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INVESTIGATING A DOSE-RESPONSE RELATIONSHIP BETWEEN HIGH-FAT DIET CONSUMPTION AND THE CONTRACTILE PERFORMANCE OF ISOLATED MOUSE SOLEUS, EDL AND DIAPHRAGM MUSCLES

Short title: EFFECT OF FATNESS ON MOUSE MUSCLE FUNCTION

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Abstract

Purpose: Recent evidence has demonstrated an obesity induced, skeletal muscle specific, reduction in contractile performance. The extent and magnitude of these changes in relation to total dose high fat diet consumption remains unclear. This study aimed to examine the dose response relationship between a high fat diet and isolated skeletal muscle contractility.

Methods: 120 female CD1 mice were randomly assigned to either control group or groups receiving 2, 4, 8 or 12-weeks of a high calorie diet (N=24). At 20 weeks soleus, EDL or diaphragm muscle was isolated (n=8 in each case) and isometric force, work loop power output and fatigue resistance were measured.

Results: When analysed with respect to feeding duration, there was no effect of diet on the measured parameters prior to 8 weeks of feeding. Compared to controls, 8-weeks feeding caused a reduction in normalised power of the soleus and 8 and 12 weeks feeding caused reduced normalised isometric force, power and fatigue resistance of the EDL. Diaphragm from the 12-week group produced lower normalised power, whereas 8 and 12-week groups produced significantly lower normalised isometric force. Correlation statistics indicated that body fat accumulation and decline in contractility will be specific to the individual and independent of the feeding duration.

Conclusion: The data indicate that a high fat diet causes a decline in muscle quality with specific contractile parameters being affected in each muscle. We also uniquely demonstrate that the amount of fat gain, irrespective of feeding duration, may be the main factor in reducing contractile performance.

Key Words: Force; Muscle Quality; Muscular Lipid; Lipid Accumulation; Power.

Abbreviations: ANOVA-Analysis of variance; CF- Cycle frequency; EDL-Extensor digitorum longus; HFD-High fat diet; PO- power output; WL – work loop.

Introduction

Recent data has indicated that 1.4 billion adults (approximately 19% of the world's population) over the age of 18 are either overweight or obese (WHO. 2017), which is disproportionally higher in adults residing in westernised nations (WOF. 2017). The increased prevalence of obesity has been closely linked with an increase in diabetes, cardiovascular disease, cancer and other potentially life-threatening illnesses and diseases (Kopelman. 2007). Furthermore, there is an emerging body of evidence suggesting that obesity significantly affects the contractile performance of skeletal muscle (Ciapaite *et al*. 2015; Tallis *et al*. 2017b; Tomlinson *et al*. 2016; Tuttle *et al*. 2012). Given that skeletal muscle is the primary site of substrate metabolism in the body (Zurlo *et al*. 1990), and that contractile function is needed to fulfil activities of daily living and increase calorie expenditure, it has been proposed that the effects of obesity on skeletal muscle may be a catalyst for obesity related disease (Tallis *et al*. 2014).

Human *in vivo* studies that have examined the effects of obesity on muscle performance have demonstrated an improvement in the absolute force producing capacity of postural and locomotor muscles (Abdelmoula *et al*. 2012; Maffiuletti *et al*. 2007; Szymura *et al*. 2011), but little effect on the contractile performance of other muscles (Capodaglio *et al*. 2009; Hulens *et al*. 2001). The improved performance in postural and locomotor muscles has been proposed to relate to a positive training adaptation induced by the elevated body mass of obese individuals. When contractile function is reported as a function of body mass, in humans, muscle function is significantly reduced in obese individuals in both sexes and across a range of ages (Aucouturier *et al*. 2007; Miyatake *et al*. 2000; Rolland *et al*. 2004; Szymura *et al*. 2011; Ward *et al*. 1997).

A recent review examining the effect of obesity on skeletal muscle function (Tallis *et al*, 2018) outlines the value of using isolated muscle to assess the effects of obesity on contractility. Although *in vivo* work has offered a valuable insight into the effects of obesity on skeletal muscle function, a more comprehensive understanding can be gained by also undertaking studies using isolated skeletal muscle. It has been suggested that human studies do not make accurate assessments of obesity associated changes in muscle quality (muscle performance normalised to muscle size) (Tallis *et al*. 2017b). Understanding obesity induced changes in muscle quality is important, as producing the highest contractile performance per quantity of tissue reduces the muscle mass required, hence lowering body mass, and decreases the cost of maintaining that muscle mass (Tallis *et al*. 2017b). It is common for *in vivo* research to report absolute changes in contractile performance, or that normalised to body mass (Abdelmoula *et al*. 2012; Miyatake *et al*. 2000; Paolillo *et al*. 2012; Ward *et al*. 1997) which provide little insight into obesity induced changes in muscle quality. Only a very small number of *in vivo* studies have used techniques such as Magnetic Resonance Imaging or Computed Tomography scans in an attempt to assess muslce quality (Blimkie *et al*. 1989), however these only provide estimates of muscle size which has led to ambiguity with respect to the effect of obesity on the intrinsic force producing capacity of the contractile proteins. Isolated skeletal muscle studies allow for a more accurate assessment of muscle quality as the whole muscle mass can be determined, and the performance of one particular muscle is measured. Furthermore, an isolated skeletal muscle approach allows assessment of the fibre type and muscle specific effect of obesity on contractile performance without the confounding effects of the central nervous system, including central inhibition. Previous work has stated that the effect of obesity on skeletal muscle fatigue cannot be accurately measured *in vivo* as the muscle of the obese

individual will be working at a greater intensity to overcome the increased body inertia (Tallis *et al*. 2017b). As such, it has been proposed that assessment of the fatigue resistance of isolated skeletal muscle allows a true examination of the effects of obesity on skeletal muscle performance.

To date, only a small number of studies have assessed the effect of obesity on isolated skeletal muscle contractility with the majority of these studies using rodents (Bott *et al*. 2017; Ciapaite *et al*. 2015; Eshima *et al*. 2017). Results demonstrate that the effect of obesity is muscle and contractile parameter specific. There is evidence to suggest that the absolute force and power producing capacity of skeletal muscle is either increased or maintained, whereas there is an obesity induced reduction in muscle quality, meaning that obesity may result in a reduction in the intrinsic force producing capacity of the contractile proteins (Tallis *et al*. 2017b). Tallis *et al* (2018), suggested that the ambiguity in findings is likely related to methodological approach (i.e. mechanical assessment method and differences in the temperature at which the experiments were performed), or feeding duration, with previous work administering HFD for durations between 3 and 16 weeks (Ciapaite *et al*. 2015; Tallis *et al*. 2017b; Thomas *et al*. 2014). Although this has not been thoroughly investigated, several proposed mechanisms, such as a reduction in protein synthesis (Akhmedov and Berdeaux. 2013; Anderson *et al*. 2008), changes in the expression of proteins involved with calcium handling (Bruton *et al*. 2002; Warmington *et al*. 2000), fibre type shift (Eshima *et al*. 2017; Shortreed *et al*. 2009) and an altered metabolic profile (Ciapaite *et al*. 2015; Seebacher *et al*. 2017; Tallis *et al*. 2017b) have been suggested as potential mechanisms for the obesity associated reduction in muscle performance. The onset and magnitude of changes in muscle performance seems likely to be muscle specific and to vary with feeding duration.

