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THE EFFECT OF OBESITY ON THE CONTRACTILE PERFORMANCE OF ISOLATED MOUSE SOLEUS, EDL AND DIAPHRAGM MUSCLES

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RUNNING HEAD: OBESITY EFFECTS ON SKELETAL MUSCLE CONTRACTILITY

ABSTRACT

Obesity affects the major metabolic and cellular processes involved in skeletal muscle contractility. Surprisingly, the effect of obesity on isolated skeletal muscle performance remains unresolved. The present study is the first to examine the muscle specific changes in contractility following dietary induced obesity using an isolated muscle work-loop (WL) model that more closely represents *in vivo* muscle performance. Following 16-week high calorific feeding, soleus (SOL), extensor digitorum longus (EDL), and diaphragm (DIA) were isolated from female (CD-1) mice and contractile performance compared against a lean control group. Obese SOL produced greater isometric force, however isometric stress (force per unit muscle area), absolute WL power and normalised WL power (watts per kg muscle mass) were unaffected. Maximal isometric force and absolute WL power of the EDL was similar between groups. For both EDL and DIA, isometric stress and normalised WL power were reduced in the obese groups. Obesity caused a significant reduction in fatigue resistance in all cases. Our findings demonstrate a muscle specific reduction in contractile performance and muscle quality that is likely related to *in vivo* mechanical role, fibre type and metabolic profile, which may in part be related to changes in MyHC expression and AMPK activity. These results infer that beyond the additional requirement of moving a larger body mass, functional performance and quality of life may be further limited by poor muscle function in obese individuals. As such, a reduction in muscle performance may be a substantial contributor to the negative cycle of obesity.

NEW AND NOTEWORTHY

The effect of obesity on isolated muscle function is surprisingly under researched. The present study is the first to examine the effects of obesity on isolated muscle performance using a method that more closely represents real word muscle function. This work uniquely establishes a muscle specific profile of mechanical changes in relation to underpinning mechanisms. These findings may be important to understanding the negative cycle of obesity and in designing interventions for improving weight status.

KEY WORDS: Muscle Quality; Muscular Lipid, Lipid Accumulation; Force; Power

121
122 **INTRODUCTION**
123
124 Obesity is a global epidemic, attributed to calorific rich foods and reduced physical activity (45).
125 Associated health complications such as metabolic syndrome, cardiovascular disease, diabetes,
126 musculoskeletal disorders, and some cancers (17) contribute to mortality, poor quality of life and
127 significant financial implications for healthcare providers (2, 18, 22). If energy intake is not balanced
128 with expenditure, adipose tissue accumulation occurs and is stored viscerally, subcutaneously and
129 ectopically in organs, including skeletal muscle (3). Skeletal muscle is the largest regulator of
130 metabolism in the body and contractility is needed to produce movement, highlighting the importance
131 of investigating the effect of elevated lipid content on this tissue. Presently it is not clear whether lipid
132 accumulation attenuates skeletal muscle contractility.
133
134 Previous *in vitro* studies suggest that obesity may improve absolute strength of 'antigravity' muscles,
135 but has little effect on musculature that is not loaded with an increased body mass (see review 39). For
136 example, Rolland, Lauwers-Cances, Pahor, Fillaux, Grandjean and Vellas (50) reports an increase in
137 the absolute force generating capacity of the knee extensors in obese elderly women, without
138 significant changes in handgrip strength. These results are not surprising given the potential training
139 adaptation that may occur due to the increased demand placed on the postural muscles during standing
140 and locomotion (20). Interestingly, the small number of studies examining muscular fatigue have
141 conflicting findings (37, 38, 40, 42, 47). Although variation in experimental methods and participants
142 (i.e. differences in muscle groups tested, mode of exercise, age and gender of population) make
143 comparisons between these studies difficult, the authors argue that the true effect of obesity on
144 muscular endurance cannot be accurately evaluated *in vivo*, as irrespective of exercise intensity,
145 musculature of the obese group will have to produce greater force to overcome greater inertia of the
146 moving limb. Further limitations also arise when relating the principle findings of this body of work
147 directly to skeletal muscle function.
148
149 The majority of human studies measure muscular strength (39), and although this is an important
150 mechanical parameter, dynamic power is needed for locomotion. Strength assessments largely involve
151 gross joint movements and are influenced by neuromuscular recruitment, making it impossible to
152 accurately examine the direct skeletal muscle and potential phenotype specific effects. Many human
153 studies examine muscle performance normalised to body mass (1, 7, 9, 43, 47, 64), which provides
154 little information regarding muscle quality (force relative to muscle mass). An obesity induced
155 reduction in muscle quality could result in an increased maintenance cost due to a larger muscle mass,
156 and consequently an increase in body mass, even before considering further lipid accumulation
157 elsewhere in the body. Although some studies have normalised muscle performance to local and more
158 commonly whole body lean mass (1, 7, 43, 47, 64), such assessments would not be as accurate at
159 evaluating muscle quality as an *in vitro* isolated muscle approach, where whole muscle mass can be
160 measured.
161
162 Obesity has been associated with a reduction in myogenesis (4, 12), degeneration in the process of
163 excitation contraction coupling and impaired calcium handling (8, 10, 19, 51), which may
164 mechanistically account for a decline in contractility and muscle quality. Importantly, skeletal muscle
165 lipid accumulation can affect metabolic capacity and phenotype composition, but the literature is
166 equivocal with evidence of a shift to a faster, slower and no change in fibre type composition (13, 14,
167 33, 34, 52, 59-61, 65). This ambiguity can largely be attributed to the different muscles tested, duration
168 of the feeding period, and limitations in methods for quantifying fibre types. It has been further
169 demonstrated that initially muscular lipid accumulation results in increases in oxidative enzymes,
170 mitochondrial function, and slow MyHC (myosin heavy chain) expression, and in the long term, causes
171 a reduction in oxidative enzymes, type I muscle fibre protein content, mitochondrial size and function
172 (13). In skeletal muscle, glucose and fatty acid metabolism as well as mitochondrial function are at
173 least partly regulated by AMP-activated protein kinase (AMPK) (46). Levels of adiponectin, a protein
174 hormone produced by adipocytes that induces AMPK activity, are decreased by obesity in skeletal
175 muscle (66), which may significantly influence skeletal muscle function.
176
177 Despite this evidence, there is a distinct dearth of literature that directly assesses the effects of obesity
178 on skeletal muscle contractility. Warmington, Tolan and McBennett (65) demonstrated little effect on
179 the isometric twitch force of both whole extensor digitorum longus (EDL) and soleus muscle isolated
180 from 5 month old genetically obese (*Ob/Ob*) mice when compared to a genetically normal control.

