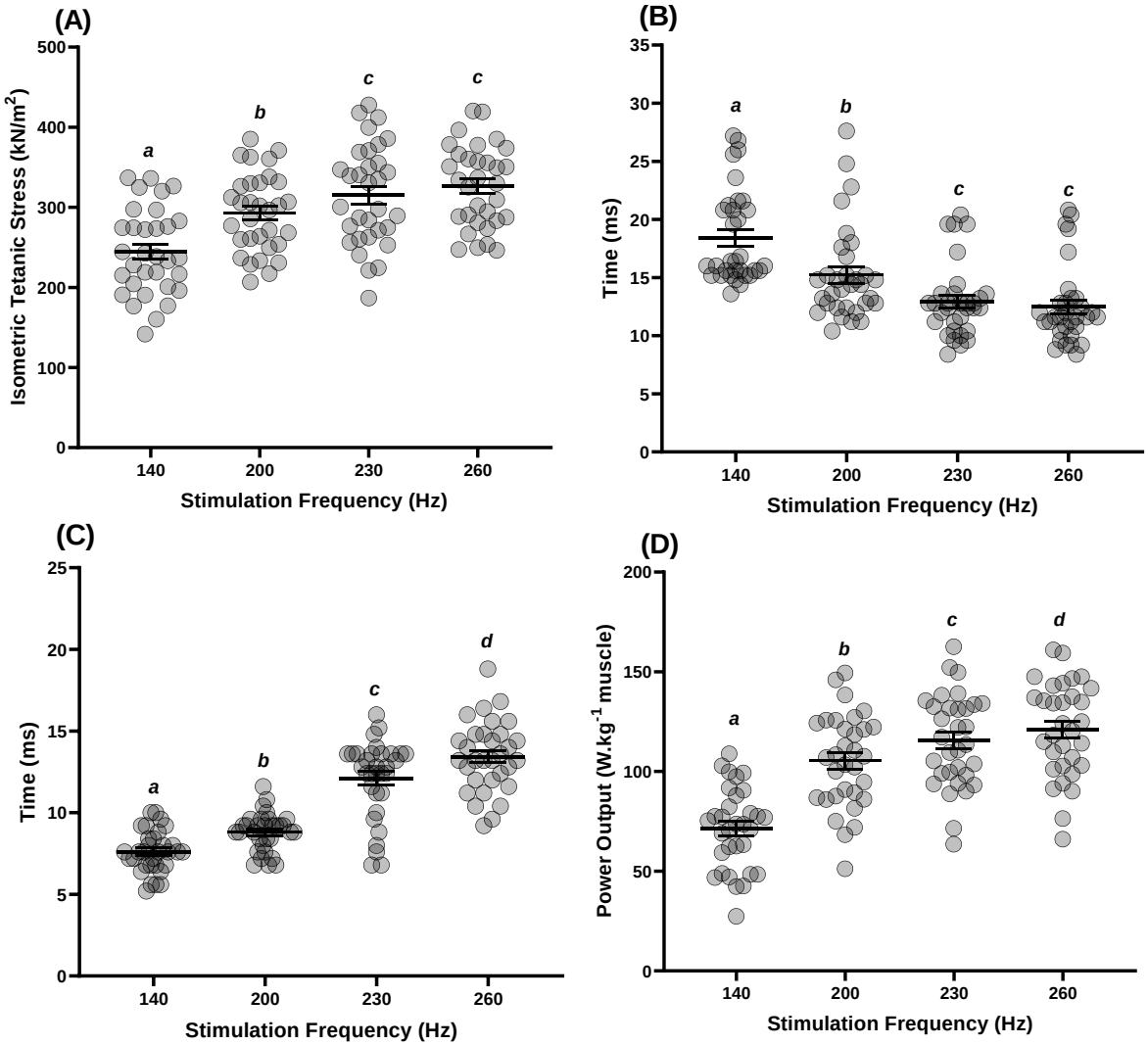

Figure 4. 3. *Effect of stimulation frequency on maximal isometric tetanic stress (A), time to half peak tetanus force (B), last stimulus to half tetanus relaxation time (C) and maximal work loop power output (D) of mouse EDL. Data presented as mean ± SEM, muscle force values normalised to muscle cross sectional area, muscle power normalised to muscle mass; N=32. Columns with different letters (a, b, c. d) are significantly different at P < 0.05.*

4.4.3 – The effects of stimulation frequency on fatigue resistance

There was a significant effect of stimulation frequency on time to fatigue to 50% of maximum power output (Fig 4.4A $P < 0.001$; 140Hz, 4.18 ± 0.20; 200Hz, 3.09 ± 0.09: 230Hz, 2.46 ± 0.03: 260Hz, 2.06 ± 0.04 s). Bonferroni multiple comparisons indicated significant differences in time to fatigue to 50% of maximum power output between all stimulation frequencies, with higher stimulation frequency resulting in quicker fatigue ($P < 0.004$, $d > 2.44$ in each case).

There was a significant effect of stimulation frequency on cumulative work produced after 50 consecutive work loops (Fig 4.4B $P = 0.042$; 140Hz, 234.6 ± 18.7; 200Hz, 244.8 ± 15.0; 230Hz, 206.6 ± 11.8: 260Hz, 182.8 ± 12.9 $J.kg^{-1}$). However, Bonferroni multiple comparisons indicated no significant differences between cumulative work produced after 50 loops between each stimulation frequency (140 vs. 200 & 230Hz $P > 0.999$, $d < 0.63$; 140 vs. 260Hz $P = 0.203$, $d = 1.14$; 200 vs. 230Hz $P = 0.569$, $d = 1.00$; 200 vs. 260Hz $P = 0.054$, $d = 1.57$; 230 vs. 260Hz $P = 0.704$, $d = 0.68$).

For recovery of maximal power output, assessed every 10 minutes for 30 minutes post 50 consecutive WL cycles, there was no significant effect of stimulation frequency (Fig 4.4C $P = 0.216$; 140Hz, 66.3 ± 4.9; 200Hz, 50.7 ± 3.9; 230Hz, 51.9 ± 6.8; 260Hz, 53.9 ± 6.7% of control power output pre fatigue after 30 minutes). There was a significant effect of time ($P < 0.001$), indicating significant recovery irrespective of stimulation frequency. There was a significant stimulation frequency*time interaction ($P = 0.013$). However, Bonferroni multiple comparisons indicate no significant differences in recovery between stimulation frequencies at each time point ($P > 0.140$).

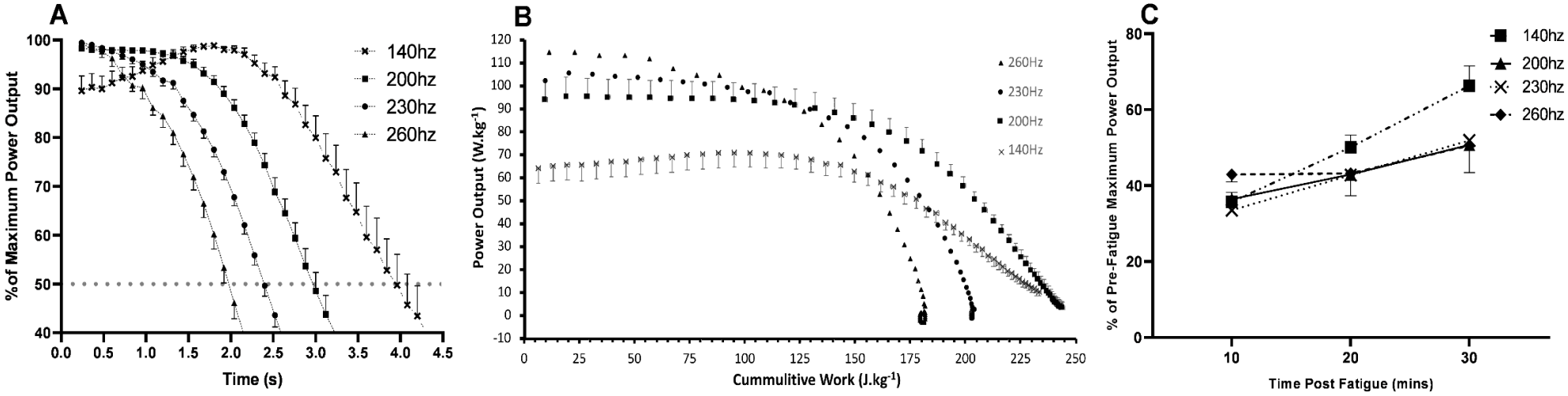

Figure 4. 4. *Effect of stimulation frequency on: net muscle power output, relative to maximum during 50 consecutive work-loop cycles at 10Hz cycle frequency (A); relationship between power output and cumulative work produced during 50 consecutive work-loop cycles (B); on recovery of maximal work loop power output of mouse EDL over 30 minutes post fatigue (C). Data are presented as mean ± SEM; N=8 at each stimulation frequency. Some error bars are omitted for clarity.*

The area within the work loop represents the net work done during the length change cycle. Typical work loop shapes, presented in Fig 4.5, demonstrate that initial net work is larger at 230 and 260Hz (Fig. 4.5C & D) when compared to 140 and 200Hz (Fig. 4.5A & B). However, from WL 10, there is greater force production during muscle re-lengthening for muscle stimulated at 230 and 260Hz distorting the work loop shape, and reducing the net work, to a much greater degree than seen at the lower stimulation frequencies. As a result, from WL 17 there is a marked reduction in the size of WL shape relative to the initial WL at the higher stimulation frequencies, whereas in contrast WL shape is comparatively well maintained at the lower stimulation frequencies.

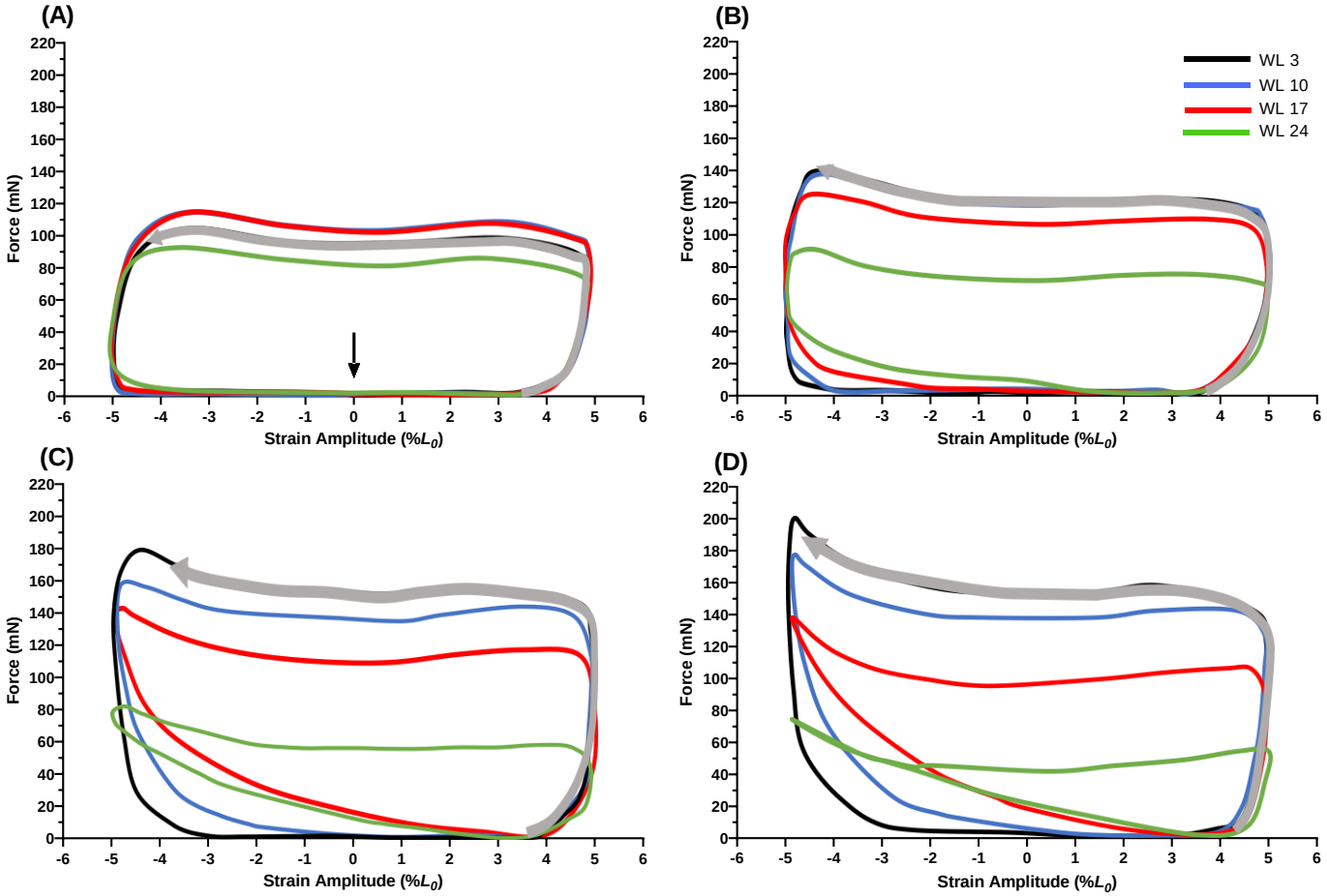

Figure 4. 5. *Effect of stimulation frequency (A, 140Hz; B, 200Hz; C, 230Hz; D, 260Hz) on typical work loop shapes during muscle fatigue at 10Hz cycle frequency for mouse EDL. Figures plotted as force against strain (%L_0). Work loops, 3, 10, 17 and 24 of the fatigue protocols are shown for each group. Work loops are performed in the anti-clockwise direction, with the work loop starting at L_0, indicated via arrow in A. The stimulation duration (grey overlay) is shown for work loop 3 and is the same for all stimulation frequencies.*

4.4 – Discussion

The novel examination of the effects of stimulation frequency on the contractile performance of fast twitch mouse EDL muscle found that stimulation frequency has large effects on force production, activation and relaxation rates, PO and pattern of fatigue, but these effects are contractile assessment specific. Furthermore, the current data indicate that a sub maximal stimulation frequency should be considered for the assessment of fatigue resistance in order to minimise the contribution of negative work to the decline in fatigue and more accurately reflect the fatigue response which occurs *in vivo.*

4.4.1 – Effect of stimulation frequency on Isometric contractile mechanics

Whole isolated EDL muscles produced a maximal isometric tetanic stress of 326 ± 9 $kN.m^{-2}$, which is comparable to values of 300 ± 23 and 332 ± 9 in mice of the same strain, age and gender, and contractile performance measured at the same experimental temperature of 37°c (Tallis et al. 2012; Hill et al. 2018). Comparisons for maximal EDL stress can also be made across the scientific literature, where EDL stress has shown to range from 200-320 $kN.m^{-2}$ (James et al. 1995; Askew and Marsh 1997; Hessel and Nishikawa 2017; Debruin et al. 2019; Hayes et al. 2019; Eshima et al. 2020), albeit direct comparisons between these studies should be made with caution given the variation in temperature, age, gender and strain of mice, which are likely to influence tetanic stress.

Higher stimulation frequency resulted in greater isometric stress and quicker activation rates, until a threshold (230Hz and above) where a further increase in stimulation frequency had little effect. This supports previous work which indicates that lower stimulation frequencies result in a reduction in tetanic stress of mouse EDL (Tallis et al. 2012) and data that show surplus stimulation beyond the optimal has little effect on the isometric force producing capacity of mammalian muscle (Buller and Lewis 1965; de Haan 1998; Vassilakos et al. 2009). These findings also support that isometric activation time decreases with stimulation frequency, in line with previous literature (Buller and Lewis 1965; de

Haan 1998; Vassilakos et al. 2009). However, force production and activation time appear to plateau at the same stimulation frequency, which differ to previous work in the soleus, where a stimulation frequency greater than that required to elicit peak force produced a shortened activation time (Vassilakos et al. 2009). The alignment of an optimised stimulation frequency for both peak force and activation time for EDL likely reflects a strategy to evoke effective rapid high force output, a requirement of fast twitch muscle (Radák 2018).

4.4.2 – Effect of stimulation frequency on work-loop power output

The present study, whilst in agreement to previous literature which indicates maximal WL PO is greater at higher stimulation frequencies (Vassilakos et al. 2009; Tallis et al. 2012, 2013), extends beyond what has previously been reported, as previous work which has considered stimulation effects on WL PO of mouse EDL did not explore stimulation frequencies beyond that used to elicit maximal isometric force. The present data identifies that whole isolated EDL muscles produced a maximal WL PO of 121 ± 4 $W.kg^{-1}$ at a 260Hz stimulation frequency, which is approximately 14-20% greater than values of 99 ± 5 $W.kg^{-1}$ and 105 ± 6 $W.kg^{-1}$ reported in previous work at the same temperature (Hill et al. 2018; Hurst et al. 2019). The greater PO per unit of tissue mass observed is likely a result of increasing stimulation frequency for WL PO, which is evident given that the WL PO values from muscle subjected to 200Hz stimulation frequency in this study (105 ± 4 $W.kg^{-1}$), are comparable to previous studies (99 ± 5 and 105 ± 6 $W.kg^{-1}$) which utilised a single fixed stimulation frequency of 200-220Hz using the same strain of mice (Hill et al. 2018; Hurst et al. 2019). Based on these findings it is plausible that previous research which has implemented the stimulation frequency for optimal isometric performance of mouse EDL may have underestimated true maximal WL PO. The greater stimulation frequency needed to elicit peak power is unlikely attributable to decreased activation time, given that there was no difference in THPT between 230 and 260Hz. Thus, the increased power likely reflects an increased ability to generate force during shortening, which maximises positive work. Whilst we are

the first to show that the stimulation frequency needed to elicit maximal WL PO of mouse EDL exceeds that needed for maximal isometric force, these findings support the suggestion that a stimulation frequency-force relationship for isometric conditions cannot reliably predict force output during dynamic contractions (James et al. 1996; de Haan 1998; Caiozzo 2002; Vassilakos et al. 2009). Based on these data we suggest future projects utilising the WL technique to achieve maximal PO in fast twitch muscle should consider utilising a stimulation frequency greater than that which evokes peak isometric force.

4.4.3 – Effect of stimulation frequency on muscle fatigue

The ability of the EDL muscle preparation to recover from fatigue and the trend of recovery was comparable to that of previous work (Hill et al. 2018, Tallis et al 2014), and did not differ between stimulation frequencies, indicating that the decline in the ability of the muscle to produce power during consecutive WL's is a result of peripheral fatigue and the contribution of any muscle damage to a decline in PO during fatigue was minimal (Askew et al. 1997b; James et al. 2004).

Similar to previous work using soleus muscle (Vassilakos et al. 2009), at the start of the fatigue protocol higher stimulation frequencies (230 and 260Hz) appeared to produce greater cumulative work than the lowest stimulation frequency (140Hz). This is unsurprising given that higher stimulation frequencies initially elicited greater WL PO. However, as the fatigue protocol progressed the lower stimulation frequencies elicited comparable cumulative work, attributed to the relative maintenance of power production throughout the fatigue protocol at those lower stimulation frequencies. By the end of 50 consecutive WL cycles, stimulation frequency did not influence cumulative work. Rate of fatigue was quicker as stimulation frequency was increased, which largely supports observations made on relatively slow twitch mouse soleus muscle (Vassilakos et al. 2009; Tallis et al. 2013). Therefore, despite the initially high WL PO observed at higher stimulation frequencies, the ability to sustain

power is significantly diminished, whereas power production is relatively well maintained at the lower stimulation frequencies.

The differences in time to fatigue and limited differences in cumulative work can in part be explained by examining the WL shapes. Whilst it may be intuitive to assume that muscle stimulated to evoke maximal power will fatigue more quickly, these data suggest that current protocols are limited in application given that the fatigue elicited is partly induced by the assessment itself and not entirely a physiological response. WL shapes indicate that as the fatigue protocol progresses, higher stimulation frequencies elicit greater force during re-lengthening, thus increase the negative work produced during the length change cycle, which reduces net work, and influences both cumulative work production and rate of fatigue. This increase in negative work during the progression of the fatigue protocol is less apparent at lower stimulation frequencies. The increased mechanical energy required to re-lengthen the muscle is likely a result of higher quantities of intracellular calcium limiting the ability of the muscle to remain predominantly passive during lengthening (Westerblad and Allen 1994). Furthermore, increased negative work may also be attributed to differences in the mechanical function of titin. Recent evidence indicates that calcium activated titin is increasingly stiffened thus requiring greater work for muscular elongation (Tahir et al. 2020; Hessel et al. 2021). Whilst the stimulation frequency used for assessment of maximal isometric force and WL PO is typically utilised for assessment of fatigue (Tallis et al. 2014, 2017; Hill et al. 2018, 2019, 2020; Hurst et al. 2019) and it is common to observe prolonged relaxation and increased negative work during fatigue protocols which employ a large number of WL cycles (Askew et al. 1997b; James et al. 2004; Tallis et al. 2014; Hill et al. 2018), current approaches may have limited *in vivo* application. The large negative work component seen in fatigue at higher stimulation frequencies is likely atypical of *in vivo* muscle mechanics, where stimulation could be altered to minimise the potential for eccentric activity, that would induce damage, and to maximise net work per cycle (Wakeling and Rozitis 2005). Whilst there

is complexity in replicating this precisely in an *in vitro* model, our data infer that a submaximal stimulation frequency likely better replicates the *in vivo* fatigue response to dynamic contractions and should be considered in the future application of the WL technique. Whilst some previous studies have reduced stimulation frequency used when determining fatigue responses (e.g. Seebacher et al. 2014), the rationale for choice of stimulation frequency is unclear. In the present study, 200Hz for the EDL appears near optimal for prolonged work output, producing a high amount of cumulative work, with the relative change in work done during shortening (as indicated by the WL shapes) being similar to that at 260 and 230Hz, but with minimal negative work.

4.4.4 – Limitations and future direction

One limitation of the present work is that WL PO continued to rise with stimulation frequency, therefore it may be anticipated that a further increase in stimulation frequency beyond the 260Hz used in the present study could elicit a further increase in PO. However, despite the difference in WL power between 230Hz and 260Hz reaching statistical significance, the effect size of differences observed in WL PO between 230 and 260Hz was small (d = 0.24), with a mean difference of ~4%. Thus, the effects of a further increase in stimulation frequency are likely to be minimal. Furthermore, stimulation frequencies greater than 260Hz should not be implemented for assessment of fatigue for the EDL, given that there is substantial negative work due to prolonged relaxation at 230Hz above, thus limiting the physiological relevance of higher stimulation frequencies.

One area of future direction is to consider the magnitude of cellular muscle damage evoked by stimulation frequency during repeated maximal contractions during fatigue. Whilst stimulation frequency did not affect the ability of the muscle to recover maximal PO, differences in muscle damage may still exist at the cellular level given that previous work indicates that the magnitude of force

production is related to magnitude of z-line disruption, identified via transmission electron microscopy imagery (Mackey et al. 2008).

4.4.5 – Conclusion

In summary, electrical stimulation frequency had significant effects on isometric properties, WL PO, and pattern of fatigue of isolated mouse EDL, although the optimal stimulation frequency for maximal performance and *in vivo* applicability is not entirely uniform across the different contractile assessments. During isometrics studies, higher stimulation frequency resulted in greater stress, quicker activation rates, and prolonged relaxation periods until a threshold where further increase in stimulation frequency had little effect, except for LSHR which continued to increase. One of the key findings from the present study is that WL PO continued to increase beyond the stimulation frequency which evoked maximal isometric force. Thus, the approach of implementing the stimulation frequency for optimal isometric force, for the assessment of power may underestimate true maximal WL PO. With respect to *in vivo* applicability of the WL technique's assessment of fatigue, our findings suggest the need for a submaximal stimulation frequency to ensure the rate and pattern of fatigue is primarily a physiological response, with minimal contribution induced by the technique itself. Based on the present data future work may wish to consider altering stimulation frequency for each contractile assessment of whole isolated EDL muscle to improve the quality of the data obtained.

4.4.6 – Thesis Progression

The findings from chapter 3 (study 1), indicating high adiposity adversely effects muscle performance across multiple modes of contractility and muscle groups, highlighting the need to focus on potential therapeutic strategies to reduce the impact of obesity on skeletal muscle contractility. However, conventional treatment strategies, such as calorific restriction and increased physical activity, are largely unsuccessful long term due to poor adherence. Some nutritional strategies, which

are relatively cost effective and require minimal lifestyle modification, mechanistically improve skeletal muscle health in obese populations, but their effect on contractility in high-fat treated muscle is rarely explored. Utilising the refined work loop protocols established in this experimental chapter (chapter 4; study 2), the main aims of experimental study 3 (chapter 5) is to examine the impact of high-fat diet on muscle contractility and, to establish if vitamin D could both improve muscle contractility in standard low-fat diet treated mice and reduce the magnitude of high-fat induced muscle contractile dysfunction.