The present study used the work loop technique to uniquely examine the effects of 2, 4, 8 or 12 weeks of HFD feeding on the mechanical performance of isolated soleus, extensor digitorum longus (EDL) and diaphragm muscle to provide an insight into the muscle specific onset and magnitude of the obesity response. This has been prioritised as an important area for future work in a recent review by Tallis *et al* (2018). The range of feeding durations used in this study was selected as previous work has shown mechanistic changes, such as altered fibre type composition, from as little as 3 weeks of a HFD consumption (Thomas *et al*. 2014). Results gained from this study will provide clarity on the magnitude of the effects of a HFD over different durations of feeding and will help rationalise previous ambiguous finding untimely providing a better understanding of the effects of obesity on isolated skeletal muscle function. Furthermore, previous studies examining the obesity effect on isolated muscle performance have primarily used assessments of isometric force (Bott *et al*. 2017; Ciapaite *et al*. 2015; Eshima *et al*. 2017; Matsakas *et al*. 2015; Shortreed *et al*. 2009; Thomas *et al*. 2014; Warmington *et al*. 2000) and to date only two studies have used the work loop technique to examine obesity effect on muscular power (Seebacher *et al*. 2017; Tallis *et al*. 2017b). Studies assessing isometric muscle force are limited with respect to their *in vivo* application given that dynamic power producing muscle activity is needed for the completion of locomotor tasks (Josephson, 1985). Although isotonic and isovelocity assessments of muscle function have been used to assed isolated skeletal muscle power in previous work (Barclay, 1996; James et al, 1996), such techniques have been criticised and suggested to substantially overestimate muscle power output (James *et al*, 1996). The work loop techniques considers muscle force generation during dynamic activity and accounts for the interaction between force produced during shortening, passive resistance to re-lengthening and changes in activation and

relaxation time using length change magnitudes and waveform that more closely replicate those used in vivo (Josephson, 1985; James *et al*, 1995). *In vivo*, locomotor muscles are used to produce and absorb work during shortening and lengthening, meaning examination of obesity effects on muscle performance using the work loop technique provides a valuable insight into the effects of obesity on a contraction type fundamental for tasks of daily living previous work has only examined the effect of obesity on work loop power at a fixed cycle frequencies (number of length changes per unit time) (Tallis *et al* .2017b; Seebacher *et al*. 2017). Here we uniquely measure obesity effects on work loop power over a range of cycle frequencies in order to gain a better understanding of obesity effects on power across and range of length change velocities. This offers a greater insight on how obesity effects range of length change velocities used during locomotor tasks *in vivo*.

Results

Morphology

Whole animal body mass, absolute gonadal fat pad mass, gonadal fat pad mass as a percentage of whole body mass and abdominal circumference were significantly affected by treatment (Table 2; ANOVA $P<0.004$ in each case). More specifically 8 and 12 weeks of feeding resulted in significantly greater body mass, absolute gonadal fat pad mass, gonadal fat pad mass as a percentage of body mass and abdominal circumference compared to lean controls (Dunnett's $P<0.01$). Muscle mass for soleus was significantly greater in treatments groups of 8 and 12 weeks of feeding when compared to lean controls (Table 2, ANOVA $P<0.001$, Dunnett's $P<0.001$ in both cases). Muscle mass of the EDL was significantly greater in treatment groups of 4, 8 and 12 weeks of feeding when compared to lean controls (Table 2, ANOVA $P<0.01$, Dunnett's $P>0.01$). Diaphragm mass is not compared between treatment groups as it was not possible to consistently remove the exact same section of the diaphragm on each occasion

Isometric Tetanus Force & Stress

For the soleus and the EDL, maximal isometric stress and force was not significantly affected by treatment (Figure 1A, 1B, 1C, 1D; ANOVA $P>0.1$ in each case). The maximal isometric stress of diaphragm was significantly lower than lean controls in the 8-week HFD treatment group (Figure 1E ANOVA $P=0.01$; Dunnett's $P=0.04$).

Time to half peak tetanus (THPT) was not significantly different between the treatment groups for soleus, EDL or diaphragm (ANOVA $P>0.5$ in each case). Similarly, time from the last stimulus to half relaxation (LSHR) for EDL and diaphragm was not significantly affected (Table 3 ANOVA $P=0.7$). However, LSHR for soleus was significantly affected by feeding duration (Table 3, ANOVA $P=0.01$, Dunnett's $P>0.04$), with LSHR being significantly greater in the 8 and 12 week feeding groups

Work loop power, fatigue and recovery.

There was no significant interaction between treatment and the optimal cycle frequency ~~CF~~ of work loop ~~WL~~ (two-way ANOVA $P=1.00$) on any of the muscles tested, the shape of the force velocity curve remained unchanged. For both the soleus and EDL, absolute work loop power output was not significantly different in any of the treatment groups when compared to the lean control (Figure 2B & D ANOVA $P>0.13$ in both muscles). When work loop power output was normalised to muscle mass: treatment group of 8 weeks of HFD for the soleus

was significantly lower than the lean control group, with the 12-week group approaching significance (Figure 1A, ANOVA P<0.019, Dunnett's P=0.03 & P=0.06 respectively). For the EDL, treatment groups of 8 and 12 weeks of HFD were significantly lower than the lean control group (Figure 2C, ANOVA, P<0.01, Dunnett's P>0.03). For the diaphragm, 12 weeks of a HFD resulted in significantly lower normalised work loop power output than the lean control group (Figure 2E, ANOVA P=0.01, Dunnett's P=0.019). Absolute work loop power output for the diaphragm was not recorded as only a section of the whole muscle was taken.

There was an overall significant difference in time to fatigue for soleus (Figure 3A, ANOVA P=0.02), however there was no differences among treatments groups and the lean control group in post hoc analysis. EDL from treatment groups 8 and 12 fatigued significantly quicker than the lean control group (Figure 3B, ANOVA, P=0.02, Dunnett's P<0.03). Diaphragm had no significant changes in time to fatigue between any of the treatment groups and the lean control group (Figure 3C, ANOVA P>0.36). The ability for the muscle to recover over a 30-minute period post fatigue protocol was not significantly affected by any treatment for all the muscles examined (ANOVA, P>0.06).

