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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

INTRODUCTION

Obesity is a global epidemic, attributed to calorific rich foods and reduced physical activity (45). Associated health complications such as metabolic syndrome, cardiovascular disease, diabetes, musculoskeletal disorders, and some cancers (17) contribute to mortality, poor quality of life and significant financial implications for healthcare providers (2, 18, 22). If energy intake is not balanced with expenditure, adipose tissue accumulation occurs and is stored viscerally, subcutaneously and ectopically in organs, including skeletal muscle (3). Skeletal muscle is the largest regulator of metabolism in the body and contractility is needed to produce movement, highlighting the importance of investigating the effect of elevated lipid content on this tissue. Presently it is not clear whether lipid accumulation attenuates skeletal muscle contractility.

Previous *in vitro* studies suggest that obesity may improve absolute strength of 'antigravity' muscles, but has little effect on musculature that is not loaded with an increased body mass (see review 39). For example, Rolland, Lauwers-Cances, Pahor, Fillaux, Grandjean and Vellas (50) reports an increase in the absolute force generating capacity of the knee extensors in obese elderly women, without significant changes in handgrip strength. These results are not surprising given the potential training adaptation that may occur due to the increased demand placed on the postural muscles during standing and locomotion (20). Interestingly, the small number of studies examining muscular fatigue have conflicting findings (37, 38, 40, 42, 47). Although variation in experimental methods and participants (i.e. differences in muscle groups tested, mode of exercise, age and gender of population) make comparisons between these studies difficult, the authors argue that the true effect of obesity on muscular endurance cannot be accurately evaluated *in vivo*, as irrespective of exercise intensity, musculature of the obese group will have to produce greater force to overcome greater inertia of the moving limb. Further limitations also arise when relating the principle findings of this body of work directly to skeletal muscle function.

The majority of human studies measure muscular strength (39), and although this is an important mechanical parameter, dynamic power is needed for locomotion. Strength assessments largely involve gross joint movements and are influenced by neuromuscular recruitment, making it impossible to accurately examine the direct skeletal muscle and potential phenotype specific effects. Many human studies examine muscle performance normalised to body mass (1, 7, 9, 43, 47, 64), which provides little information regarding muscle quality (force relative to muscle mass). An obesity induced reduction in muscle quality could result in an increased maintenance cost due to a larger muscle mass, and consequently an increase in body mass, even before considering further lipid accumulation elsewhere in the body. Although some studies have normalised muscle performance to local and more commonly whole body lean mass (1, 7, 43, 47, 64), such assessments would not be as accurate at evaluating muscle quality as an *in vitro* isolated muscle approach, where whole muscle mass can be measured.

Obesity has been associated with a reduction in myogenesis (4, 12), degeneration in the process of excitation contraction coupling and impaired calcium handling (8, 10, 19, 51), which may mechanistically account for a decline in contractility and muscle quality. Importantly, skeletal muscle lipid accumulation can affect metabolic capacity and phenotype composition, but the literature is equivocal with evidence of a shift to a faster, slower and no change in fibre type composition (13, 14, 33, 34, 52, 59-61, 65). This ambiguity can largely be attributed to the different muscles tested, duration of the feeding period, and limitations in methods for quantifying fibre types. It has been further demonstrated that initially muscular lipid accumulation results in increases in oxidative enzymes, mitochondrial function, and slow MyHC (myosin heavy chain) expression, and in the long term, causes a reduction in oxidative enzymes, type I muscle fibre protein content, mitochondrial size and function (13). In skeletal muscle, glucose and fatty acid metabolism as well as mitochondrial function are at least partly regulated by AMP-activated protein kinase (AMPK) (46). Levels of adiponectin, a protein hormone produced by adipocytes that induces AMPK activity, are decreased by obesity in skeletal muscle (66), which may significantly influence skeletal muscle function.

Despite this evidence, there is a distinct dearth of literature that directly assesses the effects of obesity on skeletal muscle contractility. Warmington, Tolan and McBennett (65) demonstrated little effect on the isometric twitch force of both whole extensor digitorum longus (EDL) and soleus muscle isolated from 5 month old genetically obese (*Ob/Ob)* mice when compared to a genetically normal control.

However, maximal isometric tetanus force was significantly reduced in the *Ob/Ob* soleus with no effect in EDL. In part, these findings were later confirmed by Bruton, Katz, Lännergren, Abbate and Westerblad (8), reporting no changes maximal isometric force, but a significant improvement in isometric force using a submaximal stimulation of whole EDL and single flexor digitorum brevis muscle fibres isolated from 3-5 month old *Ob/Ob* mice. Similarly, Ciapaite, van den Berg, Houten, Nicolay, van Dijk and Jeneson (10) reported that isometric twitch and tetanus force of whole EDL was unaltered in 12 week old mice that consumed either a high fat lard or high fat palm oil diet for 5 weeks. However, the peak contractile performance of the soleus muscle was significantly reduced in animals fed a high fat palm diet but unchanged in animals fed a high fat lard diet. These results indicate that the source of the lipid overload may be an important factor for determining skeletal muscle responses to obesity, and are particularly interesting given the role of soleus as a postural muscle.

The effect induced by genetic obesity may be different to that imposed by dietary-induced obesity. Contractile changes outlined in isolated muscle from *Ob/Ob* mice occurred in conjunction with a significant reduction in muscle mass (8, 65), where changes in contractility occurred following an elevated muscle mass in the dietary induced obesity model (10). In support, the ambiguity in obesity induced phenotype changes has been in part attributed to differing response between genetically and dietary induced obese models (14). Further work is needed to quantify the effects of dietary induced obesity on isolated skeletal muscle performance given its relevance to the real world obesity problem. In addition, previous isolated muscle work has used test temperatures between 20-26˚C (8, 10) limiting the application of findings to human skeletal muscle contractility. It should be noted that the contractile performance of skeletal muscle is greatly influenced by temperature (27) and further work is needed to evaluate change is muscle contractility using a more physiologically relevant thermal environment.

Measurements of maximal isometric stress reveal little about changes in dynamic muscular contractility, which is an important aspect of real-world muscle function. *In vivo*, locomotory muscles rarely work at constant lengths and measures of isometric force fail to consider the important integration of the force-velocity relationship, the ability of the muscle to produce work during shortening, and the passive resistance to stretch needed to accurately assess muscle power (28, 31, 32). Furthermore, demonstrated increases in muscle activation times and, more commonly, relaxation time (8, 10), will have profound effects on the ability of the muscle to produce power. The effect of obesity on the fatigue resistance of isolated skeletal muscle is also unresolved.