Chapter 5 - The Effects of a High-Fat Diet and Vitamin D on Maximal Force, Power and Fatigue Resistance of Isolated Female CD-1 Mouse Soleus and EDL Muscles

5.1 – Abstract

Obesity induced changes in skeletal muscle function likely contribute to and exacerbate obesity associated ill health and as such should be an important target for intervention. Emerging evidence reports vitamin D supplementation could be a low cost, wide reaching and appealing method to mitigate some of the adverse effects of obesity on skeletal muscle contractile performance. The present study uses the work loop technique to examine the effect of vitamin D supplementation enrichment in standard and high-fat diets, on the contractile performance of isolated mouse soleus and EDL. Four-week-old female mice (CD1; N=40 starting sample; N=37 at final sample) consumed either standard low-fat diet (SLD) or high-fat diet (HFD), with (SLD VITD, HFD VITD) or without vitamin D enrichment (20,000 IU/kg^{-1}) for 12 weeks. At ~18-weeks of age soleus and EDL (N= 8-10 per group) were isolated and absolute and normalised (relative to muscle size and body mass) maximal isometric tetanic force and work loop power output (PO) were measured across a range of contractile velocities, and the rate of fatigue and cumulative work over 50 work loop contractions were determined. Body mass and gonadal fat pad mass was significantly greater in HFD treated mice ($P < 0.001$ in both cases). Soleus absolute and normalised isometric force and PO were mostly unaffected ($P > 0.471$), except for PO normalised to body mass, which was diminished in HFD treated groups ($P < 0.001$). For the EDL, isometric force was unaffected ($P > 0.588$), but HFD resulted in a significant reduction in isometric stress ($P = 0.048$) and absolute and normalised PO ($P < 0.031$). Cumulative work production during fatiguing contractions was significantly lower in both HFD treated soleus and EDL ($P < 0.043$). Rate of fatigue was unaffected ($P > 0.060$). The present work is the first to demonstrate that a high dose of vitamin D does not influence the contractile performance of isolated fast or slow twitch muscle in either SLD or HFD treated mice. Furthermore, these results add to the growing body of literature indicating that HFD consumption adversely effects muscle function, but the adverse effects are more substantial in fast twitch muscle. Despite these findings, future investigations are needed to determine the dose and duration specific responses to vitamin D supplementation, which may culminate in improved contractile performance in HFD treated muscle.

5.2 – Introduction

Obesity has quickly become a public health crisis, due to its links with poor physical (Chu et al. 2018) and mental health (Talen and Mann 2009), and increased risk of morbidities and mortality (Abdelaal et al. 2017; Chu et al. 2018; Silvestris et al. 2018). Skeletal muscle health represents an important link to the adverse effects of obesity, as adequate muscle function is necessary for physically activity and muscle is the largest regulator of metabolism in the body (Egan and Zierath 2013). More specifically, skeletal muscle is a primary regulator of lipid metabolism (Morales et al. 2017), and plays an essential role in mediating adipose accumulation. Adverse effects of obesity on contractile function of skeletal tissue (Maffiuletti et al. 2013; Tomlinson et al. 2016; Tallis et al. 2018, 2021; Morgan et al. 2020) are linked with a negative cycle of obesity, which may exacerbate these effects (Tallis et al. 2017).

The understanding of obesity effects on skeletal muscle function has been advanced by evidence examining the effect of high fat diet (HFD) consumption on rodent isolated skeletal muscle (Ciapaite et al. 2015, Bott et al. 2017, Eshima et al. 2017, 2020, Tallis et al. 2017, Hurst et al. 2019, Hill et al. 2019). Such methods have distinct advantages over whole body *in vivo* assessments of muscle function and performance, which have been outlined in recent reviews (Tallis et al. 2015, 2018, 2021). In brief, isolated skeletal models allow for examination of direct muscle and fibre type specific effects, which cannot be determined *in vivo*. Furthermore, the heterogeneity in diet composition and duration that have been shown to influence the impact of obesity on skeletal muscle health (Tallis et al. 2018) can be more tightly controlled in animal models. Importantly, the removal of muscle from the animal allows a more accurate determination of the direct impact of HFD on muscle quality (force normalised to cross-sectional area or power normalised to muscle mass) given that the size of the contractile tissue can be more accurately determined, when compared to *in vivo* assessments. Understanding muscle quality is important as it gives an indication of HFD effects on the intrinsic force producing

capacity of muscle; given that, irrespective of any changes in absolute performance, muscles of obese individuals will be required to manoeuvre and stabilise a larger mass, such that any reduction in ability to produce force or power relative to tissue size would be exacerbated *in vivo* (Tallis et al. 2018). Furthermore, reductions in muscle quality likely have further implications for obese individuals, resulting in not only an elevated cost of tissue failure, but where larger muscles are formed this would also contribute to an already elevated bodily inertia (Tallis et al. 2017). Similarly, direct examination of the effects of HFD on muscular fatigue is important for establishing if there is a reduction in the ability to sustain repetitive muscular contractions as opposed to an increase in muscle fatigability solely occurring in obese individuals due the increase in force requirement of moving a larger mass.

There are discrepancies across the literature as to the effects of HFD consumption on isolated rodent muscle contractility (Tallis et al. 2018), in particular, absolute force and power, where reductions (Eshima et al. 2017, 2020), increases (Tallis et al. 2017; Hill et al. 2019) or no change (Hurst et al. 2019), have all been reported. Ambiguity likely stems from the varied methodological approaches taken to assess HFD effects on skeletal muscle contractility (Tallis et al. 2018), such as varying experimental test temperature (Tallis et al. 2022), duration of HFD (Hurst et al. 2019), muscle fibre type (Tallis et al. 2017; Hill et al. 2019; Eshima et al. 2020) and mode of contractility assessed (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019); all of which have shown to influence the contractile response to HFD consumption. However, when force and power are normalised to body mass and muscle size, performance remains unchanged or reduced in a muscle and contractile mode specific manner (Tallis et al. 2018). These results infer a HFD can induce a reduction in the ability to manoeuvre and control a larger mass and in the intrinsic force-producing capacity of the muscle (Tallis et al. 2017; Hurst et al. 2019; Eshima et al. 2020). Similarly, the effects of HFD consumption on fatigue at the muscular level, whilst still ambiguous, have shown reductions in fatigue resistance in a muscle and contractile mode specific manner (Tallis et al. 2017; Hurst et al. 2019). Reduced fatigue resistance at an isolated muscle

level will likely have profound effects *in vivo* as *in vivo* muscles will be required to overcome a greater bodily inertia in an obese individual (Tallis et al. 2017). HFD induced reductions in contractile function have been attributed to excessive adiposity and intramuscular lipids (Messa et al. 2020) decreased AMPK activity (Tallis et al. 2017), impaired mitochondrial function (Heo et al. 2017), chronic inflammation (Erskine et al. 2017), impaired excitation contractile coupling (Eshima et al. 2020) and impaired myogenesis (D'Souza et al. 2015), where holistically this reduction in skeletal muscle health exacerbates lipid accumulation, resulting in a negative obesity cycle. As such, strategies to promote skeletal muscle health are important in the prevention and management of obesity and its associated comorbidities.

Calorific restriction and increased physical activity are well-established lifestyle interventions for obesity management (Ross 2000; Touati et al. 2011; Foster-Schubert et al. 2012; Julian et al. 2018). However, adherence to such strategies is poor, with the annual probability of achieving a normal weight status (BMI 18-25) being <1% and ~ 0.1% in obese and morbidly obese individuals (Fildes et al. 2015); in part due to current strategies requiring large lifestyle modification, which appears unsustainable for most. Therefore, there is a need to focus on potential strategies which are appealing alternatives for the promotion of skeletal muscle function in the management of obesity, a concept supported by a recent review (Tallis et al. 2021). Therefore, nutritional supplements, which are low cost and low demanding, could play a key part in tackling the obesity epidemic (Tallis et al. 2021). One such supplement is vitamin D, with evidence suggesting chronic supplementation evokes anti-obesogenic effects (Marcotorchino et al. 2014; Benetti et al. 2018) and independently can lead to improved skeletal muscle function across populations (Rejnmark 2011; Zhang et al. 2019).

Supplementation of Vitamin D (or in its biologically active form *1α,25(OH)2D3, 24R,25-dihydroxyvitamin D3 [24R,25(OH)2D3]*) has been suggested as a potential nutritional strategy to

reduce the impact of HFD consumption on skeletal muscle contractility (Tallis et al. 2021). Vitamin D deficiency (typically defined as serum 25-hydroxyvitamin D [25(OH)D] concentrations <50 nmol/L) (Liu et al. 2018), independent of obesity, is associated with declines in skeletal muscle contractility (Toffanello et al., 2012, Visser et al., 2003, Gerdhem et al., 2005). Furthermore, deficient levels of circulating serum 25(OH)D in the blood plasma appear to promote lipogenesis and suppress lipolysis through elevated parathyroid hormone (PTH) and intracellular Ca^{2+} (Golzarand et al. 2018). An increasing body of evidence supports the notion that obesity may be a contributing factor leading to vitamin D deficiency (VDD) (Duan et al. 2020). As such, VDD itself could be one of the primary mechanisms contributing to skeletal muscle dysfunction in obese individuals. Over recent years, chronic supplementation of vitamin D has been increasingly explored in HFD rodent models (Alkharfy et al. 2012; Marcotorchino et al. 2014; Fan et al. 2016; Benetti et al. 2018; Trovato et al. 2018; Montenegro et al. 2021). There is convincing evidence to suggest vitamin D supplementation can reduce and attenuate the increase in both body mass and adiposity associated with HFD consumption (Marcotorchino et al. 2014; Fan et al. 2016; Benetti et al. 2018) likely through the inhibition of adipogenesis and increase in UCP3 activation (Fan et al. 2016). Furthermore, research indicates vitamin D supplementation may ameliorate mechanisms reported to contribute to obesity induced declines in muscle function, such as modulating inflammation (Farhangi et al. 2017; Szymczak-Pajor and Śliwińska 2019) and promoting myogenesis (Cipriani et al. 2014), although the latter has not been directly examined in an obese animal model.

There is a dearth of literature regarding optimal dose and duration for chronic supplementation of vitamin D to evoke an increase in contractile performance of isolated skeletal muscle. In apparently healthy rodents, high dose vitamin D (20,000IU/kg^{-1} diet for 4 weeks) has previously been shown to increase the isometric stress of isolated mouse soleus, when compared to a control group of mice who are suspected to be vitamin D sufficient (consuming 1,000IU/kg^{-1} diet) (Debruin et al. 2019; Hayes et

al. 2019). Improvement in muscle contractility in non-disease models may be attributed to vitamin D modulating mitochondrial function, muscle insulin signalling, contractile protein synthesis, calcium and phosphate homeostasis, and inflammation (Girgis et al., 2013, Vanlint, 2013, Montenegro et al., 2019, Szymczak-Pajor and Śliwińska, 2019), mechanisms which contribute to high functioning skeletal muscle. To date, there are no research studies which have examined the potential of vitamin D for alleviating the decline in contractile function associated with HFD consumption. Given that vitamin D supplementation appears to reduce the impact of HFD consumption on elevated adipose accumulation and inflammation and improves contractility at the muscular level in healthy rodent models, there is strong mechanistic rationale to believe vitamin D could reduce the adverse effects of HFD consumption on isolated skeletal muscle function.

Using previously established protocols for examining the impact of HFD on contractile performance of skeletal muscle (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019) the present study uniquely examined the effects of 12 weeks HFD, with and without high dose vitamin D, on the maximal isometric force, work loop power output (PO), and fatigue resistance of soleus (predominately slow-twitch) and extensor digitorum longus (EDL; predominantly fast-twitch) muscle, isolated from young adult female CD-1 mice. It was hypothesised that a HFD will induce reductions in skeletal muscle contractility, but the reductions will be lower in mice consuming a high dose of vitamin D. The results from the present work will contribute to current knowledge of the potential anti-obesogenic effects of Vitamin D, and more specifically, the role it plays in alleviating the decline in contractile performance associated with HFD consumption.

5.3 – Materials and methods

5.3.1 – Animals

The procedures outlined in this study and the use of animals was approved by the ethics committee of Coventry University (P108131), the Animal Welfare and Ethical Review Body of the University of Warwick, and the British Home Office (PP4247175), and was completed in accordance with the Animals (scientific procedures) Act 1986. Female CD-1 mice, purchased at ~4 weeks old (Charles River, Kent, UK), were housed in groups of five at the University of Warwick and kept in 12:12 hour light:dark cycles at 50% relative humidity, at 22^{o}C. Mice were provided with *ad libitum* access to food and water. At 4 weeks of age, mice were randomly split into four experimental groups (total starting sample: n = 10 per group; final sample *n* = 10 SLD VITD; *n* = 9 SLD, HFD and HFD VITD; see Table 7) and following 13 days of habituation, which included gradual transition from standard lab chow (TestDiet 5755; calories provided by protein 18.3%, fat 22.1% and carbohydrate 59.6%; gross energy 4.07 kcal/g; Vitamin D 2200IU/g) to new respective custom diets, mice consumed one of the following for 12 weeks: 1) Standard low-fat diet (TestDiet 58Y2; calories provided by protein 18.0%, fat 10.2% and carbohydrate 71.8%; gross energy 3.76 kcal/g; Vitamin D 900IU/kg) (SLD), 2) High-fat diet (TestDiet 58V8; calories provided by protein 18.3%, fat 45.7% and carbohydrate 35.5%; gross energy 4.62 kcal/g; Vitamin D 1200IU/kg) (HFD), 3) SLD enriched with Vitamin D (20,000IU/kg feed) and 4) HFD enriched with Vitamin D, as shown in table 7. Procedures were carried out blinded.

Table 7. *Treatment Groups*

Group	Abbreviation	12-wks Treatment
Standard Low-fat Diet (control)	SLD	SLD
High Fat Diet	HFD	HFD
Standard Low-fat Diet + Vitamin D	SLD VITD	SLD + VD 20,000IU/kg feed
High Fat Diet + Vitamin D	HFD VITD	HFD VD 20,000IU/kg feed

SLD VITD n=10; SLD, HFD, HFD VITD n=9 HFD = High Fat Diet; SLD = Standard Low-fat Diet; VITD = Vitamin D

Female CD-1 mice were utilised instead of inbred strains, which have also previously been used for contractile assessments e.g. C57BL/6J mouse (Shortreed et al. 2009; Thomas et al. 2014; Ciapaite et al. 2015; Eshima et al. 2017, 2020; Bott et al. 2017), as the CD-1 strain are outbred enough to display a similar genetic heterogeneity to that which is displayed in humans (Rice and O'Brien 1980; Aldinger et al. 2009). Furthermore, using female CD-1 mice allowed for a direct comparison to previous work examining the impact of HFD consumption on the contractile properties of isolated skeletal muscle (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019; Messa et al. 2020). A total of 12 weeks feeding duration and or 45% HFD was selected based on previous work that demonstrated large changes in body composition and muscle mechanics over similar durations (Matsakas et al. 2015; Tallis et al. 2017; Eshima et al. 2017, 2020; Bott et al. 2017; Hurst et al. 2019). Furthermore, typical human western diets may consist of ~30-40% fat content, making a tolerable human HFD around 50-60%; fat content in a typical rodent diet is ~10%, thus 45% rodent HFD represents similar dietary distortion found in standard and high-fat human diets (Speakman 2019). As such, 45% HFD for rodents is argued to be more replicable of an *in vivo* HFD than a 60% HFD (Nair and Jacob 2016) which has previously been utilised (Shortreed et al. 2009; Thomas et al. 2014; Eshima et al. 2017, 2020).

There is a dearth of literature examining the optimal dose and duration of vitamin D needed to evoke improvements in isolated skeletal muscle contractility, or to reduce HFD effects on a variety of health markers and mechanisms which contribute to skeletal muscle dysfunction (e.g. increased fat mass). In HFD rodent models vitamin D is typically administered via oral gavage or intraperitoneal injection (Yin et al. 2012; Fan et al. 2016; Benetti et al. 2018). However, these methods can cause significant and unnecessary stress upon the animal (Brown et al. 2000; Bonnichsen et al. 2005), which can be avoided by dosing through dietary enrichment, which has been implemented in this instance. The dose of vitamin D used (20,000IU/kg^{-1} diet) was selected based on previous evidence indicating that similar high doses attenuate the increase in overall and segmental adipose tissue associated with HFD

consumption over a 10 week feeding duration (15,000IU/kg^{-1} diet) (Marcotorchino et al. 2014), and increase levels of serum 25(OH)D in blood plasma in HFD and SLD treated mice (25,000IU/kg^{-1} diet) (Park et al. 2020). Furthermore, an identical dose (20,000IU/kg^{-1} diet) has previously shown to elicit improvements in isolated skeletal muscle contractility in healthy mice, when compared to an apparently healthy, vitamin D sufficient control group (Debruin et al. 2019; Hayes et al. 2019). Previous work indicates that 20,000IU/kg^{-1} diet of vitamin D equates to a daily intake of approximately 2,700IU/kg^{-1} body mass in mice (Hayes et al. 2019). Following a previously determined calculation for converting dose in mice to dose in humans (daily animal dose in kg^{-1} body mass ÷ metabolic scaling factor for mice = human equivalent dose in kg^{-1} body mass, i.e. 2,700IU/kg^{-1} ÷ 12.3 = 219.5IU/kg^{-1}) (Nair and Jacob 2016), the human equivalent dose (HED) of vitamin D is ~13,000IU^{-1} day for a 60kg individual.

5.3.2 – Muscle preparations

Following the 12 weeks treatment period animals were culled via cervical dislocation (in accordance with the British Home Office Animals Scientific Procedures Act 1986, Schedule 1). Animals were then weighed, to determine body mass, and nasoanal length measured using digital callipers (Fisher Scientific™ 3417, Fisher Scientific, Loughborough, UK). From this information body mass index (BMI) and Lee index of obesity (Bernardis and Patterson 1968) were calculated for each mouse.

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

Equation 1 – Rodent Body Mass Index Calculation (Sjögren et al., 2001).

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

Equation 2 - Lee Index of Obesity calculation (Bernardis and Patterson, 1968).

As per previous work (Tallis et al. 2017), the subcutaneous fat pad around the top of the hindlimbs and genitals was extracted and weighed as an indication of overall adiposity (Rogers and Webb 1980) and was later normalised to body mass for a measure of relative adiposity (Hill et al. 2019). In addition, whole extensor digitorum longus (EDL), from the right hind limb, and soleus, from the left hindlimb, were dissected in refrigerated (1-3°C) oxygenated (95% O_2, 5% CO_2) Krebs Henseleit solution (in mM: NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; $CaCl_2$ 2.54 in each case; pH 7.55 at room temperature before oxygenation). The soleus and EDL represent locomotor muscles which differ in 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) (Agbulut et al. 2003), which allow for a greater understanding of any potential muscle and fibre type-specific effects of HFD and vitamin D. For each muscle preparation, the tendon attachment at the proximal end was left intact with a small piece of bone still attached and aluminium foil T-clips were wrapped around the distal tendon/s as close to the muscle as possible to avoid slippage when the muscle was producing force (James et al. 2005; Tallis et al. 2012).

5.3.3 – Contractility measures

Upon dissection the muscle was placed in a perspex flow through chamber filled with circulated constantly oxygenated (95% O_2, 5% CO_2) Krebs Henseleit solution. The reservoirs of Krebs solution were stored in external heater/cooler baths (Grant LTD6G, Grant Instruments, Shepreth, UK), which were adjusted to maintain a physiologically relevant temperature of 37 ± 0.2°C inside the muscle bath. Temperature in the bath was continuously monitored using a digital thermometer (Traceable, Fisherbrand, Fisher Scientific, Loughborough, UK). Using the bone at the proximal end, the muscle was attached to a crocodile clip connected to a force transducer (UF1, Pioden Controls Ltd, Henwood Ashford, UK) and the T-foil clip at the distal end was attached to a crocodile clip, connected to a motor arm (V201, Ling Dynamic Systems, Royston, UK).

Isometric force

For the assessment of isometric contractile properties, platinum electrodes running parallel to the muscle within the bath received an electrical stimulation from a table top power supply to activate the muscle (PL320, Thurlby Thandar Instruments, Huntington, UK). Stimulation parameters were controlled using custom-written software (Testpoint, CEC), via a D/A board (KPCI3108, Keithley Instruments), on a standard desktop personal computer. Muscle length and stimulation amplitude (typically 12-16V for SOL and 14-18V for EDL) were optimised to elicit a maximal isometric twitch response, the magnitude of which was determined via a digital storage oscilloscope (2211 or 1002, Tektronix, Marlow, UK). Using the optimised muscle length and stimulation amplitude, stimulation frequency was manipulated (100-130Hz [typically 120Hz] and 200-230Hz [typically 230Hz] for the SOL and EDL respectively) to elicit a maximal isometric tetanus, with a 5-minute rest implemented between each tetanus (Vassilakos et al. 2009; Tallis et al. 2017). In all cases, burst duration was fixed at 350ms for SOL and 250ms for EDL. After maximal force had been achieved a control tetanus was performed at the first stimulation frequency to monitor change in contractile performance over time. From the maximal tetanus response, time to half peak tetanus force (TTHP) and last stimulus to half relaxation (LSHR) were recorded as indicative measures of activation and relaxation kinetics, respectively (Tallis et al. 2017). The muscle length optimal for isometric performance, defined as L_0, was measured using an eyepiece graticule and microscope. Estimated fibre length for the SOL and EDL were calculated as 85% and 75% of L_0, respectively (James et al. 1995).

Work-loop power output

The work-loop technique (WL), which has been used in previous research examining the effects of HFD on mouse skeletal muscle function (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019),

was utilised to examine the contractile performance of isolated muscle during cyclical length changes, which better replicate *in vivo* dynamic muscle activity when compared to other *in-vitro* assessments (Josephson 1985, 1993; James et al. 1995, 1996). The WL technique involved each muscle being subjected to four sinusoidal length changes around the previously determined L_0. During the length change cycle, muscle was stimulated to produce force primarily during the shortening phase, using the previously determined stimulation amplitude. Stimulation frequency was increased from that which resulted in maximal isometric performance to 160 and 260Hz for the SOL and EDL respectively, based on findings from chapter 4 and from previous work indicating that a greater stimulation frequency is required for maximal WL PO (Vassilakos, James, and Cox 2009). Length changes were performed via movement of the motor arm (V201, Ling 220 Dynamic Systems, Royston, UK), the position of which was measured using a Linear Variable Displacement Transformer (DFG5.0, Solartron Metrology, Bognor Regis, UK). During the length change cycle, instantaneous force and velocity were sampled at 10kHz and plotted against each other, forming a WL. The instantaneous power (instantaneous velocity* instantaneous force) calculated at each time point during the work loop is averaged over the entire work loop, providing net power output. A range of cycle frequencies (CF; rate at which each muscle undergoes sinusoidal length change cycle) were utilised to examine whether the CF that elicited maximal PO was influenced by a HFD or VITD.

Initially, CF'S of 5Hz and 10Hz, burst durations of 65ms and 50ms, phase shift of -10ms and -2ms (time of initiation of stimulation prior to muscle reaching its greatest length), and a strain of 0.10 (muscle lengthening by 5%, shortening by 10% and elongating by 5% back to L_0.) were used to examine the SOL and EDL respectively, as previous work has established that these muscles typically achieve maximal WL PO using these parameters (James et al. 1995; Tallis et al. 2017). Burst duration, phase and strain were all subject to change based on WL shape to maximise net work, e.g. where WL shape sloped downwards during re-lengthening, as the muscle was still undergoing relaxation, burst

duration was reduced to achieve maximal WL PO. Every 5-minutes, a set of four WL's were performed, and maximal net work was recorded and later used to quantify power production. Once parameters for optimal PO at 5Hz and 10Hz CF for the soleus and EDL respectively were identified, these CF's were then used as the control WL's for the remainder of the experiment. Once maximal WL PO had been achieved at the control CF's, power output was then assessed across a range of CF's to establish a PO-CF curve for each experimental group (Hill et al. 2019, 2020; Hurst et al. 2019). Establishing a PO CF curve can help determine whether a shift in the optimal CF to elicit maximal PO in each experimental group has occurred, a potential indicator of a shift in fibre type composition. The range of CF's used, and typical parameters utilised to elicit maximal net work of each muscle at each CF, are displayed in Table 8, with the exception of phase, which was typically -10ms for the SOL and -2ms for the EDL, although at higher CF's phase would occasionally be reduced to ensure stimulation occurred at the onset of shortening, e.g. at 10Hz for the soleus -6ms phase was often implemented. On rare occasions strain would be increased beyond that provided in Table 8 (0.2 increments) at lower CF's when WL's appeared rectangular in shape, again to ensure maximal net work. Except for the control CF's, the order of CF's was randomised using Microsoft Excel, Windows v. 2016. After every three CF's, and after the final CF examined, a control set of WL's were performed using the initial parameters to examine the deterioration of power over time as the performance of isolated mouse muscle will deteriorate over time due to the build-up of an anoxic core (Barclay 2005). Utilising this approach allowed for the correction of net-work for other CF's relative to the control WL's, as has been common practice in previous research (Vassilakos et al. 2009; Hurst et al. 2019; Hill et al. 2020). Once the final control WL was performed, the muscle rested for 10 minutes prior to assessment of fatigue and recovery.

