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**RESEARCH ARTICLE**

# The effect of obesity on the contractile performance of isolated mouse soleus, EDL, and diaphragm muscles

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**Tallis J, Hill C, James RS, Cox VM, Seebacher F.** The effect of obesity on the contractile performance of isolated mouse soleus, EDL, and diaphragm muscles. *J Appl Physiol* 122: 170–181, 2017. First published November 17, 2016; doi:10.1152/japplphysiol.00836.2016.— 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-wk high-calorific feeding, soleus (SOL), extensor digitorum longus (EDL), and diaphragm (DIA) were isolated from female (CD-1) mice, and contractile performance was compared against a lean control group. Obese SOL produced greater isometric force; however, isometric stress (force per unit muscle area), absolute WL power, and normalized WL power (watts per kilogram muscle mass) were unaffected. Maximal isometric force and absolute WL power of the EDL were similar between groups. For both EDL and DIA, isometric stress and normalized 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, fiber type, and metabolic profile, which may in part be related to changes in myosin heavy chain expression and AMP-activated protein kinase 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 & NOTEWORTHY** The effect of obesity on isolated muscle function is surprisingly underresearched. The present study is the first to examine the effects of obesity on isolated muscle performance using a method that more closely represents real-world 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.

muscle quality; muscular lipid, lipid accumulation; force; power

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, Ref. 39). For example, Rolland et al. (50) report 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 sex 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 normalized 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 normalized muscle performance to local and more commonly whole body lean mass (1, 7, 43, 47, 64), such assessments would not be as

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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 fiber-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 fiber types. It has been further demonstrated that initially muscular lipid accumulation results in increases in oxidative enzymes, mitochondrial function, and slow MHC (myosin heavy chain) expression and, in the long term, causes a reduction in oxidative enzymes, type I muscle fiber 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 induce 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 et al. (65) demonstrated little effect on the isometric twitch force of both whole extensor digitorum longus (EDL) and soleus (SOL) muscle isolated from 5-mo-old genetically obese (*Ob/Ob*) mice compared with a genetically normal control. However, maximal isometric tetanus force was significantly reduced in the *Ob/Ob* SOL with no effect in EDL. In part, these findings were later confirmed by Bruton et al. (8), reporting no changes in maximal isometric force, but a significant improvement in isometric force using a submaximal stimulation of whole EDL and single flexor digitorum brevis muscle fibers isolated from 3- to 5-mo-old *Ob/Ob* mice. Similarly, Ciapaite et al. (10) reported that isometric twitch and tetanus force of whole EDL were unaltered in 12-wk-old mice that consumed either a high-fat lard or high-fat palm oil diet for 5 wk. However, the peak contractile performance of the SOL 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 SOL 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 and 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, locomotor 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 (PO) and fatigue resistance of SOL (slow twitch), EDL (fast twitch), and diaphragm (DIA; mixed fiber type) muscle, isolated from young adult mice, at a test temperature of 37°C. By determining MHC expression and AMPK activity, the present study will attempt to gain a better understanding of the muscle-specific mechanisms causing the hypothesized 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 (WL) 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 ethics committee of Coventry University. At 4 wk of age, 56 CD1 female mice (Harlan) were randomly split into either an obese or a lean control group. Each group was matched for body mass and snout to anus length ($n = 28$ in each case). For the next 16 wk, 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 laboratory chow (SDS maintenance diet, Dietex International; calories provided by protein 17.5%, fat 7.4%, carbohydrate, 75.1%; gross energy 3.52 kcal/g; metabolizable 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; metabolizable 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 [weight 0.33 g)/naso-anal length (cm)] (53) of obesity for each individual.

Animals were then killed 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 hindlimbs 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 (in mM: NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; $KH_2PO_4$ 1.18; glucose 10; $CaCl_2$ 2.54 in each case; pH 7.55 at room temperature before oxygenation). The 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.

*Contractility measures.* The tendon attachment at the proximal end of both the SOL and EDL were left intact, and aluminum foil T-clips were wrapped around the distal tendon as close to the muscle as possible. For the DIA, aluminum 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 aluminum clips, the muscle preparation was attached to a force transducer (UF1, Pioden Controls) and a motor (V201, Ling Dynamic Systems) 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) via a D/A board (KPCI3108, Keithley Instruments) on a standard desktop personal computer.

