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Novelty bullets: points that summarize the key findings in the work:	We assessed contractile function of permeabilized skeletal muscle fibres in a rat model of diet-induced obesity. The high-fat, high-sucrose diet was associated with impaired force output of fibres expressing MHC I or MHC IIa in some cohorts. Other measures of contractile function were not significantly affected by diet.
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Contractility of permeabilized rat vastus intermedius muscle fibres following high-fat, high-sucrose diet consumption

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Short title: Obesity and contractile dysfunction

Abstract

Obesity is a worldwide health concern associated with impaired physical function. It is not clear if contractile protein dysfunction contributes to the impairment of muscle function observed with obesity. The purpose of this study was to examine if diet-induced obesity affects contractile function of chemically permeabilized vastus intermedius fibres of male Sprague Dawley rats expressing fast myosin heavy chain (MHC) IIa or slow MHC I. Rats consumed either a high-fat, high sucrose (HFHS) diet or a standard (CHOW) diet beginning as either weanlings (7-week duration: WEAN₇ cohort, or 14-week duration: WEAN₁₄ cohort) or young adults (12-week duration: ADULT₁₂ cohort, 24-week duration: ADULT₂₄ cohort). HFHS-fed rats had higher ($P<0.05$) whole-body adiposity (derived from dual-energy X-ray absorptiometry) than CHOW-fed rats in all cohorts. Relative to CHOW diet groups, the HFHS diet was associated with impaired force production in a) MHC I fibres in the ADULT₂₄ cohort, and b) MHC IIa fibres in the ADULT₁₂ and ADULT₂₄ cohorts combined. However, the HFHS diet did not significantly affect the Ca²⁺-sensitivity of force production, unloaded shortening velocity, or ratio of active force to active stiffness in any cohort. We conclude that diet-induced obesity can impair force output of permeabilized muscle fibres of adult rats.

Novelty Bullets:

- We assessed contractile function of permeabilized skeletal muscle fibres in a rat model of diet-induced obesity.
- The high-fat, high-sucrose diet was associated with impaired force output of fibres expressing MHC I or MHC IIa in some cohorts of rats.
- Other measures of contractile function were not significantly affected by diet.

Key Words: fibre type, obesity, skeletal muscle, pre-clinical models, myosin

List of Abbreviations

ADULT₁₂ – Group of rats starting 12-weeks of diet at 10-12 weeks of age

ADULT₂₄ – Group of rats starting 24-weeks of diet at 12 weeks of age

CHOW – Chow diet

CSA – Cross-sectional area

HFHS – High-fat, high-sucrose diet

L₀ – Optimum fibre length for steady state force production at full calcium activation

MHC – myosin heavy chain

n_H – Hill Coefficient

pCa₅₀ – Calcium concentration (log units) giving 50% of maximum steady state isometric force

V₀ – Unloaded shortening velocity of muscle fibres

VI – Vastus intermedius

WEAN₇ - Group of rats starting 7-weeks of diet at 3 weeks of age

WEAN₁₄ - Group of rats starting 14-weeks of diet at 3 weeks of age

Introduction

Obesity is associated with impairments in physical performance which makes day-to-day tasks more difficult (Evers Larsson and Mattsson 2001). Obesity-related impairments in physical function do not appear to reflect changes in the absolute capacity of muscles to produce force or power (Bollinger 2017; Garcia-Vicencio et al. 2015; Lafortuna et al. 2005; Maffiuletti et al. 2007, 2013; Paolillo et al. 2012; Tallis et al. 2018; Tomlinson et al. 2016) as absolute force output is often enhanced with obesity, particularly in young individuals and in anti-gravity muscles (reviewed in Bollinger 2017; Maffiuletti et al. 2013; Tallis et al. 2018). Rather, impairments in muscle function associated with obesity manifest as reductions in muscle force and/or power output relative to body mass (Bollinger 2017; Lafortuna et al. 2005; Maffiuletti et al. 2013; Paolillo et al. 2012; Tallis et al. 2018; Tomlinson et al. 2016), fat-free mass (Hulens et al. 2001; Lafortuna et al. 2005; Paolillo et al. 2012), and muscle size (Fabbri et al. 2017; Newman et al. 2003; Straight et al. 2021; Tallis et al. 2018; Tomlinson et al. 2014). However, the effects of obesity on muscle function appear to be age dependent, as obesity does not impair force relative to fat-free body mass or muscle size in children and adolescents (Abdelmoula et al. 2012; Blimkie et al. 1989, 1990; Maffiuletti et al. 2008; Ward et al. 1997). Impairments in muscle force output have been associated with increased adiposity at the whole body, tissue, and cellular levels (Biltz et al. 2020; Bollinger 2017; Collins et al. 2018c; Straight et al. 2021; Tallis et al. 2018; Tomlinson et al. 2016), as well as intramuscular fibrosis, intramuscular adipose tissue, lipid deposition, and alterations in cytoskeletal and sarcomeric protein expression (Biltz et al. 2020; Bollinger 2017; Collins et al. 2018c; Eshima et al. 2017; Straight et al. 2021; Tallis et al. 2018; Tomlinson et al. 2016; Valenzuela et al. 2020).