The present study examines the effects of dietary induced obesity on the maximal power output and fatigue resistance of soleus (slow twitch), EDL (fast twitch), and diaphragm (mixed fibre type) muscle, isolated from young adult mice, at a test temperature of 37˚C. By determining MyHC expression and AMPK activity, the present study will attempt to gain a better understanding of the muscle specific mechanisms causing the hypothesised decline in contractile performance. Importantly, the present work is the first to accurately determine the obesity induced muscle specific changes in quality using the work loop technique as a more accurate assessment of real life muscle function (28, 31, 32). By further examining muscle specific changes in absolute force, power and contractile performance relative to muscle mass, findings of the present work can be better applied to the real world locomotor performance of the whole animal. The results of the present work will allow a greater understanding of the specific role of skeletal muscle in the obesity induced reduction in physical activity, and the potential that a reduction in contractile performance, including muscle quality, may be a significant contributor to the obesity problem.

METHODOLOGY

ANIMAL MORPHOLOGY

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

Animals were then sacrificed by cervical dislocation (in accordance with British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1) and the subcutaneous fat pad around the top of the hind limbs and genitals was extracted and weighed. In addition, either whole SOL, EDL or DIA muscle was dissected from each individual in refrigerated (1-3°C) oxygenated (95% O_2; 5% CO_2) Krebs Henseleit 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 in each case; pH 7.55 at room temperature prior to oxygenation). The left limb muscle or right half section of the DIA was immediately snap frozen in liquid nitrogen and stored in a -80°C freezer for later biochemical analysis. The remaining limb muscle, or a ventral section of the costal DIA, was used in the study of skeletal muscle contractility.

CONTRACTILTIY MEASURES

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

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

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

The optimal muscle length (L_0) for maximal twitch force, determined by the isometric tests, was 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.

RESULTS

MORPHOLOGY

Whole animal body mass, fat pad mass, BMI and Lee index of obesity were significantly greater in the obese group when compared to controls (Table 1; Mann-Whitney $P<0.001$ for body mass & fat pad mass; T-Test $P<0.001$ for BMI & Lee Index). Body length was not significantly different (Table 1 Mann-Whitney $P=0.053$). When broken down into each treatment group whole animal body mass, BMI and Lee index of obesity were significantly greater in the obese groups when compared to the lean controls (Table 2 $P<0.05$ in all cases). For both the SOL and EDL, obese muscle mass was significantly greater (Table 2 T-Test $P<0.03$ in each case) but muscle length was not affected (Table 2 T-Test $P=0.80$ for SOL; Mann-Whitney $P=0.15$ for EDL).

Within the obese group, measures of body mass correlated well with body length and fat pad mass (Spearmans' $r> 0.4$ $P<0.03$ in each case), In addition, fat pad mass was strongly associated with greater BMI and Lee index (Spearmans' $r> 0.7$ $P<0.003$ in both cases).

ISOMETRIC STRESS

For SOL, absolute maximal isometric force was significantly higher in the obese group compared to the lean control (Fig 1B T-Test $P=0.003$), however maximal isometric stress was not significantly different (Fig 1A T-Test $P=0.38$). The absolute maximal isometric force generated by the EDL was not significantly different between the obese and the lean group (Fig 1D T-Test $P=0.20$), however maximal isometric stress was significantly reduced in the obese group (Fig 1C T-Test $P<0.005$). Similarly, the maximal isometric stress of obese DIA was significantly lower than lean controls (Fig 1E T-Test $P<0.001$). Absolute force and power was not assessed for the DIA as only a section of this muscle was used in the examination of contractile performance.

THPT was not significantly different between the obese and the lean control group for SOL, EDL or DIA (Table 3 $P>0.24$ in each case). Similarly, LSHR for EDL was not significantly different between the obese and lean group (Table 3 T-Test $P=0.67$). In the obese SOL, LSHR was significantly prolonged (Table 3 T-Test $P=0.01$), whilst in obese DIA, LSHR was significantly shorter when compared to the controls (Table 3 Mann-Whitney $P=0.02$).

WL PO & FATIGUE RESITANCE

For both the SOL and EDL, absolute WL power was not significantly different between the obese and the lean groups (Fig 2B & D T-Test $P>0.44$ in each case). When normalised to muscle mass, SOL WL power was not significantly affected (Fig 2A Mann-Whitney $P=0.30$), however normalised power was significantly reduced in the obese DIA and EDL (Fig 2C & E T-Test $P<0.006$ in each case). For the obese group there was no significant relationship between normalised WL power and body mass for EDL (Fig 3A Spearmans' $r=-0.183$ $P=0.64$), however Obese DIA extracted from animals with a larger body mass had significantly reduced performance (Fig 3B Spearmans' $r=-0.714$ $P=0.047$).

SOL from the obese group fatigued significantly faster than lean controls (Fig 4A T-Test $P=0.002$). There were no significant differences in the fatigue response for either the EDL or DIA (Fig 4B & C T-Test $P>0.8$ in each case).

PROTEIN EXPRESSION

SOL in obese mice had significantly less slow MyHC/α-Tubulin (Fig 4A T-Test $P=0.01$); fast MyHC/α-Tubulin showed a similar trend but the data were more variable so that there was no significant difference between obese and lean SOL (Fig 4B T-Test $P= 0.16$), and the ratio between 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).

DISCUSSION

Our results indicate that obesity causes a decline in the contractile performance of isolated skeletal muscle. These findings are the first to offer a detailed insight into the direct changes in absolute contractile performance and muscle quality using a methodological approach which more closely represents the environmental conditions and contractile mechanics of skeletal muscle *in vivo*. The present findings indicate that the decline in contractile performance is likely to relate to fibre type composition, metabolic profile and the *in vivo* mechanical role of each muscle.

THE EFFECT OF OBESITY ON MAXIMAL FORCE AND POWER

The absolute isometric force of the obese SOL was significantly greater than the lean control group. This result is unsurprising given the role of SOL in postural control and proposed training stimulus evoked by the elevated body mass. Similar increases in the absolute strength of 'antigravity' muscles have been reported in previous *in vivo* literature (See review by 39). Interestingly, this increase in force producing capacity did not transfer to an increase in the absolute power output produced by SOL. These findings may suggest an obesity induced favourable adaptation for the SOL in static isometric contractions needed for such activities as quiet standing, which does not necessarily transfer to an improvement in locomotor performance given that when producing power *in vivo*, SOL of the obese group would be working to move a greater whole animal body mass. Isometric stress and normalised WL power were unaffected in the SOL, possibly demonstrating that the muscle quality of the obese group was maintained. Given that normalised WL power was also unaffected, one would anticipate a similar increase in absolute power given the increase in muscle mass. Surprisingly this was not demonstrated in the statistical results and maybe attributed to the large variation in this data set.