3

181   However, maximal isometric tetanus force was significantly reduced in the *Ob/Ob* soleus with no effect
182   in EDL. In part, these findings were later confirmed by Bruton, Katz, Lännergren, Abbate and
183   Westerblad (8), reporting no changes maximal isometric force, but a significant improvement in
184   isometric force using a submaximal stimulation of whole EDL and single flexor digitorum brevis
185   muscle fibres isolated from 3-5 month old *Ob/Ob* mice. Similarly, Ciapaite, van den Berg, Houten,
186   Nicolay, van Dijk and Jeneson (10) reported that isometric twitch and tetanus force of whole EDL was
187   unaltered in 12 week old mice that consumed either a high fat lard or high fat palm oil diet for 5 weeks.
188   However, the peak contractile performance of the soleus muscle was significantly reduced in animals
189   fed a high fat palm diet but unchanged in animals fed a high fat lard diet. These results indicate that the
190   source of the lipid overload may be an important factor for determining skeletal muscle responses to
191   obesity, and are particularly interesting given the role of soleus as a postural muscle.
192
193   The effect induced by genetic obesity may be different to that imposed by dietary-induced obesity.
194   Contractile changes outlined in isolated muscle from *Ob/Ob* mice occurred in conjunction with a
195   significant reduction in muscle mass (8, 65), where changes in contractility occurred following an
196   elevated muscle mass in the dietary induced obesity model (10). In support, the ambiguity in obesity
197   induced phenotype changes has been in part attributed to differing response between genetically and
198   dietary induced obese models (14). Further work is needed to quantify the effects of dietary induced
199   obesity on isolated skeletal muscle performance given its relevance to the real world obesity problem.
200   In addition, previous isolated muscle work has used test temperatures between 20-26°C (8, 10) limiting
201   the application of findings to human skeletal muscle contractility. It should be noted that the contractile
202   performance of skeletal muscle is greatly influenced by temperature (27) and further work is needed to
203   evaluate change is muscle contractility using a more physiologically relevant thermal environment.
204
205   Measurements of maximal isometric stress reveal little about changes in dynamic muscular
206   contractility, which is an important aspect of real-world muscle function. *In vivo*, locomotory muscles
207   rarely work at constant lengths and measures of isometric force fail to consider the important
208   integration of the force-velocity relationship, the ability of the muscle to produce work during
209   shortening, and the passive resistance to stretch needed to accurately assess muscle power (28, 31, 32).
210   Furthermore, demonstrated increases in muscle activation times and, more commonly, relaxation time
211   (8, 10), will have profound effects on the ability of the muscle to produce power. The effect of obesity
212   on the fatigue resistance of isolated skeletal muscle is also unresolved.
213
214   The present study examines the effects of dietary induced obesity on the maximal power output and
215   fatigue resistance of soleus (slow twitch), EDL (fast twitch), and diaphragm (mixed fibre type) muscle,
216   isolated from young adult mice, at a test temperature of 37°C. By determining MyHC expression and
217   AMPK activity, the present study will attempt to gain a better understanding of the muscle specific
218   mechanisms causing the hypothesised decline in contractile performance. Importantly, the present work
219   is the first to accurately determine the obesity induced muscle specific changes in quality using the
220   work loop technique as a more accurate assessment of real life muscle function (28, 31, 32). By further
221   examining muscle specific changes in absolute force, power and contractile performance relative to
222   muscle mass, findings of the present work can be better applied to the real world locomotor
223   performance of the whole animal. The results of the present work will allow a greater understanding of
224   the specific role of skeletal muscle in the obesity induced reduction in physical activity, and the
225   potential that a reduction in contractile performance, including muscle quality, may be a significant
226   contributor to the obesity problem.
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244   **METHODOLOGY**

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246   ANIMAL MORPHOLOGY

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248   The procedures outlined in this study and the use of animals was approved by the ethical committee of
249   Coventry University. At 4 weeks of age, 56 CD1 female mice (Harlan, UK) were randomly split into
250   either an Obese or a Lean Control group. Each group were matched for body mass and snout to anus
251   length (n=28 in each case). For the next 16 weeks the Lean Control group were kept in cages of 8-10
252   individuals in 12:12-h light-dark cycle and were provided with water and standard lab chow (SDS
253   maintenance diet, Dietex International LTD; calories provided by Protein 17.5%, Fat 7.4%,
254   Carbohydrate, 75.1%; Gross Energy 3.52 Kcal/g; Metabolisable Energy 2.57 Kcal/g) ad libitum. The
255   Obese group were kept in identical conditions, but additionally were free to consume laboratory
256   supplied forage diet (PicoLab® Natural Sunflower; calories provided by Protein 18.0%, Fat 63.7%,
257   Carbohydrate, 18.4%; Gross Energy 5.2 Kcal/g; Metabolisable Energy 3.8 Kcal/g). Following the
258   treatment period, animals were weighed and snout to anus length measured. These data were then used
259   to calculate body mass index (BMI) and Lee index (weight0.33 (g)/Naso-Anal Length (cm); 53) of
260   obesity for each individual.

269
270   Animals were then sacrificed by cervical dislocation (in accordance with British Home Office Animals
271   (Scientific Procedures) Act 1986, Schedule 1) and the subcutaneous fat pad around the top of the hind
272   limbs and genitals was extracted and weighed. In addition, either whole SOL, EDL or DIA muscle was
273   dissected from each individual in refrigerated (1-3°C) oxygenated (95% O_2; 5% CO_2) Krebs Henseleit
274   solution (NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; $CaCl_2$ 2.54 mM
275   in each case; pH 7.55 at room temperature prior to oxygenation). The left limb muscle or right half
276   section of the DIA was immediately snap frozen in liquid nitrogen and stored in a -80°C freezer for
277   later biochemical analysis. The remaining limb muscle, or a ventral section of the costal DIA, was used
278   in the study of skeletal muscle contractility.

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281   CONTRACTILTIY MEASURES

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283   The tendon attachment at the proximal end of both the SOL and EDL were left intact and aluminium
284   foil T-clips were wrapped around the distal tendon as close to the muscle as possible. For the DIA,
285   aluminium foil T-clips were wrapped around the central tendon at one end, and at the opposing end,
286   two ribs anchoring the muscle were left intact.

287
288   Mechanical performance was measured using custom designed equipment. Each muscle preparation
289   was placed in a Perspex chamber filled with circulating oxygenated Krebs maintained at a
290   physiologically relevant 37°C. Using the intact bone or aluminium clips, the muscle preparation was
291   attached to a force transducer (UF1, Pioden Controls Ltd, UK) and a motor (V201, Ling Dynamic
292   Systems, UK) at each end via crocodile clips. The muscle was electrically stimulated to produce force
293   via parallel platinum electrodes submerged in the Krebs solution inside the muscle chamber.
294   Stimulation and length change parameters were controlled using custom written software (Testpoint,
295   CEC, Massachusetts, USA) via a D/A board (KPCI3108, Keithley Instruments, Ohio, USA) on a
296   standard desktop PC.

297
298   Initially muscle length and stimulation amplitude (typically 12-16V for SOL and DIA, 14-18V EDL)
299   were adjusted to produce a maximal isometric twitch response. Using these parameters, the muscle was
300   then subjected to a train of electrical stimuli (350ms for SOL, and 250ms EDL & DIA) with
301   stimulation frequency adjusted (usually 120Hz for SOL, 200Hz for EDL & 140Hz for DIA
302   respectively) to evoke a maximal isometric tetanus response. Time to half peak Tetanus (THPT) and
303   time from the last stimulus to half relaxation (LSHR) was measured for the maximal tetanus response
304   as a measure of activation and relaxation time. A 5-minute rest period was imposed between each
305   tetanus.

306
307   The optimal muscle length (L_0) for maximal twitch force, determined by the isometric tests, was
308   measured using an eyepiece graticule fitted to a microscope and estimates of mean fibre length were

determined as 85% of the physical length for SOL and 75% for EDL (28). No such estimates of fibre length have been reported for DIA, so the physical length measurement was use to represent L_0. This approach has been standard practice in previous work using these muscles (23, 28, 31, 55, 58).

Muscle power output was measured using the work loop (WL) technique. The method allows a more accurate assessment of muscle power and is a closer representation of the contractile mechanics used by power producing muscles *in vivo* (31, 32). Unlike other isolated muscle studies in this area that examined isometric force (8, 10, 65), the WL considers the interaction of force production during shortening, the force velocity relationship and work required to re lengthen the muscle in preparation for subsequent contraction (28, 31, 32). The *in vivo* relevance of this technique and its application has been outlined in our previous work (29, 54, 57, 58). Each muscle is subjected to a symmetrical sinusoidal length change around the previously determined optimal length and stimulated to produce force during the shortening phase. Length changes were implemented by a motor and position of the motor arm was measured using Linear Variable Displacement Transformer (DFG5.0, Solartron Metrology, UK). Instantaneous force and velocity were sampled, throughout the length change cycle, at a rate of 10 kHz and plotted against each other to form a work loop. Net work is calculated as the positive work produced during shortening, minus the work required to lengthen the muscle.