Rodent models of obesity-inducing diets offer advantages over humans with regards to control over the mode and duration of the induction period for metabolic derangement. Studies on mice with diet-induced obesity demonstrate effects of obesity on force output which depend on diet duration, age, sex, and muscle type. For example, 17-week old male C57BL/6J mice consuming a high fat diet containing palm oil for 5 weeks exhibited diminished force per muscle mass in soleus but not extensor digitorum longus muscles (Ciapaite et al. 2015). Male C57BL/6J mice that consumed a high fat diet for either 12 weeks, 4 months, or 20 months beginning at 2 months of age exhibited impaired force output relative to muscle size in the extensor digitorum but not soleus muscles (Eshima et al. 2017, 2020), but no differences in force output were seen with a 4-week high fat diet (Eshima et al. 2017). A 9-week high fat diet beginning at 70 weeks of age resulted in diminished force and power per muscle cross-sectional area (CSA) in diaphragm but not soleus or extensor digitorum muscles of female CD-1 mice (Hill et al. 2019).

The underlying cellular cause of obesity-induced contractile dysfunction is difficult to pinpoint when using *in vivo* and whole muscle *in vitro* assessments. While *in vitro* assessments of isolated muscles remove the influence of body/limb mass and inertia, as well as neuromuscular recruitment patterns, obesity could feasibly affect fascicle geometry, intramuscular force transmission, intramuscular fibrotic and adipose tissue, fibre type profiles, and have fibre-type specific effects. An intact single fibre approach could bypass these limitations, but we are not aware of any study which has taken this approach to test mechanical function. However, Eshima et al. (2020) demonstrated that calcium release is impaired in intact single fibres (predominantly comprised of types IIa and IIx myosin heavy chains (MHCs; Tarpey et al. 2018) isolated from flexor digitorum brevis of C57BL/6J mice that had consumed a high-fat diet for either 4- or 20-months in comparison to age matched controls on a chow diet. Altered calcium handling could diminish force

independent of any alteration of the intrinsic force-producing capacity of the skeletal muscle contractile proteins. Permeabilizing the membranes of single fibres permits control over metabolite and calcium concentrations, and mechanical function can be assessed at the level of the sarcomeres with relative ease. Two studies have found obesity-related impairments in contractile function of permeabilized fibres of older adult humans. A cross-sectional study by Choi et al. (2016) compared contractile function of permeabilized fibres from the vastus lateralis of older adults (65-80 years) with obesity (body mass index: 30.0 ± 0.4) and without obesity (body mass index: 22.0 ± 0.5). In this study, Choi et al. found that the older adults with obesity exhibited impairments in unloaded shortening velocity (V_0) in MHC I fibres (slow), and impaired maximal force output in MHC I fibres and MHC IIa fibres (fast) relative to the older adults who did not have obesity. Straight et al. (2019) found that adiposity was negatively correlated with single fibre force per CSA in MHC I and IIa fibres of older women, but not older men. We are not aware of any study which has examined the effects of obesity on contractility of single muscle fibres from young humans or from animals.

The purpose of this study was to examine if diet-induced obesity affects contractile function of chemically permeabilized fast and slow muscle fibres of male Sprague Dawley rats. Four cohorts of rats were examined. Each cohort consisted of a control group which consumed standard rat chow (CHOW) and a diet-induced obesity group which consumed a high-fat, high-sucrose diet (HFHS). The cohorts differed in the age of diet onset (weanling or young adulthood) and the duration of the diet (7-24 weeks). Several reports on the precise animals included in the present study have been published (Boldt et al. 2020a, 2020b, 2020c; Collins et al. 2015, 2016b, 2016c, 2017, 2018a, 2018b; Klancic et al. 2021; Rios et al. 2020) but none have broached the topic of skeletal muscle contractile function. It was hypothesized that the HFHS diet would be: 1) comparably detrimental to force output of fast and slow fibres, 2) more

detrimental to force output with a longer diet duration, and 3) more detrimental to force output when the diet is introduced in young adulthood than when the diet is introduced at the time of weaning.

Materials & Methods

Ethics

The study protocols were approved by The University of Calgary Animal Care Committee and conformed to the Guide for the Care and Use of Laboratory Animals (Protocol Numbers: AC15-0079, AC-0130, AC12-0139, AC16-0130). This study was not pre-registered.

Animals

Male rats (CD® Sprague Dawley IGS Rat; Strain Code: 001) were housed individually in a temperature (20-22° C) and humidity-controlled room on a 12:12 light-dark cycle and maintained at the University of Calgary with standard monitoring of well-being. Food and water were supplied *ad libitum*.

Cohorts

Four different cohorts of male rats were examined. Within each cohort, rats were randomly assigned into CHOW and HFHS diet groups. One cohort began the diets at the age of weaning (3 weeks old) and the diets lasted 7 weeks (WEAN₇). Another cohort began

the diets at 3 weeks of age and the diets lasted 14 weeks (WEAN₁₄). Another cohort began the diets at 10-12 weeks of age (i.e. in young adulthood (Sengupta 2013) and the diets lasted 12 weeks (ADULT₁₂). The last cohort began the diets at 12 weeks of age and the diets lasted 24 weeks (ADULT₂₄). The diet durations and ages of onset were primarily dictated by tissue availability, with samples gleaned from several inter-related, but independent projects performed in the Human Performance Lab at the University of Calgary. The changes in body mass over time for the exact animals used in this study have been published previously (WEAN₇: (Klancic et al. 2021), WEAN₁₄: (Boldt et al. 2020a), ADULT₁₂: (Collins et al. 2015) ADULT₂₄: (Rios et al. 2020)). Caloric intake was monitored in the WEAN₇ cohort (Klancic et al. 2021) and the ADULT₂₄ cohort (unpublished).