Table 8. *Typical stimulus burst durations and strains that elicited maximal net work, at each cycle frequency, during the WL assessment of Soleus and EDL.*

		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	0.1	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	0.1	0.09	0.08	0.07

Dash (-) represents CF's not used for assessment of contractile performance. Values provided were subject to change based on the shape of WL to ensure maximal WL PO.

Fatigue resistance, cumulative work, and recovery

For examination of fatigue resistance, each muscle preparation was subjected to 50 consecutive WL cycles, using the parameters that elicited maximal WL PO at 5Hz and 10Hz CF for the Soleus and EDL respectively. However, stimulation frequency was reduced to 100Hz and 200Hz for the Soleus and EDL respectively, as previous work (Vassilakos et al. 2009) and findings from chapter 4 demonstrate substantial negative work to re-lengthening the muscle at higher stimulation frequencies due to prolonged relaxation periods. However, comparable work production, with minimal negative work is observed during fatigue when utilising the submaximal stimulation frequencies selected. Large negative work is unlikely to occur *in vivo*, where muscle activation patterns would be altered to minimise the potential for eccentric activity induced damage and to maximise net work per cycle (Wakeling and Rozitis 2005). The net work of every loop was recorded and plotted relative to maximal PO recorded during the fatigue protocol. The time taken for power to fall below 50% maximal power-output was recorded, as has been used in previous studies examining fatigue resistance and dietary induced obesity using isolated mouse muscle (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019). Cumulative work, calculated as the sum of the net work performed in each cycle (Askew et al. 1997b; Vassilakos et al. 2009) was also determined to infer absolute differences in the fatigue response,

accounting for potential differences in absolute power output between experimental groups, which may promote a faster rate of fatigue. Furthermore, the combination of absolute work production and rate of fatigue likely plays a key part in exercise capacity and in the ability to complete activities of daily living which require repetitive contractions. WL shapes were plotted as force against strain ($\%L_0$) for the individual force and length data points for each work loop cycle.

The ability of each muscle preparation to recover from the fatigue protocol was monitored every 10-minutes for 30-minutes post fatigue. Every 10-minutes the muscle was subjected to one set of WL cycles using the same contractile parameters for the control WL's. Net work during recovery was expressed as a percentage of pre-fatigue maximal power output.

5.3.4 – Muscle mass and dimensions calculations

Upon completion of contractile assessments, which from the point of animal sacrifice to final contractile assessment lasted ~210 minutes, the muscle was detached from the crocodile clips and removed from the muscle chamber. The tendons, T-foil clip and bone were removed leaving only muscle tissue, which was then blotted on absorbent paper to remove the excess Krebs solution and weighed to determine wet muscle mass (TL-64, Denver Instrument Company, Arvada, CO). Mean muscle cross-sectional area was calculated from L_0, muscle mass, and an assumed density of 1060 $kg.m^{-3}$ (Méndez and Keys 1960). Isometric stress ($kN.m^{-2}$) was calculated by dividing peak tetanic force by mean muscle cross sectional area (CSA). WL PO normalised to body mass ($W.kg^{-1}$ body mass) was calculated by dividing absolute PO by body mass. WL PO normalised to muscle mass ($W.kg^{-1}$ muscle mass) was calculated as an indicative measure of muscle quality.

5.3.5 – *Statistical analysis*

Statistical analysis was performed using SPSS v.25 (SPSS Statistics for Windows, IBM Corp, Armonk, NY, USA) and GraphPad Prism v.9 (GraphPad Software, California, US). All data are presented as mean ± standard error of mean (SEM). Following appropriate checks of normality (Shapiro-Wilk) and homogeneity of variance (Levenes test), parametric analysis was performed. Mixed model Analysis of Variance (ANOVA), with HFD, treatment (between subjects) and weeks (within subjects) used as the factors, was utilised to determine the effect of HFD and vitamin D consumption on weekly body mass. Two-factor (ANOVA) were conducted with HFD (SLD vs. HFD) and treatment (Control vs. VITD) as the fixed factors to examine if differences existed in measures of animal and muscle morphology (body mass, muscle mass, muscle length and muscle CSA), isometric properties (absolute tetanic force, tetanic stress, TTHP and LSHR), time to reach 50% of maximal PO during fatigue protocol and cumulative work produced after 50 consecutive WL's. Mixed model repeated measures ANOVA, with HFD, treatment (between subjects) and CF (within subjects) used as the factors, was utilised to determine any changes in SOL and EDL absolute PO and PO normalised to body mass and muscle mass. Mixed model repeated measures ANOVA, with HFD, treatment (between subjects) and stimulation frequency (within subjects) used as the factors, was utilised to determine if the reduction in stimulation frequency used for assessment of fatigue resulted in any changes in SOL and EDL PO normalised to muscle mass and if so, were the changes in PO uniform across diet and treatment groups. Mixed model repeated measures ANOVA, with HFD, treatment (between subjects) and time (within subjects) as the factors, was utilised to examine the recovery of WL PO following the fatigue protocol. Significant interactions observed for ANOVA were explored using Bonferroni post hoc for multiple comparisons. Partial eta squared (ηp^2) was calculated to estimate effect sizes for all significant main effects. Thresholds for Partial eta squared effect size were classified as small (<0.05), moderate (0.06-0.137) or large (>0.138) (Cohen 1988). Where possible, Cohen's *d* was calculated to measure effect size of interactions observed and was then corrected for bias using Hedge's g due to differences in sample size between groups (Hedges 1981). Cohen's *d* effect size was interpreted as

trivial (<0.2), small (0.2-0.6), moderate (0.6-1.2) or large (>1.2) (Hopkins et al. 2009). The level of significance was set at $P \leq 0.05$.

5.4 – Results

5.4.1 – Morphology

There was a significant TIME*HFD interaction observed for weekly body mass (Fig 5.1. $P < 0.001$, $\eta p^2 = 0.412$). Bonferroni post hoc analysis multiple comparisons reveal HFD treated animals had significantly greater body mass from week 1 onwards ($P < 0.048$). No effect of vitamin D was observed ($P > 0.999$)

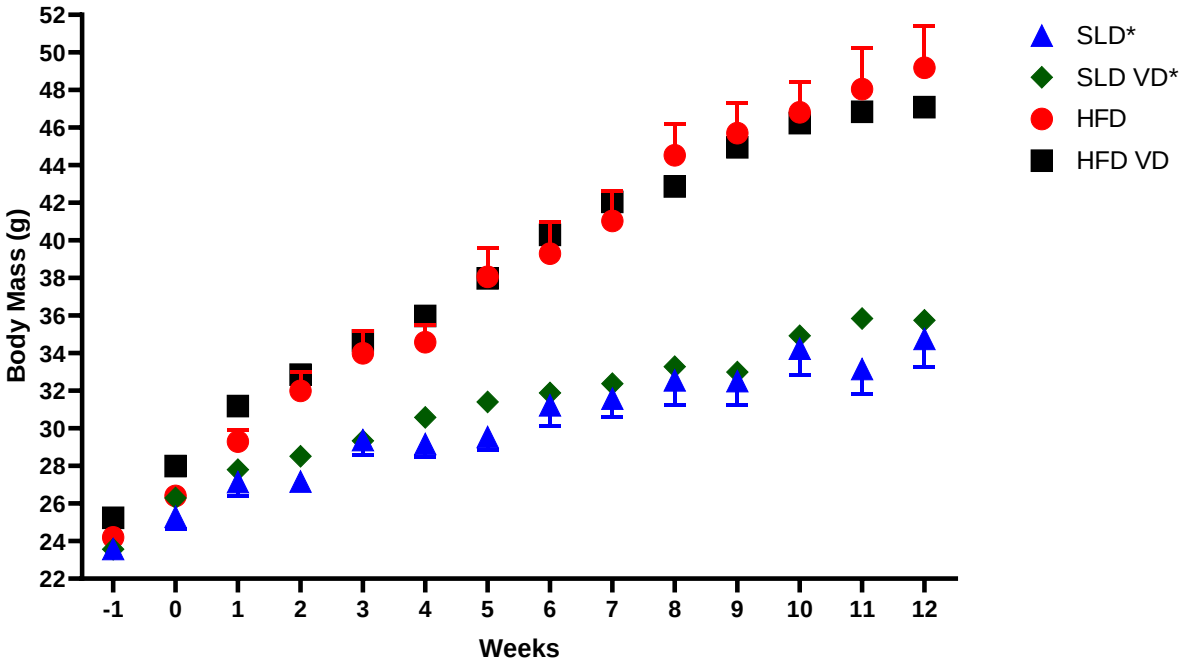

Figure 5. 1. *Effect of high-fat diet and Vitamin D on body mass over 12 weeks. Data presented as mean ± SEM; SLD, HFD, HFD VITD n=9; SLD VITD n=10; * indicates significant difference between HFD treated and SLD treated at P < 0.05; week -1 and 0 reflect habituation period where animals were gradually transitioned to their respective custom diet; some error bars are omitted for clarity.*

Final whole animal body mass, body length, BMI, Lee Index of Obesity, fat mass and Adiposity:Body mass were significantly greater in HFD groups compared to SLD groups (Table 9. $P < 0.005$, $\eta p^2 > 0.213$). No significant differences were observed for EDL or soleus muscle mass, fibre length or CSA ($P > 0.053$, $\eta p^2 < 0.112$). See Table 9 for a detailed report of the results of statistical tests for whole body and muscle morphology.

5.4.2 – Isometric Performance

There were no significant effects of HFD or vitamin D on soleus isometric properties (Table 10. $P > 0.087$, $\eta p^2 < 0.092$) or EDL THPT and tetanus force (Table 10. $P > 0.385$, $\eta p^2 < 0.024$). However, HFD groups had EDL with significantly lower tetanus stress and slower LSHR (Table 10. $P < 0.048$, $\eta p^2 > 0.117$) when compared to SLD groups. For detailed report of statistical tests for isometric properties please see Table 10.

Table 9. *Whole body and muscle specific animal morphology*

	SLD	SLD-VITD	HFD	HFD-VITD	HFD Effect		VITD Effect		Interaction	
	n = 9	*n* = 10	*n* = 9	*n* = 9	*P* value	ηp²	*P* value	ηp²	*P* value	ηp²
Whole Body										
Body Mass (g)	35.0 ± 1.5	34.4 ± 2.1	48.8 ± 1.4	48.4 ± 2.5	**< 0.001**	**0.566**	0.814	0.002	0.991	< 0.001
Body Length (mm)	101 ± 2	102 ± 2	106 ± 1	108 ± 2	**0.005**	**0.213**	0.313	0.031	0.964	< 0.001
Fat Mass (g)	1.1 ± 0.2	0.9 ± 0.3	4.6 ± 0.6	3.5 ± 0.4	**< 0.001**	**0.627**	0.152	0.061	0.328	0.029
Lee Index of Obesity	325 ± 3	317 ± 4	346 ± 4	338 ± 4	**< 0.001**	**0.458**	0.061	0.102	0.985	< 0.001
BMI ($g.cm^{-2}$)	0.34 ± 0.01	0.33 ± 0.01	0.44 ± 0.01	0.42 ± 0.01	**< 0.001**	**0.599**	0.149	0.062	0.889	< 0.001
Adiposity:Body Mass	2.8 ± 0.4	2.2 ± 0.6	9.3 ± 1.0	7.1 ± 0.6	**< 0.001**	**0.627**	0.063	0.101	0.313	0.031
EDL										
Muscle Mass (mg)	10.7 ± 0.4	10.8 ± 0.3	11.3 ± 0.2	11.4 ± 0.3	0.053	0.112	0.65	0.007	0.993	< 0.001
Fibre Length (mm)	9.2 ± 0.1	9.1 ± 0.1	8.9 ± 0.2	9.2 ± 0.2	0.496	0.015	0.306	0.033	0.22	0.047
CSA (m^2)	1.10 ± 0.04	1.11 ± 0.04	1.21 ± 0.03	1.18 ± 0.05	0.064	0.103	0.953	< 0.001	0.608	0.008
SOL										
Muscle Mass (mg)	8.7 ± 0.5	7.9 ± 0.3	8.4 ± 0.2	8.8 ± 0.3	0.422	0.021	0.457	0.012	0.13	0.072
Fibre Length (mm)	9.4 ± 0.2	9.5 ± 0.2	9.5 ± 0.2	9.4 ± 0.1	0.940	< 0.001	0.696	0.005	0.528	0.013
CSA (m^2)	0.88 ± 0.05	0.78 ± 0.03	0.84 ± 0.02	0.88 ± 0.03	0.407	0.022	0.422	0.021	0.066	0.105

Values are presented as means ± SEM; bold values indicate a significant difference at $P < 0.05$; CSA: Cross-sectional area

Table 10. *The effect of 12-week high-fat diet and vitamin D on the isometric properties of isolated mouse EDL and soleus*

	SLD	SLD-VITD	HFD	HFD-VITD	HFD Effect		VITD Effect		Interaction	
	n = 8	*n* = 10	*n* = 9 / 8	*n* = 9	*P* value	ηp²	*P* value	ηp²	*P* value	ηp²
EDL										
Tetanus Force (mN)	364 ± 28	358 ± 16	340 ± 19	354 ± 30	0.588	0.009	0.869	0.001	0.699	0.005
Tetanus Stress (kN.m^{-2})	333 ± 23	320 ± 11	283 ± 17	297 ± 16	**0.048**	**0.117**	0.975	< 0.001	0.470	0.016
THPT (ms)	11.4 ± 0.7	10.8 ± 0.7	10.4 ± 0.6	10.5 ± 0.6	0.385	0.024	0.748	0.003	0.614	0.008
LSHR (ms)	13.2 ± 0.4	13.4 ± 0.5	15 ± 0.4	14.5 ± 0.7	**0.013**	**0.178**	0.751	0.003	0.505	0.014
Soleus										
Tetanus Force (mN)	236 ± 17	208 ± 10	203 ± 12	220 ± 7	0.436	0.02	0.658	0.006	0.084	0.093
Tetanus Stress (kN.m^{-2})	278 ± 17	266 ± 9	243 ± 14	252 ± 11	0.087	0.092	0.927	< 0.001	0.428	0.019
THPT (ms)	34.5 ± 2.4	35.7 ± 1.6	36.4 ± 1.8	33.4 ± 1.3	0.935	< 0.001	0.654	0.007	0.281	0.037
LSHR (ms)	40.1 ± 2.4	47.6 ± 2.6	45.9 ± 2.2	47.8 ± 2.6	0.292	0.036	0.100	0.085	0.321	0.032

Values are presented as means ± SEM; bold values indicate a significant difference at $P < 0.05$; THPT, Time to half peak tetanus; LSHR, Last stimulus half relaxation time

5.4.3 – Maximal work loop power output

For EDL, absolute PO and PO normalised to muscle mass, there was a significant main effect of cycle frequency (CF) ($P < 0.001$, $\eta p^2 > 0.696$) indicating peak PO occurred between 8-10Hz cycle frequency (Figure 5.2 $P > 0.999$) with PO from remaining CF's significantly lower ($P < 0.002$) except for PO at 12Hz ($P > 0.264$), but PO produced at 12Hz did not differ to that produced at 6 and 14Hz ($P > 0.242$).

EDL absolute PO and PO normalised to muscle mass and body mass was significantly lower in the HFD groups when compared to SLD groups (Figure 5.2A, B & C $P < 0.031$, $\eta p^2 > 0.137$). No main effect of VITD ($P > 0.493$, $\eta p^2 < 0.015$) or HFD*VITD interaction were observed ($P > 0.070$, $\eta p^2 < 0.099$). There were no CF*HFD ($P > 0.492$, $\eta p^2 < 0.025$), CF*VITD ($P > 0.409$, $\eta p^2 < 0.030$) or CF*HFD*VITD ($P > 0.303$, $\eta p^2 < 0.037$) interactions observed.

For PO normalised to body mass there was a significant CF*HFD interaction ($P = 0.018$, $\eta p^2 = 0.092$). Bonferroni multiple comparisons revealed that in SLD groups PO produced at CF 8, 10 and 12Hz did not differ ($P > 0.999$), but PO from 10Hz was greater than 4, 14 and 16Hz ($P < 0.015$), whereas PO at 8 and 12Hz was only greater than 4 and 16Hz ($P < 0.001$). In HFD treated groups, PO produced at 8, 10, 12 and 14Hz did significantly differ ($P > 0.073$), but PO produced at 12 and 14Hz was only greater than 4Hz ($P < 0.017$), whereas PO at 8 and 10Hz was greater than that produced at 4 and 16Hz ($P < 0.002$).

For soleus, absolute PO and PO normalised to muscle mass, there was a significant main effect of CF (Figure 5.2 $P < 0.001$, $\eta p^2 > 0.913$), indicating maximal PO occurred between 3-4Hz cycle frequency ($P > 0.184$) with PO from remaining CF's being significantly lower ($P < 0.002$) except between 2 and 4Hz ($P > 0.498$). No CF*HFD ($P < 0.372$, $\eta p^2 < 0.032$), CF*VITD ($P > 0.636$, $\eta p^2 < 0.016$) or CF*HFD*VITD ($P > 0.510$, $\eta p^2 < 0.023$) interactions were observed. For PO normalised to body mass there was a significant CF*HFD interaction ($P = 0.012$, $\eta p^2 = 0.128$). However, Bonferroni multiple comparisons indicated no significant differences in the PO CF curve between groups and indicated that maximal absolute power

occurred between 3-4Hz cycle frequency (P > 0.999) with PO from the remaining CF's being significantly lower (P < 0.002), except between 2 and 4Hz (P > 0.764).

For soleus, PO normalised to body mass, HFD treated groups produced significantly less power than SLD groups (P = 0.001, ηp^2 = 0.312). For absolute PO and PO normalised to muscle mass no main effects of HFD (P > 0.471, ηp^2 < 0.017) or VITD (P > 0.722, ηp^2 < 0.004) were observed. No HFD*VITD interaction observed (P > 0.250, ηp^2 < 0.042) for absolute or normalised PO.

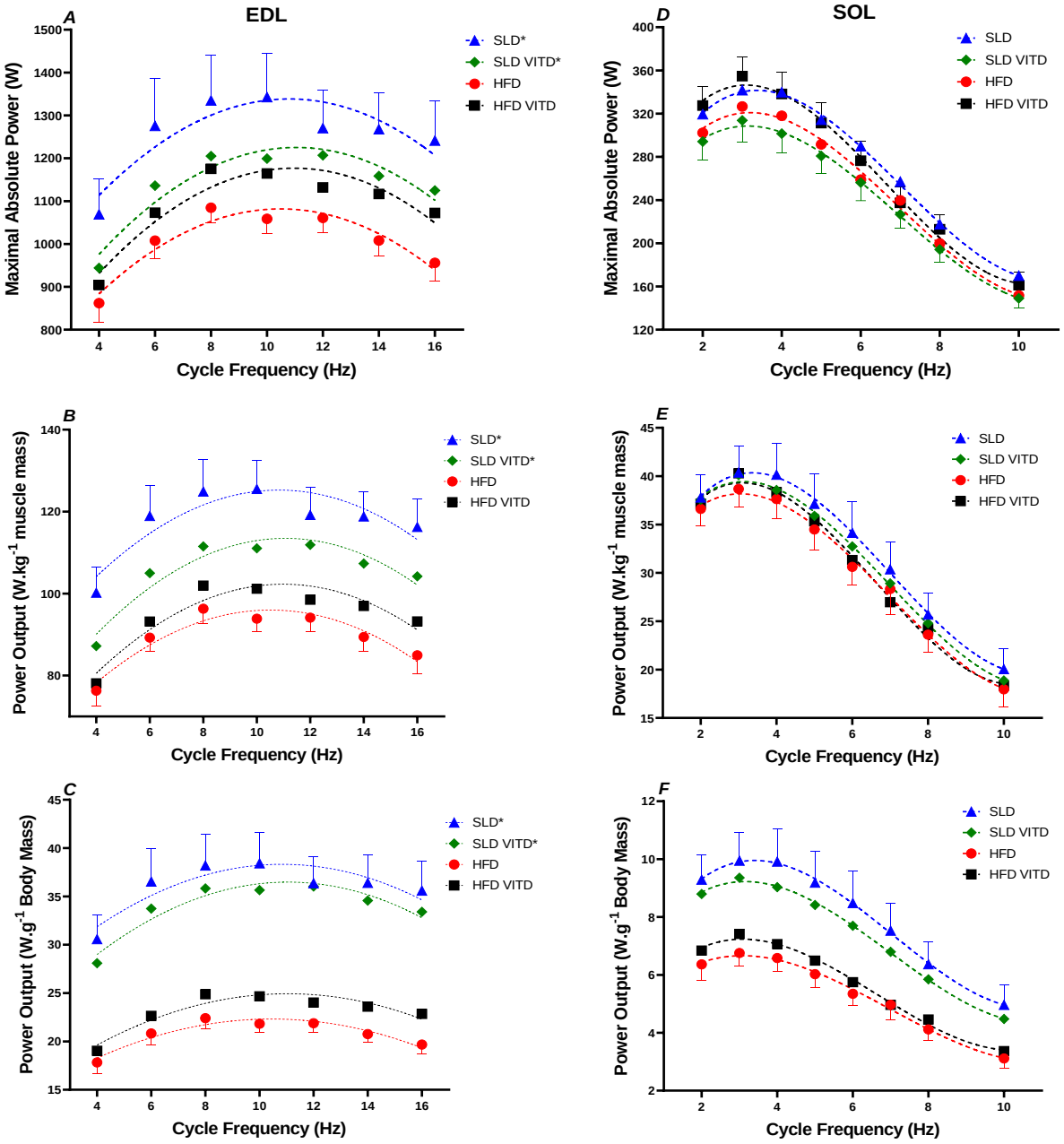

Figure 5. 2. *The effect of 12 weeks high-fat diet and Vitamin D on the power output-cycle frequency relationship for absolute power output (Watts), power output normalised to muscle mass (Watts per kg of muscle mass) and power output normalised to body mass (Watts per gram of body mass) of isolated mouse EDL (A, B and C respectively) and soleus (D, E and F respectively). For EDL n = 10 for SLD VITD, n = 9 for HFD and HFD VITD, n = 8 for SLD; for soleus n = 10 for SLD VITD, n = 9 for HFD VITD and n = 8 for SLD and HFD. Data presented as mean ± SEM; * indicates significant difference at P < 0.05; some error bars omitted for clarity.*

5.4.4 – Fatigue and Recovery

The reduction in stimulation frequency for assessment of fatigue resulted in a significant reduction in maximal WL PO normalised to muscle mass of the soleus and EDL (Figure 5.3 $P < 0.001$, $\eta p^2 > 0.725$), but the magnitude of reduction in PO was not influenced by diet or treatment ($P > 0.199$, $\eta p^2 < 0.053$).

For time to fall below 50% of maximum power output during fatigue, there were no significant effects of HFD (Figure 5.4A & D $P > 0.220$, $\eta p^2 < 0.048$), VITD ($P > 0.962$, $\eta p^2 < 0.001$) or HFD*VITD interactions observed ($P > 0.455$, $\eta p^2 < 0.018$) in either the EDL or soleus.

HFD groups produced significantly less cumulative work after 50 consecutive WL's when compared to SLD groups in both the EDL and soleus (Figure 5.4B & E $P < 0.043$, $\eta p^2 > 0.126$). There was no significant main effect of VITD ($P > 0.417$, $\eta p^2 < 0.021$) or HFD*VITD interaction observed ($P > 0.545$, $\eta p^2 < 0.012$).

For recovery of maximal power output relative to pre fatigue maximum, assessed every 10 minutes for 30 minutes post fatigue, in both the EDL and soleus, there was a significant main effect of time (Figure 5.4C & F $P < 0.020$, $\eta p^2 = 0.656$), with significant recovery of muscle power output over the 30 minutes. No TIME*HFD ($P > 0.203$, $\eta p^2 < 0.052$), TIME*VITD ($P > 0.202$, $\eta p^2 < 0.052$) or TIME*HFD*VITD interactions were observed ($P > 0.370$, $\eta p^2 < 0.028$). There were no main effects of HFD ($P = 0.228$, $\eta p^2 = 0.047$ for the EDL; $P = 0.060$, $\eta p^2 = 0.110$ for the soleus), VITD ($P = 0.790$, $\eta p^2 = 0.002$ for the EDL; $P = 0.078$, $\eta p^2 = 0.097$ for the soleus) or HFD*VITD interaction ($P > 0.542$, $\eta p^2 < 0.012$) observed.

Figures 5.5 and 5.6 illustrate typical WL shape during at a standardised time point for mouse soleus and EDL in each respective group. The area within the WL represents the net work done during the length change cycle. The area within the typical EDL WL traces (Figure 5.5) is initially smaller in HFD groups (Figure 5.5C & D) compared to SLD groups (Figure 5.5A & B), but the shape of the loop is consistent across groups. The reduction of the area within the loop, and subsequent change in WL shape exhibited during the fatigue protocol appears uniform irrespective of diet or treatment. However, typical soleus WL shapes (Figure 5.6) demonstrate that at the later stages of the fatigue

protocol (WL 30 and onward) there is greater force production during muscle re-lengthening in HFD groups (Figure 5.6C & D) when compared to SLD groups (Figure 5.6A& B). As a result, from WL 30 there is a marked reduction in the size of WL shape relative to the initial WL in HFD groups.