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

The optimal muscle length ($L_o$) for maximal twitch force, determined by the isometric tests, was measured using an eyepiece graticule fitted to a microscope, and estimates of mean fiber length were determined as 85% of the physical length for SOL and 75% for EDL (28). No such estimates of fiber length have been reported for DIA, so the physical length measurement was use to represent $L_o$. This approach has been standard practice in previous work using these muscles (23, 28, 31, 55, 58).

Muscle PO was measured using the 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 relengthen the muscle in preparation for subsequent contraction (28, 31, 32). The in vivo relevance of this technique and its application have been outlined in our laboratory's previous work (29, 54, 57, 58). Each muscle is subjected to a symmetrical sinusoidal length change around the previously determined $L_o$ 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). 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 WL. 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 optimized to elicit maximal net work at cycle frequencies of 5, 10, and 7 Hz for SOL, EDL, and DIA, respectively. Cycle frequency denotes the rate at which the WL 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 $L_o$ was used to produce maximal net work in each muscle. As such, the muscle increased in length by 5%, shortened by 10%, then was relengthened by 5% back to $L_o$. 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 relengthening phase (increased negative work). The optimal phase was typically −10 ms for SOL, −2 ms for EDL, and –5 ms 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 PO at these cycle frequencies in previous studies (23, 28, 29, 57, 58). Each muscle was subjected to four WL cycles per run, with 5-min rest intervals between each run.

Fatigue resistance was measured by subjecting each muscle to 50 consecutive WL cycles at the parameters that elicited maximal PO. The decline in maximal power was plotted against time until each muscle produced <50% of its prefatigue maximal PO. Similar methods have been employed in previous work using the WL 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 $L_o$, muscle mass, and an assumed muscle density of 1,060 kg/m$^3$ (41). Isometric stress was calculated as maximal tetanic force divided by mean muscle cross-sectional area. Muscle PO was normalized to muscle mass to express power as Watts per kilogram.

*Biochemistry.* Fast and slow MHC expression was measured to examine changes in fiber-type composition, and 5′-AMPK and phosphorylated AMPK (pAMPK) 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 1 mM, NP-40 1%, sodium-deoxycholate 1%, pH 7.5) with the addition of a protease and phosphatase inhibitor cocktail (Roche, Sydney, NSW, Australia). Protein concentrations were determined by capillary electrophoresis in a "Wes" Simple Western system (Protein Simple, Santa Clara, CA), according to the manufacturer's instructions. All antibodies were from Abcam (Cambridge, MA), and we determined concentrations of total fast (ab51263) and slow (ab11083) skeletal MHCs, AMPK-α1 + 2 (ab80039), pAMPK-α1 (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 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 were analyzed using two-tailed independent samples *t*-tests. On the small number of occasions when the data were not normally distributed, Mann-Whitney *U*-tests were performed. Where the WL 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 normalized WL power to

Table 1. *The effect of 16-wk high-fat diet on the anthropometric measures*

| | Lean | Obese |
|---|---|---|
| Body mass, g | 38.5 ± 1.00 | 52.7 ± 2.30* |
| Body length, cm | 11.3 ± 0.09 | 11.6 ± 0.09 |
| BMI, kg/m$^2$ | 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* |

Values are mean ± SE; $N = 30$ and 29 lean and obese mice, respectively, $N = 18$ and 16 for lean and obese fat pad mass, respectively. *Significant differences between lean and obese groups.

determine whether the magnitude of obesity effected muscle performance. Further Spearman's rank correlations were performed to analyze 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 compared with controls (Table 1; Mann-Whitney, $P < 0.001$ for body mass and fat pad mass; *t*-test, $P < 0.001$ for BMI and 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 compared with 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 (Spearman's $r > 0.4$, $P < 0.03$ in each case), In addition, fat pad mass was strongly associated with greater BMI and Lee index (Spearman's $r > 0.7$, $P < 0.003$ in both cases).

*Isometric stress.* For SOL, absolute maximal isometric force was significantly higher in the obese group compared with the lean control (Fig. 1*B*, *t*-test, $P = 0.003$); however, maximal isometric stress was not significantly different (Fig. 1*A*, *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. 1*D*, *t*-test, $P = 0.20$); however, maximal isometric stress was significantly reduced in the obese group (Fig. 1*C*, *t*-test, $P < 0.005$). Similarly, the maximal isometric stress of obese DIA was significantly lower than that of lean controls (Fig. 1*E*, *t*-test, $P < 0.001$). Absolute force and power were not assessed for the DIA, as only a section of this muscle was used in the examination of contractile performance.