Specific numbers of rats included in this study are as follows: CHOW WEAN₇: 8, HFHS WEAN₇: 9, CHOW WEAN₁₄: 10, HFHS WEAN₁₄: 10, CHOW ADULT₁₂: 8, HFHS ADULT₁₂: 9, CHOW ADULT₂₄: 11, HFHS ADULT₂₄: 17. All rats had at least one (and up to 6) mechanically-tested fibres included in the results.

Diet Composition

The CHOW diet (Lab Diets 5001) consisted of 28.5% protein, 13.5% fat, and 58% carbohydrates as a percentage of total calories (3.7% by weight from sucrose). The HFHS diet differed based on the age of the rats primarily to meet the additional protein requirements of rapidly growing weanling rats and then from 10 weeks of age onwards to reflect protein needs for maintenance. Rats less than 10 weeks of age were fed a diet consisting of 19% protein, 39.3% fat, and 41.7% carbohydrates as a percentage of total

calories (Dyets 103915), and then were switched to a diet consisting of 15.8% protein, 39.2% fat, and 45% carbohydrates as a percentage of total calories (~50% of total diet mass by weight from sucrose) (Dyets 102412) ≥ 10 weeks of age. The HFHS diet was selected as an obesogenic diet that approximates the type of mixed high fat/simple carbohydrate diet driving the obesity epidemic in North America and has been shown by us previously to result in significant joint and muscle damage in Sprague Dawley rats (Collins et al. 2015, 2016a, 2016b, 2018a; Pregoica et al. 2020).

Body Composition

Body mass, fat mass, percent body fat, and bone mineral content plus lean mass were determined for each animal at the endpoint of diets using dual energy X-ray absorptiometry (DXA) and analyzed with software for small animal analysis (Hologic QDR 4500; Hologic, Bedford, MA). These procedures and data from these same animals have been reported previously (Boldt et al. 2020a, 2020b; Collins et al. 2015, 2016a, 2016b; Collins et al. 2016c; Klancic et al. 2020, 2021; Rios et al. 2020), but data are re-reported to reflect the specific animals used in assessments of single fibres.

Skinned Fibre Preparation

The vastus intermedius (VI) was chosen because its fibre type profile is comparable to that of many human muscles, with a high proportion of MHC I and MHC IIa fibres (Bloemberg and Quadrilatero 2012; Dahmane et al. 2005; Hitomi et al. 2005). Muscles were excised from animals within 20 minutes of sacrifice, and chemically skinned using an adaptation of procedures used previously

(Mounier et al. 1989; Wood et al. 1975). Briefly, excised muscles were tied to wooden sticks using silk suture and stored in a skinning solution (details of solutions are listed below) for 12 h at 4°C, followed by a 1:1 skinning-glycerol solution at -20°C for 2-5 weeks. Sample size was determined based on the number of viable fibres that could be assessed in the period 2-5 weeks after tissue harvest and the random selection of fibres from the muscle samples. Samples older than 5 weeks were not mechanically tested. On the day of experiments, single muscle fibres were isolated and transferred to the glass-bottomed chamber of a Model 802B fibre testing apparatus (Aurora Scientific Inc., Aurora, Canada) filled with relaxing solution. One end of the fibre was mounted to a hook affixed to a length controller (Model 322C, Aurora Scientific Inc., Aurora, Canada) and the other end was mounted to a hook affixed to a force transducer (Model 400A, Aurora Scientific Inc., Aurora, Canada) to control length and measure force, respectively. Fibres were attached to the hooks using cellulose acetate glue. Sarcomere length was measured using the optical diffraction pattern of helium-neon laser light and adjusted to an average sarcomere length of 2.4 μm using the XYZ stage of the length controller. Dimensions of mounted fibres were taken using a calibrated reticle mounted on a dissecting microscope. Fibre cross-sectional area was calculated based on diameter and assumed circularity. Once sarcomere length was set, all subsequent length changes were performed using the length controller software.

These experiments were performed in four different years and variations in environmental factors, animal housing conditions, and test procedures may have influenced the results. Specific differences between cohorts include the experimental temperature for mechanical testing (WEAN₇: 20 $\pm$ 1°C; WEAN₁₄: 18 $\pm$ 1°C, ADULT₁₂: 18 $\pm$ 1°C; ADULT₂₄: 22 $\pm$ 1°C) and different batches of test solutions were

used to assess mechanical properties in each cohort. Experimental temperature and solutions were tightly controlled within each cohort.

Experimental Protocol

Mechanical testing began by immersing the fibre in activating solution (pCa 4.2) to check fibre quality. When force stabilized, the fibre was deactivated in a relaxing solution. Sarcomere length was checked again and reset to 2.4 μm when necessary. The force-pCa relationship and V_0 were assessed in muscle fibres from all cohorts. Active and passive stiffness were probed in all fibres from the WEAN₇, WEAN₁₂, and ADULT₂₄ cohorts, but not in the ADULT₁₂ cohort (the first cohort assessed chronologically). After mechanical testing, each fibre was immersed in 10 μL of Laemmli buffer and stored at -80°C for later assessment of MHC isoform using gel electrophoresis. As mechanical testing and data extraction were performed without knowledge of the fibre type being tested, researchers were partially blinded to the experimental condition, but not to cohort or diet group.

Force-pCa relationships and fibre stiffness

Fibres were activated in a series of solutions with incrementally higher levels of Ca^{2+} . Cross-bridge binding was probed at the force plateau achieved in each activating solution by rapidly stretching the fibres by 0.2% of their length (i.e. a stiffness test) using the length-step function in the length controller software. Force and length data were recorded continuously at 100 Hz with intermittent collection at 10 kHz during the stiffness tests. Force and stiffness data were normalized to fibre cross-sectional area. Active force was calculated by subtracting the force recorded in the relaxing solution from the force in the activating solutions. Active forces achieved

in each activating solution were normalized to peak active force and then fit to the Hill Equation (Eq. 1) using GraphPad Prism Software (v7.05, San Diego, USA).