The maximal isometric stress and normalised work loop power output was significantly reduced in the obese EDL. With respect to the limited changes in absolute isometric force and work loop power, and increase in muscle mass, these results infer that in the case of the obese EDL, larger muscles of poorer quality are formed to maintain the same absolute contractile performance as the lean counterparts. *In vivo* this would present two significant problems. Firstly, although absolute performance is maintained, larger muscles will add to the whole animal body mass thus increasing body inertia. Given this and the significant increase in body mass that will arise via adipose tissue accumulation, the maintenance of absolute force and power is likely to be inadequate given the increase in load. Similar to the EDL, isometric stress and normalised WL power of the obese DIA was significantly lower compared to the lean control group. This again suggests that there is a significant reduction in muscle quality.

As expected with dietary induced obesity models, there was a large variation in the body mass for the obese group. Interestingly, the obese DIA muscles that were extracted from animals with a higher body mass had significantly lower normalised WL power. This possibly indicates a negative relationship between the quantity of adipose tissue and muscle quality for this muscle. No such effects were demonstrated for the EDL, however it is clear from the data that this response needs to be analysed using a larger sample size. Further exploration is also needed considering the distribution of adipose tissue deposits at a whole body and muscle specific level to determine whether increased body mass is linked to increased intramuscular adipose tissue.

Given the mechanical role and fibre type composition of each muscle used in the present work, it is unsurprising to see a muscle specific response to lipid accumulation. With the SOL being composed primarily of slow oxidative fibres, it could be considered that this muscle already has a preferable metabolic profile to oxidise lipids, in comparison to EDL and DIA which have a relatively faster fibre type composition. Similar sentiments have been reported in previous literature (10). As such it is possible that lipid accumulation in the SOL would be less than the other muscle tested, thus potentially delaying the onset of degenerative mechanisms. Although it could be considered that the mechanical loading of the EDL and DIA may be increased (larger foot and thoracic cavity mass respectively), the magnitude of which is likely to be lower that the SOL due to its role in postural support.

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

THE EFFECT OF OBESITY ON FATIGUE RESISTANCE

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

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

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

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

MECHANISMS

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

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

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

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

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

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

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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REFERENCES

1. **Abdelmoula A, Martin V, Bouchant A, Walrand S, Lavet C, Taillardat M, Maffiuletti NA, Boisseau N, Duche P, and Ratel S**. Knee extension strength in obese and nonobese male adolescents. *Appl Physiol Nutr Metab* 37: 269-275, 2012.
2. **Adams KF, Schatzkin A, Harris TB, Kipnis V, Mouw T, Ballard-Barbash R, Hollenbeck A, and Leitzmann MF**. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. *N Engl J Med* 355: 763-778, 2006.
3. **Addison O, Marcus RL, Lastayo PC, and Ryan AS**. Intermuscular fat: a review of the consequences and causes. *Int J Endocrinol* 2014: 309570, 2014.
4. **Akhmedov D, and Berdeaux R**. The effects of obesity on skeletal muscle regeneration. *Front Physiol* 4: 371, 2013.
5. **Allen DG, Lamb GD, and Westerblad H**. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88: 287-332, 2008.
6. **Altringham JD, and Young IS**. Power output and the frequency of oscillatory work in mammalian diaphragm muscle: the effects of animal size. *J Exp Biol* 157: 381-389, 1991.
7. **Aucouturier J, Lazaar N, Dore E, Meyer M, Ratel S, and Duche P**. Cycling peak power in obese and lean 6- to 8-year-old girls and boys. *Appl Physiol Nutr Metab* 32: 367-371, 2007.
8. **Bruton JD, Katz A, Länneergren J, Abbate F, and Westerblad H**. Regulation of myoplasmic Ca(2+) in genetically obese (ob/ob) mouse single skeletal muscle fibres. *Pflugers Arch* 444: 692-699, 2002.
9. **Capodaglio P, Vismara L, Menegoni F, Baccalaro G, Galli M, and Grugni G**. Strength characterization of knee flexor and extensor muscles in Prader-Willi and obese patients. *BMC Musculoskelet Disord* 10: 47, 2009.
10. **Ciapaite J, van den Berg SA, Houten SM, Nicolay K, van Dijk KW, and Jeneson JA**. Fiber-type-specific sensitivities and phenotypic adaptations to dietary fat overload differentially impact fast- versus slow-twitch muscle contractile function in C57BL/6J mice. *J Nutr Biochem* 26: 155-164, 2015.
11. **Cosper PF, and Leinwand LA**. Myosin heavy chain is not selectively decreased in murine cancer cachexia. *Int J Cancer* 130: 2722-2727, 2012.
12. **D'Souza DM, Trajcevski KE, Al-Sajee D, Wang DC, Thomas M, Anderson JE, and Hawke TJ**. Diet-induced obesity impairs muscle satellite cell activation and muscle repair through alterations in hepatocyte growth factor signaling. *Physiol Rep* 3: 2015.
13. **de Wilde J, Mohren R, van den Berg S, Boekschoten M, Dijk KW, de Groot P, Muller M, Mariman E, and Smit E**. Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice. *Physiol Genomics* 32: 360-369, 2008.
14. **Denies MS, Johnson J, Maliphol AB, Bruno M, Kim A, Rizvi A, Rustici K, and Medler S**. Diet-induced obesity alters skeletal muscle fiber types of male but not female mice. *Physiol Rep* 2: e00204, 2014.
15. **Dickinson MH, Farley CT, Full RJ, Koehl MA, Kram R, and Lehman S**. How animals move: an integrative view. *Science* 288: 100-106, 2000.
16. **Ekman MJ, Klintenberg M, Bjorck U, Norstrom F, and Ridderstrale M**. Six-minute walk test before and after a weight reduction program in obese subjects. *Obesity (Silver Spring)* 21: E236-243, 2013.
17. **Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, Rimm E, and Colditz GA**. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. *Arch Intern Med* 161: 1581-1586, 2001.
18. **Fontaine KR, and Barofsky I**. Obesity and health-related quality of life. *Obes Rev* 2: 173-182, 2001.
19. **Funai K, Song H, Yin L, Lodhi IJ, Wei X, Yoshino J, Coleman T, and Semenkovich CF**. Muscle lipogenesis balances insulin sensitivity and strength through calcium signaling. *J Clin Invest* 123: 1229-1240, 2013.
20. **Garcia-Vicencio S, Coudeyre E, Kluka V, Cardenoux C, Jegu AG, Fourot AV, Ratel S, and Martin V**. The bigger, the stronger? Insights from muscle architecture and nervous characteristics in obese adolescent girls. *Int J Obes (Lond)* 40: 245-251, 2016.