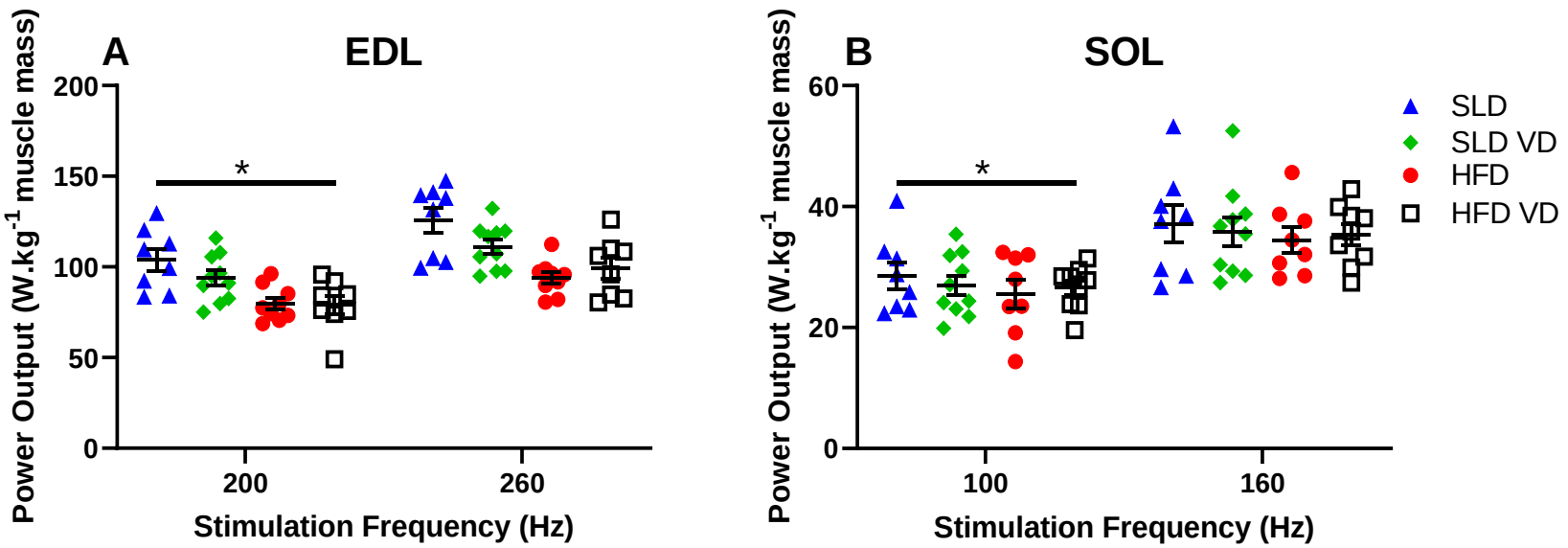

Figure 5. 3. *The effect of stimulation frequency, and 12 weeks high-fat diet and Vitamin D on maximal work loop power output normalised to muscle mass (Watts per kg of muscle mass) of isolated mouse EDL (A) and soleus (B). For EDL n = 10 for SLD VITD, n = 9 for HFD, n = 8 for SLD and HFD VITD; for soleus n = 10 for SLD VITD; n = 9 HFD VITD and n = 8 for SLD and HFD. Data presented as mean ± SEM; * indicates significant difference between stimulation frequencies P < 0.05.*

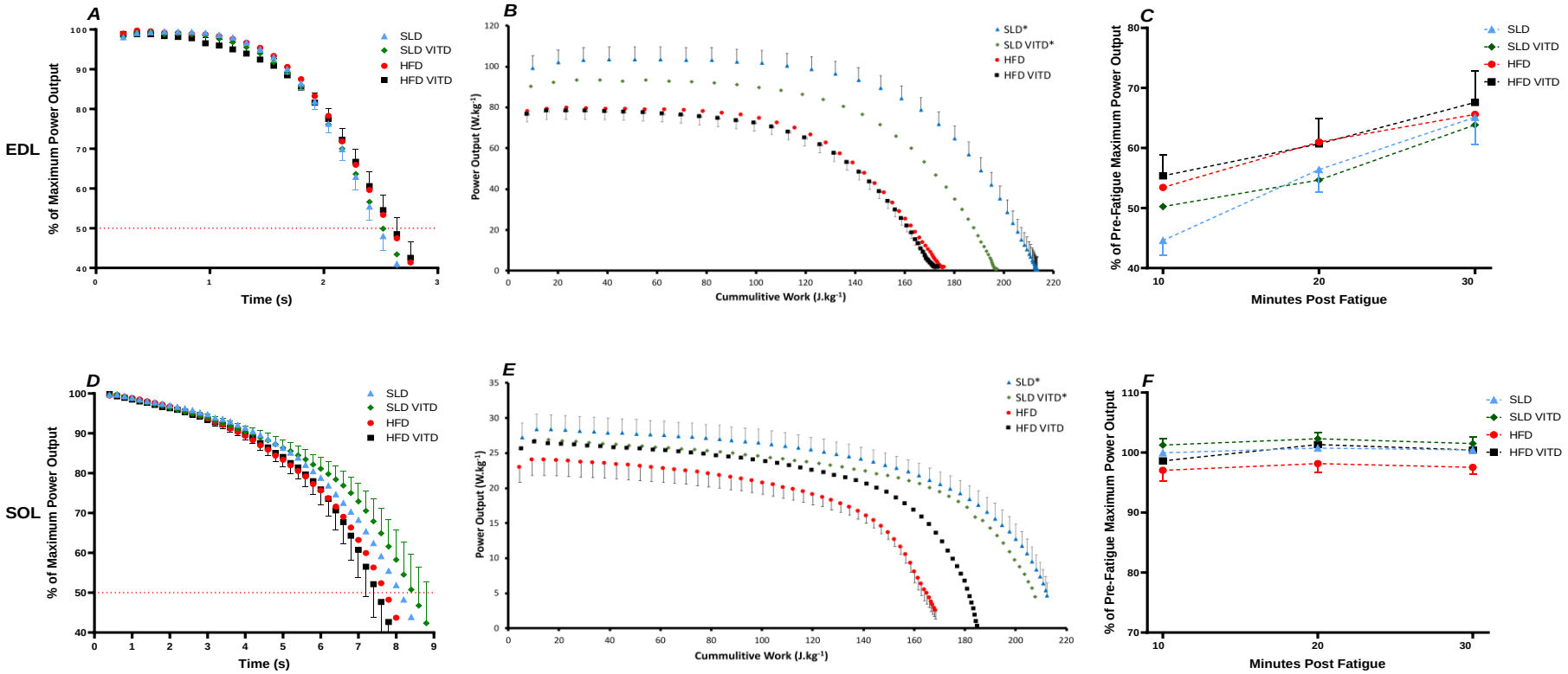

Figure 5. 4. *The effect of 12 weeks high-fat diet and Vitamin D on net muscle power output relative to maximum during fatigue (A & D), on the relationship between power output normalised to muscle mass (Watts per kg of muscle mass) and cumulative work normalised to muscle mass (Joules per kg muscle mass) produced during 50 consecutive work-loop cycles (B & E), and on the recovery of maximal work loop power output over 30 minutes post fatigue (C & F) of isolated mouse EDL (A, B & C) and soleus (D, E & F). Data are presented as mean ± SEM; For EDL n = 10 for SLD VITD, n = 9 for HFD, n = 8 for SLD and HFD VITD; for soleus n = 10 for SLD VITD; n = 9 HFD VITD and n = 8 for SLD and HFD. Data presented as mean ± SEM; * indicates significant difference between SLD and HFD treated groups at P < 0.05; some error bars omitted for clarity.*

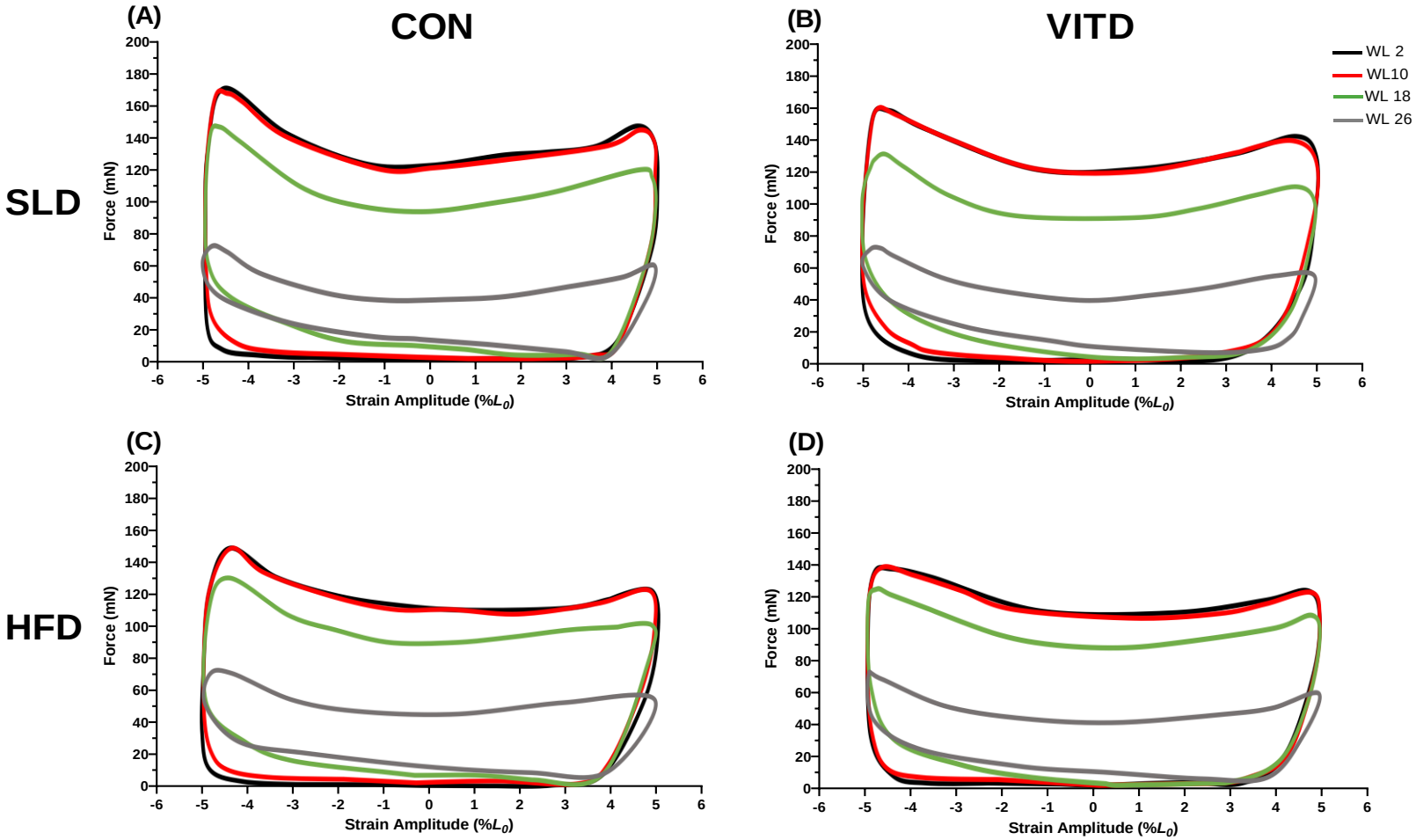

Figure 5. 5. *Effects of effect of 12 weeks high-fat diet and vitamin D (A, SLD; B, SLD VitD; C, HFD; D, HFD VitD) on typical work loop shapes during muscle fatigue at 10Hz cycle frequency for mouse EDL. Figures plotted as force against strain (%L_0). Work loops, 2, 10, 18 and 26 of the fatigue protocols are shown for each group. Work loops are performed in the anti-clockwise direction, with the work loop starting at L_0.*

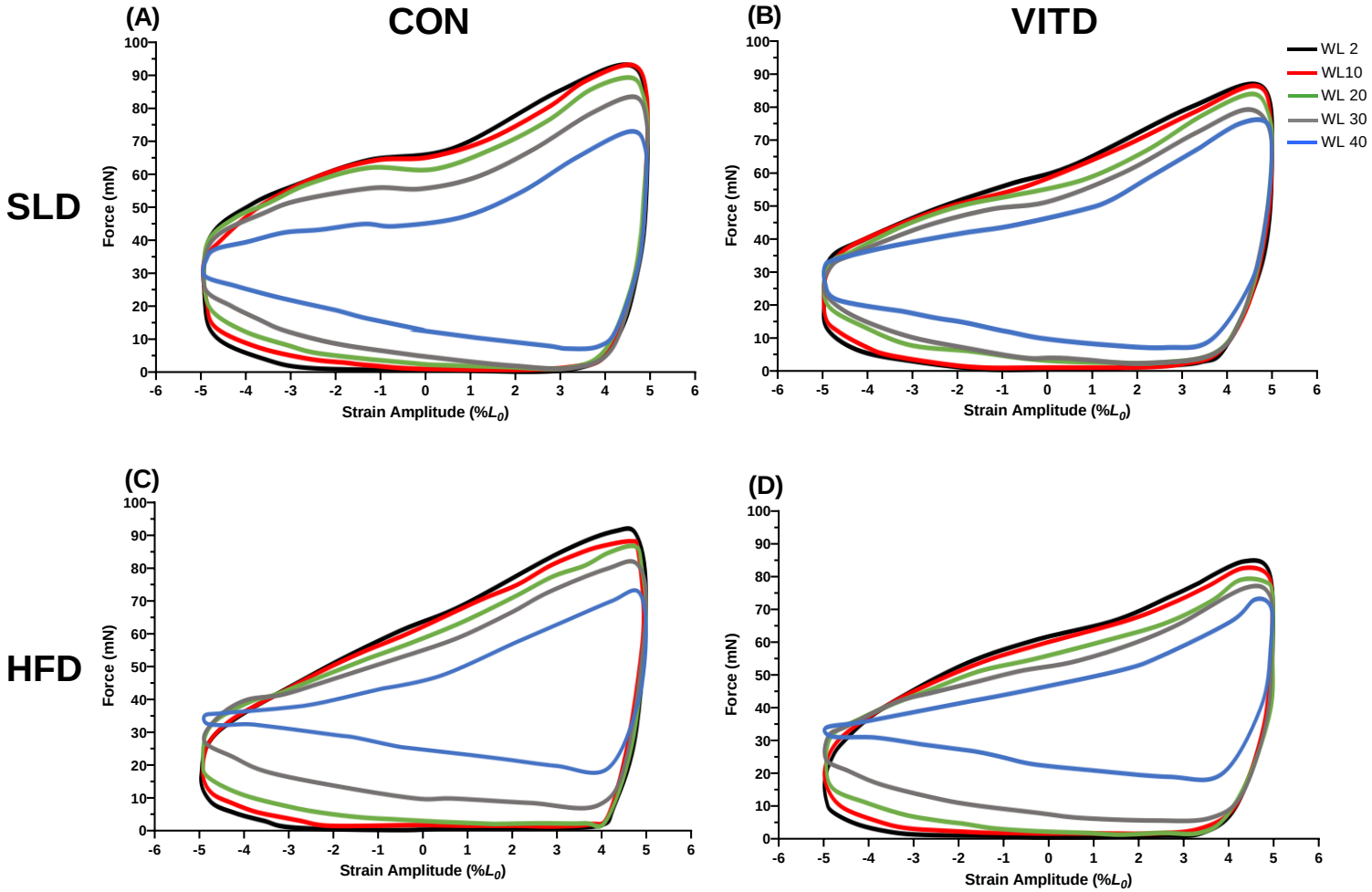

Figure 5. 6. *Effects of 12 weeks high-fat diet and vitamin D (A, SLD; B, SLD Vit-D; C, HFD; D, HFD Vit-D) on typical work loop shapes during muscle fatigue at 5Hz cycle frequency for mouse soleus. Figures plotted as force against strain (%L_0). Work loops, 2, 10, 20, 30 and 40 of the fatigue protocols are shown for each group. Work loops are performed in the anti-clockwise direction, with the work loop starting at L_0.*

5.5 – Discussion

The present study is the first to evaluate the effects of high dose vitamin D on force, power, and fatigue resistance of isolated mouse fast and slow twitch muscle, in both standard and high-fat diets. Using a methodological approach, which incorporated multiple modes of contractility, it was established that a HFD evokes muscle and contractile mode specific reductions in the contractile performance of isolated skeletal muscle. In the soleus, there was a HFD induced decline in PO normalised to body mass and cumulative work production during fatigue. However, in the EDL there was a reduction in isometric stress, absolute PO, PO normalised to muscle mass (muscle quality) and body mass, and cumulative work. Irrespective of diet, mode of contractility or muscle phenotype, a high dose of vitamin D over a 12-week period had minimal effects on the contractile performance of isolated skeletal muscle.

5.5.1 – High-fat Diet Effects on Maximal Muscle Force and Power

The contractile mode and muscle specific HFD induced reductions in contractile measures presented in these data, holistically support the trends in previous literature examining HFD effects on skeletal muscle (Ciapaite et al. 2015, Eshima et al. 2017, 2020, Tallis et al. 2017, Hill et al. 2019, Hurst et al. 2019). Whilst these findings broadly align with previous work, some differences do exist, such as a HFD induced reduction in the absolute power producing capacity of isolated mouse EDL. Differences within the present data set and to previous work are unsurprising given the muscle and contractile mode specific nature of HFD effects and the impact that varying methodological approaches, such as feeding duration, composition of diet, experimental temperature, age and strain of animal, has on contractile output (Tallis et al. 2018). The study also presents novel data regarding HFD effects on cumulative work production, which furthers understanding of obesity effects on fatigue resistance.

Peak isometric force and force normalised to cross-sectional area (CSA; stress) of the soleus was not affected by a HFD, which agrees with previous work by Hurst et al., (2019) who also used 12-week HFD feeding in female CD-1 mice. During longer feeding durations (16 weeks) in female mice, literature has identified greater absolute force of soleus from HFD animals, albeit no HFD effect on stress (Tallis et al. 2017). The magnitude of increase in body mass was originally suggested as the primary reason for an increase in absolute force observed over longer feeding durations, increasing demand on postural muscles to support and stabilise an elevated mass (Tallis et al. 2017). However, based on the present data, the concomitant effects of magnitude and duration of HFD, and duration of mechanical loading, may account for previously observed greater absolute force, as the final body masses for the HFD group from the present data (48.8g ± 1.4) were comparable to studies utilising longer feeding durations (16 weeks: 52.7g ± 2.3). Greater absolute force of soleus from HFD mice presented by Tallis et al., (2017) could be a result of the greater soleus muscle mass of HFD treated mice compared to controls, which was not found in the present study. It should be noted that the responses to HFD consumption of soleus absolute force and stress outlined here may be specific to female mice; previous work utilising isolated soleus from male mice consuming a typical HFD, irrespective of duration, report no changes in absolute soleus force or stress (Ciapaite et al. 2015; Eshima et al. 2020), but short term (5 week) high-fat palm oil diet results in diminished isometric stress (Ciapaite et al. 2015). As such, ambiguity still exists as to the effects of differing high-fat diets on the isometric performance of slow twitch muscle and how HFD responses differ between sex.

Absolute soleus PO and PO normalised to muscle mass (muscle quality) were unaffected by a HFD, supporting previous observations utilising feeding durations of 12-20 weeks (Tallis et al. 2017, Hurst et al. 2019, Tallis et al. *N.P*). Thus, despite elevated loading on postural muscles, no muscular adaptations occur to increase absolute power, which is further compounded by a substantial increase in body mass, supported by these data identifying a reduction in PO normalised to body mass.

Therefore, despite the preservation of muscle quality in HFD groups, the magnitude of change in body mass evoked by a HFD leads to a reduction in normalised PO, where *in vivo* this would result in reductions in the ability to be physically active and to perform activities of daily living, thus the potential for a reduction in quality of life and the onset of a negative obesity cycle (Tallis et al. 2018).

Whilst absolute isometric tetanus force of the EDL remained unchanged irrespective of diet, there was HFD induced reduction in stress, as has been reported in a similar study utilising the same strain and gender of mouse (Tallis et al. 2017). Despite numerous studies examining the impact of HFD consumption on EDL tetanus force and stress (Shortreed et al. 2009; Matsakas et al. 2015; Tallis et al. 2017, 2022; Eshima et al. 2017, 2020; Hurst et al. 2019), there is still little consensus regarding the HFD response, where isometric function (absolute or stress) is either reduced or unchanged.

EDL absolute and normalised PO, and muscle quality, were diminished in the HFD group. A HFD induced reduction in EDL muscle quality is supported by previous work (HFD 16 and 12 weeks respectively; Tallis et al. 2017, Hurst et al. 2019), but absolute power has previously been reported to be unchanged. Discrepancies in findings of absolute power may be attributed to increased magnitude of intramuscular lipids occurring earlier in the feeding duration in the current study when compared to previous work. Elevated intramuscular lipids occurred in the EDL after ~12 weeks of a fixed HFD (Eshima et al. 2017), but not until ~16 weeks in a high-fat forage diet (Messa et al. 2020), such as that utilised in previous work using the WL technique (Tallis et al. 2017, Hurst et al. 2019). Intramuscular lipids are not only associated with a reduction in the contractile capacity of whole isolated skeletal muscle (Biltz et al. 2020), but also linked with a reduction in the process of myogenesis (Akhmedov and Berdeaux 2013), another key factor regulating contractile performance. HFD effects on contractile performance appear more pronounced in fast twitch muscle, attributed to fast glycolytic fibres having a reduced capacity to metabolise elevated lipids levels within muscle, when compared to muscles

comprised of slow oxidative fibres, such as the soleus, where there are limited HFD effects on WL PO (Tallis et al. 2017, Hurst et al. 2019). The reduction in EDL muscle quality observed in the present study and elsewhere (Tallis et al. 2017, Hurst et al. 2019) is likely a result of the aforementioned mechanisms inhibiting maximal PO in combination with other factors such as disrupted calcium cycling (Seebacher et al. 2017; Tallis et al. 2018). Irrespective of treatment group or muscle, there were no shifts in the optimal CF needed to produce maximal WL PO, as has previously been reported in both young (Hurst et al, 2019) and old (Hill et al, 2019) female mice consuming a HFD. As such, these results infer that a HFD induced shift in fibre type composition is unlikely a contributing factor to reduced EDL power output. The data support the idea that HFD consumption effects on skeletal muscle may be contractile mode and muscle specific (Ciapaite et al. 2015; Tallis et al. 2018; Hill et al. 2019) and could promote a negative obesity cycle (Tallis et al. 2018); where a HFD leads to muscles of poorer quality, which are required to manoeuvre and support a larger mass and require a much greater metabolic cost to maintain them (Seebacher et al. 2017).

5.5.2 – High-fat Diet Effects on Fatigue Resistance

With respect to percentage decline in fatigue, these data suggest that a HFD may not influence the rate at which muscle loses power relative to maximum PO during fatiguing contractions. However, there is little consensus regarding HFD effects on isolated skeletal muscle fatigue during dynamic conditions in young adult female CD-1 mice, with no change or a reduction in the ability to maintain PO relative to maximum being reported in both the soleus and EDL (Tallis et al. 2017; Hurst et al. 2019). One notable difference which may contribute to ambiguity between the present data and previous work, is the stimulation frequency used during fatiguing contractions. The present study utilised submaximal stimulation frequencies during fatigue, based on findings from chapter 4, as utilising the stimulation frequency which evokes maximal tetanic force, as has been standard practice in previous literature, may evoke a fatigue response atypical of *in vivo* muscle mechanics. It should be

noted that in older female CD-1 mice and in other rodent (C57) and fish models (zebrafish; *danio rerio*), HFD has shown to have limited effects on the rate at which muscle loses power during consecutive WL contractions (Hill et al. 2019), or force during repeated tetani (Shortreed et al. 2009; Thomas et al. 2014; Seebacher et al. 2017; Seebacher and James 2019), supporting present observations. However, previous work has only considered HFD effects on percentage decline during fatigue from a standardised point, typically maximum PO or force. Whilst this provides an insight into the performance of the tissue during fatigue, cumulative work is also an important factor when considering *in vivo* function, as the amount of work, in addition to the rate of fatigue, will play a key part in exercise capacity and in the ability to complete activities of daily living which require repetitive contractions.