Time to half peak tetanus 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 groups (Table 3, *t*-test, $P = 0.67$). In the obese SOL, LSHR was significantly prolonged (Table 3, *t*-test, $P = 0.01$), whereas, in obese DIA, LSHR was significantly shorter compared with the controls (Table 3, Mann-Whitney, $P = 0.02$).

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

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

*Protein expression.* SOL in obese mice had significantly less slow MHC/α-tubulin (Fig. 5*A*, *t*-test, $P = 0.01$). Fast MHC/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. 5*B*, *t*-test, $P = 0.16$), and the ratio between slow and fast MHC did not differ (Fig. 5*C*, Mann-Whitney, $P = 0.82$). DIA had significantly greater slow and fast MHC/α-tubulin in obese animals compared with the lean group (Fig. 5, *G* and *H*, *t*-test, $P < 0.04$ in each case), and there was no difference in the ratio between slow and fast MHC between treatment groups (Fig. 5*I*, *t*-test, $P = 0.23$). Slow MHC/α-tubulin and fast MHC/α-tubulin were not significantly different between the obese and lean EDL (Fig. 5, *D* and *E*, *t*-test, $P = 0.83$, and Mann-Whitney, $P = 0.60$, respectively), and there was no difference in the ratio between slow and fast MHC between treatment groups (Fig. 5*F*, *t*-test, $P = 0.53$).

AMPK/α-tubulin, pAMPK/α-tubulin, and pAMPK/AMPK were significantly reduced in the obese SOL group compared with lean controls (Fig. 6, *A–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–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. 6*D*, *t*-test, $P =$

Table 2. *The effect of 16-wk high-fat diet on muscle group-specific anthropometric measures*

| | Body Mass, g | Body Length, cm | BMI, kg/m$^2$ | 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* | | |

Values are means ± SE; $N = 10$ for SOL; $N = 10$ for EDL lean (L); $N = 9$ for EDL obese (OB); $N = 8$ for DIA. *Significant differences between lean and obese groups.