Eq 1: $\text{Force} = 1 / (1 + 10^{((\text{pCa} - \text{pCa}_{50}) \cdot n_H)})$

The pCa_{50} is a measure of calcium sensitivity of force production, referring specifically to the calcium concentration resulting in 50% of peak active force. The Hill Coefficient (n_H) is a measure of the cooperativity of crossbridge binding and relates to the slope of the sigmoidal force-pCa relationship at 50% of peak active force.

Stiffness was calculated as the difference between the peak force during the stiffness test and the force immediately preceding the stiffness test divided by the stretch amplitude. Active stiffness was calculated by subtracting the stiffness of the muscle in the relaxing solution (i.e. passive stiffness) from the stiffness of the muscle in an activating solution. To gain insight into the average force per cross-bridge, the ratio of active force to active stiffness was calculated for each activation. This ratio was unreliable at active forces below ~15% of the peak active force but did not change systematically for active forces above ~15% of peak active force; data were therefore reduced by calculating the average ratio of active force to active stiffness for all activations resulting in active force above 15% of peak active force.

Force per unit CSA has been reported to diminish as the CSA of permeabilized muscle fibres increases (Elzinga et al. 1989; Gilliver et al. 2009; Krivickas et al. 2011; Miller et al. 2015). As force is typically normalized to CSA, a non-linear relationship between force and CSA is problematic when comparing different groups of muscle fibres; variability in fibre CSA will result in variability in force

per CSA, and could lead to misinterpretations of the results if there are systematic differences in CSA between groups. Krivickas et al. (2011) found that force output of permeabilized human skeletal muscle fibres has a stronger relationship with fibre diameter than with fibre CSA, they suggested that force of permeabilized muscle fibres can be normalized to fibre diameter rather than fibre CSA. We have performed an independent analysis of the relationship between force and CSA in 546 permeabilized rat skeletal muscle fibres previously assessed in our laboratory (all independent from the current study; see Supplementary Figure S1) and found force is not linearly related to fibre diameter or fibre CSA. Force per diameter was lower in fibres with CSA $3000 \mu\text{m}^2$ or lower in comparison to fibres with CSA greater than $3000 \mu\text{m}^2$; only one or two of the 2725 fibres assessed in Krivickas et al. (2011) were smaller than this $3000 \mu\text{m}^2$ threshold. Fibres with CSA close to $3750 \mu\text{m}^2$ produced more force per CSA than smaller fibres and larger fibres. To account for the non-linear relationships between force and CSA and between force and diameter, we fit the force and CSA data of the 546 fibres to a one-phase exponential association equation using GraphPad software. We then expressed the force produced by fibres in the current study relative to the force of this best fit relationship at matched fibre CSAs; absolute and relative differences in force from the best-fit relationship are reported.

Unloaded Shortening Velocity

V_0 was assessed using the slack test method (Edman 1979). Fibres were maximally activated and step-shortened by fixed proportions of fibre length ranging from 5% to 20%. The slack time of the fibre was recorded for each imposed shortening. Data were collected at

10 kHz. A graph of the imposed change in length versus measured slack time was generated, and the slope of the resultant linear relationship was taken as V_0 .

Solutions

Skinning or relaxing solution contained (in mM): potassium propionate (170), magnesium acetate (2.5), MOPS (20), K_2EGTA (5) and ATP (2.5), pH 7.0.

Washing solution contained (in mM): potassium propionate (185), magnesium acetate (2.5), MOPS (10) and ATP (2.5), pH 7.0.

Activating solutions contained (in mM): potassium propionate (170), magnesium acetate (2.5), MOPS (10), ATP (2.5) and free Ca^{2+} buffered with EGTA ($CaEGTA$ and K_2EGTA mixed in order to obtain a range of pCa values), pH 7.0.

One protease inhibitor tablet (Complete, Roche Diagnostics, Montreal, Quebec, Canada) was added for every 10 mL of prepared solution.

Myosin Heavy Chain Determination

MHC isoform compositions of whole VI and mechanically tested single fibres were determined using SDS-page gel electrophoresis techniques described previously (Joumaa et al. 2015; Smith et al. 2020). Briefly, homogenates of whole VI and single fibres were diluted in Laemmli buffer and loaded into gels containing 4.5% acrylamide in the stacking region and 7.5% acrylamide in the running region (Toursel et al. 2000). Proteins were separated using constant voltage (73 V) and temperature (4°C) using a Bio-rad Mini-

Protean III unit (USA). After 40 h, gels were stained with Coomassie blue and scanned with a GS-800 Calibrated Densitometer (Bio-Rad, USA). Myosin heavy chain identities were determined based on the band migration distance relative to a standard mixture of rat VI and extensor digitorum longus samples which contained all four skeletal MHC isoforms. MHC isoform composition of whole VI samples was quantified using ImageJ (National Institutes of Health, USA), with the optical density of each MHC band expressed as a percentage of the sum of the optical densities of all MHC bands found in that same sample.

Statistics

Measures of body composition, and MHC expression were compared between CHOW and HFHS groups using a factorial ANOVA followed by Tukey's HSD post-hoc test when appropriate. As multiple fibres were tested from each animal, the effects of diet on mechanical properties were assessed using hierarchical (nested) ANOVAs with rat number nested in diet group, thereby avoiding the effects of pseudoreplication (Eisner 2021). Fibre types were assessed separately. Cohorts were assessed in isolation, with WEAN cohorts combined, and with ADULT cohorts combined.