Electrical stimulation during the WL was delivered to the muscle at the optimal frequency and amplitude determined in the isometric tests. Strain (amplitude of length change), stimulus phase and burst duration were optimised to elicit maximal net work at cycle frequencies of 5Hz, 10Hz, and 7Hz for SOL, EDL and DIA respectively. Cycle frequency denotes the rate at which the work loops were performed and these cycle frequencies have been shown to elicit maximal PO in these muscles (6, 28). Typically, a strain of 0.10 of L_0 was used to produce maximal net work in each muscle. As such, the muscle increased in length by 5%, shortened by 10%, then was re-lengthened by 5% back to L_0. If the burst duration is too short, the muscle will not produce high amounts of force through the shortening phase (decreased positive work), too long and the muscle will be active during the re-lengthening phase (increased negative work). The optimal phase was typically -10ms for SOL, -2 ms for EDL and –5ms for DIA, indicating the start of stimulation with respect to maximal muscle length during the length change cycle. i.e. stimulation starts before maximal length is reached so that force has risen before shortening begins. The typical values for strain, burst duration and phase align with the values used to elicit maximal power output at these cycle frequencies in previous studies (23, 28, 29, 57, 58). Each muscle was subjected to four work loop cycles per run, with 5-minute rest intervals between each run.

Fatigues resistance was measured by subjecting each muscle to 50 consecutive work loop cycles at the parameters that elicited maximal power output. The decline in maximal power was plotted against time until each muscle produced less than 50% of its pre fatigue maximal power output. Similar methods have been employed in previous work using the work loop technique to examine the fatigability of muscle power (23, 58).

Finally, the muscle was detached from the equipment and tendons and bone removed. Each muscle was then blotted on absorbent paper, to remove excess Krebs solution, and placed on an electronic balance (Mettler Toledo B204-S, Zurich, Switzerland) to determine wet mass. Mean muscle cross-sectional area was calculated from L_0, muscle mass and an assumed muscle density of 1060 kg m^{-3} (41). Isometric stress was calculated as maximal tetanic force divided by mean muscle cross-sectional area. Muscle power output was normalised to muscle mass to express power as Watts.kg^{-1}.

BIOCHEMISTY

Fast and slow myosin heavy chain (MyHC) expression was measured to examine changes in fibre type composition and 5' AMP-activated protein kinase (AMPK) and phosphorylated AMPK were measured as an indicator of muscle metabolic responses. Proteins were extracted in RIPA buffer (Tris CL 20 mM, NaCl 150 mM, EDTA 1 mM, EGTA 1mM, NP40 1%, Na deoxycholate 1%, pH 7.5) with the addition of a protease and phosphatase inhibitor cocktail (Roche, Sydney, Australia). Protein concentrations were determined by capillary electrophoresis in a "Wes" Simple Western system (Protein Simple, Santa Clara, CA, USA) according to the manufacturer's instructions. All antibodies were from Abcam (Cambridge, MA, USA), and we determined concentrations of total fast (ab51263) and slow (ab11083) skeletal myosin heavy chains, AMPK alpha 1 + 2 (ab80039), phosphorylated AMPK alpha 1 (phosphorylated at T173) and 2 (phosphorylated at T172; ab133448), and α-tubulin (ab80779) as internal control (35). Note that AMPK is activated by phosphorylation so that activity of

AMPK is expressed by the ratio between phosphorylated (pAMPK) and total AMPK concentrations (63). All antibody and protein concentrations were optimized following the manufacturer's recommendations. All samples were run in duplicate and we interspersed samples from different treatments on the same plate.

STATISTICAL METHOD

Following appropriate checks of normality and homogeneity, morphological, contractile and protein data was analysed using two-tailed independent samples T-Tests. On the small number of occasions where the data was not normally distributed Mann-Whitney U tests were performed. Where the work loop power of the muscles extracted from obese animals was statistically different from lean controls, Spearman's rank correlations were performed to assess the relationship between body mass and normalised work loop power to determine if the magnitude of obesity effected muscle performance. Further Spearman's rank correlations were performed to analyse the relationship between morphological measures.

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434 **RESULTS**
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436 MORPHOLOGY
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438 Whole animal body mass, fat pad mass, BMI and Lee index of obesity were significantly greater in the
439 obese group when compared to controls (Table 1; Mann-Whitney P<0.001 for body mass & fat pad
440 mass; T-Test P<0.001 for BMI & Lee Index). Body length was not significantly different (Table 1
441 Mann-Whitney P=0.053). When broken down into each treatment group whole animal body mass, BMI
442 and Lee index of obesity were significantly greater in the obese groups when compared to the lean
443 controls (Table 2 P<0.05 in all cases). For both the SOL and EDL, obese muscle mass was significantly
444 greater (Table 2 T-Test P<0.03 in each case) but muscle length was not affected (Table 2 T-Test
445 P=0.80 for SOL; Mann-Whitney P=0.15 for EDL).
446
447 Within the obese group, measures of body mass correlated well with body length and fat pad mass
448 (Spearmans' r> 0.4 P<0.03 in each case), In addition, fat pad mass was strongly associated with greater
449 BMI and Lee index (Spearmans' r> 0.7 P<0.003 in both cases).
450
451 ISOMETRIC STRESS
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453 For SOL, absolute maximal isometric force was significantly higher in the obese group compared to
454 the lean control (Fig 1B T-Test P=0.003), however maximal isometric stress was not significantly
455 different (Fig 1A T-Test P=0.38). The absolute maximal isometric force generated by the EDL was not
456 significantly different between the obese and the lean group (Fig 1D T-Test P=0.20), however maximal
457 isometric stress was significantly reduced in the obese group (Fig 1C T-Test P<0.005). Similarly, the
458 maximal isometric stress of obese DIA was significantly lower than lean controls (Fig 1E T-Test
459 P<0.001). Absolute force and power was not assessed for the DIA as only a section of this muscle was
460 used in the examination of contractile performance.
461
462 THPT was not significantly different between the obese and the lean control group for SOL, EDL or
463 DIA (Table 3 P>0.24 in each case). Similarly, LSHR for EDL was not significantly different between
464 the obese and lean group (Table 3 T-Test P=0.67). In the obese SOL, LSHR was significantly
465 prolonged (Table 3 T-Test P=0.01), whilst in obese DIA, LSHR was significantly shorter when
466 compared to the controls (Table 3 Mann-Whitney P=0.02).
467
468 WL PO & FATIGUE RESITANCE
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470 For both the SOL and EDL, absolute WL power was not significantly different between the obese and
471 the lean groups (Fig 2B & D T-Test P>0.44 in each case). When normalised to muscle mass, SOL WL
472 power was not significantly affected (Fig 2A Mann-Whitney P=0.30), however normalised power was
473 significantly reduced in the obese DIA and EDL (Fig 2C & E T-Test P<0.006 in each case). For the
474 obese group there was no significant relationship between normalised WL power and body mass for
475 EDL (Fig 3A Spearmans' r=-0.183 P=0.64), however Obese DIA extracted from animals with a larger
476 body mass had significantly reduced performance (Fig 3B Spearmans' r=-0.714 P=0.047).
477
478 SOL from the obese group fatigued significantly faster than lean controls (Fig 4A T-Test P=0.002).
479 There were no significant differences in the fatigue response for either the EDL or DIA (Fig 4B & C T-
480 Test P>0.8 in each case).
481
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483 PROTEIN EXPRESSION
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485 SOL in obese mice had significantly less slow MyHC/α-Tubulin (Fig 4A T-Test P=0.01); fast
486 MyHC/α-Tubulin showed a similar trend but the data were more variable so that there was no
487 significant difference between obese and lean SOL (Fig 4B T-Test P= 0.16), and the ratio between
488 slow and fast MyHC did not differ (Fig 4C Mann-Whitney P=0.82). DIA had significantly greater slow

and fast MyHC/α-Tubulin in obese animals compared to the lean group (Fig 2G, H T-Test P<0.04 in each case), and there was no difference in the ratio between slow and fast MyHC between treatment groups (Fig 4I T-Test P= 0.23). Slow MyHC/α-Tubulin and Fast MyHC/α-Tubulin were not significantly different between the obese and lean EDL (Fig 4D, E T-Test P=0.83 & Mann-Whitney P=0.60 respectively), and there was no difference in the ratio between slow and fast MyHC between treatment groups (Fig 4F T-Test P=0.53)

AMPK/α-Tubulin, pAMPK/α-Tubulin and pAMPK/AMPK were significantly reduced in the obese SOL group compared to lean controls (Fig 6 A, B, C T-Test P<0.007 in each case). Conversely AMPK/α-Tubulin, pAMPK/α-Tubulin and pAMPK/AMPK were significantly greater in the obese DIA (Fig 6 G, H, I T-Test P<0.03 in each case). AMPK/α-Tubulin was lower in the obese EDL and this was approaching statistical significance (Fig 6D T-Test P=0.05), however pAMPK/α-Tubulin and pAMPK/AMPK were unchanged (Fig 6E, F T-Test P>0.05 in both cases).