Morphological data indicated that body fat accumulation is specific to the individual (table 4) and independent of the feeding duration. For example, one individual from the 2-week feeding group had a body mass of 40.96g with a FPM of 3.86g in comparison to another individual from the 12-week feeding group which had a body mass of 30.78g with a FPM of 0.5g. As such, correlations between gonadal fat pad mass and contractility measures were analysed. For soleus, there was a significant positive correlation between body mass and gonadal fat pad mass, body mass and absolute force (Table 4, R>0.42; P<0.001), and a significant negative relationship between body mass and normalised work loop power output (R=-0.3; P=0.045). For EDL, there was a significant positive relationship between body mass and absolute work loop power output (Table 4 R=0.385; P=0.011) and a significant negative relationship between body mass and time to fatigue (Table 4 R=-0.323; P=0.035). For the diaphragm, there was a significant correlation between body mass and gonadal fat pad mass (R=0.613; P<0.001) and a significant negative relationship between body mass and maximal tetanus stress and time to fatigue (Table 4. R=-0.339; P=0.025).

Discussion

The present study is the first to assess the effect of a range of high fat feeding durations on the contractile performance of skeletal muscle using a method which more closely replicates *in vivo* muscle function. When considered with respect to feeding duration, the results indicated that HFD consumption for a period shorter than 8 weeks has no significant effect on animal morphology or muscle isometric performance, results that agree with previous findings showing short durations (3-8 weeks) of feeding have little effect on muscle contractility (Eshima *et al*. 2017; Shortreed *et al*. 2009; Thomas *et al*. 2014). Only after 8 weeks of a HFD consumption affected contractile performance, with this duration being the first associated with a significant increase in whole body mass, gonadal fat pad mass (Table 2) and gonadal fat pad mass as a percentage of body mass (Table 2). These data therefore indicate that changes in skeletal muscle performance are related to changes in body composition. When gonadal fat pad mass was correlated with skeletal muscle mechanical performance variables and considered irrespective of feeding duration, it was evident that for some individuals changes in contractile performance

ensued prior to 8 weeks HFD consumption. As such, the present findings infer that quantity of fat accumulation rather than feeding duration is the key factor driving obesity associated changes in muscle contractility and rationalising previous ambiguous findings in this area of research where studies report outcomes following fixed feeding durations.

Obesity effects on force and power

There were no changes in peak isometric force or peak isometric stress (force/cross section area) of SOL in any of treatment groups when compared to the lean control group. This is contradictory to the findings of previous work which has demonstrated obese individuals have increased absolute force of postural muscles (Tallis *et al*. 2017b). In human studies, this has been attributed to an added training effect from increased body mass (Maffiuletti *et al*. 2013). One possible reason for the discrepancy in our results is the body mass of the animals used; body masses of the 8 and 12-week treatment groups were 41.2±7.1 and 38.7±6 respectively. However, the body mass of the obese animals in the work by Tallis *et al* (2017b), was 52.7±2.30, with this additional body mass likely from the longer duration of feeding the animal received (16 weeks). A greater body mass is likely to evoke a greater training stimulus, given the higher force requirement needed to overcome body inertia. Absolute power output was also unaffected by treatment in the present study, however when normalised to muscle mass, power output from the 8-week HFD group was significantly less than their lean counterparts and was approaching significance (P=0.06) in the 12-week HFD group. This reduction in the normalised power of soleus was not evident in our previous work using a longer feeding duration (Tallis *et al*. 2017b), further exacerbating the complexity with respect to the effect of feeding duration and fat gain has on muscle performance.

When compared to controls, isometric stress, absolute force or absolute power output in the EDL or soleus was unchanged in the HFD groups. Shortreed *et al* (2009), also reported no force changes in the EDL after 8 weeks of feeding. Previous literature has reported a decrease in both tetanic force and stress (Eshima *et al*. 2017; Matsakas *et al*. 2015), although variation in methodology (both age and assessment temperature) may account for these discrepancies. In the EDL normalised power output was significantly reduced in animals from HFD treatment groups of 8 and 12-week duration, indicating that the increase in muscle mass induced by obesity was not associated with an improvement in contractile performance. These data also demonstrate that the reduction in muscle quality may be reduced earlier than 8 weeks and that the magnitude of the decline is dependent on fat accumulation rather than feeding duration (see table 4). As such, findings from the present study infer that an obesity associated reduction in muscle quality is likely to occur following much shorter feeding durations than those previously cited (Tallis *et al*. 2017b). When considered *in vivo*, larger muscles of poorer quality will be produced, raising metabolic demand in protein synthesis and further increasing body mass and body inertia.

Our results infer that the magnitude and onset of the obesity associated changes in contractile performance are muscle specific, and likely related to mechanical function, anatomical location and fibre type distribution. These findings are the first to indicate that obesity may cause a reduction in skeletal muscle quality across all muscles, which has not been the case in previous work. For example, Tallis *et al* (2017b), demonstrated that muscle quality was relatively well maintained in the soleus, which is not the case in the present findings. Such results further highlight the complexity and ambiguity in relation to this area of research. However, we propose that the changes

on body composition, or more specifically fatness, are the most important mediator of obesity associated changes in muscle performance. Given the rate of fat accumulation is not uniform across all individuals, assessing the effects of obesity using HFD during a fixed feeding duration and failing to consider individual results may be the cause of ambiguity in recent findings.

Correlation data (table 4) between morphological and contractility measurements plotted against fat pad mass support the theory that fatness rather than feeding duration is the main influence on the decline on muscle performance.

Obesity effects on fatigue resistance

The effect of obesity on fatigue resistance of skeletal muscle has been previously assessed *in vivo* (Maffiuletti *et al*. 2007). However, *in vivo* work does not give an accurate assessment of skeletal muscle fatigue as muscle from an obese individual will have to move a bigger mass resulting in faster fatigue. Only one study has previously assessed the effect of obesity on the fatigue of muscle power output. Tallis *et al* (2017b), reported that soleus fatigued significantly quicker in the obese group when compared to the lean controls, given the postural support role of the Sol this would have detrimental effects on sustained locomotion. Results from the current study showed little change in time to fatigue for the soleus and no change in the diaphragm. Contrary to Tallis *et al* (2017b), the present study reported a significant reduction in time to fatigue in the EDL in both 8 and 12-week treatment groups, as a longer duration of feeding was used in the Tallis *et al* (2017b) study, it is possible the fiber type of the EDL began to take on the metabolic formula of the adjacent fibers in preparation to alter fiber type in accordance with the nearest neighbor theory (Pette and Staron. 1997). As per previous work (Tallis *et al*. 2017b) it would be expected that when such muscle performance is considered *in vivo*, that all muscles would experience greater fatigue given the obesity induced reduction in normalized work loop power and the elevated segmental mass. This point is particularly important when considering the *in vivo* role of the diaphragm; reduced fatigue resistance could lead to reduced oxygen delivery, deteriorating fatigability for all muscles. Although significant differences were shown in the time to fatigue in the EDL, correlation data (table 4) shows individuals from all treatment groups can be found at both ends of the trend line, again suggesting that fatness, not duration of feeding if the key underlying issue.