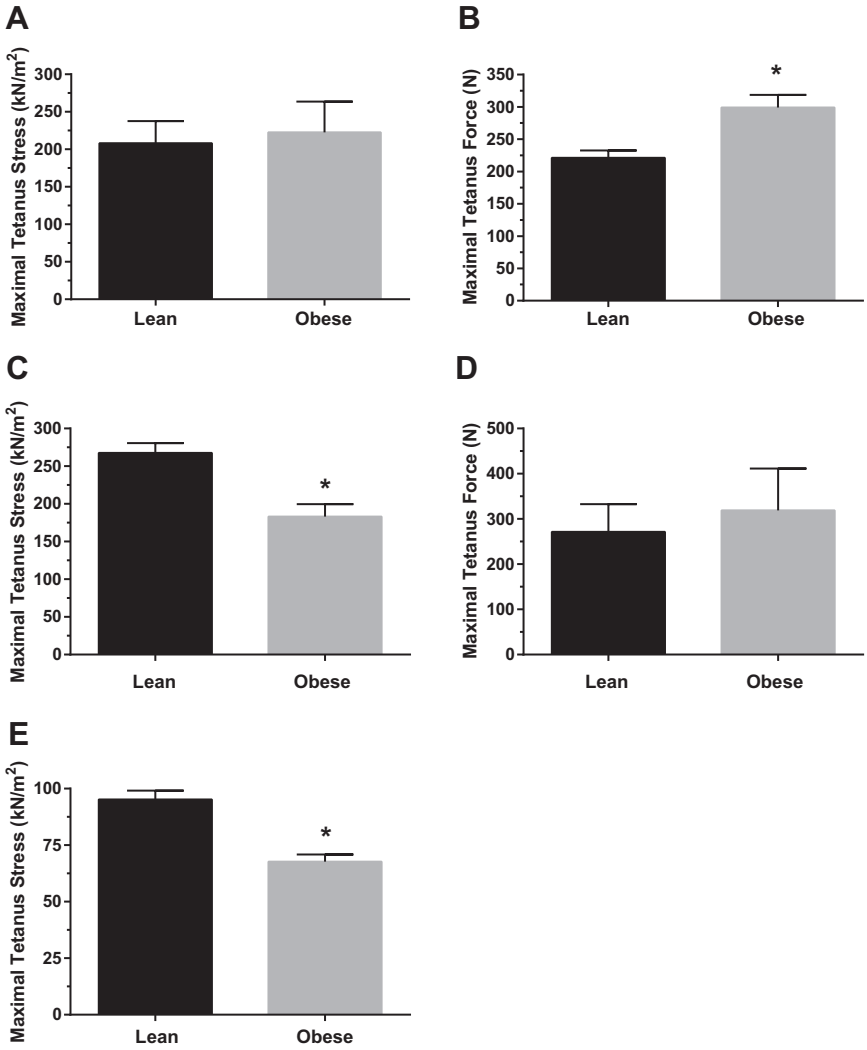


Fig. 1. The effect of 16-wk high-fat diet (HFD) on the maximal isometric tetanus stress (*A*, *C*, and *E*) and absolute isometric tetanus force (*B* and *D*) of isolated mouse SOL (*A* and *B*), EDL (*C* and *D*), and DIA (*E*). Values are means ± SE; $N = 10$ for SOL; $N = 10$ for EDL lean; $N = 9$ for EDL obese; $N = 8$ for DIA. *Significant differences.

0.05); however, pAMPK/α-tubulin and pAMPK/AMPK were unchanged (Fig. 6, *E* and *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 fiber-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 that of 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 Ref. 39). Interestingly, this increase in force-producing capacity did not transfer to an increase in the absolute PO produced by SOL. These findings may suggest an obesity-induced favorable 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 normalized WL power were unaffected in the SOL, possibly demonstrating that the muscle quality of the obese group was maintained. Given that

Table 3. *The effect of 16-wk high-fat diet on isometric THPT and LSHR of isolated mouse SOL, EDL, and DIA*

| | 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* |

Values are means ± SE; $N = 10$ for SOL; $N = 10$ for EDL lean; $N = 9$ for EDL obese; $N = 8$ for DIA. THPT, time to half peak tetanus; LSHR, last stimulus to half tetanus relaxation. *Significant differences between lean and obese groups.

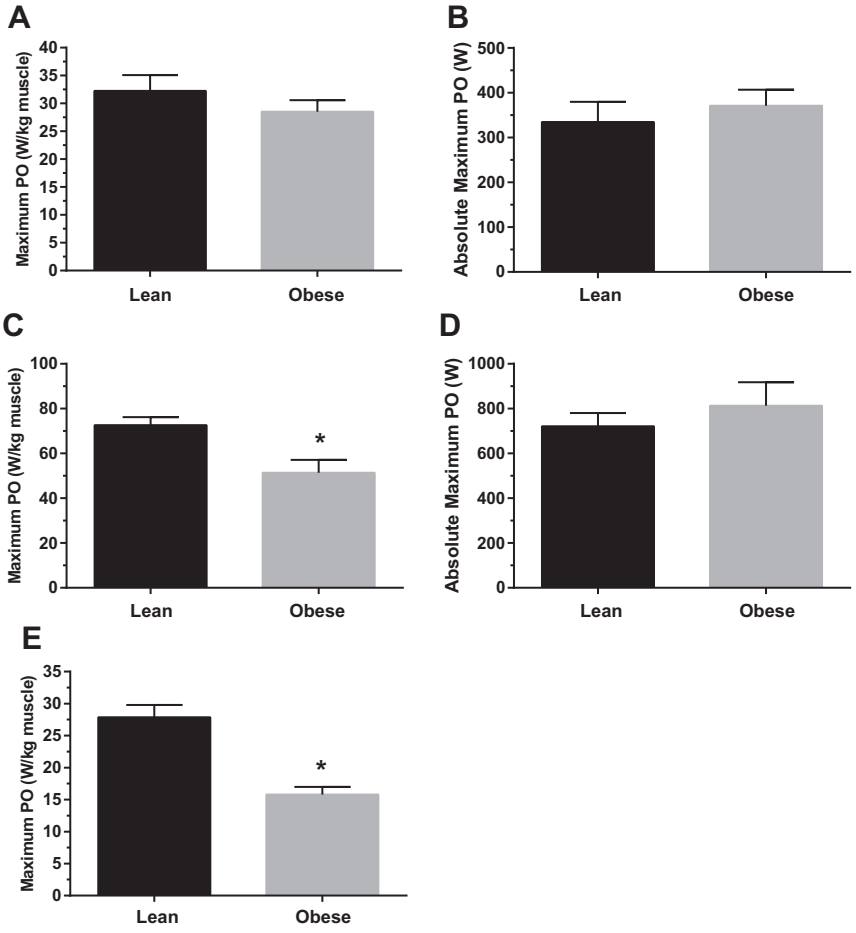


Fig. 2. The effect of 16-wk HFD on the maximal normalized WL PO (*A*, *C*, and *E*) and absolute WL PO (*B* and *D*) of isolated mouse SOL (*A* and *B*), EDL (*C* and *D*), and DIA (*E*). Values are means ± SE; $N = 10$ for SOL; $N = 10$ for EDL lean; $N = 9$ for EDL obese; $N = 8$ for DIA. *Significant differences.

normalized 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 may be attributed to the large variation in this data set.

