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RESEARCH ARTICLE

High-fat diet affects measures of skeletal muscle contractile performance in a temperature-specific manner but does not influence regional thermal sensitivity

Jason Tallis*, Rob S. James, Emma L. J. Eyre, Val M. Cox and Josh Hurst

ABSTRACT

The present study examined whether high-fat diet (HFD) consumption for 20 weeks had a temperature-specific effect on the contractile performance and regional thermal sensitivity of isolated mouse soleus and diaphragm muscle. Four-week-old female CD-1 mice were randomly selected to consume either a standard laboratory diet or a standard laboratory diet in conjunction with a HFD for 20 weeks. Peripheral soleus and core diaphragm were isolated from each animal and maximal isometric force and work loop power were assessed at 20, 28, 35 and 40°C. Increasing temperature to 35°C resulted in greater isometric stress, lower activation and relaxation time, and higher work loop power in both muscles. A further increase in temperature to 40°C did not affect isometric force but increased work loop power output of the soleus. Conversely, isometric force of the diaphragm was reduced and work loop power maintained when temperature was increased to 40°C. HFD consumption resulted in greater isometric force and absolute work loop power of the soleus and reduced isometric stress of the diaphragm, effects that were less apparent at lower temperatures. When the relationship between temperature and each measure of contractile function was examined by linear regression, there was no difference in slope between the control or HFD groups for either the soleus or diaphragm. These results indicate that whilst contractile function initially increases with temperature, the temperature to elicit maximal performance is muscle and contractile mode specific. Furthermore, HFD effects on contractile function are temperature specific, but HFD does not influence the relationship between temperature and performance.

KEY WORDS: Obesity, Work loop, Isometric, Force, Power

INTRODUCTION

Obesity is a global epidemic (WHO, 2000), associated with poor health, reduced quality of life and increased mortality (Abdelaal et al., 2017; Vásquez et al., 2014; Pimenta et al., 2015; Flegal et al., 2013; Aune et al., 2016). More specifically, obesity has been associated with increased risk of cardiovascular disease, insulin resistance, non-alcoholic fatty liver disease, subfertility and cancer (Abdelaal et al., 2017), and the direct effects of obesity on skeletal muscle function may act as a catalyst to negative health outcomes (Tallis et al., 2018, 2017b). *In vitro* assessments of skeletal muscle isolated from rodents following consumption of a high-fat diet (HFD) have been important in developing an understanding of obesity effects on skeletal muscle function, indicating muscle and contractile mode-specific responses (Tallis et al., 2018). One important area that has received little attention, however, is the interaction between temperature and HFD on skeletal muscle contractile function and whether obesity induced by HFD consumption influences the thermal sensitivity of skeletal muscle.

Mammals are endothermic, tightly regulating body temperature to optimise metabolic processes and skeletal muscle contractile function, important for optimising locomotor function and sustaining life (James and Tallis, 2019). Despite the tight regulation of core temperature, skeletal muscle is subject to temperature fluctuations influenced by the environment and heat generated through sustained activity. Human peripheral muscle may undergo fluctuations in temperature of as much as 15°C (Ducharme et al., 1991; Ranatunga et al., 1987). Furthermore, exercise can increase muscle temperature by 2–5°C (Yaicharoen et al., 2012; Mangum et al., 2018). Whilst these examples may represent the extremes, it is evident that skeletal muscle may function across a temperature range. This temperature variation is likely to impact locomotor function, given the profound effects of temperature on skeletal muscle function. Typically, peak force, shortening velocity, the speed of activation and relaxation, and as a consequence, mechanical work increase with temperature (James et al., 2015, 2012; Olberding and Deban, 2017; Rall and Woledge, 1990; Frueh et al., 1994; Lännergren and Westerblad, 1987; Prezant et al., 1990; Ranatunga, 1998). In many animals, mechanical work begins to level off towards the peak of the physiologically relevant range of temperatures (James et al., 2015, 2012; Lännergren and Westerblad, 1987). Despite this general trend, the impact of temperature on skeletal muscle function has been shown to be muscle specific. Our previous research indicates that mouse diaphragm tetanus activation and relaxation time, and work loop (WL) power output were more sensitive to changes in temperature than the more peripheral soleus muscle (James et al., 2015), an effect attributed to the tighter regulation of core temperature than that at the periphery. It is yet to be established whether these effects are apparent after body compositional changes brought about through the consumption of a HFD.