A supplemental analysis was performed to assess our ability to detect well-established differences in pCa_{50} , n_H and V_0 between MHC I fibres and MHC IIa fibres (see Schiaffino and Reggiani 2011). In this analysis, MHC I and MHC IIa fibres were compared within each cohort and diet group using hierarchical (nested) ANOVAs, nesting rat number within fibre type. Diet groups were assessed separately. Cohorts were assessed in isolation, with WEAN cohorts combined, and with ADULT cohorts combined.

Statistical testing was performed using GraphPad Prism software version 9.0.2 (San Diego USA). Differences were considered statistically significant at $\alpha=0.05$. No data obtained were excluded from analysis.

Results

Body Composition

HFHS rats had significantly ($P<0.05$) higher body mass than CHOW rats in the WEAN₁₄, ADULT₁₂, and ADULT₂₄ cohorts, but not in the WEAN₇ cohort (Fig 1A). HFHS rats had higher fat mass, and percent body fat relative to CHOW rats in all four cohorts (Fig. 1B, C). No significant differences in fat-free mass (i.e. bone mineral content + lean mass) were observed between CHOW and HFHS rats within any cohort (Fig 1D). Differences in body mass, fat mass, % body fat, and fat-free mass between cohorts were noted (Fig. 1A-D).

Fibre Type Composition

Diet was not associated with any significant differences in MHC isoform distribution in whole VI, but significant differences were seen between cohorts as shown in Fig. 2.

Mechanical Properties

Mechanical data are summarized in Table 1. Few MHC IIx, MHC IIb, and mixed MHC fibres were identified in the pools of mechanically tested fibres. As such, only findings from apparently pure MHC I and MHC IIa fibres are reported. Within each fibre type and cohort, CHOW and HFHS fibres had comparable values for V_0 , pCa_{50} , n_H , and the ratio of active force to active stiffness (an indicator of average force per cross-bridge). Normalized force-pCa curves for MHC I and MHC IIa fibres in each cohort are shown in Fig. 3. As a test of our ability to detect differences in contractile properties between groups of fibres, MHC I and MHC IIa fibres are expected to differ in pCa_{50} , V_0 and n_H values (reviewed in Schiaffino and Reggiani 2011). Consistent with these expectations, we found that MHC IIa fibres had lower pCa_{50} values, higher V_0 values, and higher n_H values than MHC I fibres within each diet group and cohort, with the exception of n_H in the HFHS rats of the ADULT₁₂ cohort where no significant difference was found between MHC I and MHC IIa fibres (refer to Table 1).

After accounting for fibre cross-sectional area, diet did not affect the peak force output of single fibres from the WEAN₇ cohort, the WEAN₁₄ cohort, or the WEAN₇ and WEAN₁₄ cohorts combined (Table 1). Force per fibre CSA was 20% lower ($P<0.05$) in the MHC I fibres from the ADULT₂₄ HFHS group relative to MHC I fibres from the ADULT₂₄ CHOW group, and 24% lower ($P<0.05$) in MHC IIa fibres of HFHS-fed rats in the ADULT₁₂ and ADULT₂₄ cohorts combined relative to those of CHOW-fed rats in the ADULT₁₂ and ADULT₂₄ cohorts combined.

As force per unit CSA decreases as the CSA of permeabilized muscle fibres increases (Elzinga et al. 1989; Gilliver et al. 2009; Krivickas et al. 2011; Miller et al. 2015), variability in CSA within groups or between-group differences in fibre CSA may be a

confounding factor when comparing different groups of fibres. To better account for differences in fibre CSA, we generated a best-fit one-phase exponential association curve of legacy data from our lab ($n=546$ fast and slow rat skeletal muscle fibres; see Supplementary Figure S1). This best-fit curve is our best approximation of the relationship between force and CSA of permeabilized fibres that have been assessed in our laboratory. We then related the force output of each fibre in the current study to the force of the best-fit curve of the legacy data at matched CSAs. In this analysis we found that diet had no significant effects on force output of fibres from the WEAN cohorts (alone or in combination), regardless of whether the force differences were expressed as a percentage or as an absolute difference from the best-fit curve (Fig. 4). However, differences were seen in the ADULT cohorts. Specifically, we found that MHC I fibres from HFHS-fed rats in the ADULT₂₄ cohort had significantly lower force output than MHC I fibres from CHOW-fed rats in the ADULT₂₄ cohort when force output was expressed as a percentage of the best-fit curve ($P=0.031$), but not when force was expressed as an absolute difference from the best-fit curve ($P=0.06$) (Fig. 5). The HFHS diet was also associated with lower force output in MHC IIa fibres from the ADULT₁₂ and ADULT₂₄ cohorts assessed together, regardless of whether force was expressed as a percentage or as an absolute difference from the best-fit curve (Fig 5).