The maximal isometric stress and normalized WL PO were significantly reduced in the obese EDL. With respect to the limited changes in absolute isometric force and WL 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. First, 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,

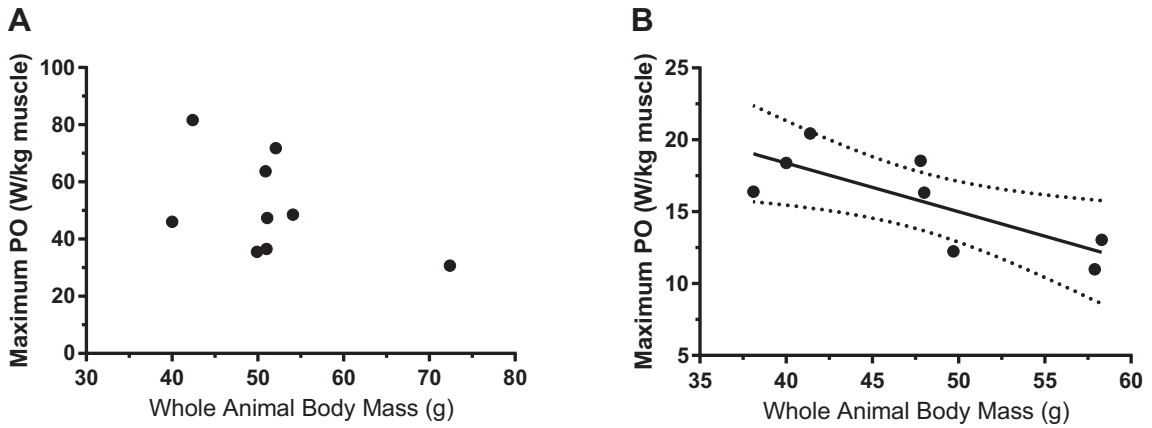


Fig. 3. The relationship between whole animal body mass and normalized work-loop power for the obese EDL (*A*) and DIA (*B*) experimental groups. $N = 9$ for EDL; $N = 8$ for DIA. *B*: the lines represent a first-order polynomial fitted to the data using a least squares regression and the 95% confidence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