There is growing evidence to support a muscle and contractile mode-specific impact of HFD consumption (Tallis et al., 2018). A HFD associated increase in body mass has been shown to increase the absolute force or power-producing capacity of postural muscles (Tallis et al., 2017b; Hill et al., 2019), whilst force and power normalised to body mass and muscle mass (i.e. muscle quality) have been shown to decrease (Tallis et al., 2017b; Hill et al., 2019; Hurst et al., 2018; Seebacher et al., 2017; Eshima et al., 2017; Ciapaite et al., 2015). Despite these emerging trends, methodological discrepancies between published work have resulted in

Research Centre for Sport, Exercise and Life Sciences, Coventry University, Coventry, CV1 5FB, UK.

*Author for correspondence (ab0289@coventry.ac.uk)

J.T., 0000-0001-8904-2693; R.S.J., 0000-0002-3969-8261; E.L.J.E., 0000-0002-4040-5921; V.M.C., 0000-0002-7836-6120; J.H., 0000-0002-0658-3740

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inconsistent findings (Tallis et al., 2018). More specifically, HFD feeding duration, the nutritional composition of the diet, the contractile modality assessed, and the temperature at which the experiments were performed are likely to influence the result. For example, previous work has examined HFD effects on isolated muscle function at temperatures ranging between 20 and 37°C (Bott et al., 2017; Tallis et al., 2017b; Ciapaite et al., 2015). Given that temperature substantially influences the contractile function of skeletal muscle (James, 2013; James and Tallis, 2019), the variation in temperatures used to assess effects of HFD probably influences the outcomes of such studies. Assessing the interaction between temperature and HFD on muscle function will allow improved interpretation and comparison between previous studies examining the effect of HFD on skeletal muscle function and is important in considering the broader impact of HFD on muscle function, given that muscles operate across a temperature range.

Furthermore, HFD consumption may influence skeletal muscle thermal sensitivity. Obesity is associated with high body heat content, related to physiological mechanisms resulting in greater heat production and impaired heat loss (Savastano et al., 2009). Obesity is associated with greater heat production as a consequence of an increased fat-free mass, a higher vasoconstriction threshold, and greater subcutaneous adipose tissue resulting in impaired thermal conductivity and increased heat insulation (Savastano et al., 2009; Kasai et al., 2003). In obese individuals, the thermal cost of locomotion is probably also increased, given the need for greater muscular activity to overcome elevated body inertia. Obese individuals may also demonstrate decreased skin blood flow during exercise (Vroman et al., 1983), which may constrain heat dissipation that can be achieved by directing blood to the periphery. Furthermore, obese individuals may be at a thermoregulatory disadvantage given their reduced surface area to mass ratio (Verbraecken et al., 2006), reducing the surface area for cutaneous heat loss (Savastano et al., 2009). Though by no means unanimous, there is evidence indicating that body mass index (BMI) is positively associated with body temperature (Eriksson et al., 1985; Bastardot et al., 2019; Hoffmann et al., 2012). Whilst any change may be modest in magnitude, even small changes in temperature may impact skeletal muscle function. Impaired heat dissipation may have more profound effects for muscle of the periphery, where temperature fluctuations may be less severe than in non-obese counterparts, given the insulating properties of the increased surrounding adipose tissue. In support of this idea, an increased, and probably more stable, peripheral muscle temperature reported in overweight and obese individuals (Jalil et al., 2019; Savastano et al., 2009) may result in peripheral muscle becoming more of a thermal specialist in individuals with high adiposity compared with the same muscle in leaner individuals (James et al., 2015; Donley et al., 2012).

As such, the present work examined whether the effects of 20-weeks of HFD consumption on the contractile function of isolated skeletal muscle are temperature specific and determined whether HFD influences the thermal sensitivity of contractile performance. Based on the available evidence, it was hypothesised that HFD effects on contractile function would be temperature specific and that greater whole-body fat accumulation brought about through HFD consumption would result in more thermally specialised muscle.