Whilst the rate of fatigue was unaffected by HFD, total cumulative work was reduced in the HFD soleus and EDL. The reduction in cumulative work is not a result of the sub maximal stimulation frequency leading to a greater reduction in PO in the HFD group, as the magnitude of change in maximal PO through a reduction in stimulation frequency did not differ between diet and treatment groups. A reduction in cumulative work in HFD EDL is likely a consequence of the reduction in acute PO. Therefore, despite fatiguing at a similar rate as SLD EDL, work per contraction is lower culminating in lower work production throughout the time course of fatigue. In addition to this, time to fatigue is plotted as a percentage decline relative to maximum power output. As such, if HFD EDL were required to work at the same absolute intensity as SLD EDL, it would likely fatigue more quickly given that it would be required to work at a greater percent of its maximum (Tallis et al. 2017). The same justification for a reduction in soleus cumulative work is unlikely given there were no differences in acute PO, suggesting other mechanisms account for a reduced capacity to perform work. There is no evidence, from data presented in the present study, that HFD caused increased relaxation time during acute maximal tetanic activations, as has been previously reported in HFD treated soleus (Tallis et al.

2017; Hurst et al. 2019). However, it is still possible that a HFD induced reduction in the function of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) (Funai et al. 2013), which is responsible for the movement of Ca^{2+} from the cytoplasm back into the sarcoplasmic reticulum, impairs calcium reuptake during fatiguing contractions of the soleus. Consecutive fatiguing contractions have been shown to increase relaxation time in some muscles (Allen et al. 2008); if this response is exacerbated by a HFD, it would likely cause a reduction in cumulative work as the magnitude of negative work would increase per WL cycle, i.e., the muscle is active during lengthening, thus decreasing net-work to a greater extent at each subsequent contraction. This is supported by the soleus WL shapes (Figure 5.6) - as the fatigue protocol progresses, HFD treated muscle had a larger negative work component during re-lengthening, indicative of increased relaxation time, reducing work production during each cycle. However, independent of changes in SERCA, HFD induced reductions in the efficacy of actin-myosin cross-bridge cycling (Schilder et al. 2011; Ciapaite et al. 2015) may play an important role in a reduction in cumulative work in both the soleus and EDL, and in the case of the soleus, without influencing acute power production.

5.5.3 – Vitamin D and High-fat Diet Effects on Maximal Force, Power and Fatigue Resistance

The administration of high doses of vitamin D has been shown, in previous work, to beneficially alter mechanisms associated with a HFD induced decline in skeletal muscle contractility (Marcotorchino et al. 2014; Manna et al. 2017; Benetti et al. 2018). However, the dose of vitamin D used in the present study did not elicit any changes in whole body or muscle morphology, or any of the contractile parameters assessed, in either SLD or HFD. There are limited comparisons to be made regarding the potential for vitamin D to alleviate obesity induced declines in contractile performance, in fact, only one previous study has directly considered this (Kim et al. 2020). This previous research study reported that a high dose of vitamin D attenuated the reduction of *in vivo* grip strength and sensorimotor function in 24 week old, male, P-62 deficient mice (genetic obesity model) (Kim et al.

2020). However, it is difficult to make a direct comparison to the present data, given the differences in the methodological approach, such as different model of obesity (dietary vs genetic), gender and age of mouse, different assessment of contractile function (*in vivo* vs isolated muscle) and different quantity, duration, and mode of administration of vitamin D (75IU every 3 days for 10 weeks via oral gavage), which are all factors that could influence the effect of vitamin D on contractile function.

The positive effects of vitamin D supplementation on contractile function are often reported to occur in VDD individuals (Beaudart et al. 2014). However, the present study compares the effects of high dose vitamin D against a standard low dose utilised in control chow, meaning control mice may not have been VDD. Although, this cannot be confirmed given circulating serum levels of 25(OH)D in blood plasma were not measured in this instance. These results could suggest that once adequate vitamin D status is achieved, improvements in contractile function associated with vitamin D supplementation plateau. However, this would appear unlikely as previous research has established high dose of vitamin D can evoke improvements in isolated skeletal muscle contractility when compared to mice consuming a standard dose of vitamin D enriched in to lab chow (Debruin et al. 2019; Hayes et al. 2019).

Much like previous work which assessed vitamin D supplementation on isometric properties of isolated muscle utilising the same dose (20,000IU/Kg^{-1} feed) in SLD C57/BL6 mice, no large changes in calcium handling were observed (Debruin et al. 2019, 2020; Hayes et al. 2019) as inferred by lack of difference in measurers of THPT and LSHR. Data is also presented to show that high dose Vitamin D had little effect on isometric force, WL PO or fatigue resistance of either the soleus or EDL, in either dietary group. Previous observations support limited vitamin D effects on EDL force output in SLD treated mice (Ray et al. 2016; Debruin et al. 2019, 2020; Hayes et al. 2019). However, we are the first

to show minimal effects of high dose vitamin D on soleus stress, as prior work indicates improved soleus stress after 4 weeks in SLD treated female mice (Debruin et al. 2019; Hayes et al. 2019) but diminished after 8 weeks in SLD treated male mice (Debruin et al. 2020) when supplemented with high dose vitamin D (20,000IU/Kg^{-1} feed). It could be that excess vitamin D supplementation adversely effects muscle function, as has been reported in both human and animal studies, where large single bolus doses have been associated with impaired isolated muscle contractile performance (Hayes et al. 2019) and increased risk of falls in the elderly (Sanders et al. 2010). Although the dose provided in this study, whilst high, was not given in one bolus dose and nor does the data show a reduction in contractile performance irrespective of muscle or mode of contractility. Whilst speculative, based on previous evidence and the data presented in this study, there could well be an "inverted-U" relationship with duration of high dose vitamin D supplementation and isolated skeletal muscle contractility (Bollen et al. 2022). Initially high dose of vitamin D may be beneficial for isolated skeletal muscle contractility until an optimal quantity of circulating vitamin D is achieved, from that point on there is a downward trajectory in muscle performance. However, more data is needed to explore the effect of dose and duration of vitamin D on isolated muscle contractility.

Whilst the present study provides a valuable insight into the effects vitamin D on isolated muscle mechanics, these results are only generalisable to the specific dose and duration used, in female mice. As such, future work is needed to establish the optimal dose and duration of vitamin D to evoke improvements in isolated skeletal muscle mechanics in both SLD and HFD treated mice. One plausible explanation for differences between our data and previous work may well be sex specific responses, given there may be sex specific determinants in the modulation of 25(OH)D levels (Jungert and Neuhäuser-Berthold 2015; Dupuis et al. 2021) - there is limited available evidence for how this effects the contractile response. Overall previous studies provide evidence that vitamin D supplementation can, in some instances, promote improvement in mechanisms responsible for optimal skeletal muscle

performance, including attenuation of mechanisms responsible for HFD induced declines in contractile performance such as reduced AMPK activity and decreased lipid metabolism (Marcotorchino et al. 2014; Manna et al. 2017; Benetti et al. 2018). However, in the present study, high dose vitamin D did not elicit any improvement in the force and power producing capacity or fatigue resistance of isolated locomotor muscles in either dietary group.

Despite the findings presented in this study, high dose vitamin D could still be beneficial for contractile performance when supplemented long term. As suggested in previous work, the responses may be dose/duration dependent, whereby excess vitamin D may cause vitamin D dysregulation and result in deleterious (Debruin et al. 2020), or in the case of these data, no effects on contractile performance. Whilst 8-week high dose was found to reduce isometric stress of the soleus by ~50% compared to a control dose, when high dose vitamin D was supplemented with exercise it attenuated the loss in stress associated with vitamin D alone and even enhanced fatigue resistance compared to exercise only (Debruin et al. 2020). The authors suggest that high dose vitamin D alone results in vitamin D dysregulation, through alterations in the activity of key metabolic enzymes 1,25-alpha-hydroxylase and 24-alpha-hydroxylase involved in vitamin D metabolism. However, exercise induced enhancement of mitochondrial function appeared to abate the increase in metabolic stress evoked by high dose vitamin D, ultimately promoting improvements in contractile function (Debruin et al. 2020). Therefore, it may be that high dose vitamin D is beneficial in attenuating a HFD diet induced decline in muscle contractility when combined with an added stimulus which evokes beneficial alterations in mitochondria, be that exercise or in combination with other nutritional strategies. Although, this suggestion is somewhat ambiguous, given previous research reports that high dose vitamin D supplementation enhances mitochondrial function, through an increase in UCP3 (Fan et al. 2016). As such, future work is still needed to elucidate both the mechanistic and contractile response to vitamin D, utilising multiple doses and durations in both SLD and HFD treated, male and female mice.

The effects of surplus vitamin D on isolated muscle function appear dependent upon a number of factors such as dose, duration, sex and physical activity. It could be suggested the variability in responses excludes vitamin D as a suitable candidate for improving skeletal muscle function, particularly as large bolus doses have shown to reduce isolated soleus stress (Hayes et al. 2019) and increase risk of falls and fractures in elderly women (Sanders et al. 2010). However, there is clear evidence demonstrating that chronic supplementation of high dose vitamin D can improve isolated muscle performance (Debruin et al. 2019; Hayes et al. 2019) and evoke mechanisms which directly oppose HFD induced declines in muscle function (Marcotorchino et al. 2014; Manna et al. 2017; Benetti et al. 2018). Given the available evidence, coupled with the need to explore alternative therapeutic strategies to alleviate the adverse effects of obesity, supplementation of vitamin D warrants further investigation; understanding and identification of suitable dosing strategies could contribute to a reduction in the adverse effects of obesity on overall health and specifically skeletal muscle health (Tallis et al. 2021), which are current public health priorities.

5.5.4 – Limitations and Future Direction

One limitation of this study is that the exact levels of vitamin D achieved are unknown in each individual as serum 25 (OH) D concentration in blood plasma was not measured. Restrictions of the home office licence for the presented studies prohibits cardiac puncture prior to schedule 1 procedure, and attempts to extract blood samples post schedule 1 yielded insufficient quantities of blood for analysis. As such, serum 25 (OH) D concentration in blood plasma could not be quantified in the present study. However, previous research shows that an identical high dose and duration (20,000IU/kg diet for 12 weeks) elicits ~4 fold increase in serum 25 (OH) D3 concentration when compared to a control dose (1000IU/kg diet) in C57 mice (Rowling et al. 2007). Thus, we would expect that high dose vitamin D groups would have substantially greater quantities of circulating vitamin D compared to control doses.

Given improved contractile performance has been observed over shorter feeding durations utilising a high dose vitamin D (Debruin et al. 2019; Hayes et al. 2019), future work should consider dose and duration response to vitamin D to establish optimal feeding protocols in both male and female mice. Optimisation of feeding protocols to improve contractile performance, in combination with attenuation of the mechanisms associated with a HFD induced reduction in contractile performance, have the potential to culminate in improved skeletal muscle health in HFD treated mice.

5.5.5 – Conclusion

The present study demonstrates that HFD effects are muscle and contractile mode specific, which are likely influenced by both magnitude of adiposity and duration of feeding. For acute force and power performance there are limited effects of HFD on isolated mouse soleus, but reductions in EDL stress and absolute and normalised PO. During fatiguing contractions, whilst percentage decline relative to maximum PO remained unchanged between groups, total cumulative work was reduced in HFD treated soleus and EDL, although it would appear the mechanisms responsible for this decline are not entirely uniform between muscles. Whilst high dose vitamin D did not attenuate any of the HFD induced declines in contractile performance or alter contractile performance in SLD treated mice in this present study, future work is needed to consider the impact of various doses and durations to determine if vitamin D can be effective in reducing the impact of obesity of skeletal muscle function.

5.5.6 – Thesis Progression

As established in both chapter 3 and 5, high adiposity and high fat diet consumption can adversely affect skeletal muscle contractility, with findings relatively uniform across whole muscle groups in humans and isolated rodent muscle. Despite vitamin D previously showing modulation of

mechanisms involved in optimal muscle function, and attenuation of mechanisms attributed to obesity muscular dysfunction, 20,000IU/kg^{-1} diet vitamin D did not influence the contractile performance of isolated mouse soleus or EDL. However, there are other nutritional compounds which have shown to reduce the adverse effects of high-fat diet consumption on skeletal muscle health and, albeit limited, show some improvement in muscle contractility in models which share similar mechanistic alterations to obesity, such as aging. Therefore, using the work loop protocol refined in chapter 4, the aim of chapter 6 (study 4) is to examine the impact of RES on whole body and muscle morphology, to determine if it could be used to reduce adverse effects of high-fat diet consumption on the contractile performance of isolated skeletal muscle, particularly in fast twitch EDL, where high-fat diet effects appear most substantial.

Chapter 6 - The Effects of a High-Fat Diet and Resveratrol on Maximal Force, Power and Fatigue Resistance of Isolated Female CD-1 Mouse Soleus and EDL Muscles

6.1 – Abstract

Conventional treatment strategies to abate the adverse effects of obesity on the contractile function of skeletal muscle are substantially limited by long term adherence. Resveratrol, a cost effective and low demanding alternative to current strategies, exhibits numerous anti-obesogenic responses, including attenuation of mechanisms contributing to the reduction in contractile performance associated with obesity. However, the effects of resveratrol on isolated skeletal muscle mechanics in HFD treated mice are yet to be explored. This study will examine the effects of high-fat diet and resveratrol consumption on the contractile properties of isolated mouse soleus and EDL muscle. Four-week-old female mice (CD1; N=40 starting sample; N=38 final sample) were randomly assigned to standard low-fat diet (SLD) or high-fat diet (HFD), with (SLD RES, HFD RES) or without resveratrol for 12-weeks. At ~18-weeks of age soleus and EDL (N= 8-10 per group) were isolated and absolute and normalised (relative to muscle size and body mass) maximal isometric tetanic force and work loop power output (PO) were measured across a range of contractile velocities, and the rate of fatigue and cumulative work over 50 work loop contractions were determined. Body mass and fat mass were significantly greater in HFD treated mice ($P < 0.002$ in both cases), but HFD RES attenuated the increase in fat mass compared to HFD only ($P = 0.039$). Soleus absolute and normalised isometric force and PO did not differ between groups ($P > 0.584$) except for absolute tetanus force, which was significantly greater in HFD RES Vs. HFD only ($P = 0.030$), and PO normalised to body mass, which was diminished in HFD treated groups ($P < 0.001$). For the EDL, absolute and normalised isometric force did not differ between groups ($P > 0.091$). The HFD induced reduction in absolute and normalised EDL PO, and cumulative work production ($P < 0.019$) was attenuated with resveratrol supplementation, with HFD RES producing comparable PO and work during fatigue as SLD and SLD RES treated EDL ($P > 0.101$). Recovery from fatigue did not differ between groups ($P > 0.144$). Resveratrol had little effect on the contractile properties of SLD treated muscle. However, the data infers resveratrol attenuates the HFD induced reduction in contractile performance of fast twitch EDL. This data suggests that RES may be an appropriate strategy to alleviate the decline in muscle function associated with obesity.

6.2 – Introduction

Obesity induced reductions in skeletal muscle function have been proposed to be one of the catalysts for ill health in obese populations, and may also evoke a negative cycle of obesity (Tallis et al. 2018) . A negative obesity cycle is defined as a reduction in contractile performance limiting physical capacity and energy expenditure which contributes to further lipid accumulation, potentially exacerbating the adverse effects described (Tallis et al. 2018). Recent research exploring the effects of obesity on skeletal muscle contractility, including the data presented in chapter 3 and 5, report muscle and contractile mode specific reductions in muscle performance (Maffiuletti et al. 2013; Tomlinson et al. 2016; Bollinger 2017; Tallis et al. 2018, 2021; Straight et al. 2021). Obesity induced declines in skeletal muscle function are proposed to evoke a reduction in functional movement, postural control and reduced capacity to be physically active (Maffiuletti et al. 2013; Teasdale et al. 2013; Tomlinson et al. 2016; Bollinger 2017; Tallis et al. 2018, 2021). Given these adverse effects, combatting obesity induced reductions in skeletal muscle contractility is a public health priority (Wolfenden et al. 2019). Whilst lifestyle interventions, such as calorie restriction and increased physical activity are effective in reducing the adverse effects of obesity, poor adherence severely limits their long-term effectiveness (Hall and Kahan 2018). Therefore, further exploration of strategies which could help reduce the impact of obesity on skeletal muscle function, which are sustainable and feasible for most, are needed to help negate a negative obesity cycle. One such strategy which has received considerable attention for its anti-obesogenic properties, is nutritional supplementation of Resveratrol (RES) (Tallis et al. 2021).

Recent studies have explored the anti-obesogenic properties of resveratrol (3,5,4’-trihydroxystilbene), a natural polyphenolic compound, sourced in trace amounts from grapes, wine, and peanuts (Fernández-Quintela et al. 2016; Carpéné et al. 2019). There is theoretical evidence to suggest resveratrol may be a successful nutritional strategy in promoting skeletal muscle health in obese

individuals (Tallis et al. 2021). In middle aged (40 – 60 years old) obese male humans, short term (30 days of 150mg daily) supplementation of RES has been shown to increase AMPK activity, increase SIRT1 and PGC-1α protein levels, reduce intramyocellular lipid levels and inflammatory markers (Timmers et al. 2011) and reduce adipocyte size (Konings et al. 2014). Longer term RES supplementation (90 days of 1,500mg daily) is associated with positive changes in body composition including a reduction in overall adiposity (Méndez-del Villar et al. 2014). The therapeutic effects of RES in dietary induced obese animal models also show promise. It has been proposed that RES directly targets adipose tissue and lipid metabolism of the liver and skeletal muscle, inducing decreased adipogenesis, increased apoptosis, and stimulation of the lipolytic and oxidative pathways, culminating in reduced adiposity in HFD treated mice (Lagouge et al. 2006; Kim et al. 2011; Aguirre et al. 2014; Wang et al. 2015).

In skeletal muscle, RES has been shown to increase AMPK activity, mitochondrial function, reduce chronic inflammation and, via elevated lipid metabolism, reduce intramyocellular lipid in HFD treated rodents (Baur et al. 2006; Lagouge et al. 2006; Kim et al. 2011; Wang et al. 2015; Shabani et al. 2020). Dysregulation of these mechanisms is associated with HFD induced changes in skeletal muscle function (Tallis et al, 2018), making the dietary supplementation of RES a strong candidate for investigation. This concept is further supported by the small number of studies that have examined the effect of RES on skeletal muscle function. Evidence suggests that a range of doses and durations of RES supplementation improves *in vivo* absolute and relative forelimb grip strength, and fatigue resistance of young and old non-obese rodents (Lagouge et al. 2006; Dolinsky et al. 2012; Wu et al. 2013; Kan et al. 2018; Zhou et al. 2019), and *in situ* specific peak tension and peak tension normalised for body mass of triceps surae complex in mdx mice (Gordon et al. 2014). Furthermore, in HFD treated animals, RES has been shown to improve *in vivo* relative grip strength when compared to a HFD only group (Lagouge et al. 2006). However, there is no available evidence directly exploring the effects of

RES on a HFD induced decline in isolated muscle contractile performance. RES can alter adipose morphology, mediate mechanisms which promote obesity and HFD-induced muscle dysfunction (Baur et al. 2006; Lagouge et al. 2006; Kim et al. 2011; Wang et al. 2015; Shabani et al. 2020), and has shown potential to improve muscle function in multiple non-obese rodent models (Lagouge et al. 2006; Dolinsky et al. 2012; Wu et al. 2013; Kan et al. 2018; Zhou et al. 2019). As such, RES could be a viable nutritional strategy to alleviate the decline in contractile performance associated with HFD consumption.

Using previously established protocols for examining the impact of HFD on contractile performance of skeletal muscle (Tallis et al. 2017; Hill et al. 2019; Hurst et al. 2019) the present study uniquely examined the effects of 12 weeks of RES and a HFD on the maximal isometric force, work loop (WL) power output (PO), and fatigue resistance of soleus (predominantly slow-twitch) and extensor digitorum longus (EDL; predominantly fast-twitch) muscle, isolated from young adult female CD-1 mice. It was hypothesised that the magnitude of body mass and adipose accumulation, and reduction in contractile performance evoked via HFD would be reduced by RES consumption, particularly in fast twitch skeletal muscle, where the impact of a HFD has previously shown to be the most substantial. The results from the present work provide important insight into the potential anti-obesogenic effects of RES and more specifically, the role it could play in alleviating the decline in contractile performance associated with HFD consumption.

6.3 – Materials and methods

The experimental approach for contractile assessments is provided in detail in chapter 5. Therefore, the focus of this section is to detail and outline the use of RES and the statistical approach taken. The present study utilised the same controls (SLD and HFD) as chapter 5, to reduce the number of animals needed to complete both set of experiments, falling in line with NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research; NC3Rs, 2022)

6.3.1 – Animals

The procedures outlined in this study and the use of animals was approved by the ethics committee of Coventry University (P108131), the Animal Welfare and Ethical Review Body of the University of Warwick, and the British Home Office (PP4247175), and was completed in accordance with the Animals (scientific procedures) Act 1986. Female CD-1 mice, purchased at ~4 weeks old (Charles River, Kent, UK), were housed in groups of five at the University of Warwick and kept in 12:12 hour light:dark cycles at 50% relative humidity and 22^{o}C. Mice were provided with *ad libitum* access to food and water. At 4 weeks of age, mice were randomly split into four experimental groups (total starting sample: n = 10 per group, final sample: *n* = 10 SLD RES and HFD RES; *n* = 9 SLD and HFD) and following 13 days of habituation, which included gradual transition from standard lab chow (TestDiet 5755; calories provided by protein 18.3%, fat 22.1% and carbohydrate 59.6%; gross energy 4.07 kcal/g) to their new respective custom diets, mice consumed one of the following diets for 12 weeks: 1) Standard low-fat diet (TestDiet 58Y2; calories provided by protein 18.0%, fat 10.2% and carbohydrate 71.8%; gross energy 3.76 kcal/g) (SLD); 2) High-fat diet (TestDiet 58V8; calories provided by protein 18.3%, fat 45.7% and carbohydrate 35.5%; gross energy 4.62 kcal/g) (HFD); 3) SLD enriched with RES (MegaResveratrol; 99% Trans-Resveratrol 4g/kg feed); and 4) HFD enriched with RES, as shown in Table 11.

Table 11. *Treatment Groups*

Group	Abbreviation	12-wks Treatment
Standard Low-fat Diet (control)	SLD	SLD
High Fat Diet	HFD	HFD
Standard Low-fat Diet + Resveratrol	SLD RES	SLD + 4g/kg feed of Resveratrol
High Fat Diet + Resveratrol	HFD RES	HFD + 4g/kg feed of Resveratrol

SLD RES, HFD RES n=10; SLD, HFD n = 9; HFD = High Fat Diet; SLD = Standard Low-fat Diet; RES = Resveratrol

The justification for a 12-week 45% HFD feeding duration has been outlined in chapter 5. In brief, 12-week HFD, and comparable feeding durations, have previously demonstrated large changes in body composition and muscle mechanics (Matsakas et al. 2015; Tallis et al. 2017; Eshima et al. 2017, 2020; Bott et al. 2017; Hurst et al. 2019). Research studies examining the potential anti-obesogenic properties of RES have implemented a range of doses and durations, but the optimal dose for improved skeletal muscle contractility is unknown. Therefore, the dosing strategy implemented in this study (4g/kg^{-1} diet for 12 weeks) was selected based on previous evidence indicating that an identical dose, over similar feeding durations (10-15weeks), elicits: A reduction in adiposity in HFD treated rodents compared to control rodents consuming standard lab chow (Lagouge et al. 2006; Mendes et al. 2016; Shabani et al. 2020); improves voluntary *in vivo* fatigue resistance (endurance test) in SLD and HFD treated mice (Lagouge et al. 2006), and SLD treated rats (Dolinsky et al. 2012); and improves *in vivo* relative grip strength in HFD treated mice compared to HFD controls (Lagouge et al. 2006). Previous evidence reports that RES at 4g/kg^{-1} diet provides a daily dose of ~400mg/kg^{-1} body mass in mice (Lagouge et al. 2006). Therefore, using a previously established method for determining human equivalent dose (HED) from a mouse model (daily animal dose/kg^{-1} body mass ÷ metabolic scaling factor for mice = HED/kg^{-1} body mass, i.e. 400mg/kg^{-1} ÷ 12.3 = 32.5mg/kg^{-1}) (Nair and Jacob 2016), the HED of RES used in the study is ~2000mg daily for a 60kg individual.