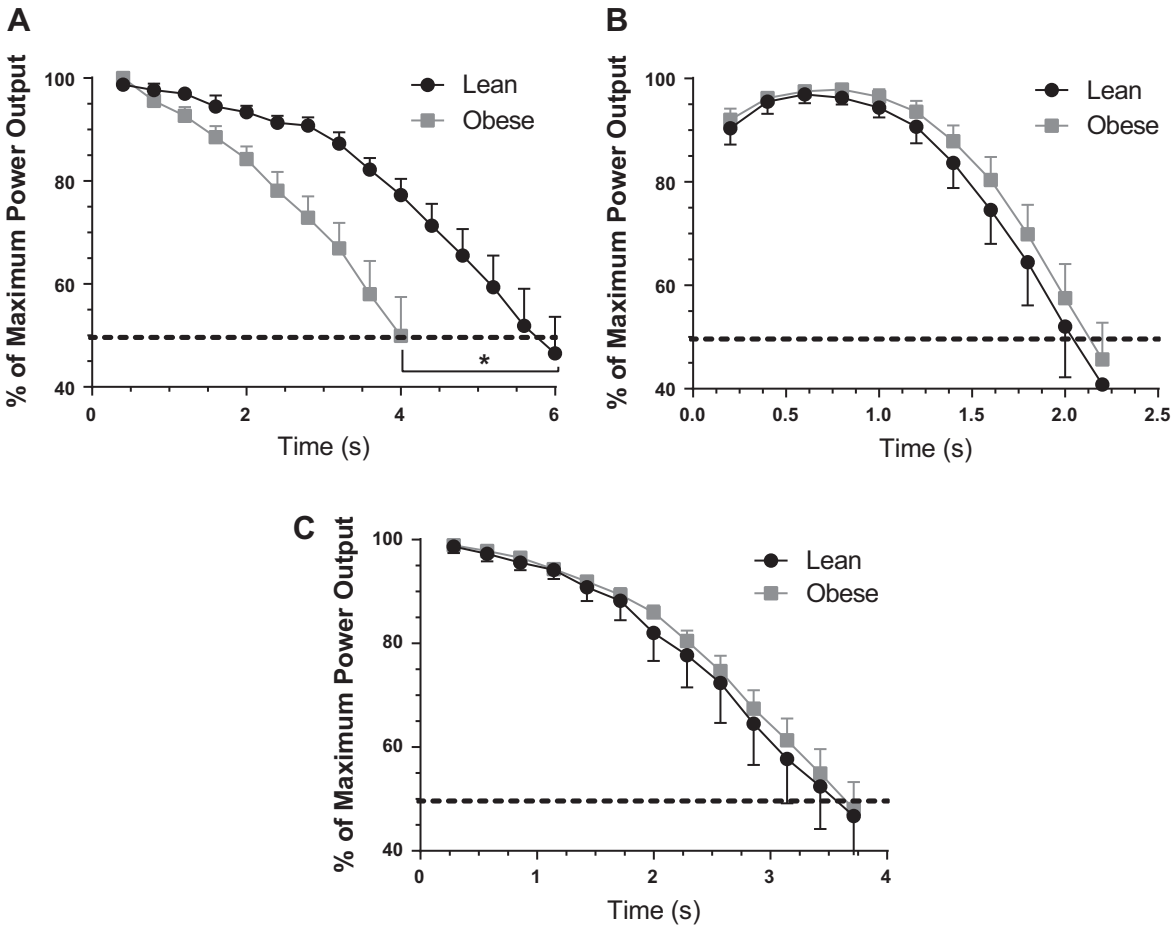


Fig. 4. The effect of 16-wk HFD on the fatigue resistance of maximally stimulated mouse SOL (*A*), EDL (*B*), and DIA (*C*). Values are means ± SE; $N = 10$ for SOL; $N = 10$ for EDL lean; $N = 9$ for EDL obese; $N = 8$ for DIA. *Significant differences.

the maintenance of absolute force and power is likely to be inadequate, given the increase in load. Similar to the EDL, isometric stress and normalized WL power of the obese DIA were significantly lower compared with 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 normalized 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 analyzed 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 fiber-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 fibers, it could be considered that this muscle already has a preferable metabolic profile to oxidize lipids, in comparison to EDL and DIA, which have a relatively faster fiber-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 in 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), its magnitude is likely to be lower than the SOL due to its role in postural support.

It is clear from the contractile evidence demonstrated here and in 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