21. **Goodpaster BH, He J, Watkins S, and Kelley DE**. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86: 5755-5761, 2001.
22. **Hammond RA, and Levine R**. The economic impact of obesity in the United States. *Diabetes Metab Syndr Obes* 3: 285-295, 2010.
23. **Higgins MF, James RS, and Price MJ**. The effects of sodium bicarbonate (NaHCO3) ingestion on high intensity cycling capacity. *J Sports Sci* 31: 972-981, 2013.
24. **Hofmann M, Schober-Halper B, Oesen S, Franzke B, Tschan H, Bachl N, Strasser EM, Quittan M, Wagner KH, and Wessner B**. Effects of elastic band resistance training and nutritional supplementation on muscle quality and circulating muscle growth and degradation factors of institutionalized elderly women: the Vienna Active Ageing Study (VAAS). *Eur J Appl Physiol* 116: 885-897, 2016.
25. **Houmard JA, Pories WJ, and Dohm GL**. Is there a metabolic program in the skeletal muscle of obese individuals? *J Obes* 2011: 250496, 2011.
26. **Ivey FM, Tracy BL, Lemmer JT, NessAiver M, Metter EJ, Fozard JL, and Hurley BF**. Effects of strength training and detraining on muscle quality: age and gender comparisons. *J Gerontol A Biol Sci Med Sci* 55: B152-157; discussion B158-159, 2000.
27. **James RS**. A review of the thermal sensitivity of the mechanics of vertebrate skeletal muscle. *J Comp Physiol B* 183: 723-733, 2013.
28. **James RS, Altringham JD, and Goldspink DF**. The mechanical properties of fast and slow skeletal muscles of the mouse in relation to their locomotory function. *J Exp Biol* 198: 491-502, 1995.
29. **James RS, Kohlsdorf T, Cox VM, and Navas CA**. 70 microM caffeine treatment enhances in vitro force and power output during cyclic activities in mouse extensor digitorum longus muscle. *Eur J Appl Physiol* 95: 74-82, 2005.
30. **James RS, Wilson RS, and Askew GN**. Effects of caffeine on mouse skeletal muscle power output during recovery from fatigue. *J Appl Physiol (1985)* 96: 545-552, 2004.
31. **James RS, Young IS, Cox VM, Goldspink DF, and Altringham JD**. Isometric and isotonic muscle properties as determinants of work loop power output. *Pflugers Arch* 432: 767-774, 1996.
32. **Josephson RK**. Mechanical power output from striated muscle during cyclical contraction. *Journal of Experimental Biology* 114: 493-512, 1985.
33. **Kemp JG, Blazev R, Stephenson DG, and Stephenson GM**. Morphological and biochemical alterations of skeletal muscles from the genetically obese (ob/ob) mouse. *Int J Obes (Lond)* 33: 831-841, 2009.
34. **Kriketos AD, Baur LA, O'Connor J, Carey D, King S, Caterson ID, and Storlien LH**. Muscle fibre type composition in infant and adult populations and relationships with obesity. *Int J Obes Relat Metab Disord* 21: 796-801, 1997.
35. **Lee I, Hüttemann M, Liu J, Grossman LI, and Malek MH**. Deletion of heart-type cytochrome c oxidase subunit 7a1 impairs skeletal muscle angiogenesis and oxidative phosphorylation. *J Physiol* 590: 5231-5243, 2012.
36. **Machann J, Bachmann OP, Brechtel K, Dahl DB, Wietek B, Klumpp B, Haring HU, Claussen CD, Jacob S, and Schick F**. Lipid content in the musculature of the lower leg assessed by fat selective MRI: intra- and interindividual differences and correlation with anthropometric and metabolic data. *J Magn Reson Imaging* 17: 350-357, 2003.
37. **Maffiuletti NA, Jubeau M, Agosti F, De Col A, and Sartorio A**. Quadriceps muscle function characteristics in severely obese and nonobese adolescents. *Eur J Appl Physiol* 103: 481-484, 2008.
38. **Maffiuletti NA, Jubeau M, Munzinger U, Bizzini M, Agosti F, De Col A, Lafortuna CL, and Sartorio A**. Differences in quadriceps muscle strength and fatigue between lean and obese subjects. *Eur J Appl Physiol* 101: 51-59, 2007.
39. **Maffiuletti NA, Ratel S, Sartorio A, and Martin V**. The Impact of Obesity on In Vivo Human Skeletal Muscle Function. *Current Obesity Reports* 2: 251-260, 2013.
40. **Mayer JM, Nuzzo JL, Chen R, Quillen WS, Verna JL, Miro R, and Dagenais S**. The impact of obesity on back and core muscular endurance in firefighters. *J Obes* 2012: 729283, 2012.
41. **Mendez J, Keys, A.** Density and composition of mammalian muscle. *Metabolism* 9: 184-188, 1960.
42. **Minetto MA, Botter A, Sprager S, Agosti F, Patrizi A, Lanfranco F, and Sartorio A**. Feasibility study of detecting surface electromyograms in severely obese patients. *J Electromyogr Kinesiol* 23: 285-295, 2013.