Mechanisms for an obesity associated change in skeletal muscle contractile performance

There are a number of mechanisms which have been suggested to affect the decline in muscle performance due to obesity, such as a change in fiber type composition (Eshima *et al*. 2017; Shortreed *et al*. 2009), a reduction in protein synthesis (Akhmedov and Berdeaux. 2013; Anderson *et al*. 2008), altered calcium handling (Bruton *et al*. 2002; Warmington *et al*. 2000) and changes to metabolic profile (Ciapaite *et al*. 2015; Seebacher *et al*. 2017; Shortreed *et al*. 2009; Tallis *et al*. 2017b). The variation in mechanisms proposed for how obesity contributes to a decline in muscular contractility may at least partially be due to variation in methodology used in previous literature and the dearth of research which attempts to couple mechanistic changes with effects on contractile performance. The changes in mechanisms relating to muscle function are likely to be muscle specific given that the changes in performance are, there is also some relation to the magnitude of fatness. These mechanisms may be further exacerbated by long duration feeding.

The findings regarding changes in fiber type expression as a result of obesity in rodents are equivocal, with some literature reporting no change (de Wilde *et al*. 2008; Denies *et al*. 2014; Shortreed *et al*. 2009; Tallis *et al*. 2017b; Trajcevski *et al*. 2013), and some reporting a shift to either slow twitch or fast twitch fibers (Eshima *et al*. 2017; Kemp *et al*. 2009; Shortreed *et al*. 2009; Tanner *et al*. 2002; Thomas *et al*. 2014; Warmington *et al*. 2000). Part of this ambiguity may be related to evidence derived from dietary induced obesity models compared genetically obese rodent models. Generally, studies using OB/OB rodents show a shift to slower, more oxidative fibers (Warmington *et al*. 2000) with a lower muscle mass and those using dietary induced obesity demonstrate a shift towards faster fibers (Eshima *et al*. 2017; Thomas *et al*. 2014) and a larger muscle mass (Ciapaite *et al*. 2015; Tallis *et al*. 2017b). Although fiber type composition and metabolic capacity was not analyzed, a decrease in slow twitch fibers or altered AMPK activation may go some way in explaining the reduction in time to fatigue in obese individuals, however a reduction in contractile performance without a change in fiber type composition has also been reported (Tallis *et al*. 2017b). Ciapaite *et al* (2015), reported a decrease in tetanus force in the soleus with no change in MyHC, however an increase in slow Tnnt1 isoform and decrease in fast Tnnt3 isoform was reported. This change would increase Ca^{2+} sensitivity and decrease Ca^{2+} cooperativity of force production, therefore, reducing force production of the muscle (Brotto *et al*. 2006). Changes in both fibre type and speed of actin-myosin cycling need further investigation.

Obesity has been associated with chronic inflammation leading to elevated pro-inflammatory cytokines TNF-α and IL-6 which has been linked to a decline in skeletal muscle contractile protein synthesis (Akhmedov and Berdeaux. 2013; D'Souza *et al*. 2015; Tallis *et al*. 2017b). The increase in muscle mass and decrease in normalised muscle power output from the obese group in the current study suggest that although skeletal muscle remodeling is continued in obese individuals, the quality of the contractile proteins being synthesized is likely to be reduced.

Broader applications of the findings

Elevated fatness has the potential to cause individuals to fall into a negative cycle, as body mass increases and muscle quality decreases, obese individuals may have their movement reduced due to earlier fatigue and reduced ability to produce muscular power. Obese individuals may also have reduced ability to oxidize fat due to metabolic changes, further adding to the negative cycle. This lack of movement will not only decrease quality of life, but also lead to further decreases in calorie expenditure which may lead to further increases in body mass. An obesity associated reduction in the performance of the diaphragm, may affect pulmonary function and oxygen delivery to working tissue thus further limiting muscle performance.

This data has shown important results with respect of the effect of obesity on muscle function at the muscle level, but to consider effect on whole body functional performance, contractile function in relation to elevated body inertia should be considered. The effect of a reduction in muscle quality on locomotor performance has already been outlined. In addition, the limited effects on the absolute force and power of soleus and EDL muscles would also present substantial problems for functional performance *in vivo* given that such musculature is expected to cause movement in a system that has a greater inertia. As a result, the decline in fatigue resistance will also be on a greater magnitude given that musculature of the obese group would have to work at a greater intensity to sustain

movement at the same speed as their lean counterparts. Importantly, this data adds growing evidence that indicates that the obesity associated reduction in locomotor performance is caused by factors further to elevated inertia induced by greater fat mass, but a significant reduction in the decline of the contractile performance of skeletal muscle. Furthermore, the effects seen at a muscle level may be further exaggerated as obesity has reportedly caused changes in neuromuscular recruitment (Yoshida *et al*. 2012).

Limitations and future work

One limitation for this study is normalising work loop power to whole muscle mass in attempt to assess muscle quality. Goodpaster *et al* (2001), reported that skeletal lipid content can double in obese individuals; this would mean a smaller proportion of the muscle mass would be contractile protein; therefor these results may overestimate the decline in contractile performance. Normalising work loop power to lean muscle mass would allow for a more accurate representation of muscle quality. Although obese individuals may have a higher intramuscular lipid content than their lean counterparts, Machann *et al* (2003) reported this difference was limited with intramuscular fat in the soleus rising from 2.5% in lean individuals to 3.8% in obese individuals. It would be possible to quantify the volume of the contractile machinery in the muscle fibre using transmission electron microscopy (Singh *et al,* 2009). This would provide a more detailed assessment of muscle quality in an obese model.

The current study used gonadal fat pad mass as an indicative measure of whole animal body fat. Although this is a valuable measurement for determining differences in fatness between the experimental groups, evidence has indicated that mechanistic responses to obesity may be influenced by the specific location of lipid accumulation (Wronska *et al*, 2012). The effect of lipid accumulation location on skeletal muscle contractile performance is yet to be explored and should be a specific focus of future work. In particular, a focus on muscular lipid accumulation and skeletal muscle function should be preauthorised.

The current study, reports the effect of a range of short term feeding durations on muscle contractility, however, it would be valuable to look at the effects of longer durations of feeding which reflect a ‘lifetime’ of a high fat diet and more sustained obesity. This would also take into consideration both obesity and sarcopenic effects. Given that this study replicates the negative effects of a high fat diet on skeletal muscle contractility, reversibility of these effects should be investigated further. Both exercise and calorie restrictive diet models should be implemented on dietary induced obese animals.

As mentioned in a recent review article (Tallis *et al*, 2018), a key direction for future work should be to add to the dearth of littering examining the underpinning mechanisms which cause the decline in performance onset by obesity.