6.3.2 – Statistical analysis

Statistical analysis was performed using SPSS v.25 (IBM SPSS Statistics for Windows, IBM Corp, Armonk, NY, USA) and GraphPad Prism v.9 (GraphPad Software, California, US). All data are presented as mean ± standard error of mean (SEM). Following appropriate checks of normality and homogeneity of variance (Levenes), parametric analysis was performed. Mixed model repeated measures Analysis of Variance (ANOVA; with HFD, RES treatment [between subjects] and weeks [within subjects] used as the factors), was utilised to determine the effect of HFD and RES consumption on weekly body mass. Two factor Analysis of variance (ANOVA) tests were conducted, with HFD (SLD vs. HFD) and treatment (Control vs. RES) as the fixed factors, to examine if differences existed in measures of animal and muscle morphology (body mass, muscle mass, muscle length and muscle CSA), isometric properties (absolute tetanic force, tetanic stress, TTHP and LSHR), time to reach 50% of maximal PO during fatigue protocol and cumulative work produced after 50 consecutive WL's. Mixed model repeated measures ANOVA, with HFD, RES treatment (between subjects) and cycle frequency (within subjects) used as the factors, was utilised to determine any changes in SOL and EDL absolute PO and PO normalised to body mass and muscle mass. Mixed model repeated measures ANOVA, with HFD, RES treatment (between subjects) and stimulation frequency (within subjects) used as the factors, was utilised to determine if the reduction in stimulation frequency used for assessment of fatigue resulted in any significant changes in SOL and EDL PO normalised to muscle mass and if so, were the changes in PO uniform across diet and treatment groups. Mixed model repeated measures ANOVA, with HFD, RES treatment (between subjects) and time (within subjects) as the factors, was utilised to examine the recovery of WL PO following the fatigue protocol. Significant interactions observed for ANOVA were explored using Bonferroni post hoc for multiple comparisons. Partial eta squared (ηp^2) was calculated to estimate effect sizes for all significant main effects. Thresholds for Partial eta squared effect size were classified as small (<0.05), moderate (0.06-0.137) or large (>0.138) (Cohen 1988). Where appropriate, Cohen's *d* was calculated to measure effect size of interactions observed and was then corrected for bias using Hedges g due to the appropriate sample size (Hedges 1981). Cohen's *d*

effect size was interpreted as trivial (<0.2), small (0.2-0.6), moderate (0.6-1.2) or large (>1.2) (Hopkins et al. 2009). The level of significance was set at $P \leq 0.05$.

6.4 – Results

6.4.1 – Morphology

There was a significant TIME*HFD interaction observed for weekly body mass (Fig 6.1 $P < 0.001$, $\eta p^2 = 0.537$). Bonferroni multiple comparisons revealed that HFD treated groups had significantly greater body mass from week 2 onwards when compared to SLD treated groups ($P < 0.002$).

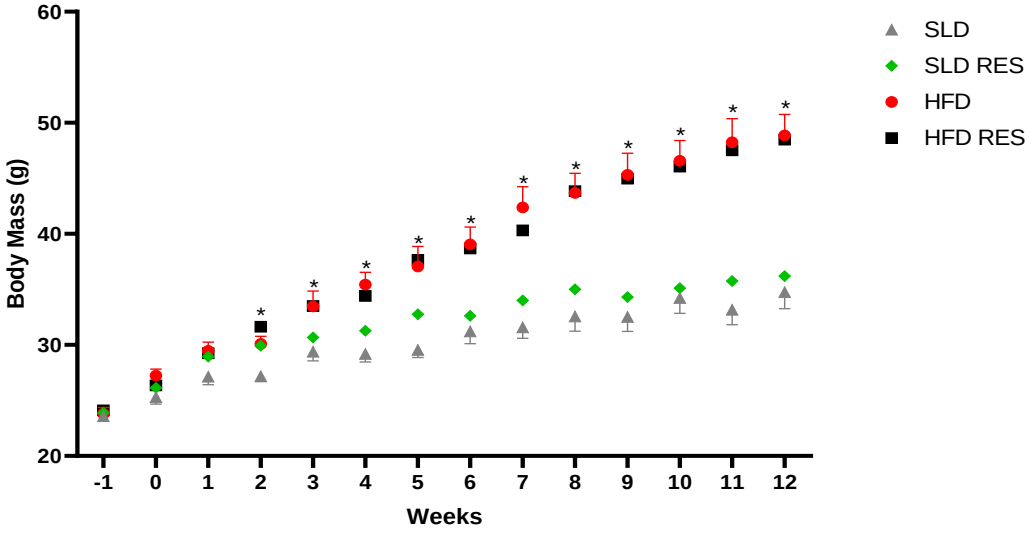

Figure 6. 1. *Effect of high-fat diet and resveratrol on body mass over 12 weeks. Data presented as mean ± SEM for HFD and SLD, just mean shown for other groups, for clarity; SLD and HFD* n*=9; SLD RES and HFD RES* n*=10; * indicates significant difference between HFD treated and SLD treated at P < 0.05; week -1 and 0 reflect habituation period where animals were gradually transitioned to their respective custom diet.*

Final whole animal body mass, body length, BMI, and Lee Index of Obesity were significantly higher in HFD groups compared to SLD groups (Table 12. $P < 0.003$, $\eta p^2 > 0.232$). For fat mass and Adiposity:Body mass a significant HFD*RES interaction was observed ($P < 0.038$, $\eta p^2 > 0.121$). Bonferroni multiple comparisons revealed that fat mass and Adiposity:Body mass was significantly higher in HFD treated compared to SLD ($P < 0.002$, $d > 2.09$) and HFD only compared to HFD RES ($P < 0.039$, $d > 0.98$), but no

differences between SLD treated groups were observed ($P > 0.999$, $d < 0.28$). No significant differences were observed for EDL or soleus muscle mass, fibre length or CSA ($P > 0.066$, $\eta p^2 < 0.099$).

6.4.2 – Isometric performance

For EDL, there were no significant HFD (Table 13. $P > 0.457$, $\eta p^2 < 0.017$), RES ($P > 0.483$, $\eta p^2 < 0.017$) or HFD*RES interactions ($P > 0.091$, $\eta p^2 < 0.083$) observed for absolute tetanus force or tetanus stress. A significant HFD*RES interaction was observed for time to half peak tetanus ($P = 0.033$, $\eta p^2 = 0.131$), although Bonferroni pairwise comparisons indicate no differences ($P > 0.09$, $d < 0.87$, except for SLD RES vs HFD RES where $P = 0.052$, $d = 1.07$). Last stimulus to half relaxation was significantly longer in HFD treated groups compared to SLD treated ($P < 0.001$, $\eta p^2 = 0.283$).

For soleus, there was a significant HFD*RES interaction for absolute tetanus force ($P = 0.030$, $\eta p^2 = 0.139$). Bonferroni multiple comparisons revealed that the HFD RES group produced significantly higher (~24%) absolute tetanus force compared to HFD group ($P = 0.017$, $d = 1.64$), but no other differences were observed ($P > 0.106$, $d < 0.80$). There were no significant HFD ($P > 0.584$, $\eta p^2 < 0.009$), RES ($P > 0.151$, $\eta p^2 < 0.063$) or HFD*RES interactions ($P > 0.160$, $\eta p^2 < 0.061$) observed for tetanus stress, time to half peak tetanus or last stimulus to half relaxation.

Table 12. *Whole body and muscle specific animal morphology*

	SLD	SLD-RES	HFD	HFD-RES	HFD Effect		RES Effect		Interaction	
	n = 9	n = 10	n = 9	n = 10	*P value*	ηp^2	*P* value	ηp^2	*P* value	ηp^2
Whole Body										
Body Mass (g)	35.0 ± 1.5	36.7 ± 1.5	48.8 ± 1.4	47.8 ± 2.0	**< 0.001**	**0.599**	0.843	0.001	0.443	0.017
Body Length (mm)	101 ± 2	101 ± 1	106 ± 1	107 ± 1	**< 0.001**	**0.317**	0.555	0.01	0.702	0.004
Fat Mass (g)	1.1 ± 0.2 [a]	1.2 ± 0.2 [a]	4.6 ± 0.6 [b]	3.2 ± 0.3 [c]	< 0.001	0.611	0.108	0.074	**0.038**	**0.121**
Lee Index of Obesity	325 ± 3	329 ± 6	346 ± 3	339 ± 4	**0.003**	**0.232**	0.759	0.003	0.225	0.043
BMI ($g.cm^{-2}$)	0.34 ± 0.01	0.36 ± 0.02	0.44 ± 0.01	0.42 ± 0.02	**< 0.001**	**0.429**	0.908	< 0.001	0.21	0.046
Adiposity:Body Mass (%)	2.8 ± 0.4 [a]	3.2 ± 0.5 [a]	9.3 ± 1.0 [b]	6.5 ± 0.5 [c]	< 0.001	0.591	0.086	0.084	**0.027**	**0.136**
EDL										
Muscle Mass (mg)	10.7 ± 0.4	11.1 ± 0.3	11.3 ± 0.2	11.2 ± 0.2	0.246	0.041	0.559	0.01	0.348	0.027
Fibre Length (mm)	9.2 ± 0.1	9.2 ± 0.3	8.9 ± 0.2	8.9 ± 0.1	0.089	0.089	0.779	0.002	0.898	0.001
CSA (mm^2)	1.10 ± 0.04	1.14 ± 0.05	1.21 ± 0.03	1.19 ± 0.02	0.066	0.099	0.737	0.003	0.459	0.017
Soleus										
Muscle Mass (mg)	8.7 ± 0.5	8.5 ± 0.4	8.4 ± 0.2	9.2 ± 0.4	0.594	0.009	0.526	0.013	0.238	0.043
Fibre Length (mm)	9.4 ± 0.2	9.5 ± 0.2	9.5 ± 0.2	9.4 ± 0.1	0.736	0.004	0.78	0.002	0.724	0.004
CSA (mm^2)	0.88 ± 0.05	0.84 ± 0.03	0.84 ± 0.02	0.92 ± 0.04	0.855	0.001	0.244	0.042	0.155	0.0062

Values are presented as mean ± SEM; bold values indicate a significant difference at $P < 0.05$; Values with different letters (*a, b, c*) are significantly different at $P < 0.05$ utilised for when significant interactions were observed; CSA: Cross-sectional area

Table 13. *The effect of 12-week high-fat diet and resveratrol on the isometric properties of isolated mouse EDL and soleus*

	SLD	SLD-RES	HFD	HFD-RES	HFD Effect		RES Effect		Interaction	
	n = 8	n = 10	n = 9 / 8	n = 10	*P* value	ηp^2	*P* value	ηp^2	*P* value	ηp^2
EDL										
Tetanus Force (mN)	363 ± 27	346 ± 15	340 ± 19	388 ± 22	0.689	0.005	0.483	0.015	0.156	0.06
Tetanus Stress ($kN.m^{-2}$)	332 ± 22	307 ± 17	283 ± 17	327 ± 17	0.457	0.017	0.652	0.007	0.091	0.084
THPT (ms)	11.4 ± 0.7	10.0 ± 0.4	10.4 ± 0.6	11.5 ± 0.5	0.629	0.007	0.782	0.002	**0.033**	**0.131**
LSHR (ms)	13.2 ± 0.4	13.6 ± 0.3	15.0 ± 0.4	14.6 ± 0.3	**< 0.001**	**0.283**	0.967	< 0.001	0.354	0.026
Soleus										
Tetanus Force (mN)	236 ± 17 ^{ab}	222 ± 15 ^{ab}	203 ± 12 ^{a}	252 ± 6 ^{b}	0.959	< 0.001	0.212	0.048	**0.03**	**0.139**
Tetanus Stress ($kN.m^{-2}$)	278 ± 17	265 ± 19	243 ± 14	281 ± 14	0.584	0.009	0.478	0.016	0.16	0.061
THPT (ms)	34.5 ± 2.4	33.8 ± 1.1	36.4 ± 1.8	32.1 ± 1.0	0.927	< 0.001	0.151	0.063	0.296	0.034
LSHR (ms)	40.1 ± 2.4	46 ± 3.0	45.9 ± 2.2	42.8 ± 3.3	0.681	0.005	0.653	0.006	0.166	0.059

Values are presented as mean ± SEM; bold values indicate a significant difference at $P < 0.05$; Values with different letters (*a, b*) are significantly different at $P < 0.05$ utilised for when significant interactions were observed; THPT: Time to half peak tetanus; LSHR: Last stimulus half relaxation time

6.4.3 – Maximal work loop power output

For EDL, maximal PO and PO normalised to muscle mass there was a significant main effect of CF (Fig 6.2A & B. $P < 0.001$, $\eta p^2 = 0.675$), indicating that maximal PO occurred between 8 and 12Hz ($P > 0.999$), with the PO values from the remaining cycle frequencies being significantly lower ($P < 0.04$).

For maximal PO normalised to body mass there was a significant HFD*CF (Fig 6.2C. $P < 0.030$, $\eta p^2 = 0.080$) interaction observed. Bonferroni multiple comparisons reveal that the PO CF curves for EDL PO normalised to body mass did not significantly differ, except for at 12Hz where in SLD treated mice, PO was significantly greater than at 4Hz ($P < 0.001$), whereas in HFD treated mice, PO was significantly higher at 12Hz compared to 4Hz ($P < 0.001$) and 16Hz ($P = 0.031$). For EDL maximal PO, PO normalised to muscle mass and PO normalised to body mas, there was a significant HFD*RES interaction (Fig 6.2A, B & C. $P < 0.037$, $\eta p^2 > 0.125$). Bonferroni pairwise comparisons reveal all outcomes were significantly lower in HFD treated only group compared to all other groups ($P < 0.019$, $d > 1.05$), and the other groups did not significantly differ ($P > 0.101$, $d < 0.32$, except for SLD treated groups vs HFD RES for PO normalised to body mass where $P > 0.102$, $d < 0.85$).

For soleus, maximal PO and PO normalised to muscle mass there was a significant main effect of CF (Fig 6.2D & E. $P < 0.001$, $\eta p^2 = 0.675$), which identified maximal PO occurred between 3 and 4Hz ($P > 0.084$) with the PO values from the remaining cycle frequencies being significantly lower ($P < 0.04$), except between 2 and 4Hz for absolute PO, albeit the difference was approaching significance ($P = 0.055$).

For maximal soleus PO normalised to body mass there was a significant HFD*CF interaction (Fig 6.2F. $P = 0.008$, $\eta p^2 = 0.139$). Bonferroni pairwise comparisons indicated that in SLD groups PO did not differ between 2 and 5Hz ($P > 0.999$) and 3 and 4Hz ($P > 0.999$), but all other cycle frequencies were lower ($P < 0.01$). In HFD groups PO did not differ between 2 and 4Hz ($P > 0.999$) and 3 and 4Hz ($P = 0.088$), but all other cycle frequencies were significantly different ($P < 0.012$).

For soleus, absolute PO and PO normalised to muscle mass there were no significant main effects of HFD ($P > 0.262$, $\eta p^2 < 0.039$), RES ($P > 0.141$, $\eta p^2 < 0.066$) or HFD*RES interaction (Fig 6.2D & E $P > 0.436$, $\eta p^2 < 0.019$). For PO normalised to body mass, HFD groups produced significantly lower PO when compared to SLD groups (Fig 6.2F $P < 0.001$, $\eta p^2 = 0.324$). There was no effect of RES ($P = 0.423$, $\eta p^2 = 0.020$) or HFD*RES interaction ($P = 0.379$, $\eta p^2 = 0.024$).

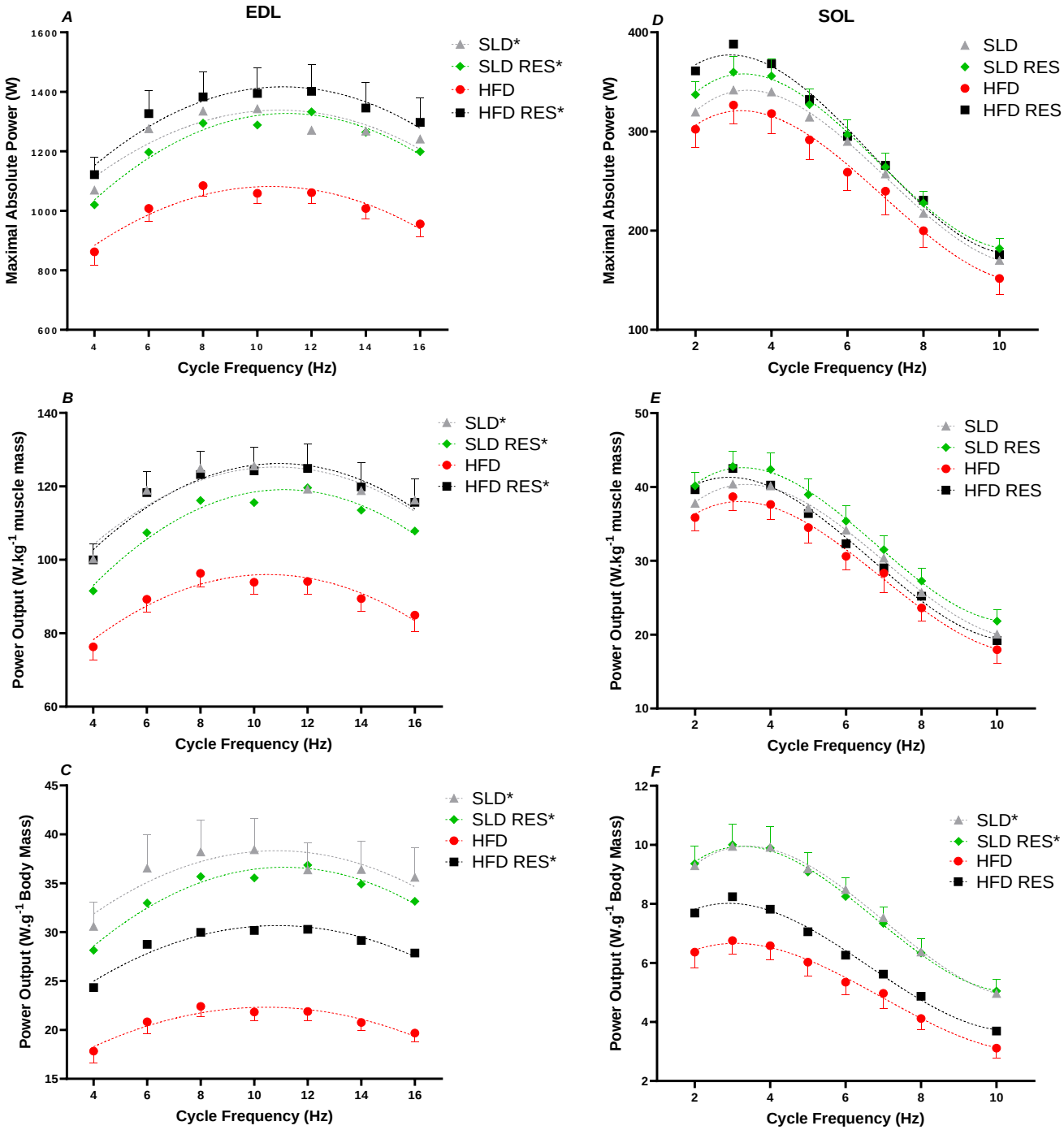

Figure 6. 2. *The effect of 12 weeks high-fat diet and resveratrol on the work loop power output-cycle frequency relationship for absolute power output (Watts), power output normalised to muscle mass (Watts per kg of muscle mass) and power output normalised to body mass (Watts per gram of body mass) of isolated mouse EDL (A, B and C respectively) and soleus (D, E and F respectively). EDL n = 10 for SLD RES and HFD RES, n = 8 for SLD and n = 9 for HFD; soleus n = 10 for SLD RES and HFD RES and n = 8 for SLD and HFD. Data presented as mean ± SEM; * indicates significant difference at P < 0.05; some error bars omitted for clarity.*

6.4.4 – Fatigue and recovery

As per chapter 5, the lower stimulation frequency used for assessment of fatigue resulted in a significant reduction in maximal WL PO normalised to muscle mass of the soleus and EDL (Fig 6.3 $P < 0.001$, , $\eta p^2 > 0.799$), but the magnitude of reduction in PO was not influenced by diet or RES ($P > 0.211$, $\eta p^2 < 0.048$).

For the EDL, time to 50% fatigue was significantly lower in RES treated groups when compared to control groups (Fig 6.4A $P = 0.035$, $\eta p^2 = 0.132$). No significant HFD effect ($P = 0.799$, $\eta p^2 = 0.002$) or HFD*RES interaction were observed ($P = 0.162$, $\eta p^2 = 0.060$). For the soleus, there were no significant effects of HFD (Fig 6.4D $P = 0.166$, $\eta p^2 = 0.059$), RES ($P = 0.915$, $\eta p^2 < 0.001$) or HFD*RES interaction ($P = 0.966$, $\eta p^2 < 0.005$).

For the EDL, a significant RES*HFD interaction was observed for cumulative work after 50 consecutive WL's (Fig 6.4B $P = 0.049$, $\eta p^2 = 0.116$). Bonferroni multiple comparisons indicated that cumulative work in HFD treated only group was lower compared to all other groups ($P > 0.037$, $d > 1.03$), but there were no other differences ($P > 0.477$, $d < 0.34$). For the soleus, HFD groups produced significantly lower cumulative work after 50 consecutive WL's when compared to SLD groups (Fig 6.4E $P = 0.014$, $\eta p^2 = 0.175$). There was no significant main effect of RES ($P = 0.315$, $\eta p^2 = 0.031$) or a RES*HFD interaction ($P = 0.963$, $\eta p^2 < 0.001$).

For recovery of maximal PO of the EDL and soleus, there were no significant time*HFD (Fig 6.4C & E $P > 0.657$, $\eta p^2 < 0.007$), time*RES ($P > 0.773$, $\eta p^2 < 0.003$) or time*HFD*RES ($P > 0.219$, $\eta p^2 < 0.049$) interactions. There was a significant main effect of time ($P < 0.001$, $\eta p^2 = 0.630$) for the EDL, indicating significant recovery of maximal PO every 10 minutes. This was not observed in the soleus ($P = 0.08$, $\eta p^2 = 0.079$), where maximal PO did not significantly change over the 30 minutes recovery period. The average recovery of each group being ~97-100% of pre fatigue maximum PO during the first set of WL's 10 minutes post fatigue, indicative of a physiological reduction in PO during the fatigue test rather than damage to the muscle preparation. For both the EDL and soleus, there were no significant

effects of HFD ($P > 0.144$, $\eta p^2 < 0.079$), RES ($P > 0.181$, $\eta p^2 < 0.057$) or HFD*RES interactions ($P > 0.829$, $\eta p^2 < 0.005$) on the ability of the muscle to recover maximal PO following the respective fatigue protocols.

Figures 6.5 and 6.6 illustrate typical WL shape during at a standardised time point for mouse soleus and EDL in each respective group. The area within the WL represents the net work done during the length change cycle. The area within the typical EDL WL traces (Figure 6.5) appears initially smaller in the HFD only groups (Figure 6.5C) compared to all other groups (Figure 6.5A, B & D), but the shape of the loop is consistent across groups. The reduction of the area within the loop, and subsequent change in WL shape exhibited during the fatigue protocol appears uniform irrespective of diet or treatment. However, typical soleus WL shapes (Figure 6.6) demonstrate that at the later stages of the fatigue protocol (WL 30 and onward) there is greater force production during muscle re-lengthening in HFD groups (Figure 6.6C & D) when compared to SLD groups (Figure 6.6A& B). As a result, from WL 30 there is a marked reduction in the size of WL shape relative to the initial WL in HFD groups.