43. **Miyatake N, Fujii M, Nishikawa H, Wada J, Shikata K, Makino H, and Kimura I**. Clinical evaluation of muscle strength in 20-79-years-old obese Japanese. *Diabetes Res Clin Pract* 48: 15-21, 2000.
44. **Moffat C, and Harper ME**. Metabolic functions of AMPK: aspects of structure and of natural mutations in the regulatory gamma subunits. *IUBMB Life* 62: 739-745, 2010.
45. **Nguyen DM, and El-Serag HB**. The epidemiology of obesity. *Gastroenterol Clin North Am* 39: 1-7, 2010.
46. **O'Neill HM, Holloway GP, and Steinberg GR**. AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: implications for obesity. *Mol Cell Endocrinol* 366: 135-151, 2013.
47. **Paolillo FR, Milan JC, Bueno Pde G, Paolillo AR, Borghi-Silva A, Parizotto NA, Arena R, Kurachi C, and Bagnato VS**. Effects of excess body mass on strength and fatigability of quadriceps in postmenopausal women. *Menopause* 19: 556-561, 2012.
48. **Pataky Z, Armand S, Muller-Pinget S, Golay A, and Allet L**. Effects of obesity on functional capacity. *Obesity (Silver Spring)* 22: 56-62, 2014.
49. **Pinto RS, Correa CS, Radaelli R, Cadore EL, Brown LE, and Bottaro M**. Short-term strength training improves muscle quality and functional capacity of elderly women. *Age (Dordr)* 36: 365-372, 2014.
50. **Rolland Y, Lauwers-Cances V, Pahor M, Fillaux J, Grandjean H, and Vellas B**. Muscle strength in obese elderly women: effect of recreational physical activity in a cross-sectional study. *Am J Clin Nutr* 79: 552-557, 2004.
51. **Schilder RJ, Kimball SR, Marden JH, and Jefferson LS**. Body weight-dependent troponin T alternative splicing is evolutionarily conserved from insects to mammals and is partially impaired in skeletal muscle of obese rats. *J Exp Biol* 214: 1523-1532, 2011.
52. **Shortreed KE, Krause MP, Huang JH, Dhanani D, Moradi J, Ceddia RB, and Hawke TJ**. Muscle-specific adaptations, impaired oxidative capacity and maintenance of contractile function characterize diet-induced obese mouse skeletal muscle. *PLoS One* 4: e7293, 2009.
53. **Stephens DN**. Does the Lee obesity index measure general obesity? *Physiol Behav* 25: 313-315, 1980.
54. **Tallis J, Duncan MJ, and James RS**. What can isolated skeletal muscle experiments tell us about the effects of caffeine on exercise performance? *Br J Pharmacol* 172: 3703-3713, 2015.
55. **Tallis J, Higgins MF, Cox VM, Duncan MJ, and James RS**. Does a physiological concentration of taurine increase acute muscle power output, time to fatigue, and recovery in isolated mouse soleus (slow) muscle with or without the presence of caffeine? *Can J Physiol Pharmacol* 92: 42-49, 2014.
56. **Tallis J, James RS, Cox VM, and Duncan MJ**. The effect of a physiological concentration of caffeine on the endurance of maximally and submaximally stimulated mouse soleus muscle. *J Physiol Sci* 63: 125-132, 2013.
57. **Tallis J, James RS, Cox VM, and Duncan MJ**. The effect of physiological concentrations of caffeine on the power output of maximally and submaximally stimulated mouse EDL (fast) and soleus (slow) muscle. *J Appl Physiol (1985)* 112: 64-71, 2012.
58. **Tallis J, James RS, Little AG, Cox VM, Duncan MJ, and Seebacher F**. Early effects of ageing on the mechanical performance of isolated locomotory (EDL) and respiratory (diaphragm) skeletal muscle using the work-loop technique. *Am J Physiol Regul Integr Comp Physiol* 307: R670-684, 2014.
59. **Tanner CJ, Barakat HA, Dohm GL, Pories WJ, MacDonald KG, Cunningham PR, Swanson MS, and Houmard JA**. Muscle fiber type is associated with obesity and weight loss. *Am J Physiol Endocrinol Metab* 282: E1191-1196, 2002.
60. **Trajcevski KE, O'Neill HM, Wang DC, Thomas MM, Al-Sajee D, Steinberg GR, Ceddia RB, and Hawke TJ**. Enhanced lipid oxidation and maintenance of muscle insulin sensitivity despite glucose intolerance in a diet-induced obesity mouse model. *PLoS One* 8: e71747, 2013.
61. **Wade AJ, Marbut MM, and Round JM**. Muscle fibre type and aetiology of obesity. *Lancet* 335: 805-808, 1990.
62. **Wakeling JM, and Rozitis AI**. Motor unit recruitment during vertebrate locomotion. *Journal of Animal Biology* 55: 41-58, 2005.
63. **Walter I, Hegarty B, and Seebacher F**. AMP-activated protein kinase controls metabolism and heat production during embryonic development in birds. *J Exp Biol* 213: 3167-3176, 2010.
64. **Ward DS, Trost SG, Felton G, Saunders R, Parsons MA, Dowda M, and Pate RR**. Physical activity and physical fitness in African-American girls with and without obesity. *Obes Res* 5: 572-577, 1997.

65. **Warmington SA, Tolan R, and McBennett S**. Functional and histological characteristics of skeletal muscle and the effects of leptin in the genetically obese (ob/ob) mouse. *Int J Obes Relat Metab Disord* 24: 1040-1050, 2000.
66. **Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, and Kadowaki T**. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8: 1288-1295, 2002.
67. **Yoshida Y, Marcus RL, and Lastayo PC**. Intramuscular adipose tissue and central activation in older adults. *Muscle Nerve* 46: 813-816, 2012.

FIGURES

Figure 1 – The effect of 16 weeks HFD on the maximal isometric tetanus stress and absolute isometric tetanus force of isolated mouse SOL (A & B), EDL (C & D) and DIA (E) [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]

Figure 2 – The effect of 16 weeks HFD on the maximal normalised WL PO and absolute WL PO of isolated mouse SOL (A & B), EDL (C & D) and DIA (E) [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]

Figure 3 - The relationship between whole animal body mass and normalised work loop power for the obese EDL (A) and DIA (B) experimental groups [N=9 for EDL; N=8 for DIA; The lines represent a first-order polynomial fitted to the data using a least squares regression and the 95% confidence limits of this line]

Figure 4 - The effect of 16 weeks HFD on the fatigue resistance of maximally stimulated mouse SOL (A), EDL (B) and DIA (C) [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]

Figure 5 – The effect of 16 weeks HFD on fast and slow MyHC expression of mouse SOL, EDL and DIA [Data represented as Mean±SE; N=6 in each case; * represent significant differences]

Figure 6 - The effect of 16 weeks HFD on the AMPK activity of mouse SOL, EDL and DIA [Data represented as Mean±SE; N=7 for SOL; N=6 for EDL; N=6 for DIA; * represent significant differences]