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555 **DISCUSSION**
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557 Our results indicate that obesity causes a decline in the contractile performance of isolated skeletal
558 muscle. These findings are the first to offer a detailed insight into the direct changes in absolute
559 contractile performance and muscle quality using a methodological approach which more closely
560 represents the environmental conditions and contractile mechanics of skeletal muscle *in vivo*. The
561 present findings indicate that the decline in contractile performance is likely to relate to fibre type
562 composition, metabolic profile and the *in vivo* mechanical role of each muscle.
563
564 THE EFFECT OF OBESITY ON MAXIMAL FORCE AND POWER
565
566 The absolute isometric force of the obese SOL was significantly greater than the lean control group.
567 This result is unsurprising given the role of SOL in postural control and proposed training stimulus
568 evoked by the elevated body mass. Similar increases in the absolute strength of 'antigravity' muscles
569 have been reported in previous *in vivo* literature (See review by 39). Interestingly, this increase in force
570 producing capacity did not transfer to an increase in the absolute power output produced by SOL.
571 These findings may suggest an obesity induced favourable adaptation for the SOL in static isometric
572 contractions needed for such activities as quiet standing, which does not necessarily transfer to an
573 improvement in locomotor performance given that when producing power *in vivo*, SOL of the obese
574 group would be working to move a greater whole animal body mass. Isometric stress and normalised
575 WL power were unaffected in the SOL, possibly demonstrating that the muscle quality of the obese
576 group was maintained. Given that normalised WL power was also unaffected, one would anticipate a
577 similar increase in absolute power given the increase in muscle mass. Surprisingly this was not
578 demonstrated in the statistical results and maybe attributed to the large variation in this data set.
579
580 The maximal isometric stress and normalised work loop power output was significantly reduced in the
581 obese EDL. With respect to the limited changes in absolute isometric force and work loop power, and
582 increase in muscle mass, these results infer that in the case of the obese EDL, larger muscles of poorer
583 quality are formed to maintain the same absolute contractile performance as the lean counterparts. *In*
584 *vivo* this would present two significant problems. Firstly, although absolute performance is maintained,
585 larger muscles will add to the whole animal body mass thus increasing body inertia. Given this and the
586 significant increase in body mass that will arise via adipose tissue accumulation, the maintenance of
587 absolute force and power is likely to be inadequate given the increase in load. Similar to the EDL,
588 isometric stress and normalised WL power of the obese DIA was significantly lower compared to the
589 lean control group. This again suggests that there is a significant reduction in muscle quality.
590
591 As expected with dietary induced obesity models, there was a large variation in the body mass for the
592 obese group. Interestingly, the obese DIA muscles that were extracted from animals with a higher body
593 mass had significantly lower normalised WL power. This possibly indicates a negative relationship
594 between the quantity of adipose tissue and muscle quality for this muscle. No such effects were
595 demonstrated for the EDL, however it is clear from the data that this response needs to be analysed
596 using a larger sample size. Further exploration is also needed considering the distribution of adipose
597 tissue deposits at a whole body and muscle specific level to determine whether increased body mass is
598 linked to increased intramuscular adipose tissue.
599
600 Given the mechanical role and fibre type composition of each muscle used in the present work, it is
601 unsurprising to see a muscle specific response to lipid accumulation. With the SOL being composed
602 primarily of slow oxidative fibres, it could be considered that this muscle already has a preferable
603 metabolic profile to oxidise lipids, in comparison to EDL and DIA which have a relatively faster fibre
604 type composition. Similar sentiments have been reported in previous literature (10). As such it is
605 possible that lipid accumulation in the SOL would be less than the other muscle tested, thus potentially
606 delaying the onset of degenerative mechanisms. Although it could be considered that the mechanical
607 loading of the EDL and DIA may be increased (larger foot and thoracic cavity mass respectively), the
608 magnitude of which is likely to be lower that the SOL due to its role in postural support.

609 It is clear from the contractile evidence demonstrated here and that of previous literature (20, 50), that
610 an increased load may evoke a substantial training stimulus to promote muscle adaptation. However,
611 one would expect a progressive resistance training program to evoke increases in both contractile
612 protein quantity (mass) and quality (24, 26, 49), which was not demonstrated in our obese model. This
613 may inadvertently point to defects in the process of myogenesis which has previously been reported as
614 a consequence of obesity (4, 12).

616 THE EFFECT OF OBESITY ON FATIGUE RESISTANCE

618 Although a number of *in vivo* studies demonstrated an obesity associated reduction in the ability to
619 sustain locomotor performance (16, 48), and more specifically skeletal muscle force production (38), in
620 reality this evidence tells us little about the direct effect of obesity on skeletal muscle performance. It is
621 likely that skeletal muscle of an obese experimental group will fatigue much faster *in vivo* than a lean
622 group, irrespective of exercise intensity, due to elevated body inertia. To date the effect of obesity on
623 the fatigue resistance of isolated skeletal muscle has only been examined by Bruton, Katz, Lännergren,
624 Abbate and Westerblad (8), who demonstrated reduced fatigue resistance of single flexor digitorum
625 brevis fibres but no effect on the whole EDL of *Ob/Ob* mice following a bout of repeated tetanic
626 stimulations at a submaximal intensity. The present findings uniquely examine the isolated skeletal
627 muscle fatigue resistance following dietary induced obesity and using dynamic contractions to estimate
628 changes in muscle power output.

630 Despite the acute contractile performance of the SOL being reasonably well maintained, when
631 subjected to a bout of repeated WL contractions, the obese SOL fatigued significantly faster than the
632 lean control group. Such findings would indicate a significant limitation to sustained locomotory
633 performance. Mechanistically, this may relate to the demonstrated reduction in slow MyHC expression.
634 The ability to release and reuptake Ca^{2+} from and to the Sarcoplasmic Reticulum (SR) dictates the rate
635 and magnitude of force production and relaxation. An obesity induced increase in tetanus relaxation
636 time may point to a change in Ca^{2+} kinetics, particularly as previous research has demonstrated an
637 obesity associated reduction in the function of SERCA, which is responsible for the movement of Ca^{2+}
638 from the cytoplasm back into the SR (19). If the muscle is still active during the re-lengthening phase
639 of the work loop, this will significantly increase the work required to lengthen the muscle (negative
640 work) and as a consequence, decrease the net work produced. An elevated relaxation time has been
641 reported as a consequence of fatiguing contractions in normal conditions in some muscles (5), which
642 given the present data is likely to be further exacerbated in the obese condition. Furthermore, obesity
643 has been associated with a reduction in the efficacy of actin-myosin cross bridge cycling that may
644 possibly occur independently of changes in SERCA (10, 51).

646 Although the pattern of fatigue would appear to be unaffected in EDL and DIA, these data are plotted
647 from 100% of maximal power obtained for each of the obese and the lean group. As such one should
648 consider that the 100% power values for the obese group would be significantly lower in the obese
649 group compared to the controls as outlined in the acute data. As such, if muscles of each group were to
650 work at the same absolute intensity, the obese group would be working closer to maximum power
651 compared to the lean group and subsequently will fatigue more quickly.

653 This is the first evidence to demonstrate that the reduction in endurance capacity seen *in vivo* (16, 48)
654 can be in part be attributed to a reduction in the fatigue resistance of skeletal muscle. The reduction in
655 muscle fatigue resistance is likely to be further magnified *in vivo* by the elevated body inertia that will
656 arise from an increase in body mass. The reduction in the fatigue resistance of the DIA could have
657 further substantial consequences for *in vivo* performance. Limiting pulmonary function will
658 subsequently affect the quantity of oxygen delivered to muscle throughout the body, and as a result the
659 capacity to regenerate ATP. The delivery of oxygen to muscle is also fundamental for lipid oxidation,
660 thus potentially limiting the ability to unitise lipid as an energy source during physical activity and thus
661 further exacerbating accumulation.