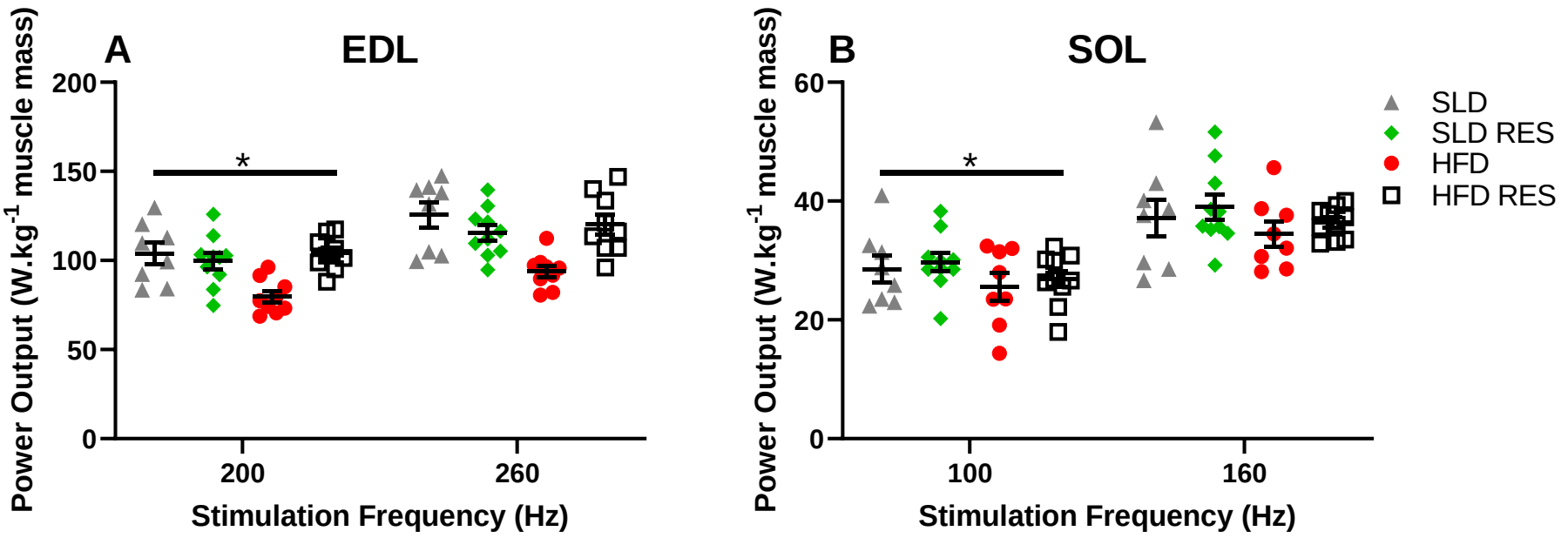

Figure 6. 3. *The effect of stimulation frequency, and 12 weeks high-fat diet and resveratrol on maximal work loop power output normalised to muscle mass (Watts per kg of muscle mass) of isolated mouse EDL (A) and soleus (B). EDL n = 10 for SLD RES, n = 8 for SLD and n = 9 for HFD and HFD RES; soleus n = 10 for SLD RES and HFD RES and n = 8 for SLD and HFD. Data presented as mean ± SEM; * indicates significant difference between stimulation frequencies P < 0.05.*

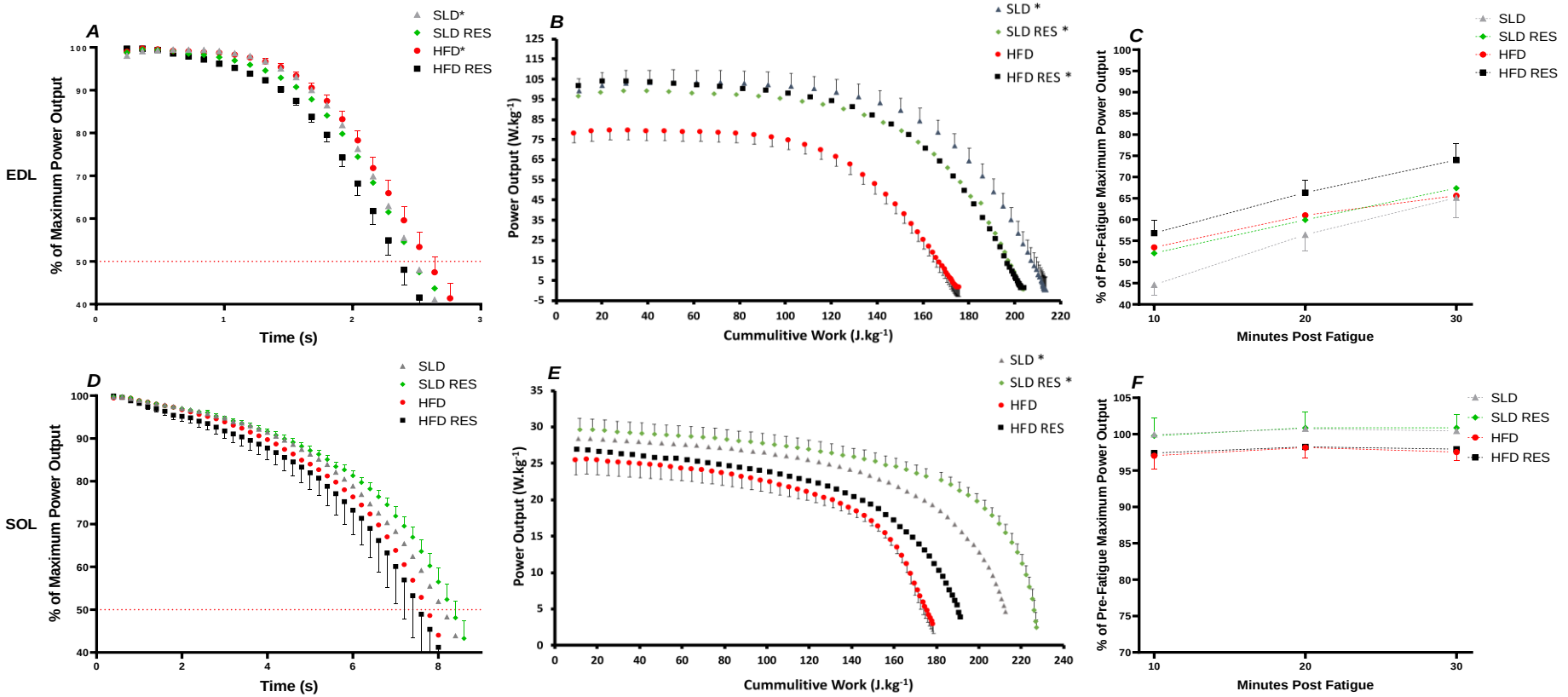

Figure 6. 4. *The effect of 12 weeks high-fat diet and resveratrol on net muscle power output relative to maximum power output during fatigue (A & D), on the relationship between power output normalised to muscle mass (Watts per kg of muscle mass) and cumulative work normalised to muscle mass (Joules per kg muscle mass) produced during 50 consecutive work-loop cycles (B & E), and on the recovery of maximal work loop power output over 30 minutes post fatigue (C & F) of isolated mouse EDL (A, B & C) and soleus (D, E & F). EDL n = 10 for SLD RES, n = 8 for SLD and n = 9 for HFD and HFD RES; soleus n = 10 for SLD RES and HFD RES and n = 8 for SLD and HFD. Data presented as mean ± SEM; * indicates significant difference at P < 0.05; some error bars omitted for clarity.*

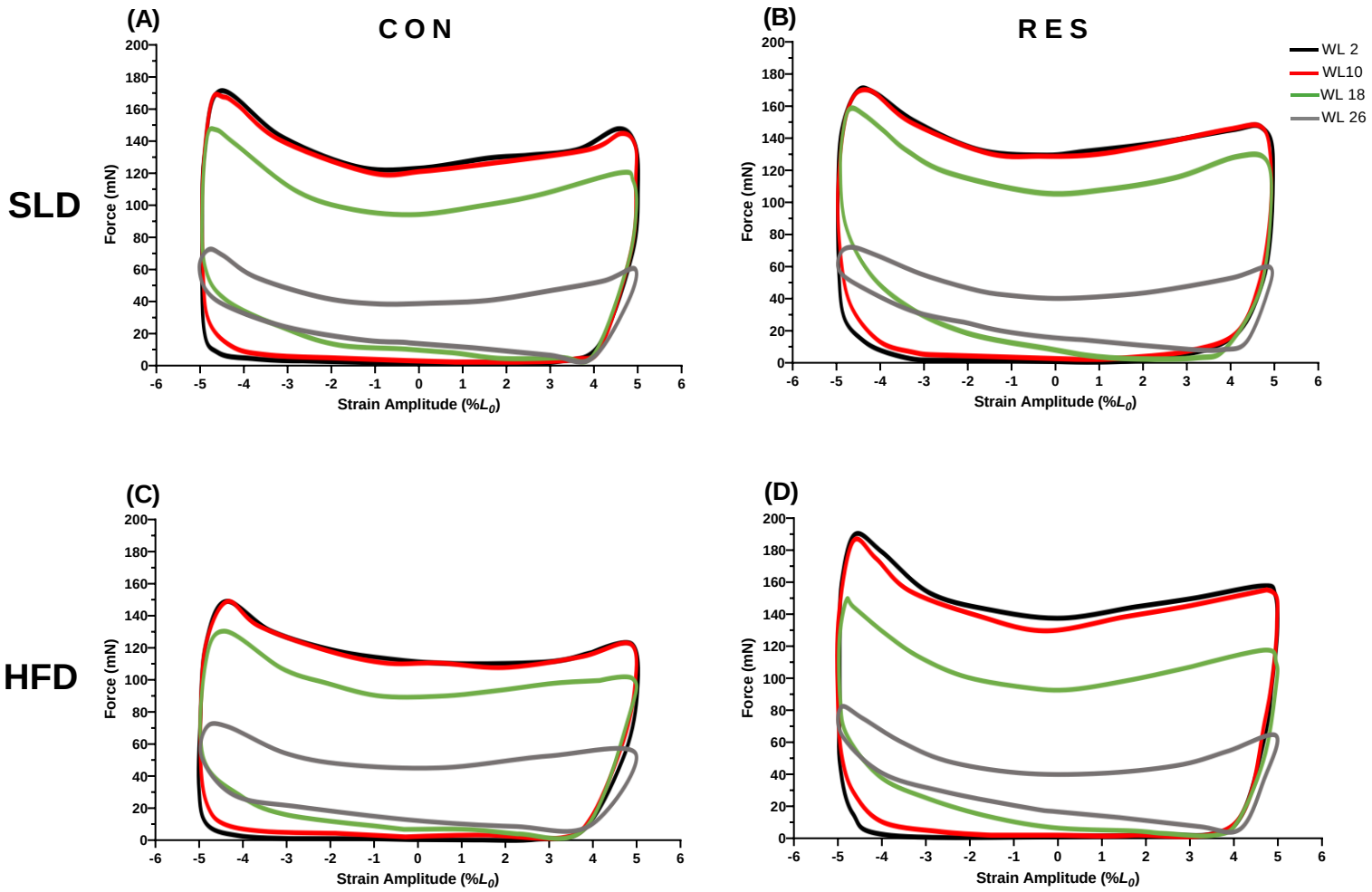

Figure 6. 5. *Effects of 12 weeks high-fat diet and resveratrol (A, SLD; B, SLD RES; C, HFD; D, HFD RES) on typical work loop shapes during muscle fatigue at 10Hz cycle frequency for mouse EDL. Figures plotted as force against strain (%L_0). Work loops, 2, 10, 18 and 26 of the fatigue protocols are shown for each group. Work loops are performed in the anti-clockwise direction, with the work loop starting at L_0.*

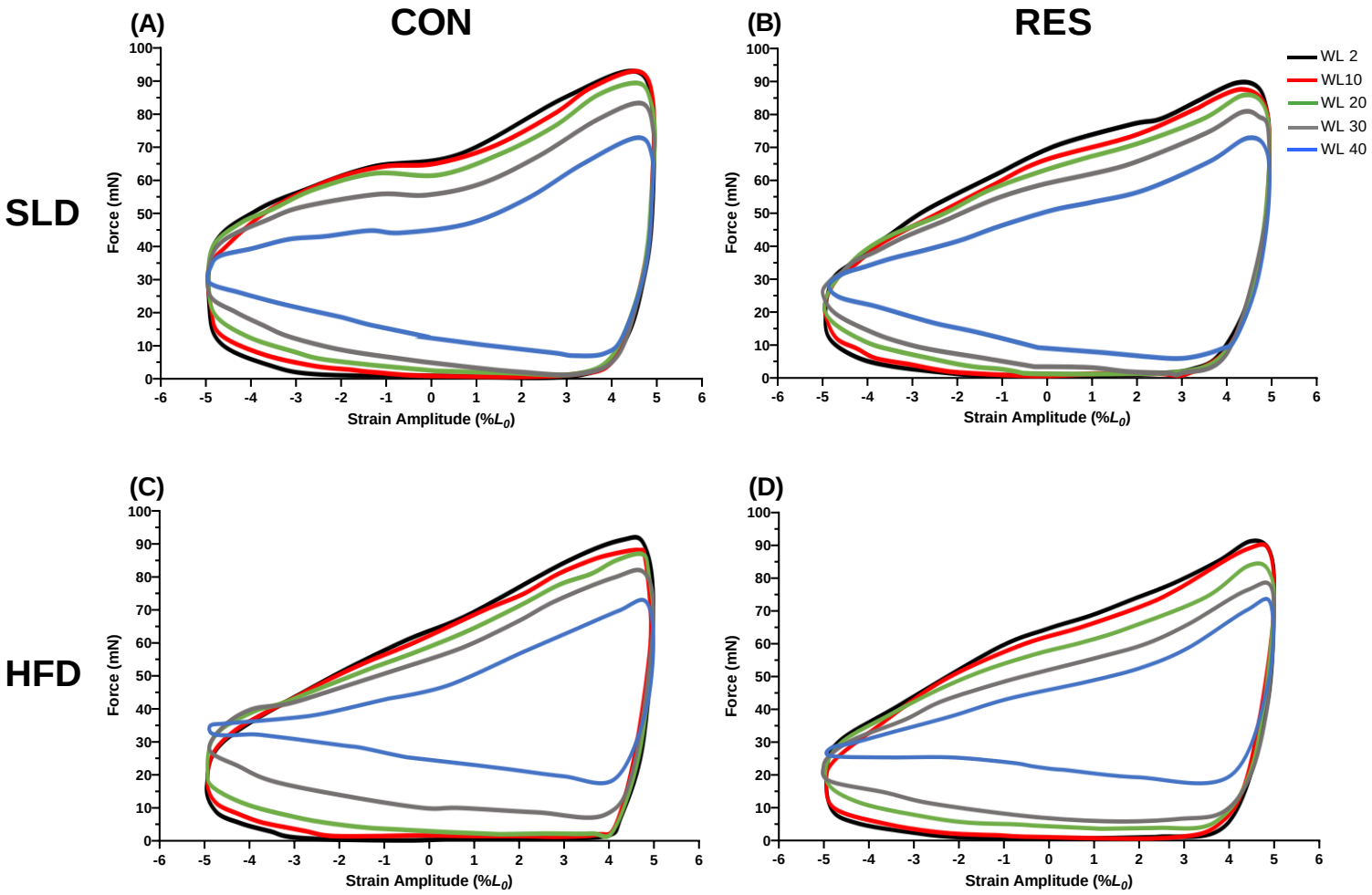

Figure 6. 6. *Effects of 12 weeks high-fat diet and resveratrol (A, SLD; B, SLD RES; C, HFD; D, HFD RES) on typical work loop shapes during muscle fatigue at 5Hz cycle frequency for mouse soleus. Figures plotted as force against strain (%L_0). Work loops, 2, 10, 20, 30 and 40 of the fatigue protocols are shown for each group. Work loops are performed in the anti-clockwise direction, with the work loop starting at L_0.*

6.5 – Discussion

The present work examined the effect of 12 weeks of HFD and RES supplementation on maximal force, power and fatigability of isolated soleus and EDL. Using a methodological approach which considers and reflects *in vivo* skeletal muscle mechanics (Josephson 1985; James et al. 1995), the present study provides a detailed investigation into the anti-obesogenic properties of RES and the effects RES has on the contractile performance of isolated skeletal muscle in both SLD and HFD groups of female mice. These present results indicate that HFD consumption has little effect on the isometric properties of isolated skeletal muscle, but leads to reductions in normalised WL PO and cumulative work during fatiguing contractions of both the soleus and EDL, and in the case of EDL, reductions in absolute PO and muscle quality (PO normalised to muscle mass). Whilst RES had little effect on contractile performance in SLD treated muscle, when synergistically consumed with a HFD, RES attenuated the previously described HFD induced reductions in contractile performance of the EDL. This data infers that RES may be an effective nutritional strategy to abate HFD induced reductions in fast twitch skeletal muscle function.

6.5.1 – Resveratrol and High-fat Diet Effects on Maximal Force and Power

The effects of HFD on the contractile properties of isolated soleus and EDL are described in detail in chapter 5 as the same controls were used for both treatment studies. As such, the discussion will focus on the effects of RES on isolated muscle mechanics. Supplementation of RES consumed with SLD had little effect on acute force and power production. However, when consumed synergistically with a HFD, RES had contractile mode and muscle specific effects on contractile measures, which are more apparent in fast twitch muscle. In the soleus, RES did not attenuate any HFD effects on PO normalised to body mass or cumulative work production during fatigue but did evoke an increase in absolute soleus tetanus force in the HFD RES group when compared to HFD only. The effect may be explained by RES inciting processes involved with myogenesis (Montesano et al. 2013), promoting

more effective adaptation to the elevated load placed on the postural muscles, compared to the HFD where no effect was observed. The non-significant but average increase in CSA (~9.1%; *d* = 0.73) in HFD RES soleus compared to HFD supports the notion of effective adaptation to larger body mass and in part could explain increased absolute force; providing a plausible explanation for why despite an increase in absolute force, no effect on isometric stress was identified. As such, HFD RES may produce a larger soleus of equal quality. However, it should be noted that stress was ~14.7% greater, with a moderate effect size (*d* = 0.88), in HFD RES compared to a HFD, but did not reach statistical significance. Thus, the potential of RES improving isometric force in slow phenotype HFD treated muscle remains ambiguous and requires further exploration.

In fast twitch EDL muscle, RES may help to alleviate the adverse effects of a HFD on absolute and normalised PO and muscle quality. This novel finding could have important implications, providing evidence to support the supplementation of RES as a cost-effective initial step, which requires minimal lifestyle modification, in combating the obesity induced reduction in muscle function. In obese individuals, improved contractile capacity could negate the reduction in the ability to be physically active and perform activities of daily living, thus limiting the accumulation of adipose tissue, improving quality of life and reversing a negative cycle of obesity. Although, future research is still needed to examine if the specific effects identified in isolated rodent tissue translate to other species i.e., humans.

The attenuation of HFD induced reductions in contractile performance through consumption of RES are likely more pronounced in isolated EDL since HFD effects are more substantial in fast twitch muscle. Mechanistically, RES could attenuate the adverse effects of HFD on EDL PO through altering fibre type composition (fast to slow) (Wen et al. 2020; Huang et al. 2020). Previous observations suggest HFD effects on maximal PO may be in part due to a slow to fast shift in fibre type composition (Seebacher et al. 2017; Tallis et al. 2018). However, this would not appear to be a driving mechanism

in this instance as the CF's used to elicit maximal PO (Fig 6.2), and the shape of the power output-cycle frequency curves appeared unaffected irrespective of diet or treatment. This idea is supported by previous work utilising isolated skeletal muscle from female CD-1 mice, reporting no changes in myosin heavy chain composition between lean and HFD treated EDL (Tallis et al. 2017; Messa et al. 2020). Therefore, based on these data indicating reduced accumulation of adipose in HFD when supplemented with RES (see Table 12), it is more likely that one of the key factors reducing the HFD induced reduction in EDL PO is through RES limiting the magnitude of adipose (Lagouge et al. 2006; Kim et al. 2011; Cho et al. 2012; Shabani et al. 2020) and intramuscular lipid accumulation (Shabani et al. 2020). Given the magnitude of change in EDL PO between HFD and HFD RES groups, enhanced lipid metabolism alone is unlikely to be the only mechanism attenuating contractile performance. As such, RES may act to offset the HFD induced reduction in 5'-adenosine monophosphate-activated protein kinase (AMPK) activity (Shabani et al. 2020), a whole body regulatory system which controls several mechanisms which contribute to skeletal muscle health, including lipid metabolism and protein synthesis (Wang et al. 2018). Research demonstrates that obesity decreases and inhibits AMPK activity, which can promote fat deposition through impaired lipid metabolism, increased chronic low-grade inflammation and oxidative stress, and impaired processes involved in myogenesis (Steinberg et al. 2006; Fu et al. 2013; Tallis et al. 2018; Lyons and Roche 2018). Consequently, enhanced AMPK activity reported in HFD treated muscle when supplemented with RES (Shabani et al. 2020), at a comparable dose as the present research ($4g.kg^{-1}$ of diet), could be a driving mechanism for maintained EDL PO. However, to date there is no available evidence directly linking the effects of RES on contractile performance with the mechanisms mediating muscle function. As such, reasons for why RES abates the adverse effects of a HFD on EDL PO remain speculative and warrant further investigation.

6.5.2 – Resveratrol and High-fat Diet Effects on Fatigue Resistance

RES had no effects on soleus fatigue and recovery. In EDL, time to fatigue was higher in RES treated muscle when compared to controls, although, this effect was not apparent when considering total cumulative work. In fact, HFD RES may alleviate the reduction in cumulative work established in HFD. The preservation of acute power in HFD RES EDL appears to translate into greater cumulative work during fatiguing contractions, as greater work is produced per WL cycle. For *in vivo* considerations, cumulative work is an important variable as the adverse effects of HFD on cumulative work are likely to be compounded when the isolated muscle, which already had diminished ability to produce work over consecutive contractions, is required to move a larger body mass. A reduction in cumulative work has important implications for a negative obesity cycle, as this will likely to lead to diminished exercise capacity and reduced ability to complete activities of daily living which require consecutive contractions. In addition to preserving absolute EDL PO and muscle quality, the present findings demonstrate that RES attenuates a HFD induced reduction in EDL cumulative work. As such our findings supports the suggestion that RES could play a part in reversing a negative obesity cycle by offsetting the decline in physical function and exercise capacity (Tallis et al. 2021)

6.5.3 – Limitations and Future Direction

One limitation of the present study is providing supplementation of RES through dietary enrichment in grouped cages, opposed to a method whereby accurate dosage per animal can be monitored i.e., oral gavage or individual housing. Dosing through direct methods ensures you can accurately determine the dose of supplement being provided to each individual mouse. However, both individual housing and oral gavage can induce significant stress upon the animal (Brown et al. 2000; Bonnichsen et al. 2005; Manouze et al. 2019), which in this instance can be mitigated by providing the RES as part of their diet, in cages containing multiple mice. Whilst it was not possible to determine the exact quantity of RES consumed, the approximation based on previous research which

reported that RES enriched in to a diet at 4g/kg, as used in the present study, equates to approximately 400mg/kg body mass (Lagouge et al. 2006).

Whilst the present study provides an insight into the potential therapeutic use of RES for alleviating the detrimental effects of a HFD on contractile performance of skeletal muscle, there are still several avenues for future research. Firstly, dose response for RES should be examined to determine the optimal dose for improved muscle contractility. Recent research also identified that HFD induced reductions in muscle performance are contractile mode and muscle specific, whereby changes in concentric and eccentric muscle quality are not concurrent (Shelley et al. 2021). This is likely a result of the different physiological mechanisms resulting in concentric and eccentric force production. Adequate eccentric force is important for maintaining balance, deceleration and absorbing impact, which occurs during activities such as stair descents, descending into a seated position and during activities which require dynamic balance (Delbaere et al. 2003; Nishikawa et al. 2018a). As such, future work should consider the therapeutic effects of RES on a HFD induced reduction in eccentric performance of locomotor muscles. Furthermore, for both HFD and RES consumption, future work should consider longer durations of feeding to understand the responses to long term HFD consumption, to better replicate *in vivo* obesity, where many individuals will have been obese for most of their adult life. Finally, the present data indicates RES may reduce the impact of HFD consumption on contractile performance of fast twitch EDL, therefore, future work should explore the therapeutic effects of RES on skeletal muscle health in other disease models which share a similar mechanistic response as a HFD, such as aging (Pérez et al. 2016).

There are other animal models which should be recognised and could be used in future research studies to confirm and expand upon the results presented here and elsewhere in this thesis. The mouse lemur (*Microcebus murinus*), a non-human primate, has been suggested as an ideal animal

model to explore the effects of ageing due to their phylogenetic proximity to humans (Ezran et al. 2017). When held in captivity mouse lemur life expectancy can reach 9-11 years old (compared to ~3 years old in the wild) (Castanet et al. 2004); from 5 years of age mechanistic changes associated with ageing in humans begin to occur in mouse lemurs (Bons et al. 2006). Given ageing and obesity share similar mechanistic responses (Pérez et al. 2016) future research may wish to consider utilising mouse lemur as a model to explore HFD effects on muscle performance and potential therapeutic strategies to alleviate the detrimental effects of obesity. A zebrafish model (*Danio rerio*) is another animal model which has been extensively used and has provided important insight into the effects of dietary-induced obesity on muscle performance (Seebacher et al. 2017, 2019). Many of the signaling pathways associated with energy homeostasis are similar between humans and zebrafish (Song and Cone 2007; Oka et al. 2010), highlighting zebrafish as another suitable animal model which could be used in future work to expand upon the present results. However, the use of a mouse model (*mus musculus*) was used in the present thesis for numerous reasons including: hindlimb mouse muscle are close analogues to human lower limb muscle (Charles et al. 2016); mouse model has been extensively used to examine the impact of HFD feeding on skeletal muscle health, which allows for direct comparison to previous work; whilst there is opportunity for further refinement of protocols to examine isolated muscle contractile performance, the protocols used for isolated mouse muscle are well established when compared to other animal models; like zebrafish, signaling pathways associated with energy homeostasis are similar between humans and rodents (Oka et al. 2010).

6.5.4 – Conclusion

In summary, HFD effects were shown to be muscle and contractile mode specific, causing limited effects on isometric properties in EDL and soleus, and soleus PO, but a reduction in absolute and normalised EDL PO and cumulative work in both fast and slow twitch muscle. These results show that when consumed with a SLD, RES has limited effects on contractile function, irrespective of mode of contractility or muscle fibre type composition. However, when consumed with a HFD, RES reduced

the accumulation of adipose tissue and ameliorated the reduction in absolute and normalised power output, and cumulative work during fatiguing contractions, of fast-twitch EDL. These findings suggest RES could be a low-cost and appealing nutritional strategy to offset the obesity induced decline in skeletal muscle function, potentially improving physical function and may be the first step in reducing the impact of a negative obesity cycle.

Chapter 7 – Summary of Key Findings and General Conclusions

The data presented in this thesis provide an important and novel insight into the contractile mode and muscle specific effects of high adiposity and high fat diet (HFD) consumption on skeletal muscle contractility, utilising a combination of *in vivo* and *in vitro* methods. Furthermore, the findings from this thesis have developed the work loop protocol, improving the *in vivo* replicability of data on contractile mechanics obtained utilising isolated skeletal muscle. Finally, this thesis provides a detailed account of the effects of vitamin D and RES upon whole animal and muscle morphology, and isolated skeletal muscle contractility, in SLD and HFD conditions.