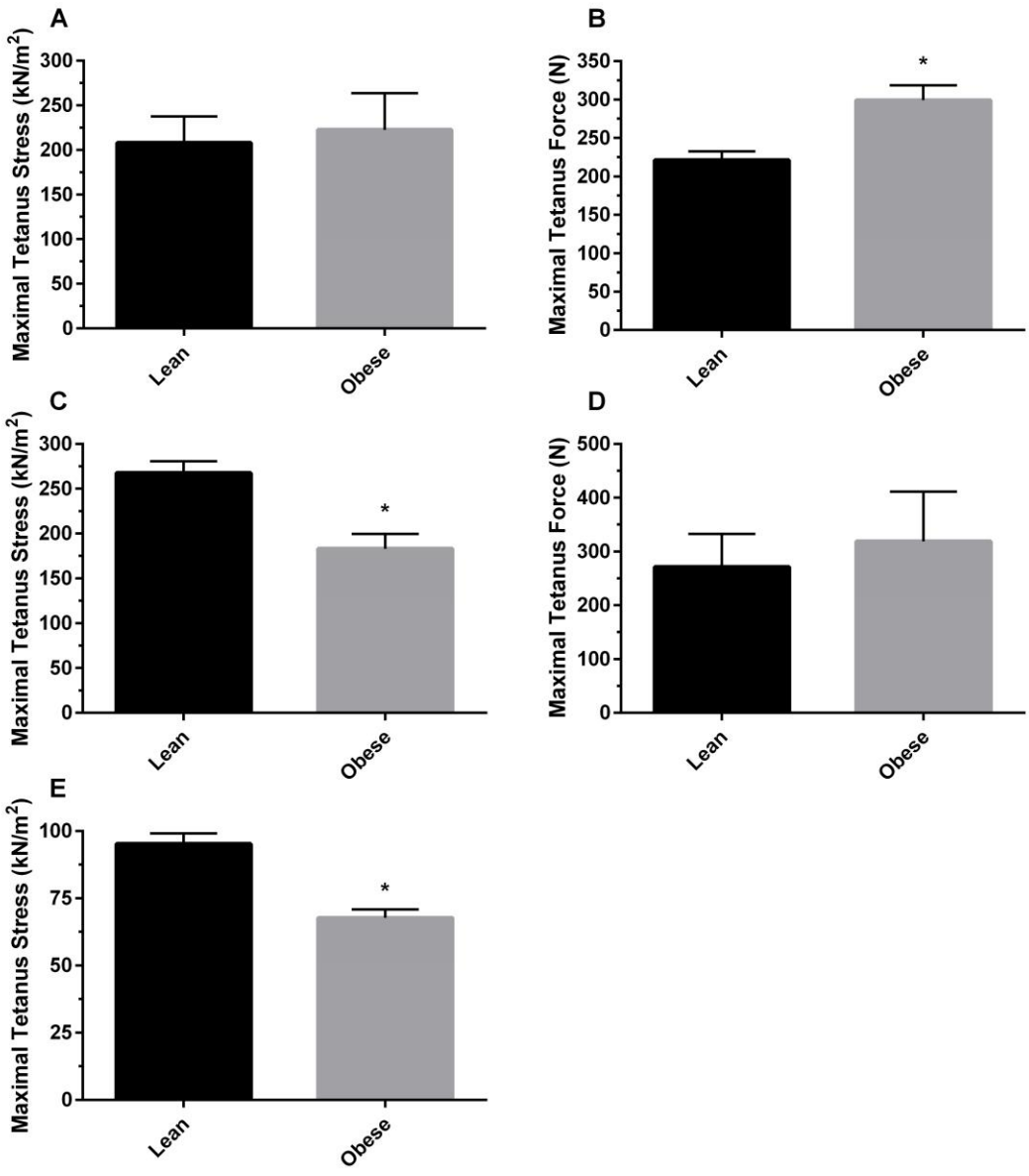
A
Maximal Tetanus Stress (kN/m2)
300
250
200
150
100
50
0
Lean
Obese
B
Maximal Tetanus Force (N)
350
300
250
200
150
100
50
0
*
Lean
Obese
C
Maximal Tetanus Stress (kN/m2)
300
250
200
150
100
50
0
*
Lean
Obese
D
Maximal Tetanus Force (N)
500
400
300
200
100
0
Lean
Obese
E
Maximal Tetanus Stress (kN/m2)
100
75
50
25
0
*
Lean
Obese

FIGURE 1.