663 MECHANISMS

665 Obesity has been shown to affect a number of important metabolic and cellular processes involved with
666 force production. This data is vital in our understanding of mechanistic changes that occur, but given
667 the dearth of research exploring both contractility and underpinning mechanisms, there is difficulty in
668 mapping the muscle specific changes in contractile performance with specific mechanisms.

669

670 Although the reduction in slow MyHC expression may help to explain the reduced fatigue resistance in
671 the SOL, the normalised force and power of the EDL and DIA occurred without a change in the ratio of
672 fast and slow MyHC expression. Evidence examining the effect of obesity on muscle fibre type
673 composition is varied (as discussed by 14). Although there is evidence demonstrating a shift to both a
674 faster and slower phenotype in obese experimental groups (14, 33, 34, 52, 59, 61, 65), equally there is
675 evidence reporting no change (13, 14, 52, 60). Interestingly, Warmington, Tolan and McBennett (65)
676 demonstrate that despite a shift to a slower fibre type, the maximal force generating capacity of the
677 muscle was unchanged in *Ob/Ob* mice. Interpretation and comparison of the evidence reported in
678 previous literature is however subject to the same methodological discrepancies identified in studies
679 measuring muscle performance. The present findings infer that fibre type shifts may play are role, but
680 do not substantially explain the reduction in the obesity induced change in contractile performance.
681 Given that the present data only examines 16 weeks of feeding, changes in fibre type expression could
682 elicit more significant mechanical consequences following longer feeding periods.

683

684 AMPK is an important regulator of energy homeostasis in the body, and more specifically, skeletal
685 muscle (44, 46). A change in AMPK activity would result in a reduction in the ability to regenerate
686 ATP via both glycolysis and fatty acid oxidation (44), and consequently may affect the contractile
687 performance. Principally a change in muscular AMPK activity is likely to have little effect on the
688 ability of the muscle to produce one off maximal force and power as the energy for this is expected to
689 come from the small quantity of available ATP. However, the demonstrated reduced pAMPK/AMPK
690 expression may help to further explain the significantly reduced time to fatigue in the obese SOL. The
691 increase in pAMPK/AMPK in the obese DIA had little effect on the pattern of fatigue. Interestingly, in
692 the obese DIA, when compared to the obese SOL, the results of the DIA may call into question the
693 contribution of this mechanism to the given decline in obese SOL fatigability. However, it is likely that
694 the demand for ATP per unit mass of tissue is much less in the obese DIA compared to the lean DIA
695 given the significant reduction in normalised maximal power. Interestingly, despite a reduction in
696 AMPK concentration in the obese EDL, pAMPK/AMPK was unchanged as was the pattern of fatigue.

697

698 These findings in part support previous evidence demonstrating an obesity related changes in the
699 metabolic profile of skeletal muscle (10, 13, 25). As indicated by (13), this response is likely to be
700 muscle specific and its complexity relate to fibre type and duration of high fat diet consumption.
701 Although there were some favourable effects for DIA, this had little effect on contractile performance.
702 Irrespective of the muscle specific application, insufficient APMK activity in the muscle may further
703 exacerbate lipid accumulation via reduced lipid oxidation and as such, may further promote the decline
704 in contractile function through this and other mechanisms (46).

705

706 As previously outlined, literature has demonstrated an obesity associated decline in muscle protein
707 synthesis (4, 12). Degradation in the normal process of contractile protein maintenance and
708 regeneration would have significant implications on mechanical performance. The increased quantity
709 of both fast and slow myosin heavy chain expression in the obese DIA would conceivably contradict
710 this previous evidence. These results would infer that the quantity of lean tissue was greater in the
711 obese DIA, however the normalised contractile performance of this muscle was significantly reduced.
712 In addition, the increase in the absolute force of SOL and the proposed similar concentrations on lean
713 mass between obese and lean EDL (i.e. no change in slow and fast MyHC expression) were not
714 coupled with improved or maintained muscle quality respectively. This suggests that although
715 plasticity in skeletal muscle modelling is continued, the quality of the contractile protein produced is
716 significantly reduced, thus supporting the demonstrated reduction in protein synthesis previously
717 reported (4, 12).

718

719 The results of the present work demonstrate a complex and muscle specific interaction in the down
720 regulation of important processes that evoke contractility, which likely gives rise to the muscle specific
721 decline in performance outlined in this study. The contribution of each of the proposed mechanisms is
722 still unknown and is likely to change with obesity status. Furthermore, the demonstrated reduction in
723 skeletal muscle performance may be further exacerbated *in vivo* given the reported changes in
724 neuromuscular recruitment (67). As such a mechanism for the reduction in muscle quality and
725 subsequent compensatory increase in size may be due to obesity induced denervation affecting the
726 ability to efficiently recruit fibres. However, given the lack of studies in this area, it is not clear whether
727 a reduction in recruitment is a cause or a consequence of the skeletal muscle obesity response.

728

LIMITAIONS & FUTURE DIRECTION

Although isometric stress and normalised WL power provide an accurate assessment of muscle quality per unit of muscle mass, it is considered that in the obese group a smaller proportion of the total mass will be contractile protein due to the greater infiltration and accumulation of lipids. Normalising contractile performance to lean tissue mass would allow further consideration of how much the change in muscle quality is related to changes in lipid accumulation. However, there are significant methodological problems with accurately obtaining measure of muscular lipid and contractile mass. Previous work has indicated that obesity can cause a two fold increase in skeletal muscle lipid content (21). Machann, Bachmann, Brechtel, Dahl, Wietek, Klumpp, Haring, Claussen, Jacob and Schick (36) further demonstrated a muscle specific increase in the lipid content of skeletal muscle of obese individuals. The lipid content of the tibialis anterior (relatively fast twitch fibre composition) increased from 1.6% in normal weight individuals to 2.8% in obese individuals, and from 2.5% to 3.8% in the SOL. Given these findings, and that fat is less dense than lean muscle mass, it is likely the potential elevation in lipid content in the obese muscles of the present study will only be a minor contributor to the significant increase in muscle mass. As such, lipid storage itself is likely to only play a small role in the obesity associated reduction in muscle quality.

Although the sinusoidal length change waveform used in the present study provides an approximation of *in vivo* cyclical muscle activities, it is a simplification of the length change waveforms used in real-life locomotion (15). In particular during fatiguing contractions, the pattern of fibre stimulation and length change waveforms are likely to be manipulated throughout movement (62). Therefore, if a muscle is active as it begins to re-lengthen (i.e. producing too much eccentric force), the duration of stimulation is likely to be reduced in order to lessen the elevated negative work and any associated muscle damage. That considered, the model used in this study is appropriate to assess the decline in the ability of the muscle to produce maximal power during repeated contractions and is representative of the protocol used in other isolated skeletal muscle studies (23, 29, 30, 55, 56, 58).

We followed published protocols in determining MyHC concentrations (35). However, there is a suggestion in the literature (11) that using low salt buffers such as RIPA buffers underestimates the concentrations of MyHC. Our measures of MyHC concentrations may therefore be an underestimate, but this will not affect our comparisons between obese and lean individuals, or between the relative abundance of slow and fast MyHC within muscles.

These results offer an important insight into the effects of obesity on the contractile performance of isolated skeletal muscle, however future work should consider examining contractile performance following a varied range of feeding periods. It is clear from the evidence in the literature that skeletal muscle mechanistic response is likely to change depending on duration of feeding and as a result it should be considered that the contractile performance will alter accordingly. Such work would be valuable in determining the muscle specific onset of obesity related changes in muscle performance and the potentially more severe implications of feeding regimes longer than that used in the present work. Given the importance of the present findings, it would also be of interest to repeat this work in an ageing animal model given the recent popularity in studies examining the relationship between obesity and sarcopenia.