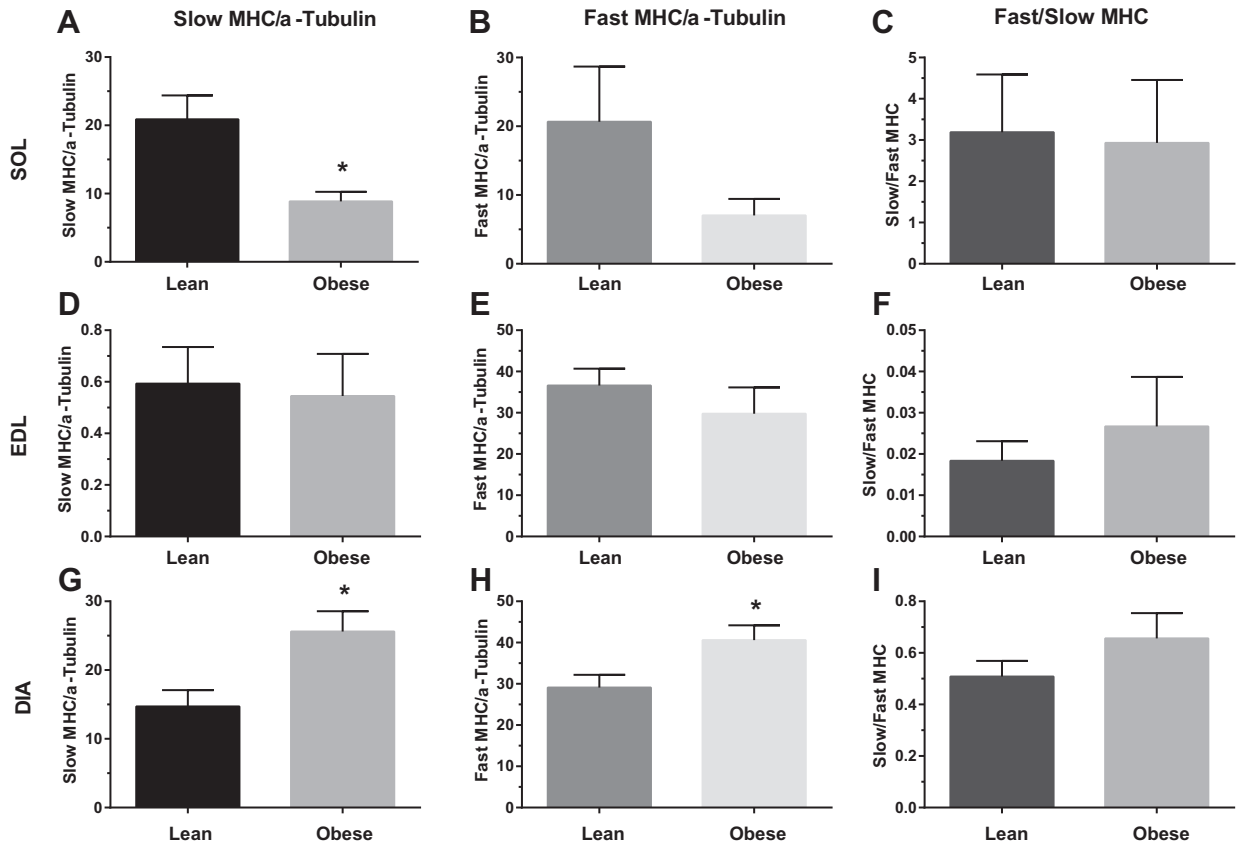


Fig. 5. The effect of 16-wk HFD on fast and slow MHC expression of mouse SOL (*A–C*), EDL (*D–F*), and DIA (*G–I*). *A*, *D*, and *G*: slow MHC/α-tubulin. *B*, *E*, and *H*: fast MHC/α-tubulin. *C*, *F*, and *I*: ratio of fast to slow MHC. Values are means ± SE; $N = 6$ in each case. *Significant differences.

much faster in vivo than that of 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 et al. (8), who demonstrated reduced fatigue resistance of single flexor digitorum brevis fibers 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 PO.

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 locomotor performance. Mechanistically, this may relate to the demonstrated reduction in slow MHC expression. The ability to release and reuptake $Ca^{2+}$ from and to the sarcoplasmic reticulum 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 sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase, which is responsible for the movement of $Ca^{2+}$ from the cytoplasm back into the sarcoplasmic reticulum (19). If the muscle is still active during the relengthening phase of the WL, 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 independent of changes in sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase (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 with 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 with 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, 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 deliv-