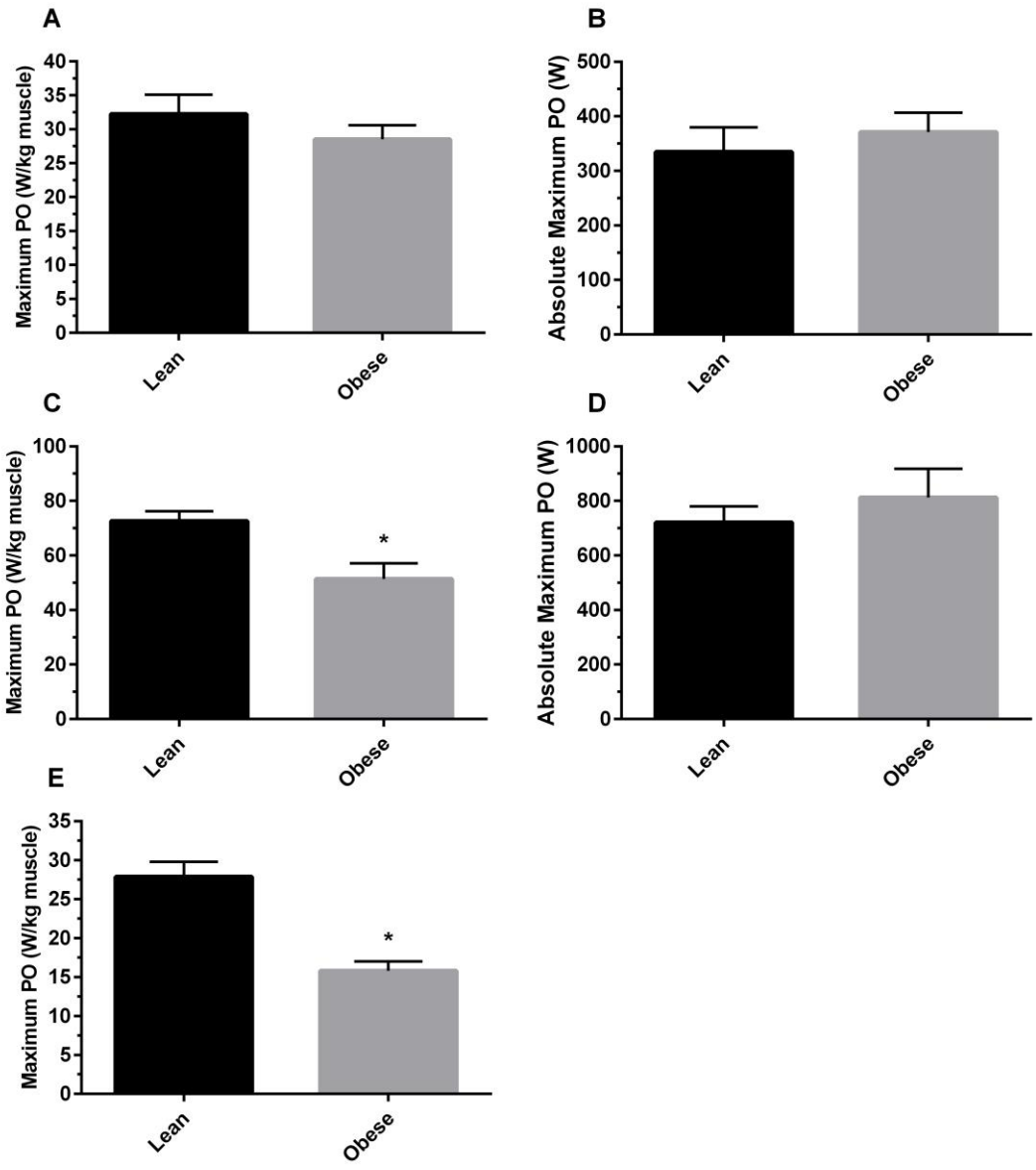
A
Maximum PO (W/kg muscle)
40
35
30
25
20
15
10
5
0
Lean
Obese
B
Absolute Maximum PO (W)
500
400
300
200
100
0
Lean
Obese
C
Maximum PO (W/kg muscle)
100
80
60
40
20
0
*
Lean
Obese
D
Absolute Maximum PO (W)
1000
800
600
400
200
0
Lean
Obese
E
Maximum PO (W/kg muscle)
35
30
25
20
15
10
5
0
*
Lean
Obese

FIGURE 2.

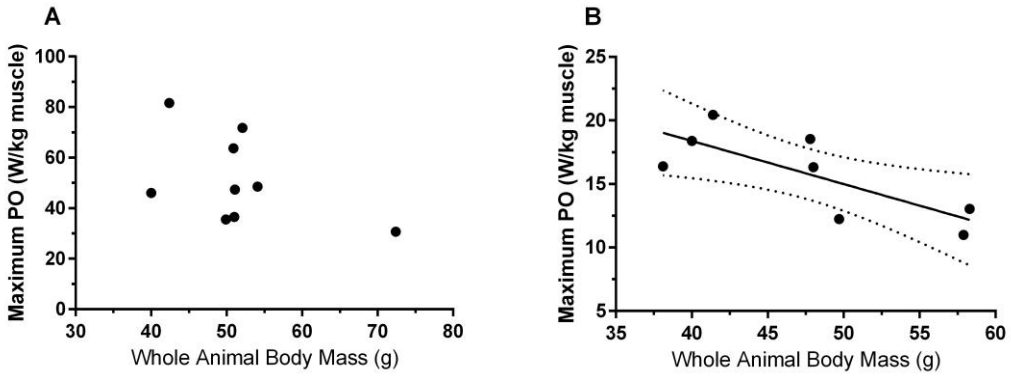
A
Maximum PO (W/kg muscle)
100
80
60
40
20
0
30
40
50
60
70
80
Whole Animal Body Mass (g)
B
Maximum PO (W/kg muscle)
25
20
15
10
5
35
40
45
50
55
60
Whole Animal Body Mass (g)

FIGURE 3.

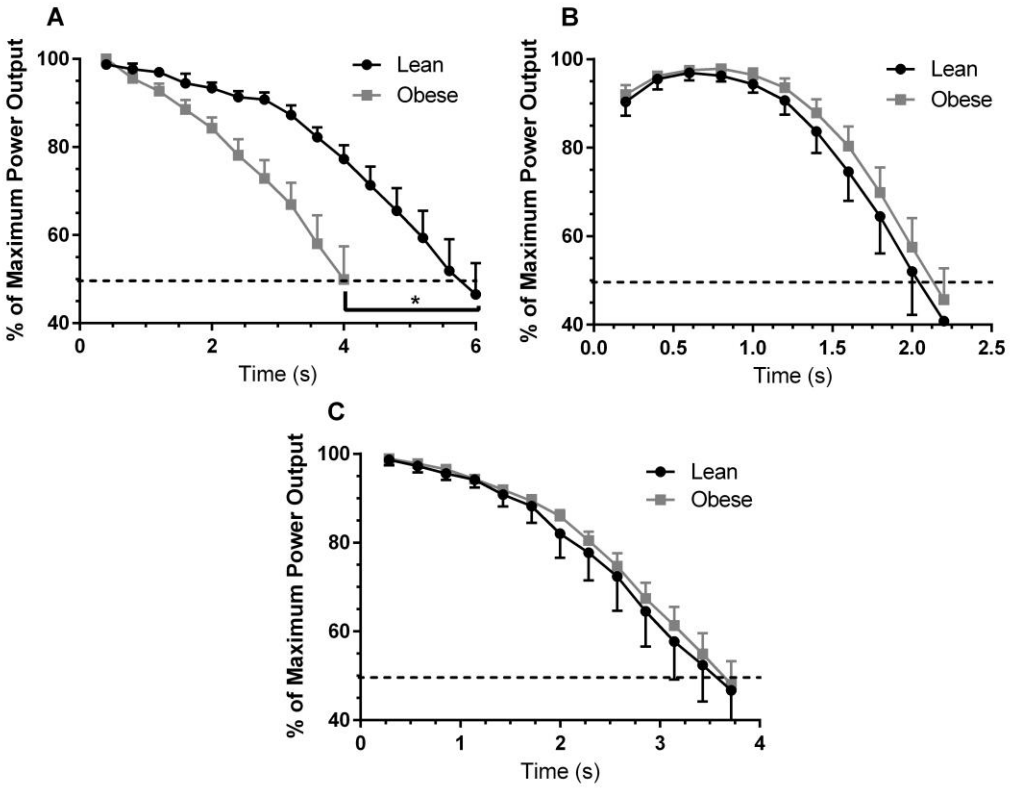
A
% of Maximum Power Output
100
80
60
40
0
2
4
6
Time (s)
Lean
Obese
*
B
% of Maximum Power Output
100
80
60
40
0.0
0.5
1.0
1.5
2.0
2.5
Time (s)
Lean
Obese
C
% of Maximum Power Output
100
80
60
40
0
1
2
3
4
Time (s)
Lean
Obese

FIGURE 4.