CONCLUSION

The present findings demonstrate a muscle specific reduction in the contractile performance of isolated skeletal muscle which is likely related to a combination of *in vivo* mechanical role, fibre type expression and metabolic profile. The increase in the absolute isometric force of the SOL is unsurprising given the role of this muscle in postural support, however this increase occurred without a change in muscle quality (normalised force and power), potentially demonstrating detrimental effects of obesity on skeletal muscle plasticity and myogenesis. Although the absolute contractile performance of the EDL was maintained, muscle quality was significantly reduced. As such, in order for the obese group to maintain the same performance as the lean counterparts, larger muscles of lower quality were produced, thus further adding to *in vivo* force and power requirements needed to support and overcome the elevated whole animal body mass. The results are the first to assess the effect of obesity on fatigue resistance during power production in isolated skeletal muscle and demonstrate that obese mice would be unlikely to maintain the same absolute power output in SOL, EDL and DIA muscles for as long as lean animals. These results indicate that irrespective of the increase in body inertia, the reduction in

locomotory performance demonstrated *in vivo* can be in part attributed to a reduction in the fatigue resistance of skeletal muscle. The present results confirm that mechanistically, significant changes in contractile performance can occur in EDL and DIA without a change in fibre type composition. Although there is some previous evidence alluding to changes in metabolic profile, Ca^{2+} handling and protein synthesis, future work should focus on establishing the onset and extent of these mechanisms in relation to changes in contractile mechanics. In summary, a reduction in the contractile performance of skeletal muscle could be a significant catalyst to the negative cycle of obesity. Reducing the capacity to locomote and maintain adequate pulmonary function, is likely to contribute to a reduction in quality of life, exercise capacity and will sustain a significant calorific imbalance.

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856 **AKNOWLEDGMENTS**
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FUNDING

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1126
1127 **FIGURES**
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1129 Figure 1 – The effect of 16 weeks HFD on the maximal isometric tetanus stress and absolute isometric
1130 tetanus force of isolated mouse SOL (A & B), EDL (C & D) and DIA (E) [Data represented as
1131 Mean±SE; N=10 for SOL; N=10 for EDL lean; N=9 for EDL obese; N=8 for DIA; * represent
1132 significant differences]
1133
1134 Figure 2 – The effect of 16 weeks HFD on the maximal normalised WL PO and absolute WL PO of
1135 isolated mouse SOL (A & B), EDL (C & D) and DIA (E) [Data represented as Mean±SE; N=10 for
1136 SOL; N=10 for EDL lean; N=9 for EDL obese; N=8 for DIA; * represent significant differences]
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1138 Figure 3 - The relationship between whole animal body mass and normalised work loop power for the
1139 obese EDL (A) and DIA (B) experimental groups [N=9 for EDL; N=8 for DIA; The lines represent a
1140 first-order polynomial fitted to the data using a least squares regression and the 95% confidence limits
1141 of this line]
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1143 Figure 4 - The effect of 16 weeks HFD on the fatigue resistance of maximally stimulated mouse SOL
1144 (A), EDL (B) and DIA (C) [Data represented as Mean±SE; N=10 for SOL; N=10 for EDL lean; N=9
1145 for EDL obese; N=8 for DIA; * represent significant differences]
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1147 Figure 5 – The effect of 16 weeks HFD on fast and slow MyHC expression of mouse SOL, EDL and
1148 DIA [Data represented as Mean±SE; N=6 in each case; * represent significant differences]
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1150 Figure 6 - The effect of 16 weeks HFD on the AMPK activity of mouse SOL, EDL and DIA [Data
1151 represented as Mean±SE; N=7 for SOL; N=6 for EDL; N=6 for DIA; * represent significant
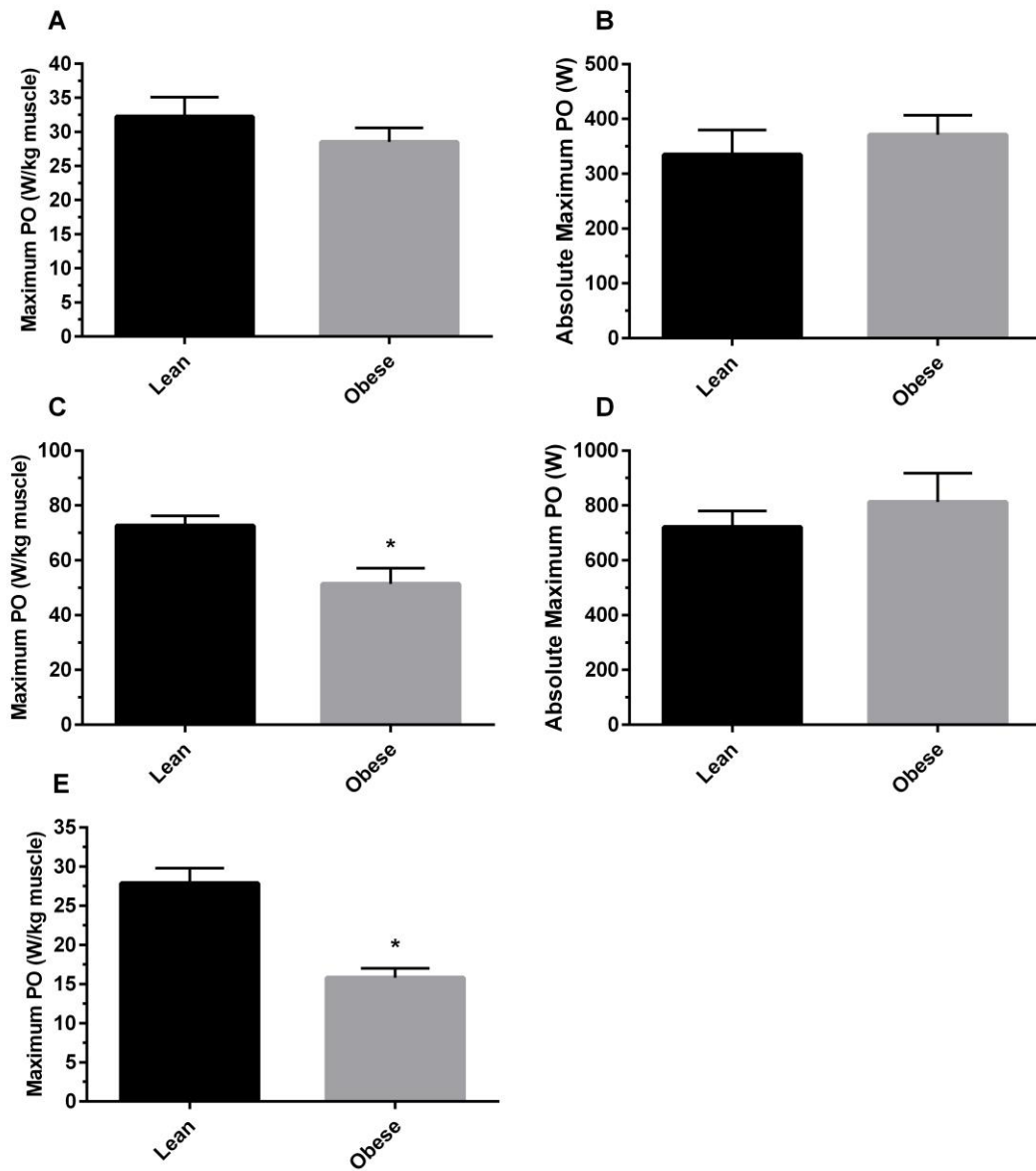1152 differences]
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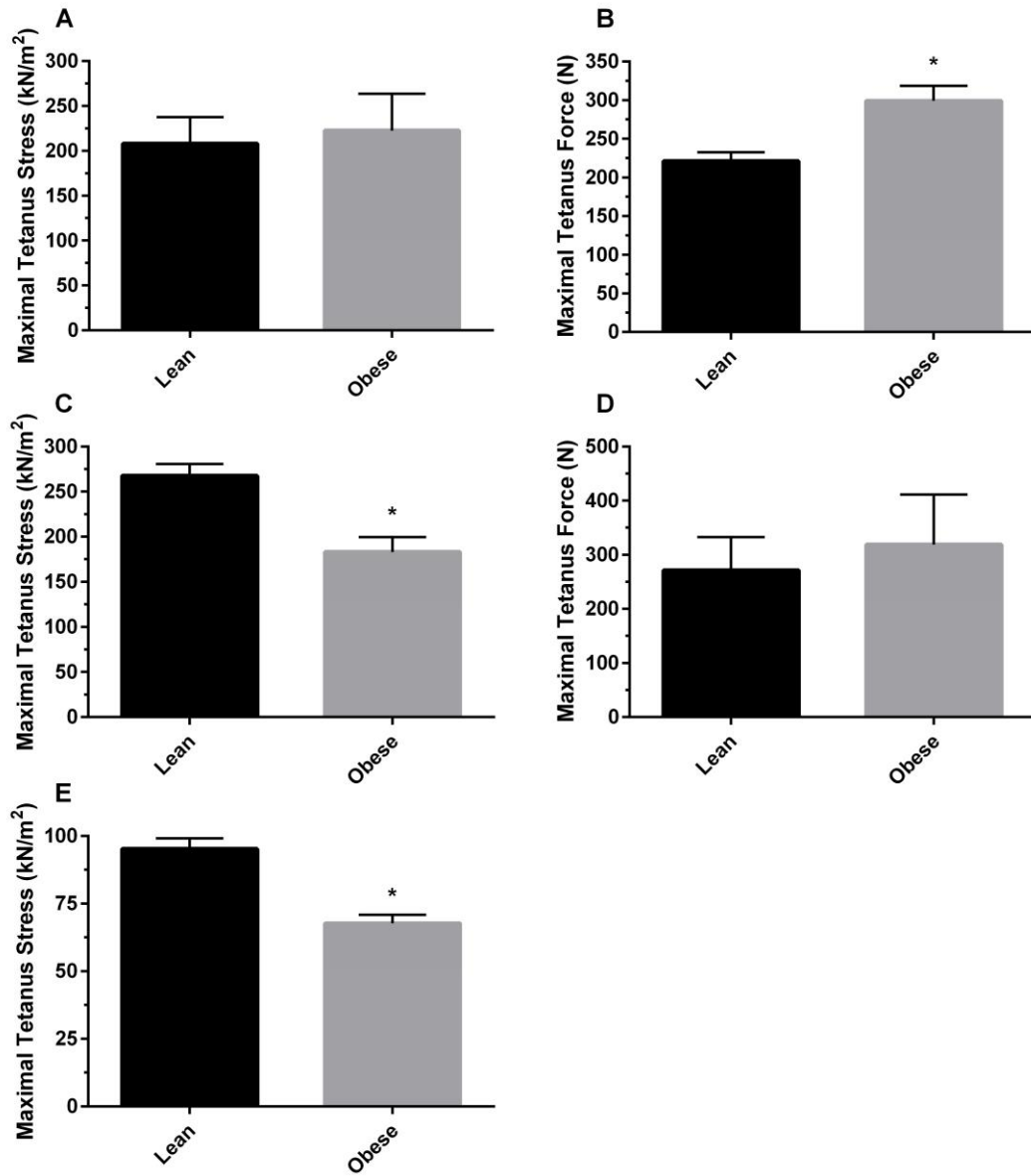
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1189 **FIGURE 1.**