A summary of the key findings presented in the thesis are described below in chronological order:

1. High adiposity did not influence absolute torque production in human males irrespective of age (18-30 or 60-80 years old), mode of contractility (concentric or eccentric), contractile velocity (60, 120 or 180° s^{-1}) or muscle group (elbow [EE, EF] and knee [KE, KF] extensors and flexors) assessed using isokinetic dynamometry. The lack of adaptation in absolute torque despite an increase in body mass may be related to a reduction in activation capacity and impaired myogenesis in muscle from obese individuals. ***Chapter 3***

2. High adiposity resulted in a reduction in concentric and eccentric torque normalised to body mass from lower limb musculature and a reduction in concentric torque normalised to body mass from upper limb musculature of both young and older males. High adiposity only diminished eccentric torque normalised to body mass from the EF in young males. Such evidence offers a detailed insight into the muscle, age, and contractile mode specific response to obesity. ***Chapter 3***

3. Concentric muscle quality (torque normalised to segmental muscle mass) was generally diminished by high adiposity in each muscle group assessed, expect for the EE where no effect

was observed. However, high adiposity only diminished eccentric muscle quality in the EF and KF. These effects were uniform across young and older male adults. The data from this thesis indicates that reductions in eccentric muscle quality are not concurrent with changes in concentric muscle quality, likely due to differences in how obesity effects the physiological mechanisms resulting in concentric and eccentric force production. ***Chapter 3***

4. Rate of fatigue (percentage decline in torque relative to maximum) was generally unaffected by adiposity, irrespective of age, mode of contractility or muscle group assessed in young and old male adults. Whilst the effects of obesity on fatigue resistance *in vivo* remain ambiguous, the findings presented in this thesis support the suggestion that increased fatigue during whole body locomotor tasks is in part due to having to overcome an elevated body inertia ***Chapter 3***

5. The stimulation frequency needed to evoke maximal work loop power output (260Hz) of isolated mouse EDL, exceeded that needed to evoke maximal isometric force (230Hz). As such, previous research examining the impact of HFD consumption on isolated skeletal muscle contractility, which has implemented a fixed stimulation frequency for all contractile assessments (typically for isometric and concentric power output), may have underestimated true maximal power output ***Chapter 4***

6. Increasing stimulation frequency evoked an increased rate of fatigue (percentage decline in power output relative to maximum), but total cumulative work remained unchanged irrespective of stimulation frequency. The highest stimulation frequencies (230 and 260Hz) evoked a large negative work component, atypical of *in vivo* fatigue mechanics. Excessive negative work is less prevalent at lower stimulation frequencies (140 and 200Hz). Stimulation frequency of 200Hz appears near optimal for replicating maximal *in vivo* fatigue mechanics; it

evokes a high amount of cumulative work, with the relative change in work done during shortening (as indicated by the WL shapes) being like the highest stimulation frequencies, but with minimal negative (eccentric) work. Sub maximal stimulation frequency for assessment of fatigue should be adopted across isolated skeletal muscle work. This is particularly important for the assessment of HFD effects on fatigue resistance, where the previously reported reduction in rate of fatigue may have been contributed to by mechanical fatigue. ***Chapter 4***

7. HFD consumption for 12 weeks in young CD-1 female mice resulted in an increase in whole animal body mass and fat pad mass, but did not influence morphology of the Soleus or EDL ***Chapter 5 &6***

8. HFD induced reductions in contractile performance of isolated skeletal muscle were contractile mode and muscle specific, much like that reported *in vivo* within experimental chapter 3. Absolute isometric force was unaffected by HFD in both the soleus and EDL. However, absolute power output was diminished in HFD treated EDL but not soleus. Isometric stress and power output normalised to muscle mass remained unaffected by a HFD in the soleus. However, in HFD EDL, isometric stress was either lower (Chapter 5) or unchanged (Chapter 6) and power output normalised to muscle mass was significantly reduced. HFD resulted in impaired power output normalised to body mass in both the soleus and EDL. The findings from this thesis suggest the impact of a HFD on contractile performance is more substantial in fast twitch muscle; attributed to a reduced capacity to metabolise lipid compared to muscle with a more predominant slow-twitch phenotype, resulting in greater lipid accumulation ***Chapter 5 &6***

9. In both the soleus and EDL, cumulative work production was significantly impaired by HFD consumption, although rate of fatigue remained unchanged. The mechanisms for a HFD

induced reduction in cumulative work reported in this thesis does not appear uniform between muscles. Based on work loop shapes plotted from the fatigue data, reduced cumulative work in the EDL is likely a consequence of the reduction in acute absolute power output, reducing the amount of work performed per cycle. In the soleus, where acute power output is unaffected by a HFD, reduced cumulative work appears to be a result of the accumulation of increased relaxation time, gradually increasing negative work during muscle elongation, becoming substantial after several consecutive contractions. ***Chapter 5 &6***

10. Vitamin D supplementation did not affect whole body or muscle morphology in either dietary group (standard or HFD). Furthermore, irrespective of muscle (soleus or EDL), dietary composition, and mode of contractility (isometric force, concentric power output or fatigue resistance) a high dose of vitamin D did not significantly affect contractile performance. It may be that the high dose of vitamin D resulted in alterations in the key metabolic processes responsible for vitamin D metabolism, ultimately evoking vitamin D dysregulation. ***Chapter 5***

11. Resveratrol supplementation in HFD treated mice reduced central adipose accumulation compared to HFD only treated counterparts. Resveratrol supplementation in HFD treated mice did not affect overall body mass or morphological characteristics of isolated soleus or EDL. It has been proposed that RES reduces adipose accumulation in HFD treated mice via stimulation of the lipolytic and oxidative pathways. ***Chapter 6***

12. Absolute isometric force of soleus from HFD and resveratrol treatment group was significantly greater than that of HFD only soleus, although there was no effect on isometric stress. This could imply an attenuation of a HFD induced reduction in muscle activation capacity and

myogenesis. Aside from this, resveratrol had limited effects on the contractile performance of isolated soleus. ***Chapter 6***

13. Resveratrol consumption with a HFD alleviated the reduction in absolute and normalised power output, and cumulative work production of EDL associated with HFD only. The attenuation of power output and cumulative work in fast twitch muscle through resveratrol consumption is likely a combination of alterations to mechanisms involved in HFD muscle dysfunction, such as AMPK activity and lipid metabolism. This novel data presented in this thesis could have important implications as resveratrol may be a cost and time effective initial step in offsetting the decline in contractile function associated with obesity.***Chapter 6***

14. Despite evidence from this thesis identifying both HFD and resveratrol induced changes in power production of EDL, neither changed the shape of the work loop power output-cycle frequency curve, indicating limited changes in intrinsic rate specific mechanical properties of muscle, such as maximal shortening velocity. As such, it is likely neither treatment resulted in significant alterations in EDL fibre type composition. ***Chapter 5 &6**

7.1 – General Discussion

7.1.1 – Recap

Obesity can lead to, and exacerbate, declines in physical and mental health (Larsson et al. 2002), and provides substantial economic burden on government and health care providers (Kjellberg et al. 2017). Whilst the effects of obesity on the whole-body system are multifactorial, the effect it has on the musculoskeletal system could play a key role in reducing the ability to remain physically active, and may exacerbate potential adverse health effects (Tallis et al. 2018). Detailed understanding of the adverse effects of obesity on skeletal muscle function are not only integral to our understanding of

how obesity affects physical function but provides the opportunity to identify and highlight specific strategies to alleviate any potential reductions in contractile performance. Research examining obesity and skeletal muscle contractility typically focuses on a single mode of contractility or single muscle/ muscle group, which is limited in its application given that different modes of contractility play important roles in different activities of daily living (Choi 2016) and muscles/muscle groups vary in both form (e.g., architecture and fibre type distribution) and function (Frontera and Ochala 2015). Furthermore, current lifestyle interventions to abate the adverse effects of obesity appear unsustainable for most people in the longer term (Fildes et al. 2015; Hall and Kahan 2018). Therefore, nutritional supplements offer a cost and time effective alternative to current strategies to mitigate the adverse effects of obesity (Tallis et al. 2021), but their effect on contractile function are seldom explored. Utilising a range of experimental models, this thesis provides an in-depth analysis of the age, contractile mode, and muscle/muscle group specific effects of obesity on muscle function and a detailed insight into the effects of vitamin D and resveratrol on the contractile properties of isolated fast and slow twitch muscle from HFD and SLD treated mice.

7.1.2 – High Adiposity and High-fat Diet Effects on the Contractile Function of Skeletal Muscle

This thesis examined the effects of high adiposity and HFD consumption in human and rodent models, in muscles from a range of anatomical locations, which differ in function and are composed of different fibre types, across multiple contractile modalities. This approach has been valuable as it has allowed for a holistic understanding of the impact of obesity on skeletal muscle contractility both *in vivo* and at the isolated muscle level. Whilst this thesis presents data indicating that the effects of high adiposity and HFD consumption on skeletal muscle contractility are highly complex and not entirely uniform across age groups, muscles/muscle groups or mode of contractility assessed, there are still general trends observed across the experimental models used. Firstly, the data presented here identifies that

high adiposity or HFD consumption in most cases has no effect on absolute function, irrespective of age (**chapter 3**) or mode of contractility (concentric and eccentric torque: **chapter 3**; isometric force and concentric power output: **chapter 5 & 6**). There is evidence from humans and rodent models contrary to observations presented in this thesis, indicating that obesity and HFD consumption can lead to in an increase in absolute function of some muscles, occurring as compensatory mechanism evoked by a sustained mechanical loading and need to overcome a greater bodily inertia for locomotion (Maffiuletti et al. 2013; Tomlinson et al. 2016). However, as presented in this thesis, there is substantial evidence to indicate high adiposity and HFD result in no change, or to a lesser extent, a reduction in absolute contractile performance (Hulens et al. 2001; Lafortuna et al. 2005; Paolillo et al. 2012; Pajoutan et al. 2016; Hurst et al. 2019). Unchanged or reduced absolute performance is likely a consequence of reduction in activation capacity (Tomlinson et al. 2014a) and reduction in the processes involved in myogenesis (O'Leary et al. 2018), the latter limiting the ability of muscle to adapt and respond to the increase in bodily inertia. The adverse effects of obesity on contractile function appear more profound in fast twitch fibres, likely due to an unfavourable metabolic profile for oxidising lipids (Tallis et al. 2017), hence a reduction in absolute power output of predominantly fast twitch EDL but unchanged in predominantly slow twitch soleus (**Chapter 5 & 6**). It should be noted that a reduction in absolute power output of the EDL appears related to the magnitude of adipose and intramuscular lipid accumulation, which could be the catalyst for a host of systemic effects, such as chronic inflammation, culminating in reduced absolute power production. An increase in intramuscular lipids of isolated mouse EDL occurs earlier in fixed HFD feeding (~12 weeks) (Eshima et al. 2017) than a high fat forage diet (~16 weeks) (Messa et al. 2020), which is likely why the reduction in EDL absolute power output occurs earlier (12 weeks) in the present thesis compared to previous research, where absolute power output of the EDL remains unchanged (Tallis et al. 2017; Hurst et al. 2019).

Skeletal muscle function normalised to body mass was adversely affected by obesity in both human and isolated rodent muscle, with very few exceptions; consistent across age, contractile mode, and muscles/muscle groups. The reduction in contractile performance normalised to body mass is consistent across the literature, proposed to have severe consequences on functional capacity and may limit the ability of individuals to be physically active and perform activities of daily living (Maffiuletti et al. 2013; Tomlinson et al. 2016; Bollinger 2017; Tallis et al. 2018). Complexity and ambiguity exist around the effects of obesity on muscle quality (contractile function normalised to muscle size) (Tallis et al. 2018), although data presented in this thesis support the notion that obesity can adversely affect the intrinsic force producing capacity in some skeletal muscle (Tallis et al. 2017; Erskine et al. 2017). Changes in muscle quality are not always uniform across mode of contractility, muscles or muscle groups. These unique responses are highlighted in both experimental models, where in a human model concentric muscle quality (torque normalised to segmental muscle mass) is diminished in both the KF and KE, but eccentric muscle quality is only diminished in the KF (**chapter 3**). In isolated rodent tissue, a reduction in muscle quality is observed in the EDL but not the soleus. As such, the data suggests that high adiposity and HFD consumption can create larger (**chapter 3**) or equal (**chapter 5 & 6)** sized muscles of poorer quality, which are required to stabilise, support, and move a larger mass and require a much greater metabolic cost to maintain (Tallis et al., 2018).

The current results demonstrate that high adiposity or HFD consumption has limited effects on rate of fatigue relative to maximum torque or power, irrespective of contractile mode (**chapter 3**) or muscle/muscle group (**chapter 3, 5 & 6**). Assessing the relative decline in contractile performance over the time course of the fatiguing contractions provides information regarding the impact of high adiposity and HFD consumption on the rate of fatigue. Previous research considering rate of fatigue is limited and ambiguous, but in general supports these findings that obesity has limited effects on rate of fatigue in human (Maffiuletti et al. 2008; Minetto et al. 2012) or rodent models (Shortreed et al.

2009; Seebacher et al. 2014; Thomas et al. 2014; Tallis et al. 2017; Hill et al. 2019; Seebacher and James 2019; Hurst et al. 2019). Whilst relative decline provides an insight into rate of fatigue, cumulative work was also determined (**chapter 5 & 6**) to infer absolute differences in the fatigue response. This approach accounts for any differences in absolute performance, including differences which are not detectable during acute performance but could become apparent during repeated contractions. Despite rate of fatigue being unaffected by high adiposity or HFD consumption (**chapter 3, 5 & 6**), cumulative work was reduced in HFD treated EDL and soleus (**chapter 5 & 6**), which could have substantial impact on activities which require repetitive contractions, particularly when placed back into the body where the muscle, which already had diminished capacity to perform cumulative work, is required to repeatedly overcome a greater inertia to complete the task.

Whilst previous work has considered both absolute and relative function, data presented in this thesis are the first to demonstrate that obesity induced reductions in eccentric performance are not concurrent with changes in concentric function and are not uniform between upper and lower limb musculature. Furthermore, data from this thesis is the first to demonstrate that the effects of obesity and HFD consumption are largely comparable in both human and rodent models. The data collected across both experimental models indicates that faster muscle fibres and locomotor muscles which have a lesser contribution to postural control, are more susceptible to a high adiposity or HFD induced declines in contractile function.

7.1.3 – Isolated Skeletal Muscle Contractile Assessments

The benefits of isolated skeletal muscle models, and in particular the use of the WL technique, have been well established in the literature (Tallis et al. 2018; Nishikawa et al. 2018b) and discussed in detail in this thesis (chapter 4). However, there are still certain limitations of current WL protocols that have yet to be addressed, which in part relate to the *in vivo* replicability of fatigue data. One of

the main reasons for utilising isolated skeletal muscle models is to ascertain the effects of HFD consumption on skeletal muscle contractility which are difficult to accurately obtain using *in vivo* models, and to translate these findings to *in vivo* function. Therefore, before exploring the adverse effects of HFD consumption and potential therapeutic strategies to alleviate any decline in contractile performance, one aim of this thesis was to further refine WL protocols, specifically identifying optimal stimulation parameters for different contractile assessments.

Typically, experiments utilising the WL model will implement the stimulation frequency that yielded maximal isometric force, for assessment of power and fatigue (James et al. 1995; Ahn et al. 2003; Choi and Widrick 2009; Seebacher et al. 2014; Hessel and Nishikawa 2017; Kissane et al. 2018). However, this approach is limited for two primary reasons. Firstly, the stimulation force relationship for isometric activity cannot reliably predict force output for dynamic conditions (de Haan 1998; Caiozzo 2002). Secondly, high stimulation frequency during assessments of fatigue has been shown to evoke a large negative work component atypical of *in vivo* fatigue mechanics (Kissane et al. 2018; Hill et al. 2018), whereas fibre length and stimulation would likely be manipulated *in vivo* to reduce negative work in power producing muscles to minimise risk of muscle damage (Wakeling and Rozitis 2005). This thesis was the first to consider the effects of a range of stimulation frequencies on force, power, and fatigue resistance of isolated mouse EDL. There are two key findings from this chapter (**chapter 4**), which influenced the protocol used in later chapters (**chapter 5 & 6**). Firstly, the stimulation frequency needed to evoke maximal WL PO (260Hz) exceeded that needed for maximal isometric force (230Hz), meaning previous work has possibly underestimated true maximal WL PO. Secondly, sub maximal stimulation frequencies (140 & 200Hz) evoke a fatigue response more representative of *in vivo* fatigue and produce comparable cumulative work to the higher stimulation frequencies needed to evoke maximal force and PO. Based on the rate of fatigue, cumulative work production, and plotted WL shapes, 200Hz appeared to be near optimal for assessment of the fatigue response in isolated mouse EDL in this thesis.

7.1.4 – Nutritional Strategies to Reduce the Impact of HFD Consumption on Skeletal Contractile Function

This thesis is the first to consider the efficacy of nutritional supplementation, in the form of vitamin D and RES, for alleviating the detrimental decline in isolated skeletal muscle contractility associated with HFD feeding. Despite previous research providing mechanistic rationale to believe vitamin D could be effective in attenuating a HFD induced decline in muscle function through reducing adipose accumulation and chronic inflammation (Marcotorchino et al. 2014; Farhangi et al. 2017; Benetti et al. 2018), and increasing insulin signalling and contractile protein synthesis (Vanlint 2013), there were no changes in contractile performance when supplemented with a high dose (20,000IU/kg^{-1}) for 12 weeks. Based on previous data, it could be suggested that vitamin D can mechanistically improve skeletal muscle health (Latham et al. 2021) without evoking improved contractility (Carswell et al. 2018). However, it may be that high dose vitamin D over longer feeding durations (> 8 weeks) evokes dysregulation in the metabolic processes involved with vitamin D metabolism (Debruin et al. 2020) which is not prevalent over shorter durations (4 weeks) where improvements in tetanic stress of isolated soleus have been observed (Debruin et al. 2019; Hayes et al. 2019) in healthy rodent models. In previous work when exercise was combined with high dose vitamin D supplementation during longer feeding durations, vitamin D has been shown to evoke additional increases in soleus stress when compared to exercise alone. The authors suggested that exercise mediates vitamin D dysregulation through improved mitochondrial function (Debruin et al. 2020). Therefore, despite these results, the effects of vitamin D on isolated skeletal muscle contractility remain ambiguous and are likely influenced by dose, duration, and additional stimuli.

RES reduced the magnitude of increase in fat pad mass by 31% when compared to HFD only, similar to previous observations (Lagouge et al. 2006; Kim et al. 2011; Shabani et al. 2020), but did not affect overall body mass or muscle mass of soleus or EDL. The reduction in absolute and normalised PO and

cumulative work of HFD treated mouse EDL was attenuated with RES consumption. Whilst RES did not reduce the increase in body mass in HFD treated animals, maintained contractile performance of fast twitch muscle and reduction in the accumulation of adipose could help reduce the risks or severity of disease associated with obesity. This could have a substantial impact on the development and exacerbation of obesity and its related co-morbidities, offsetting any potential negative cycle of obesity. RES did not affect contractile performance in SLD treated muscle or reduce the impact of a HFD on soleus cumulative work. Therefore, it could be hypothesised that RES may induce attenuation of contractile performance in fast twitch muscle by targeting adipose and more specifically, a reduction in intramuscular lipids (Shabani et al. 2020). As previously described, the impact of a high adiposity and HFD consumption appears more substantial in fast twitch muscle, in part due to an unfavourable metabolic profile for oxidising elevated intramuscular lipids (Tallis et al. 2017).

7.2 – Limitations

Whilst the present thesis provides a detailed insight into the effects of obesity on skeletal muscle contractility, and potential nutritional strategies to abate any adverse effects of HFD consumption on force, power and fatigue resistance of isolated skeletal muscle, there are still several limitations which should be highlighted. A discussion of limitations has been presented in detail for each individual experimental chapter. Below is an overview of limitations which cover multiple chapters, which have not been presented within the respective chapters to avoid repetition.

7.2.1 - Chapter 4, 5 & 6

One limitation of the present thesis, and of many studies using the WL technique (Josephson 1985, 1993), is the use of sinusoidal length change waveforms. Sinusoidal length change waveforms are commonly used for WL assessments (James et al. 2004; Choi and Widrick 2009; Tallis et al. 2014; Hessel and Nishikawa 2017; Stoehr et al. 2020) as they provide a close approximation of the dynamic

in vivo cyclical muscle activities in some species, such as fish during steady swimming and steady insect flight (Josephson and Ellington 1997; Ellerby et al. 2000). However, in other species, muscles *in vivo* can often undergo complex length changes during real-life locomotion (Dickinson et al. 2000) and estimates of the muscle length change used by mouse EDL during running suggest deviation from a sinusoidal length change waveform (James et al. 1995).

Another limitation of this thesis is the tendon at the proximal end of the tissue (soleus and EDL) remained intact during contractile assessments. As such, it is possible that length change during repetitive fatiguing contractions would gradually reduce because of tendon creep evoked by sustained mechanical stress (Wren et al. 2003). However, the length of tendon that is unclamped was minimal and its contribution to length change is likely minimal.

7.3 – Future Direction

Based on the findings presented in this thesis there are number of exciting opportunities to advance our knowledge on obesity effects on muscle contractility, and investigations into the strategies which may help alleviate any adverse responses.

Whilst **chapter 4** has provided important insight needed to better optimise PO via the WL technique, there is still opportunity to further refine the method. One important area of future direction is to consider optimising muscle length for dynamic contractions. At present, the muscle length optimal for isometric performance is often implemented as the starting length in the WL assessment of mammalian muscle (James et al. 2005; Hessel and Nishikawa 2017; Kissane et al. 2018; Hill et al. 2020). Whilst previous work has considered the effect of starting length on WL PO of the soleus and EDL, reporting reduced power at lengths 10 and 20% greater and less than L_0 (James et al. 1995), this was only performed at a single cycle frequency. Therefore, future work should consider the effects of starting length across a range of CF's, adjusting muscle length in smaller increments, and systematically adjusting each contractile parameter to achieve maximal net work at each length.

Results from **chapter 3** indicate that high adiposity induced changes in eccentric function are not concurrent with changes in concentric function. The reasons for which are attributed to differences in the physiological mechanisms resulting in concentric and eccentric function. Recent studies have begun to directly combine the underpinning mechanisms and contractility data together to develop our understanding of obesity induced changes in isometric and concentric force production (Tallis et al. 2017), which has helped identify potential therapeutic strategies. However, there is a dearth of literature considering the mechanisms involved in a HFD induced reduction of eccentric force. With an emerging body of evidence highlighting the important role of the giant protein titin in eccentric force production (Herzog et al. 2016), an initial avenue of exploration could be to examine obesity induced changes in the function of titin and its direct link to contractile performance.

In line with combining mechanistic and contractility data for eccentric function, future work should consider the direct mechanisms responsible for the attenuation of fast twitch contractile performance in HFD RES treated mice. Whilst previous research examining the mechanistic changes in skeletal muscle associated with RES in HFD treated animals can be drawn upon to elucidate the findings from **chapter 5**, the exact mechanisms responsible for RES attenuating the HFD induced decline in contractile performance of fast twitch muscle remains speculative. Understanding the mechanisms responsible for maintained contractile performance in fast twitch muscle could help target other strategies to abate the adverse effects of obesity or lead to RES being used in other disease models which share similar mechanistic alterations evoked by obesity.

This thesis only used isolated skeletal muscle from female mice, which limits the generalisability of data. Previous research indicates HFD effects on morphological characteristics are sex-specific (DeNies et al. 2014). Furthermore, in aging models, which share similar mechanistic responses to obesity, declines in skeletal muscle contractility are more substantial in male mice (Hill et al. 2020). Therefore, future work should consider the effects of HFD consumption in both sexes to establish whether changes in contractile performance are uniform between male and female mice.

When taking into consideration the findings from **chapter 5,** indicating no effect of high dose vitamin D on the contractile performance of isolated skeletal muscle, and previous research indicating improved soleus stress over 4 weeks (Debruin et al. 2019; Hayes et al. 2019), but diminished over 8 weeks (Debruin et al. 2020), it would appear that response to vitamin D supplementation is time and dose sensitive. Therefore, future work should consider a range of doses over various feeding durations to establish an optimum feeding protocol to evoke improvements in contractile performance.

Considering findings from **chapter 6**, which indicates that dietary enrichment of RES reduces the impact of HFD consumption on isolated skeletal muscle contractility, future work should consider RES as a potential therapeutic strategy in other disease models which share similar responses to obesity. This could include an aging model, where reductions in contractile function are in part attributed to intramuscular lipid content and chronic inflammation (McGregor et al. 2014; Zembron-Lacny et al. 2019), mechanisms which have previously shown to improve with RES consumption (Baur et al. 2006; Lagouge et al. 2006; Kim et al. 2011; Wang et al. 2015; Shabani et al. 2020). Furthermore, the efficacy of RES to attenuate a reduction in contractile performance in obese individuals should be conducted in humans.

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