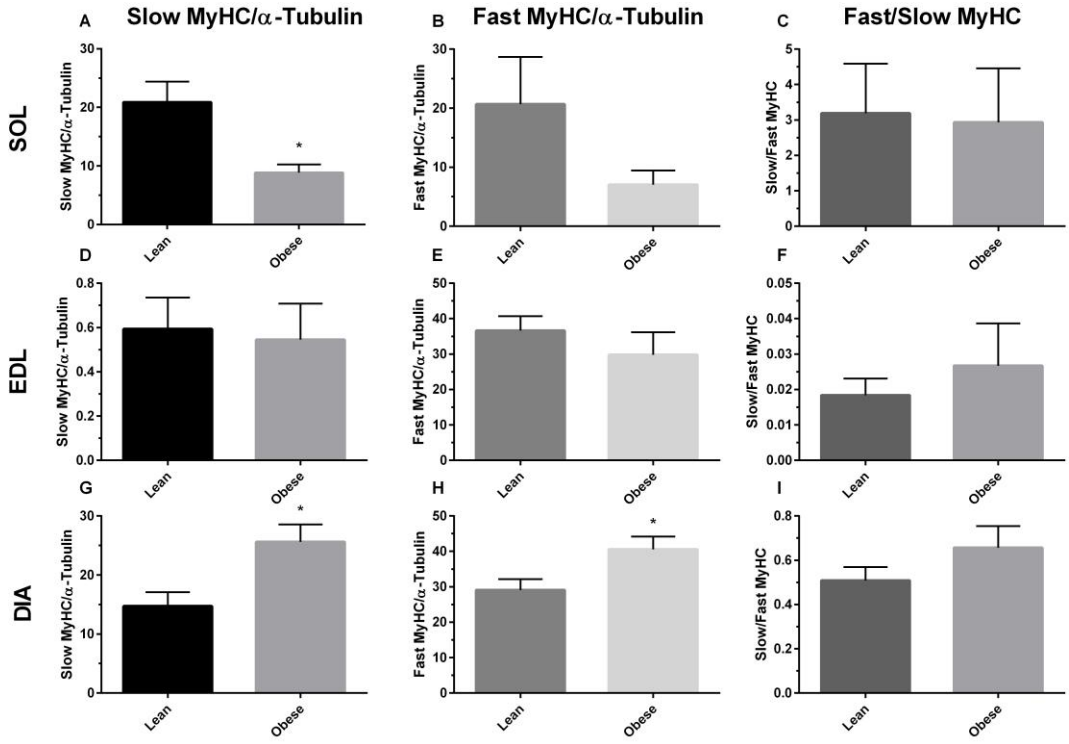
A
Slow MyHC/α-Tubulin
B
Fast MyHC/α-Tubulin
C
Fast/Slow MyHC
SOL
EDL
DIA
D
E
F
G
H
I
Slow MyHC/α-Tubulin
Fast MyHC/α-Tubulin
Slow/Fast MyHC
Lean
Obese
*

FIGURE 5.

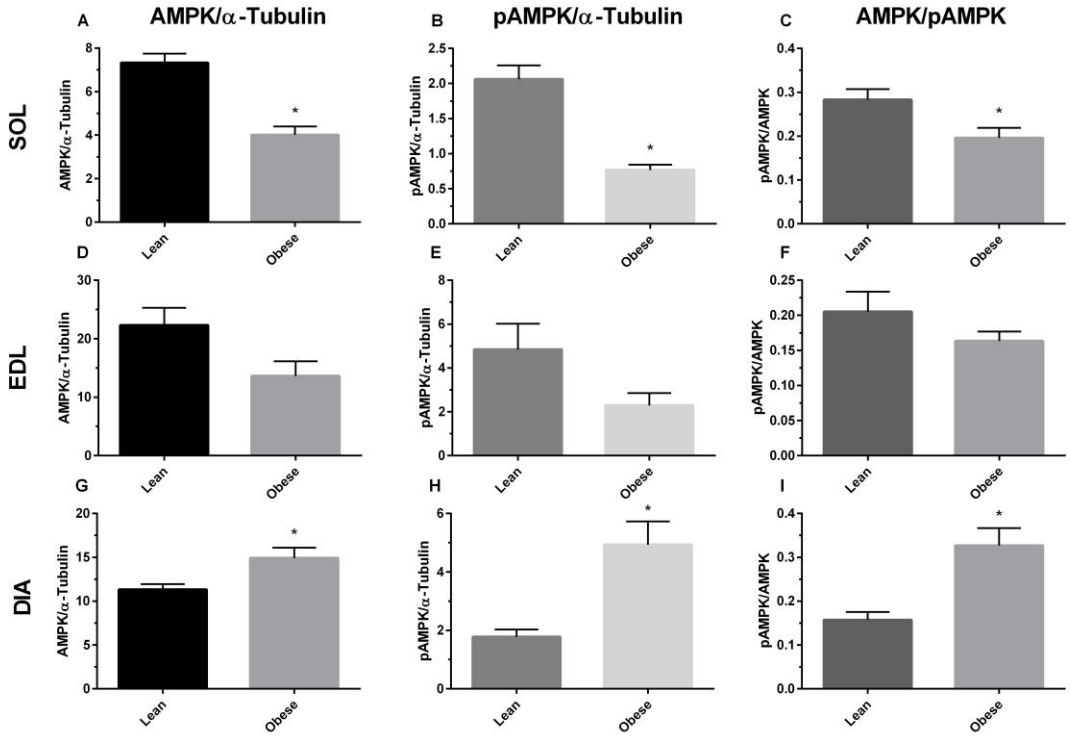
A
AMPK/α-Tubulin
B
pAMPK/α-Tubulin
C
AMPK/pAMPK
SOL
D
E
F
EDL
G
H
I
DIA
Lean
Obese
*

FIGURE 6.

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]

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]

	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*

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*		

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*