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FIGURE 2.
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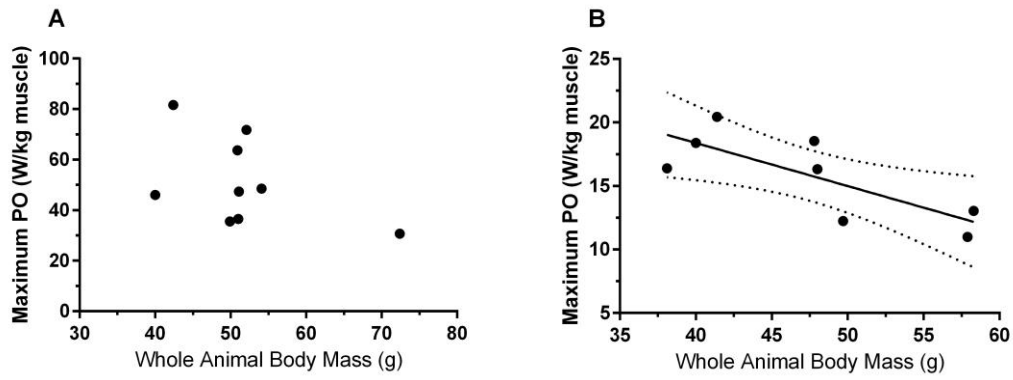1216 **FIGURE 3.**
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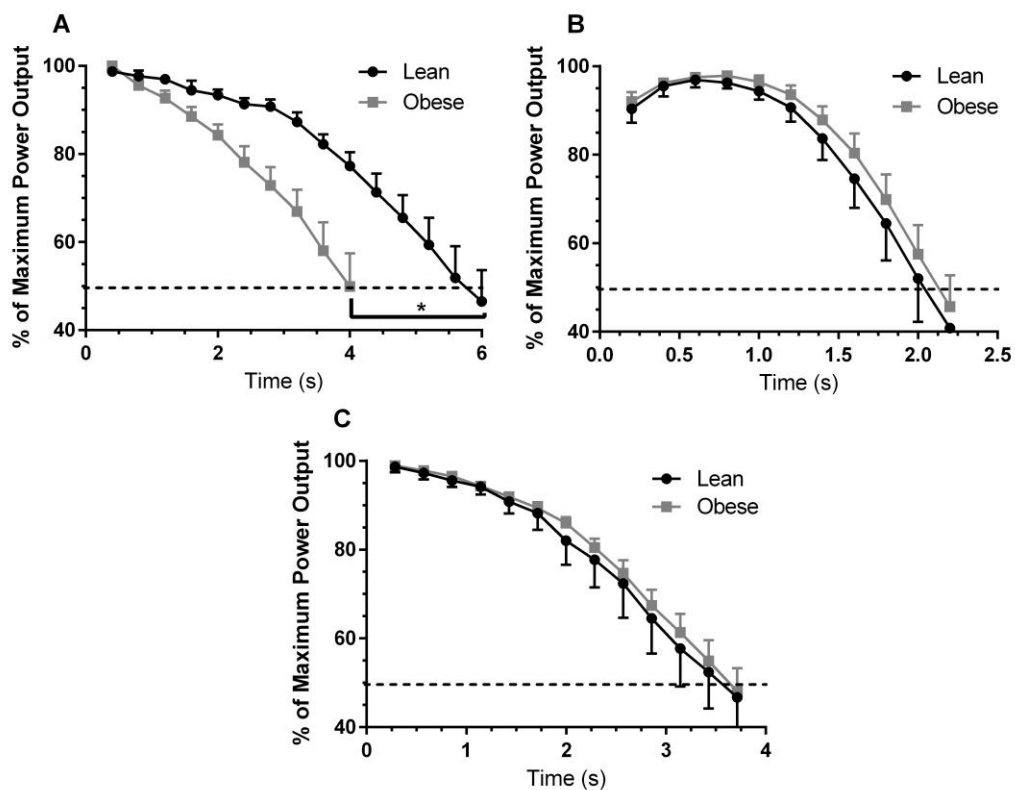1255
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1263 **FIGURE 4.**
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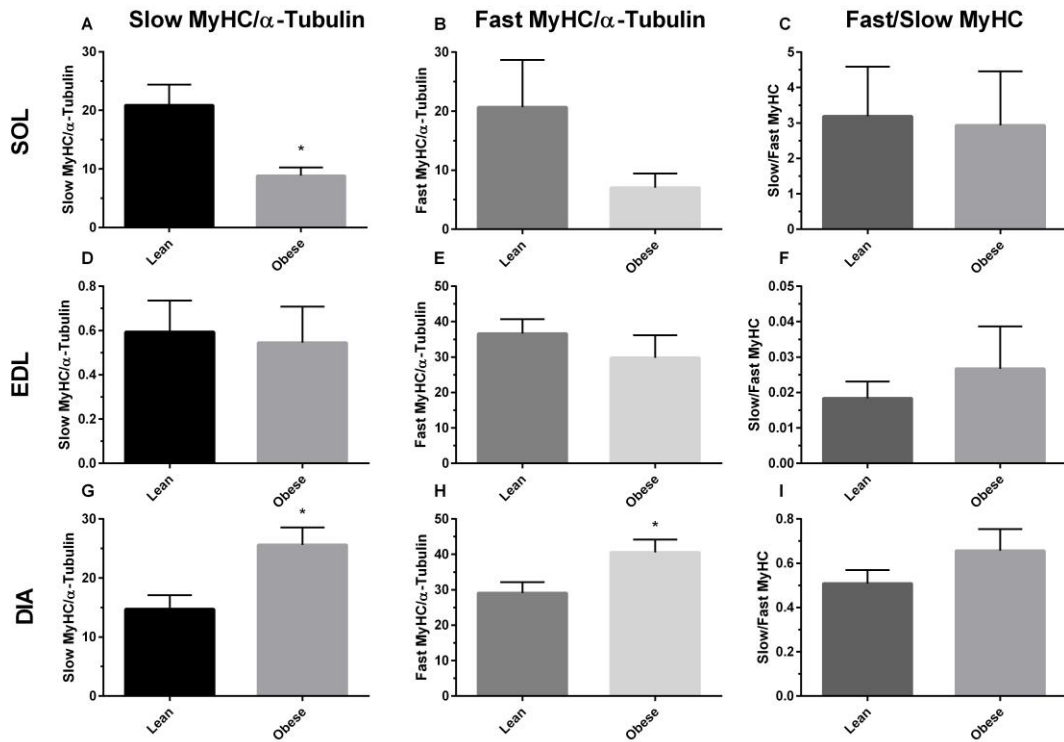1288
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A Slow MyHC/α-Tubulin   **B** Fast MyHC/α-Tubulin   **C** Fast/Slow MyHC