Discussion

The purpose of this study was to examine if diet-induced obesity affects contractile function of chemically permeabilized fast and slow muscle fibres of young male Sprague Dawley rats. The HFHS diet significantly increased whole-body adiposity in all four cohorts of rats. After accounting for fibre size, the HFHS diet was associated with reduced force output of MHC I fibres of the

ADULT₂₄ cohort, and MHC IIa fibres of the ADULT₁₂ and ADULT₂₄ cohorts in a combined analysis. We are confident that the observed diet-induced impairments in force output are not manifestations of group differences in the CSA of mechanically tested fibres because normalization procedures using linear and non-linear force vs CSA relationships to normalize force yielded comparable results. As the reductions in force output were not accompanied by reductions in the ratio of active force to active stiffness, the mechanism underlying diet-induced force deficits is not likely to be a reduction in the average force per cross-bridge. The ratio of active force to the stiffness and/or number of bound cross-bridges appears to be independent of CSA in permeabilized human skeletal muscle fibres (Miller et al. 2015), suggesting that variability in fibre CSA is unlikely to be a confounding factor in the current assessment of the ratio of active force to active stiffness. Alternative explanations could include a diet-induced impairment in the ability of contractile proteins to form weakly-bound cross-bridges, or perhaps a reduction in the number of contractile proteins per unit volume. Swelling of myofilament lattice spacing could account for a reduced amount of contractile proteins per unit volume, but this would tend to increase the pCa₅₀ (i.e. lower the sensitivity of the contractile proteins to calcium) (Wang and Fuchs 1995). As diet did not significantly affect the pCa₅₀ values, we are not inclined towards this explanation. An alternative possibility is that non-contractile tissue (such as intramyofibrillar lipid) took up space within the fibre, contributing to CSA but not to active force or active stiffness. This explanation is consistent with findings that specific force output is diminished by intracellular fat content (Biltz et al. 2020; Choi et al. 2016). However, we are not aware of any work which has demonstrated the presence of non-contractile regions or diminished actin or myosin content of permeabilized fibres in different diet groups, nor are we aware of any study which has assessed changes in

myofilament lattice spacing. An assessment of how obesity or potential lipid deposition affects sarcomere structure, including myofilament lattice spacing would be a valuable addition to the literature.

Methodological concerns give us hesitation in making conclusions regarding the influence fibre type, age, diet type, and diet duration have on contractile characteristics. We believe that these questions should be left as matters for ongoing study and debate.

Nonetheless, the current data indicate that while force output of permeabilized fibres can be impaired by a HFHS diet, this is not always the case. Our observations that force output was diminished in MHC I fibres of ADULT₂₄ cohort, but not the other cohorts, and MHC IIa of the combined ADULT cohorts but not the combined WEAN cohorts are consistent with previous findings that the effects of obesity on contractile function are exacerbated by age (Abdelmoula et al. 2012; Blimkie et al. 1989, 1990; Bollinger 2017; Maffiuletti et al. 2008, 2013; Tallis et al. 2018; Ward et al. 1997) and diet duration (Eshima et al. 2017). Moreover, the diet-induced declines in force per CSA of MHC I fibres of the ADULT₂₄ cohort (20% decline) and MHC IIa fibres of the ADULT₁₂ and ADULT₂₄ cohorts combined (24% decline) are comparable in magnitude to the force deficits seen in older humans with obesity (Choi et al. 2016). As the VI is a postural muscle in rodents (Hitomi et al. 2005), our findings indicate that anti-gravity muscles are not immune to the detrimental effects of obesity; the work of Ciapiate et al.(2015) have seen this in the mouse soleus. As we did not find enough MHC IIx or IIb fibres to report, it is unclear if fibres with faster MHC isoforms were affected by the HFHS diet, whether in the VI or in other muscles; more work is needed.

As V_0 , n_H , and pCa_{50} exhibited statistically significant differences between fibre types, any undetected change in these parameters induced by the HFHS diet and associated adiposity must be considered small in comparison. The absence of an effect of the HFHS diet on unloaded shortening velocity differs from the results of Choi et al. (2016) who found that MHC I fibres of older humans with obesity had a lower V_0 than MHC I fibres of older humans without obesity.

In summary, contractile protein function is one of many factors that could contribute to altered neuromusculoskeletal function associated with obesity. We found that a HFHS diet increased whole-body adiposity of all groups of rats tested without significantly affecting the MHC isoform distribution in the VI. Force output was impaired in permeabilized MHC I fibres of the VI of HFHS-fed rats in the ADULT₂₄ cohort, but not the ADULT₁₂, WEAN₇ or WEAN₁₄ cohorts. Force output of permeabilized MHC IIa fibres of the VI of HFHS-fed rats in the ADULT₁₂ and ADULT₂₄ cohorts, but not the WEAN₇ or WEAN₁₄ cohorts, or in any cohort individually. Data from stiffness tests suggest that the observed contractile dysfunction is related to a decline in the number of force-producing cross-bridges rather than a change in the average force per cross-bridge. These results indicate that dysfunction of the contractile proteins can indeed be a factor in obesity-related muscle dysfunction seen at the tissue and whole-body levels, but more work is needed to characterize how diet duration and ages of diet onset affect the changes in contractile function in different fibre types, as well as the underlying cause of the contractile dysfunction. Finally, we introduce an analysis method which can account for the non-linear relationship between force output and CSA of permeabilized fibres which should be considered in future studies using skinned fibres.

Competing Interests

The authors declare that there are no competing interests. The funding agencies had no role in the design, analysis, or interpretation of the data contained within this manuscript.

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Data Availability Statement

Data underlying this manuscript are available upon request to the corresponding author.

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Figure Legends

Figure 1: Body composition measured by DXA. Individual data points are shown; bars are means $\pm$ SD. * - Different from CHOW of the same cohort ($P<0.05$). † - Different from the same diet group in the WEAN₇ cohort ($P<0.05$). ‡ - Different from the same diet group in the WEAN₁₄ cohort ($P<0.05$). # - Different from the same diet group in the ADULT₁₂ cohort ($P<0.05$). Diet had no effect on bone mineral content + lean mass but significant differences were seen between cohorts as listed at the top of panel D ($P<0.05$, main effects of ANOVA). Body composition data from these animals have been featured in previous reports, though different subsets of animals have been used. Linked studies: WEAN₇ - (Klancic et al. 2021); WEAN₁₄ - (Boldt et al. 2020a); ADULT₁₂ - (Collins et al. 2015, 2016a, 2016b, 2016c); ADULT₂₄ - (Boldt et al. 2020b; Rios et al. 2020).