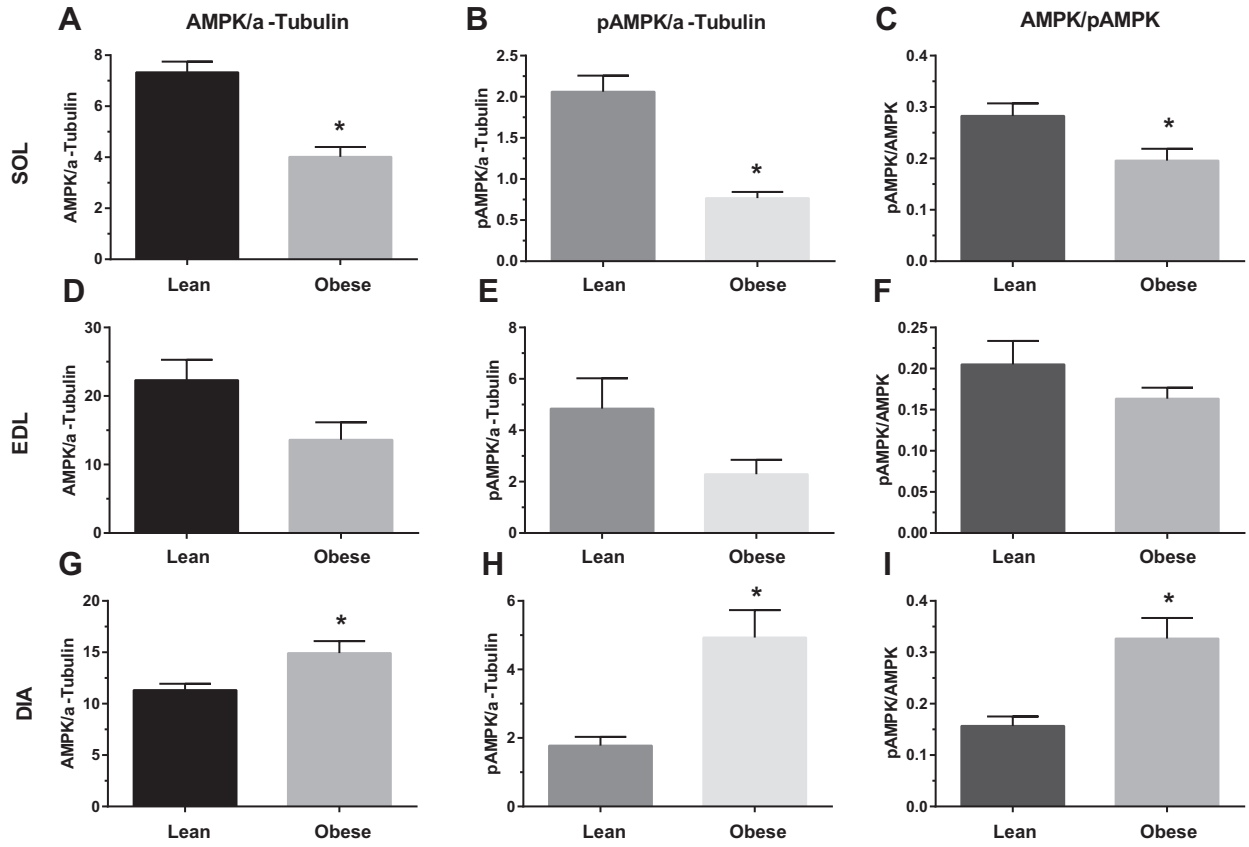


Fig. 6. The effect of 16-wk HFD on the AMPK activity of mouse SOL (*A–C*), EDL (*D–F*), and DIA (*G–I*). *A*, *D*, and *G*: AMPK/α-tubulin. *B*, *E*, and *H*: pAMPK/α-tubulin. *C*, *F*, and *I*: ratio of AMPK to pAMPK. Values are means ± SE; $N = 7$ for SOL; $N = 6$ for EDL; $N = 6$ for DIA. *Significant differences.

ered 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 unitize 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. These data are 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 MHC expression may help to explain the reduced fatigue resistance in the SOL, the normalized force and power of the EDL and DIA occurred without a change in the ratio of fast and slow MHC expression. Evidence examining the effect of obesity on muscle fiber-type composition is varied (as discussed by Ref. 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 et al. (65) demonstrated that, despite a shift to a slower fiber 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 are, however, subject to the same methodological discrepancies identified in studies measuring muscle performance. The present findings infer that fiber-type shifts may play a role, but do not substantially explain the reduction in the obesity-induced change in contractile performance. Given that the present data only examine 16 wk of feeding, changes in fiber-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, compared with 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 with the lean DIA, given the significant reduction in normalized 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 obesity-related changes in the metabolic profile of skeletal muscle (10, 13, 25). As indicated by Ref. 13, this response is likely to be muscle specific, and its complexity related to fiber type and duration of high-fat-diet consumption. Although there were some favorable 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 MHC 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 normalized 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 MHC expression) were not coupled with improved or maintained muscle quality, respectively. This suggests that, although plasticity in skeletal muscle modeling 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 downregulation 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 fibers. 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.

*Limitations and future direction.* Although isometric stress and normalized 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. Normalizing 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 twofold increase in skeletal muscle lipid content (21). Machann, et al. (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 fiber 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 fiber 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 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 MHC concentrations (35). However, there is a suggestion in the literature (11) that using low-salt buffers such as RIPA buffers underestimates the concentrations of MHC. Our measures of MHC 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 MHC 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 aging 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, fiber 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 (normalized 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 PO 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 locomotor 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 fiber-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 and exercise capacity and will sustain a significant calorific imbalance.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

## AUTHOR CONTRIBUTIONS

J.T., J.T., C.H., and F.S. performed experiments; J.T., J.T., C.H., R.S.J., and F.S. analyzed data; J.T., J.T., R.S.J., and F.S. interpreted results of experiments; J.T. and J.T. prepared figures; J.T. and J.T. drafted manuscript; J.T., J.T., C.H., R.S.J., V.M.C., and F.S. edited and revised manuscript; J.T., J.T., C.H., R.S.J., V.M.C., and F.S. approved final version of manuscript.

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