MATERIALS AND METHODS

Animal and muscle preparation

Following ethics approval from the Coventry University Ethics Committee, 4 week old CD1 female mice (n=17; Charles River) were randomly assigned (using Microsoft Excel, Windows 2016) to either a control or HFD group. Throughout the experiment, all mice were housed in groups of 8–10 and were kept in a 12 h light:12 h dark cycle. All animals had access to water and standard lab chow (SDS RM-1 Maintenance; calories provided by protein 17.49%, fat 7.42%, carbohydrate 75.09%; gross energy 3.52 kcal g^{-1}; metabolisable energy 2.57 kcal g^{-1}) *ad libitum*. Mice in the HFD group had *ad libitum* access to a forage diet of husked sunflower seeds (Advanced Protocol PicoLab, Fort Worth, TX, USA; calories provided by protein 17.95%, fat 63.66%, carbohydrate 18.39%; gross energy 5.24 kcal g^{-1}; metabolisable energy 3.80 kcal g^{-1}) in addition to the standard chow. Following 20 weeks on the respective diets, and at 24 weeks of age, animals were killed by cervical dislocation in accordance with British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Body mass was measured to the nearest 0.01 g using an electronic balance. Nasoanal length was measured to the nearest 0.1 mm using digital callipers (Fisher Scientific™ 3417, Loughborough, UK) and body circumference around the abdomen was measured to the nearest 0.1 cm with a textile tape measure. The gonadal fat pad was dissected and weighed to the nearest 0.01 mg as a marker of whole-body adiposity (Rogers and Webb, 1980).

The isolation of skeletal muscle and assessment of contractile function followed our published protocols (Hurst et al., 2018; Hill et al., 2020, 2019; Tallis et al., 2017b; James et al., 2015; Tallis et al., 2014a; Vanhooydonck et al., 2014; James et al., 1995). Whole soleus muscle (n=8 for control; n=9 for HFD) and a ventral section of the costal diaphragm (n=8 for control; n=9 for HFD) were rapidly dissected from each animal in refrigerated (1–3°C), oxygenated (95% O_2; 5% CO_2) and frequently changed Krebs–Henseleit solution (mmol l^{-1}: NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; $CaCl_2$ 2.54; pH 7.55 at room temperature prior to oxygenation). The soleus and diaphragm represent a peripheral and a core muscle, respectively, and were chosen to allow comparison with our previous work examining the thermal sensitivity of non-obese mice (James et al., 2015) and effects of HFD consumption (Bott et al., 2017; Ciapaite et al., 2015; Hurst et al., 2018; Tallis et al., 2017b; Hill et al., 2019; Eshima et al., 2017). For soleus, an aluminium foil T-clip was wrapped around the distal tendon and a small piece of bone was left at the proximal end to allow the muscle to be anchored into the apparatus used to assess contractile function. Similarly for the diaphragm, an aluminium foil T-clip wrapped around the central tendon and two ribs at the opposing end were left intact.

Experimental set-up

Contractile function was assessed using custom-built apparatus. Each muscle was placed into a Perspex chamber filled with continually circulating oxygenated (95% O_2; 5% CO_2) Krebs–Henseleit solution. A central reservoir of Krebs–Henseleit solution was kept in a heater/cooler (Grant LTD6G, Grant Instruments, Shepreth, UK), where the temperature of the solution could be manipulated and was circulated using two peristaltic pumps (101U/R, Watson & Marlow, Falmouth, UK). The muscle was anchored in the bath via crocodile clips that were attached at one end to a force transducer (UF1, Pioden Controls Ltd, Henwood, Ashford, UK), and at the other end to a motor arm (V201, Ling Dynamic Systems, Royston, UK). The motor arm was used to subject the muscle to controlled length change cycles during the assessment of WL power. The position of the motor arm was detected via a Linear Variable Differential Transformer (LVDT, DFG5.0, Solartron Metrology). The muscle was electrically

stimulated to produce force via parallel platinum electrodes submerged in the Krebs–Henseleit solution inside the Perspex chamber. The amplitude of the stimulation was controlled by an external power source (PL320, Thurlby Instruments, Huntingdon, UK). Visual representation of the force and length data was provided by a storage oscilloscope (2211, Tektronix, Marlow, UK). Length change and stimulation parameters were controlled by a custom-written program in TestPoint (TestPoint, CEC, Billerica, MA, USA), via a digital-to-analog board (KPCI3108, Keithley Instruments, Cleveland, OH, USA). Data were sampled at 10 kHz.