Figure 2: Fibre type distributions in whole rat vastus intermedius. Individual data points are shown; bars are means $\pm$ SD. *- Significant difference between the indicated cohorts ($P<0.05$). No significant effects of diet or interaction effects were found. MHC – myosin heavy chain.

Figure 3: Force-pCa curves with best fit sigmoidal relationships for each fibre type, diet group and cohort.

Figure 4: The effects of diet on peak force of muscle fibres in the WEAN₇ and WEAN₁₄ cohorts, accounting for the non-linear relationship between force and fibre CSA. MHC I data are plotted in the left column, MHC IIa data are plotted in the right column. Top Row: Individual data points are from current experiments. The black line corresponds to the best-fit exponential equation relating peak force and cross-sectional area of 546 permeabilized rat skeletal muscle fibres from past experiments in our lab (all MHC

isoforms pooled; refer also to Supplementary Figure S1). Middle Row: The absolute difference in force between the current data and the force of the exponential fit at matched CSA (Calculated as: Measured Force – Force of exponential fit at matched CSA). Bottom Row: The percent difference in force between the current data and the exponential equation at matched CSA (Calculated as: $100\% \cdot (\text{Measured Force} - \text{Force of exponential fit at matched CSA}) \cdot (\text{Force of exponential fit at matched CSA})^{-1}$). ALL WEAN refers to the WEAN₇ and WEAN₁₄ cohorts combined. No statistically significant differences were found between diet groups.

Figure 5: The effects of diet on peak force of muscle fibres in the ADULT₁₂ and ADULT₂₄ cohorts, accounting for the non-linear relationship between force and fibre CSA. MHC I data are plotted in the left column, MHC IIa data are plotted in the right column. Top Row: Individual data points are from current experiments. The black line corresponds to the best-fit exponential equation relating peak force and cross-sectional area of 546 permeabilized rat skeletal muscle fibres from past experiments in our lab (all MHC isoforms pooled; refer also to Supplementary Figure S1). Middle Row: The absolute difference in force between the current data and the force of the exponential fit at matched CSA (Calculated as: Measured Force – Force of exponential fit at matched CSA). Bottom Row: The percent difference in force between the current data and the exponential equation at matched CSA (Calculated as: $100\% \cdot (\text{Measured Force} - \text{Force of exponential fit at matched CSA}) \cdot (\text{Force of exponential fit at matched CSA})^{-1}$). ALL ADULT refers to the ADULT₁₂ and ADULT₂₄ cohorts combined. * - HFHS $\neq$ CHOW, $P < 0.05$.

Table 1: Contractile characteristics of permeabilized vastus intermedius fibres of rats on a chow diet or a high-fat, high-sucrose diet.

Cohort	Diet	MHC Isoform (n)	CSA of Tested Fibres (µm ²)	Force/CSA (mN · mm ⁻²)	V ₀ (L ₀ · s ⁻¹)	pCa ₅₀ (pCa Units)	n _H	Active Force · Active Stiffness ⁻¹ (kPa · (kPa · µm ⁻¹) ⁻¹)
WEAN ₇	CHOW	I (19)	2050±740	123±31	1.32±0.61	6.27±0.10	5.09±1.24	26.8±6.1
WEAN ₇	HFHS	I (15)	1500±600	109±42	1.56±0.96	6.25±0.05	4.68±1.01	30.7±9.4
WEAN ₇	CHOW	IIa (14)	1410±550†	120±56	2.22±0.91†	6.16±0.07†	6.20±1.47†	30.7±10.3
WEAN ₇	HFHS	IIa (18)	1270±600	112±48	2.20±1.18†	6.15±0.06†	5.97±1.02†	33.7±11.4
WEAN ₁₄	CHOW	I (19)	3510±1450	143±52	0.97±0.21	6.23±0.07	3.01±0.58	18.3±3.9
WEAN ₁₄	HFHS	I (10)	4550±2770	146±63	0.98±0.16	6.29±0.05	3.00±0.39	17.1±5.1
WEAN ₁₄	CHOW	IIa (11)	4550±740†	136±37	1.46±0.45†	6.11±0.05†	4.79±0.89†	25.8±9.5†
WEAN ₁₄	HFHS	IIa (18)	4340±1380	150±37	1.46±0.23†	6.12±0.03†	4.67±0.78†	23.5±4.7†
ADULT ₁₂	CHOW	I (14)	4930±1600	172±82	1.01±0.30	6.37±0.06	3.21±0.67	-
ADULT ₁₂	HFHS	I (14)	4260±1650	180±63	0.94±0.22	6.43±0.06	4.38±1.46	-
ADULT ₁₂	CHOW	IIa (12)	4790±1370	150±47	1.68±0.66†	6.22±0.08†	5.23±1.52†	-
ADULT ₁₂	HFHS	IIa (9)	6870±3170†	107±38†	1.61±0.82†	6.25±0.05†	4.15±1.58	-
ADULT ₂₄	CHOW	I (25)	5300±1300	159±40	2.70±0.82	6.23±0.06	3.66±0.52	30.0±6.5
ADULT ₂₄	HFHS	I (17)	6080±2000	127±33*	2.57±1.20	6.19±0.05	4.05±0.89	35.9±6.9
ADULT ₂₄	CHOW	IIa (8)	5250±1220	151±21	3.87±1.44†	6.14±0.08†	4.77±0.94†	37.2±7.9†
ADULT ₂₄	HFHS	IIa (17)	7270±1670*	117±42	3.65±1.41†	6.10±0.04†	5.00±0.99†	37.1±9.2
All WEAN	CHOW	I (38)	2780±1360	146±72	1.18±0.51	6.25±0.08	4.02±1.41	22.4±6.6
All WEAN	HFHS	I (25)	2660±2270	129±55	1.30±0.76	6.26±0.05	4.01±1.16	25.2±10.4
All WEAN	CHOW	IIa (25)	2800±1710	123±45	1.85±0.81†	6.14±0.06†	5.55±1.40†	28.5±10.0†
All WEAN	HFHS	IIa (36)	2800±1880	130±46	1.92±1.00†	6.14±0.05†	5.30±1.10†	29.4±8.8
All ADULT	CHOW	I (39)	5170±1400	163±58	2.09±1.06	6.28±0.09	3.50±0.61	-
All ADULT	HFHS	I (32)	5260±2040	151±56	1.79±1.25	6.30±0.13	4.20±1.17	-
All ADULT	CHOW	IIa (20)	4970±1310	150±42	2.60±1.51	6.19±0.09†	5.04±1.32†	-
All ADULT	HFHS	IIa (26)	7130±2250*†	114±43*†	2.95±1.57†	6.16±0.08†	4.71 ±1.26	-