Conclusion

In summary, our findings offer a complex and novel insight into the effects of a HFD on skeletal muscle mechanics. The data suggests that although feeding duration may have some influence, it is the amount of fat gained during a HFD that affects muscle contractility. An increase in muscle mass in the soleus and EDL along with no change in absolute tetanic force or absolute power output shows a decrease in muscle quality as bigger

muscles are required to produce the same amount of force. Such effects would be exacerbated *in vivo* when considering the additional body mass gained during obesity, which could lead to a negative cycle of further weight gain, reduced mobility and a reduction in quality of life. Our data suggest that it may be possible for some individuals to see substantial negative changes at low durations of a HFD if considerable fat gain occurs, compromising both function performance and health. The decline in muscular performance due to obesity may be caused by a range of morphological and biochemical changes and future work should aim to correlate these changes to better identify the underpinning mechanisms.

Material and Methods

Animals

Following ethics approval from Coventry University, 150 female CD1 mice (Charles River, UK; Harlan Laboratories, UK) were randomly assigned to 5 different experimental groups - 4 treatment groups of differing feeding durations and 1 lean control group. The treatment groups were given 2, 4, 8 or 12 weeks of a high fat forage diet. Mice were housed in a 12-hour light/dark cycle at 50% humidity. Animals were aged matched and kept in cages containing 8-10 individuals. All animals were given water and standard lab chow (SDS RM-1 Maintenance) *ad libitum*. Mice in the treatment groups also had *ad libitum* access to a HFD, in the form of a laboratory supplied forage diet (Advanced Protocol PicoLab Natural Sunflower). Nutritional information for both the lab chow and the sunflower seeds are given in Table 1.

Mice where aged to 20 weeks of age, with the high fat diet delivered at the defined period prior to 20 weeks (e.g. 12 weeks of feeding began at 8 weeks of age). Once an experimental group was of age they were weighed and sacrificed via cervical dislocation in accordance with British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Gonadal fat pad mass was dissected and weighed as an indicative measure of total body fat (Rodgers *et al*, 1980). Either the rib cage or hind limbs were removed and placed in chilled oxygenated (95% O_2; 5% CO_2) Krebs- Henseleit solution ([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; pH 7.55 at room temperature [James *et al*. 2005]). The target muscle was then isolated from either the right hind limb or the rib cage and dissected under microscope.

Dissection and preparation

The limb containing the target muscle (EDL or soleus) or the rib cage containing the whole, intact diaphragm (n=8-10 per experimental group) was isolated and pinned out on an sylgard dish containing frequently changed, chilled, oxygenated (95% O_2, 5%CO_2) Krebs-Henseleit solution. These muscles were used due to their different anatomical location, function and fibre type composition, as well as to replicate previous literature where these muscles are commonly used for contractile performance studies. Once the muscle (whole Sol, whole EDL, section of the DIA) had been isolated, aluminium foil T clips were used to wrap around the tendons at the distal end of the muscle whilst a small piece of bone was left at the proximal end of the muscle, these were placed into crocodile clips to secure the muscle in the bath.

Experimental set up

The equipment used to assess contractile performance was custom built, controlling changes in both muscle length and stimulation parameters. Each muscle was placed in an organ bath and attached at one end to a force transducer (UF1, Pioden Controls Ltd, UK) and at the other to a motor arm (V201, Ling Dynamic Systems, UK). Position of the motor arm was detected via a Linear Variable Displacement Transformer (LVDT, DFG5.0, Solartron Metrology, UK). The motor arm was used to change the length of the muscle during assessments of work loop power. The muscle was immersed in circulated oxygenated Krebs solution (37^{o}C ± 0.2) which was pumped from a central reservoir maintained at a constant temperature by a heater/cooler (Grant LTD6G, Grant Instruments Ltd, UK). A digital thermometer (Checktemp C, Harvard Apparatus, UK) was used inside the bath to monitor temperature throughout the duration of the experiment.

The muscle was stimulated to produce force via parallel platinum electrodes which were submerged in the Krebs solution inside the bath, the amplitude of the stimulation was controlled by an external power source (PL320, Thurlby Thandar Instruments, Huntingdon, UK). Visual representation of the force and length data was provided by a storage oscilloscope (2211, Tektronix, Marlow, UK). Length change and stimulation parameters were controlled by a custom programme within Testpoint software (Testpoint, CEC, Massachusetts, USA).

Experimental testing of skeletal muscle

The procedure for assessing the contractile performance of isolated skeletal muscle aligns with that of previous work (James *et al*. 1996; Tallis *et al*. 2014; Tallis *et al*. 2017b). Once the muscle was successfully clamped in the bath it was allowed 10 minutes to equilibrate to the new temperature. Following this, the resting length of the muscle and stimulation amplitude (typically 14-16V, 14-16V and 12-14V for soleus, EDL and diaphragm respectively) was altered to attain maximal twitch force. Assessment of isometric tetanus force was then measured. Electrical stimulation was delivered at a fixed burst duration (350ms, 200ms and 250ms for soleus, EDL and diaphragm respectively), and stimulation frequency (typically 120-140Hz, 200-220-Hz and 120-140Hz for soleus, EDL and diaphragm respectively) was optimised to elicit maximal tetanus force. Each tetanus assessment was separated by a 5-minute recovery period. Measures of activation time (time to half peak tetanus; THPT) and relaxation time (time from last stimulus to half tetanus relaxation; LSHR) were obtained from the peak isometric tetanus force trace.

The muscle length that elicited maximal isometric force was measured by an eyepiece graticule fitted to a microscope and was defined as L_0. As per previous work, mean muscle fiber length was calculated as 85% and 75% of L_0 for soleus and EDL muscle respectively (James *et al*. 1995). Due to the variances in each diaphragm dissection, no such estimation of muscle fibre length exists. Therefore, the exact diaphragm preparation length reading gained from the graticule was used as per previous studies (Tallis *et al*. 2014; Tallis *et al*. 2017a).

Post isometric optimisation and a 5-minute rest, the work loop technique was used. This technique assesses the ability of the muscle to produce power whilst undergoing cyclical length changes (James *et al*. 2005; James *et al*. 1995; Josephson. 1985). During the work loop, optimal length and stimulation parameters determined during the isometric assessments were used. Each muscle was subjected to 4 sinusoidal length changes at a symmetrical strain of 0.10 (10% of L_0), lengthening the muscle from its original L_0 length by 5% and then shortening the muscle to 5% less than L_0, before returning to L_0 length. Cycle frequency, phase and burst duration were altered

to attain maximal net work loop power and to construct a power output- cycle frequency curve. Between each work loop, a 5-minute rest period was used to ensure recovery (Tallis *et al*. 2012).

The cycle frequency determines the speed at which the muscle undergoes changes in length. Due to the differences in fibre type composition, the different muscles tested produced maximal power at different length change velocities. Previous work demonstrates that the cycle frequency to elicit maximal work loop power is 5Hz, 7Hz and 10Hz for soleus, diaphragm and EDL respectively in healthy young female mice (Altringham and Young. 1991; James *et al*. 1995). Given that the optimal cycle frequency to elicit maximal power may be affected by obesity, here we assessed work loop power over a range of cycle frequency (2, 3, 4, 5, 6, 7, 8 and 10 Hz for soleus; 4, 6, 8, 10, 12, 14 and 16 Hz for EDL; 3, 4, 5, 6, 7, 8, 10 and 12 Hz for diaphragm). This methodological approach has been used in previous studies (James *et al*. 1996; James *et al*. 1995; Tallis *et al*. 2014; Tallis *et al*. 2017b). To optimise work at each cycle frequency, strain was also altered. Typically, in order to obtain maximal work loop power strain had to be reduced slightly at higher cycle frequency and increased slightly at lower cycle frequency.