Maximal isometric force and work loop power were measured at four temperatures (20, 28, 35 and 40°C), in one of the following run orders. A: 35°C→40°C→35°C→28°C→20°C→35°C, or B: 28°C→20°C→28°C→35°C→40°C→28°C. These run orders were chosen to maintain tissue viability and two distinct sets were selected to mitigate an order effect on the measured outcome variables. For each run order, a control temperature (A=35°C; B=28°C) was selected and performance monitored over time via repeats of measurements at these temperatures to control for the degradation of muscle performance over time. This is standard practice for experiments examining temperature effects on isolated skeletal muscle function (James et al., 2015, 2012). Direct operating temperatures of specific skeletal muscle have not been investigated in rodents. The range of temperatures was selected as it has previously been used to assess the impact of temperature and thermal sensitivity of isolated mammalian skeletal muscles (James et al., 2015; Rummel et al., 2021). Furthermore, it reflects the range of temperatures that has previously been used to assess the effect of HFD on isolated skeletal muscle function (Bott et al., 2017; Ciapaite et al., 2015; Hurst et al., 2018; Tallis et al., 2017b) and more generally in research assessing the contractile function of isolated skeletal muscle (Rossi et al., 2001; Head et al., 2011; Hill et al., 2020; Askew et al., 1997). In each case, the temperature inside the Perspex chamber was monitored using a digital thermometer (Checktemp C, Harvard 216 Apparatus, Cambridge, UK) and maintained with ±0.2°C of the target temperature. Prior to the assessment of contractile function, each muscle was allowed to acclimate to any new test temperature for 10 min. This period was deemed adequate as there was no systematic change between the initial and subsequent assessment of WL assessments.

Isometric measurements

Initially, each muscle was subjected to a series of isometric twitch activations where muscle length and then stimulation amplitude (12–16 V) were optimised to evoke a maximal isometric twitch response. Stimulation current (160 mA) and pulse width (1.2 ms) were fixed. Muscle length was then measured using an eyepiece graticule fitted to a microscope. L_0 was calculated as 85% of muscle length for the soleus (James et al., 1995). Given that no such estimates of mean fibre length exist for the diaphragm, the physical length was used (Hill et al., 2020; Tallis et al., 2017a, 2014b). Using a fixed burst duration (350 ms for soleus; 250 ms diaphragm), stimulation frequency (120–140 Hz for both muscles) was manipulated to evoke maximal tetanic force. Time to half-peak tetanus (THPT) and time from last stimulus to half tetanus relaxation (LSHR) were measured from the tetanus that elicited the highest force. Each tetanus activation was separated from the next by 5 min to allow sufficient recovery.

Assessment of WL power output

Using the previously determined, unique set of muscle length and stimulation parameters gained from twitch and tetanus assessments, power output was determined using the WL technique. The WL technique assesses the ability of the muscle to produce power whilst undergoing cyclical length changes and can provide a better representation of real-world muscle function compared with assessments of isometric force and other methods of determining the power output of isolated skeletal muscle (Josephson, 1985, 1993; James et al., 1995, 1996). Starting at L_0, each muscle was subject to four sinusoidal length change cycles per set at an initial total symmetrical strain of 0.10 (i.e. 10% of L_0). Length change cycle frequency and stimulus burst duration were manipulated to achieve maximal WL power. Muscle force was plotted against muscle length for each cycle to generate a WL, the area of which equated to the net work produced by the muscle during the cycle of length change (Josephson, 1985). Cycle frequency affects the speed of the length change cycle, and WL power was assessed at cycle frequencies ranging between 2 and 6 Hz for the soleus and 3 and 8 Hz for the diaphragm, the range in which WL power has been shown to be maximal for each muscle (Hurst et al., 2018; James et al., 1995; Hill et al., 2020). Phase shift, the time that stimulation begins relative to peak muscle length, was −10 and −5 ms for the soleus and diaphragm, respectively (Tallis et al., 2012; Hill et al., 2020). The burst duration (initially 65 ms for soleus and 55 ms for diaphragm at 5 Hz and 7 Hz cycle frequency, respectively; Tallis et al., 2012; Hill et al., 2020) and strain (range 0.08–0.014) were manipulated at each cycle frequency until maximum work was achieved. The burst duration denotes the period of electrical stimulation applied to the muscle. Given that net work during a length change cycle is the product of work done during shortening (positive work) minus the work done during lengthening (negative work) (Josephson, 1985), the optimal burst duration maximises work through the whole WL cycle. The aim is to promote high work production during shortening (positive work), whilst avoiding excessive force production during lengthening (negative work), which can occur if the burst duration is too long.