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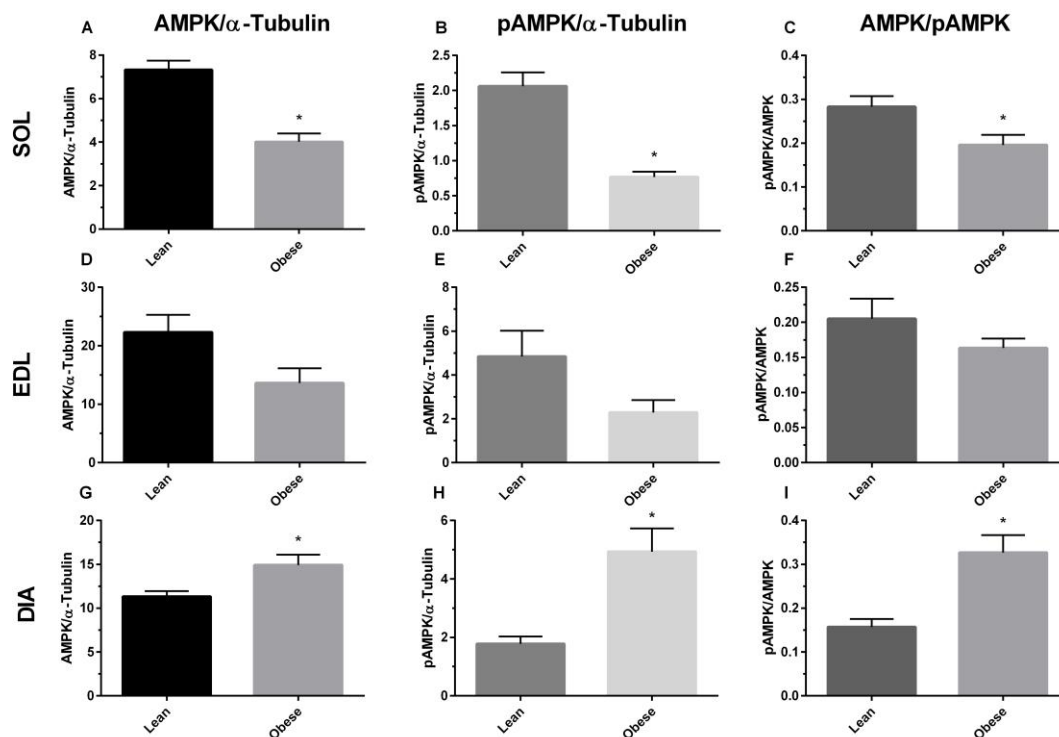1320
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1331 **FIGURE 6.**

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TABLES

Table 1 - The effect of 16 weeks HFD on the anthropometric measures [Data represented as Mean±SE; N= 30 & 29 lean & obese respectively, N=18 & 16 for lean and obese fat pad mass respectively; * indicate significant differences between Lean and Obese groups]

Table 2 - The effect of 16 weeks HFD on muscle group specific anthropometric measures [Data represented as Mean±SE; N=10 for SOL; N=10 for EDL lean; N=9 for EDL obese; N=8 for DIA; * represent significant differences between Lean and Obese groups]

Table 3 – The effect of 16 weeks HFD on isometric time to half peak tetanus (THPT) and last stimulus to half tetanus relaxation (LSHR) of isolated mouse SOL, EDL and DIA [Data represented as Mean±SE; N=10 for SOL; N=10 for EDL lean; N=9 for EDL obese; N=8 for DIA; * represent significant differences between Lean and Obese groups]

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1425 **TABLES**
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Table 1 - The effect of 16 weeks HFD on the anthropometric measures [Data represented as Mean±SE; N= 30 & 29 lean & obese respectively, N=18 & 16 for lean and obese fat pad mass respectively; * indicate significant differences between Lean and Obese groups]

	Lean	Obese
BM (g)	38.5±1.00	52.7±2.30[*]
Body Length (cm)	11.3±0.09	11.6±0.09
BMI	0.30±0.01	0.39±0.01[*]
Lee Index	0.30±0.00	0.32±0.02[*]
Fat Pad Mass (g)	0.73±0.08	5.24±0.52[*]

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Table 2 - The effect of 16 weeks HFD on muscle group specific anthropometric measures [Data represented as Mean±SE; N=10 for SOL; N=10 for EDL lean; N=9 for EDL obese; N=8 for DIA; * represent significant differences between Lean and Obese groups]

	BM (g)	Body Length (cm)	BMI	Lee Index	Muscle Mass (mg)	Muscle Length (mm)
SOL L	39.7±1.93	11.2±0.24	3.15±0.13	0.30±0.01	10.1±0.02	8.96±0.07
SOL OB	52.0±1.90*	11.5±0.20	3.93±0.15*	0.32±0.01*	13.0±0.80*	9.07±0.19
EDL L	35.5±1.2	11.2±0.08	2.84±0.00	0.29±0.00	10.0±0.03	9.26±0.40
EDL OB	57.5±5.61*	11.6±0.14*	4.21±0.35*	0.33±0.01*	14.4±1.97*	8.45±0.25
DIA L	40.9±1.34	11.6±0.09	3.05±0.02	0.30±0.00		
DIA OB	47.7±2.71*	11.0±0.15	3.59±0.15*	0.31±0.00*		

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Table 3 – The effect of 16 weeks HFD on isometric time to half peak tetanus (THPT) and last stimulus to half tetanus relaxation (LSHR) of isolated mouse SOL, EDL and DIA [Data represented as Mean±SE; N=10 for SOL; N=10 for EDL lean; N=9 for EDL obese; N=8 for DIA; * represent significant differences between Lean and Obese groups]

	THPT (ms)		LSHR (ms)	
	Lean	Obese	Lean	Obese
SOL	39.1±3.5	34.4±1.1	47.2±2.5	57.1±2.6*
EDL	17.0±1.1	17.4±1.1	13.6±1.4	14.3±0.9
DIA	26.1±1.8	25.0±1.1	28.1±1.8	23.8±0.8*

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