In order to optimise the net work produced during the work loop cycle, stimulation parameters were adjusted. Burst duration equates to the number of electrical stimuli which the muscle receives during the work loop. The optimal burst duration is one that maximises net work during the whole work loop cycle. If the burst duration is too short, then maximal work will not be achieved due to limited Ca^{2+} release limiting the duration of force production during shortening. If the burst duration is too long, then the muscle will be too active during muscle lengthening and at rest, causing increased work done on the muscle during lengthening and as result decreased net work (net work = work done by the muscle during shortening –work done on the muscle during lengthening (Josephson. 1993). The phase shift denotes the time at which the electrical stimulation is applied, relative to peak muscle length. Negative values were used (-10 ms for soleus, -5 ms for Diaphragm and -2 ms for EDL). The value of -10 ms used for the soleus meant that 10 ms prior to maximal length, stimulation of the muscle began. This meant that 55 ms (based on a burst duration of 65) of stimulation occurred during shortening. The values for phase are typical of previous work (Tallis *et al*. 2014; Tallis *et al*. 2017b). The values for strain, cycle frequency, burst duration and stimulus phase were altered after interpreting the work loop shapes and net work value.

It is recognised that the performance of isolated mouse muscle will decrease very slowly over time due to the development of an anoxic core (Barclay. 2005). In accordance with previous studies using a similar protocol (James *et al*. 1996; James *et al*. 1995), a series of 'control' assessments of work were made at various time points across the protocol. As such the performance of the muscle across the time course of the experiment could be corrected by considering the small decline in muscle performance over time.

Following the final work loop, each muscle was rested for 10 minute before being subjected to 50 consectutive workloops (at 5Hz, 7Hz and 10Hz cycle frequency for SOL, DIA and EDL respectively) to assess fatigue resistance. Net work was analysed for every second work loop until the muscle began to produce negative work. This protocol has been used previously to assess fatigabilty in isolated skeletal msucle (Tallis *et al*. 2014; Tallis *et al*. 2017b).

Each muscle underwent a 30-minute recovery period with 4 work loops being performed at each of 10, 20 and 30 minutes. These were used to measure the ability of the muscle to recover following fatigue (Tallis *et al*. 2014;

Tallis *et al*. 2017b). Each experiment lasted between 2.5 and 3 hours, this was dependant time taken to optimise work loop power at each cycle frequency.

Muscle Mass Measurements

Upon completion, the muscle was removed from the crocodile clips and the remaining tendon, bone and aluminium clips were removed. Any excess fluid remaining on the muscle was removed by blotting the tissue on absorbent tissue paper. The muscle was then weighed to attain wet muscle mass to the nearest 0.0001g. Muscle cross sectional area was then calculated from the wet muscle mass, fibre length and an assumed muscle density of 1060 kg m^{-3} (Mendez and Keys. 1960) Isometric stress was calculated as force divided by mean muscle cross-sectional area. Muscle power output was normalised to muscle mass to express power as $W.kg^{-1}$.

Statistical Methods

Following appropriate checks of normality and homogeneity, data for animal morphology, isometric stress, force, work loop power output, and fatigue was analysed using a one-way ANOVA with planned contrasts with all treatment groups compared against the lean control group in SPSS (SPSS, IL, USA), and Dunnett's post hoc analysis was used with $P<0.05$ to indicate significant differences between treatment groups. Two-way ANOVA were used to assess the effects of feeding duration and cycle frequency on maximal power output. Correlations were performed on all muscles between fat free mass and body mass, isometric force, isometric stress, absolute work loop power output, normalised work loop power output and fatigue resistance.

Acknowledgements

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Conflict of interests

The authors have no conflict of interest to declare.

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552 Tables:

553 **Table 1: Nutritional value of the diets given to the animals**.

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

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TABLE 2 - THE EFFECT OF HFD FEEDING DURATION ON THE WHOLE ANIMAL AND MUSCLE MORPHOLOGY

Treatment Group	Control		2 week		4 week		8 week		12 week	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body Mass (g)	33.3	4.1	36.4	4.9	35.9	5.6	41.2*	7.1	38.7*	6.0
Body Length (cm)	10.4	0.4	10.6	0.3	10.8	0.4	10.9	0.5	10.9	0.3
Gonadal Fat Pad Mass (g)	0.91	0.79	1.91	1.01	1.99	1.28	2.92*	2.39	2.30*	1.39
Gonadal Fat Pad Mass (% of body mass)	2.86	0.5	5.39	0.6	5.06	0.6	7.34*	1.0	6.00*	0.5
Circumference (cm)	7.48	0.49	8.52	0.79	8.49	0.84	9.24*	1.03	9.03*	0.93
SOL Mass (mg)	8.50	0.2	10.1	0.5	9.90	0.4	10.8*	0.5	12.3*	0.4
EDL Mass (mg)	10.4	0.6	11.9	0.7	12.9*	0.3	12.6*	0.3	13.7*	0.3

*[Data represented as Mean + SEM; N= 24 for groups control, 2 and 4weeks; N= 30 for groups 8 and 12 weeks; * indicate significant differences between treatment group and lean control group]*

***TABLE 3* – THE EFFECT OF DIFFERENT DURATIONS OF A HFD ON ISOMETRIC TIME FROM LAST STIMULUS TO HALF TETANUS RELAXATION (LSHR) OF ISOLATED MOUSE SOLEUS, EDL AND DIAPHRAGM**

Treatment Group	Control		2 weeks		4 weeks		8 weeks		12 weeks	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Soleus (ms)	45.0	2.9	45.9	4.8	47.8	6.2	52.7*	5.2	55.1*	4.0
EDL (ms)	12.9	0.9	12.8	1.0	13.2	1.2	14.6	0.7	14.4	1.1
Diaphragm (ms)	25.1	2.5	25.8	2.1	25.5	3.0	26.2	2.8	24.9	3.1

*[Data represented as Mean + SEM N= 8 for SOL, DIA and EDL in groups Control, 2 and 4 weeks, and N= 10 for Soleus, Diaphragm and EDL in groups 8 and 12 weeks; * represents significant difference between treatment group and lean control group]*

***TABLE 4* – CORRELATION BETWEEN GONADAL FAT PAD MASS AND: BODY MASS, TETANUS FORCE, TETANUS STRESS, ABSOLUTE POWER OUTPUT, NORMALISED POWER OUTPUT AND FATIGUE FOR SOLEUS, EDL AND DIAPHRAGM. ALL EXPERIMENTAL GROUPS WERE POOLED FOR THIS ANALYSIS.**