Values are means ± SD. * - $P < 0.05$ vs CHOW group of same myosin heavy chain (MHC) isoform. † - $P < 0.05$ vs MHC I of the same diet group. Note that there were differences in experimental temperature between cohorts: WEAN₇: 20±1°C; WEAN₁₄: 18±1°C, ADULT₁₂: 18±1°C; ADULT₂₄: 22±1°C. See also the data visualizations in Figures 3, 4, and 5.

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Figure 1: Body composition measured by DXA. Individual data points are shown; bars are means $\pm$ SD. * - Different from CHOW of the same cohort ($P < 0.05$). + - Different from the same diet group in the WEAN₇ cohort ($P < 0.05$). ‡ - Different from the same diet group in the WEAN₁₄ cohort ($P < 0.05$). # - Different from the same diet group in the ADULT₁₂ cohort ($P < 0.05$). Diet had no effect on bone mineral content + lean mass but significant differences were seen between cohorts as listed at the top of panel D ($P < 0.05$, main effects of ANOVA). Body composition data from these animals have been featured in previous reports, though different subsets of animals have been used. Linked studies: WEAN₇ - (Klancic et al. 2021); WEAN₁₄ - (Boldt et al. 2020a); ADULT₁₂ - (Collins et al. 2015, 2016a, 2016b, 2016c); ADULT₂₄ - (Boldt et al. 2020b; Rios et al. 2020).

201x150mm (300 x 300 DPI)

Figure 2: Fibre type distributions in whole rat vastus intermedius. Individual data points are shown; bars are means ± SD. *- Significant difference between the indicated cohorts ($P<0.05$). No significant effects of diet or interaction effects were found. MHC – myosin heavy chain.

201x143mm (300 x 300 DPI)

Figure 3: Force-pCa curves with best fit sigmoidal relationships for each fibre type, diet group and cohort.

238x100mm (300 x 300 DPI)

Figure 4: The effects of diet on peak force of muscle fibres in the WEAN₇ and WEAN₁₄ cohorts, accounting for the non-linear relationship between force and fibre CSA. MHC I data are plotted in the left column, MHC IIa data are plotted in the right column. Top Row: Individual data points are from current experiments. The black line corresponds to the best-fit exponential equation relating peak force and cross-sectional area of 546 permeabilized rat skeletal muscle fibres from past experiments in our lab (all MHC isoforms pooled; refer also to Fig S1). Middle Row: The absolute difference in force between the current data and the force of the exponential fit at matched CSA (Calculated as: Measured Force – Force of exponential fit at matched CSA). Bottom Row: The percent difference in force between the current data and the exponential equation at matched CSA (Calculated as: 100% · (Measured Force – Force of exponential fit at matched CSA) · (Force of exponential fit at matched CSA)⁻¹). ALL WEAN refers to the WEAN₇ and WEAN₁₄ cohorts combined. No statistically significant differences were found between diet groups.

102x146mm (300 x 300 DPI)

Figure 5: The effects of diet on peak force of muscle fibres in the ADULT₁₂ and ADULT₂₄ cohorts, accounting for the non-linear relationship between force and fibre CSA. MHC I data are plotted in the left column, MHC IIa data are plotted in the right column. Top Row: Individual data points are from current experiments. The black line corresponds to the best-fit exponential equation relating peak force and cross-sectional area of 546 permeabilized rat skeletal muscle fibres from past experiments in our lab (all MHC isoforms pooled; refer also to Fig S1). Middle Row: The absolute difference in force between the current data and the force of the exponential fit at matched CSA (Calculated as: Measured Force – Force of exponential fit at matched CSA). Bottom Row: The percent difference in force between the current data and the exponential equation at matched CSA (Calculated as: 100% · (Measured Force – Force of exponential fit at matched CSA) · (Force of exponential fit at matched CSA)⁻¹). ALL ADULT refers to the ADULT₁₂ and ADULT₂₄ cohorts combined. * - HFHS ≠ CHOW, *P* < 0.05.

102x142mm (300 x 300 DPI)