		Body mass	Tetanus Force	Tetanus Stress	Absolute PO	Normalised PO	Fatigue (% of max)
SOLEUS	R Value	0.792	0.326	0.081	0.151	-0.216	0.191
	P Value	0.001*	0.03*	0.606	0.331	0.164	0.221
EDL	R Value	0.155	0.068	0.082	-0.008	-0.267	-0.357
	P Value	0.321	0.664	0.603	0.957	0.074	0.019*
DIAPHRAGM	R Value	0.613		-0.334		-0.021	-0.413
	P Value	0.001*		0.03*		-0.891	0.005*

[N=44 for each group; * represents significant correlation between body mass and variable]

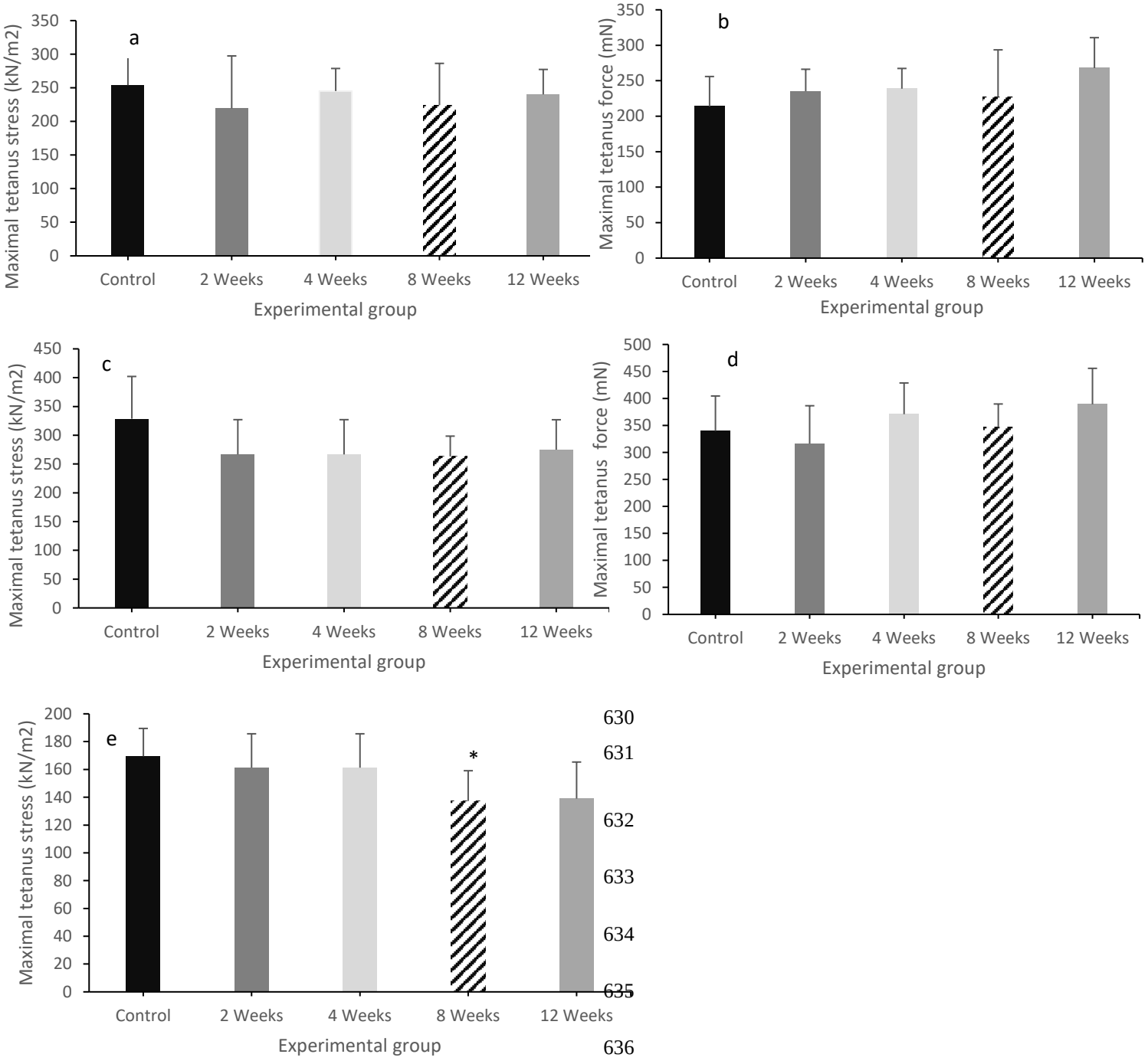
Legends to figures:

Figure 1 – The effect of different durations of a HFD on the maximal isometric tetanus force and stress of isolated mouse Soleus (a & b), EDL (c & d) and Diaphragm (e) [Data represented as Mean+SEM N= 8 for Soleus, Diaphragm and EDL in groups Control, 2 and 4 weeks, and N= 10 for Soleus, Diaphragm and EDL in groups 8 and 12 weeks; * represent significant differences between treatment group and lean control group]

Figure 2 – The effect of different durations of a HFD on the normalised net WL PO and absolute net WL PO of isolated mouse Soleus (a & b), EDL (c & d) and Diaphragm (e) over a range of cycle frequencies. [Data represented as Mean; N= 8 for Soleus, Diaphragm and EDL in groups Control, 2 and 4 weeks, and N= 10 for Soleus, Diaphragm and EDL in groups 8 and 12 weeks * represents significant difference between treatment group and lean control group]

Figure 3 - The effect of different durations of a HFD on the fatigue resistance of maximally stimulated mouse Soleus (a), EDL (b) and Diaphragm (c) [Data represented as Mean±SEM; N= 8 for Soleus, Diaphragm and EDL in groups Control, 2 and 4 weeks, and N= 10 for Soleus, Diapragm and EDL in groups 8 and 12 weeks]

629 Figures: Figure One:



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642 Figure Two:

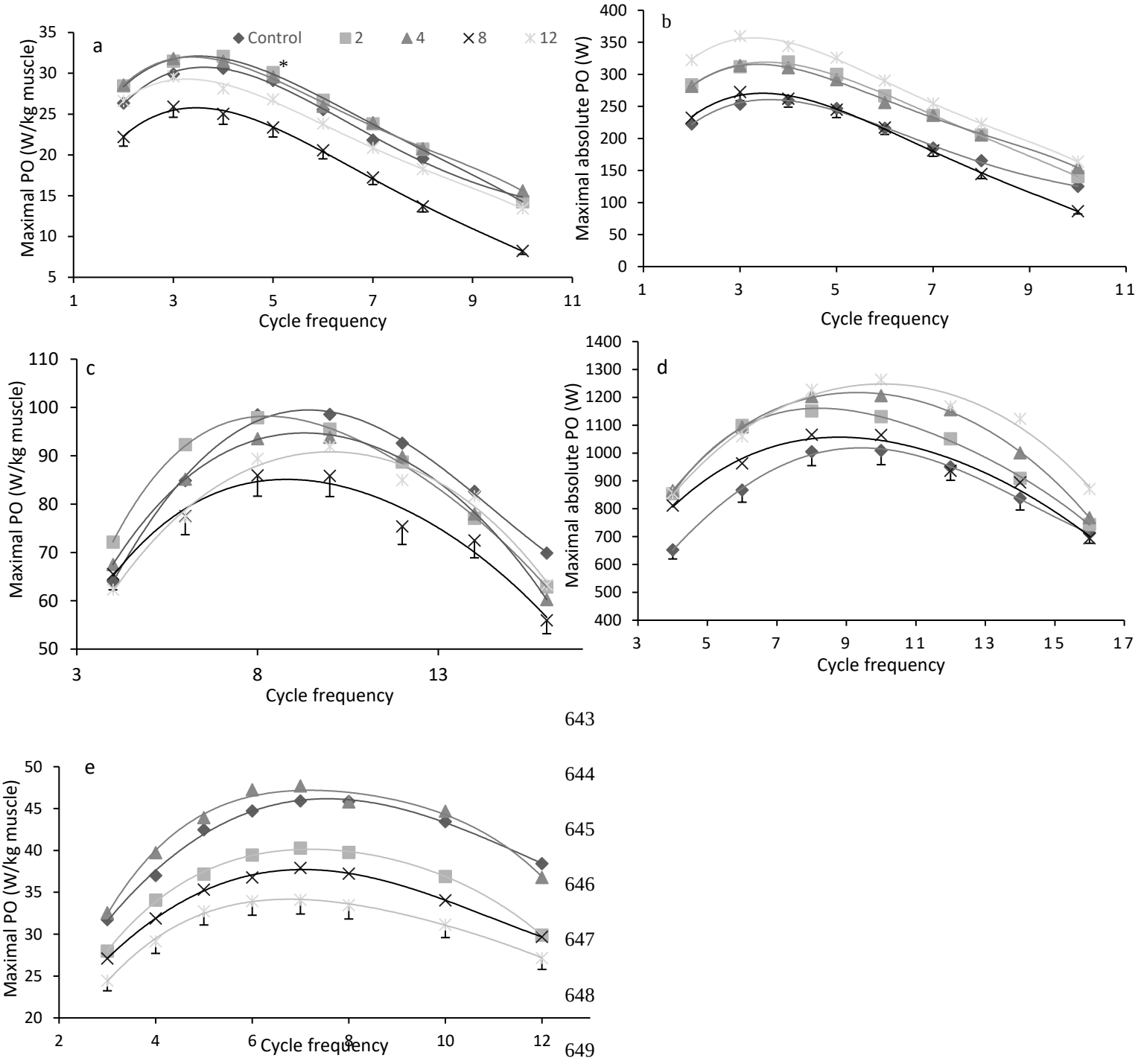

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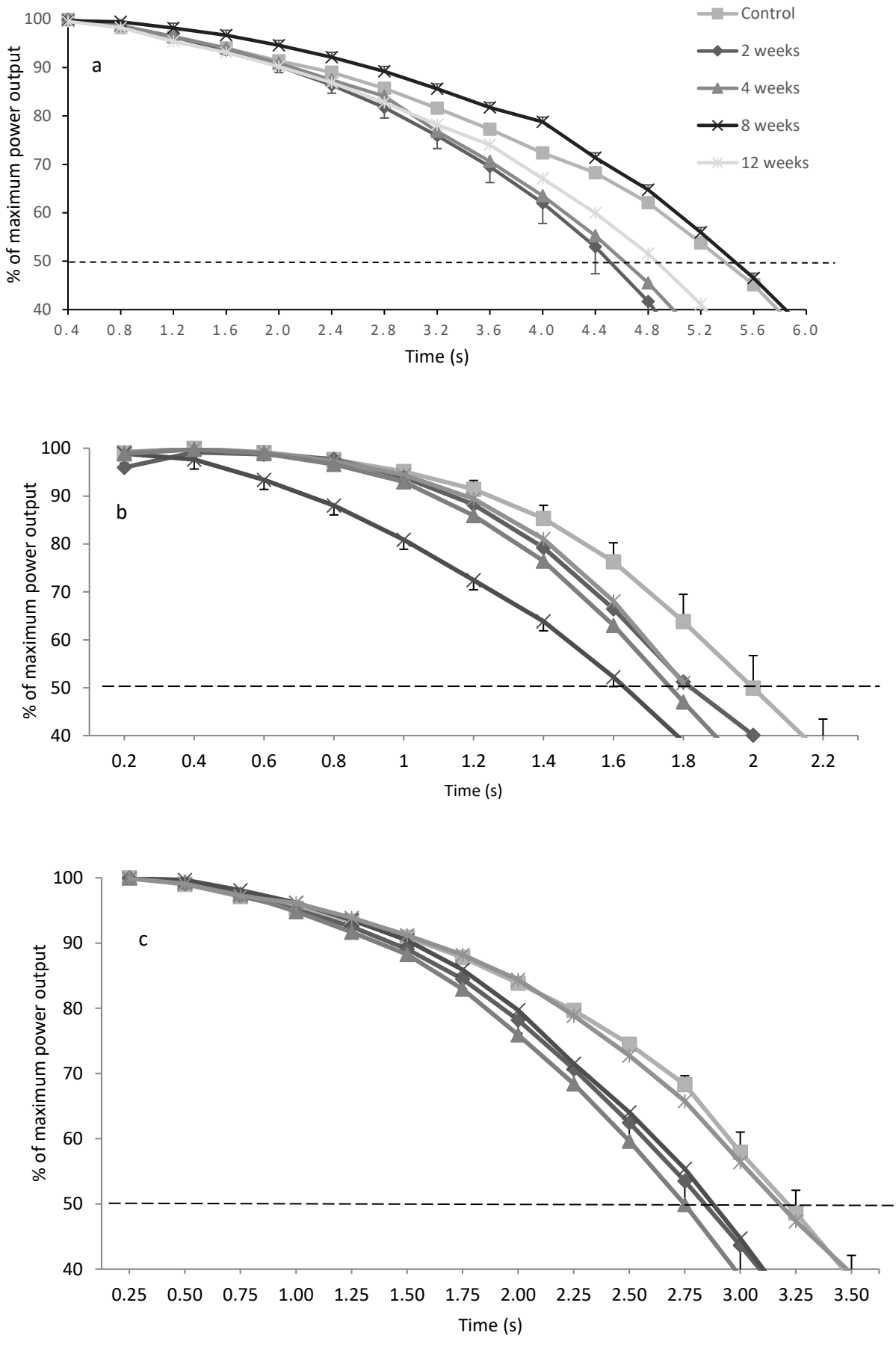
Figure Three:



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