Considering that the performance of isolated skeletal muscle will slowly deteriorate over time as a result of the development of an anoxic core (Barclay, 2005), as with similar experimental approaches (James et al., 2015, 2011, 2012), a series of ‘control’ assessments of isometric force and WL power were made. After WL power was optimised at a given temperature, contractile function was reassessed at the temperature used for the initial assessment. This allowed for monitoring of the muscle performance over time and isometric force and WL power to be corrected to account for the deterioration in performance, with the assumption that the changes were linear.

Muscle mass measurement and dimension calculation

Upon completion of the experiment, the muscle was disconnected from the apparatus, the foil T-clip removed from the bones and tendon, and the muscle blotted on absorbent paper to remove excess Krebs–Henseleit solution. Wet muscle mass was determined to the nearest 0.0001 g using an electronic balance (Mettler-Toledo B204-S, Greifensee, Switzerland). Mean muscle cross-sectional area was calculated from L_0, muscle mass and an assumed muscle density of 1060 kg m^{-3} (Mendez and Keys, 1960). Isometric stress was calculated as force divided by mean muscle cross-sectional area. Muscle power output was normalised to muscle mass and expressed as power output in watts per kilogram muscle mass.

Statistical analysis

Following appropriate checks of normality and homogeneity of variance, parametric statistical analysis was performed. Independent

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Table 1. Animal and skeletal muscle morphology

	Body mass (g)	Fat pad mass (g)	Circumference (cm)	Body length (cm)	Soleus mass (mg)	Soleus fibre length (mm)
Control	31.52±1.03	0.73±0.19	8.44±0.16	10.01±0.19	8.95±0.39	9.09±0.19
HFD	51.56±1.82	5.46±0.42	10.46±0.15	11.1±0.13	10.96±0.42	9.39±0.15
P	<0.001	<0.001	<0.001	<0.001	0.004	0.281
g	4.27	4.57	4.19	2.71	1.59	0.52

Data are means±s.e.m. for mice from the control (n=8) and high-fat diet (HFD; n=9) groups, with *P*-values and Hedges' *g*.

samples *t*-tests were used to assess differences in whole-body and muscle morphology between the control and HFD groups. Mixed-model ANOVA with treatment (control and HFD) as the between-subjects factor, and temperature (20, 28, 35 and 40°C) as the within-subject factor, were used to assess data obtained from the isometric and WL assessments. Violations of sphericity were corrected using Greenhouse–Geisser where applicable. Significant main effects for temperature and interactions were explored with Bonferroni-corrected pairwise comparisons. For ANOVA, partial eta squared (η_p^2) was calculated as an estimate of effect size and was interpreted as small (>0.01), medium (>0.06) or large (>0.14) (Richardson, 2011). On a small number of occasions, for data analysed using ANOVA, normality was violated. However, ANOVA is still considered a robust statistical method in such cases (Jaijee et al., 2018; Blanca et al., 2017). For *t*-tests and pairwise comparisons involving measures of treatment, Cohen's *d* was calculated and corrected for bias using Hedges' *g* (Lakens, 2013). Hedges' *g* effect size was interpreted as trivial (<0.2), small (<0.6), moderate (<1.2) or large (>1.2) (Hopkins et al., 2009). To assess for differences in thermal sensitivity, isometric force, THPT, LSHR and absolute WL power at each temperature were calculated as a percentage of the performance at 20°C. This approach was taken to control for potential effects of HFD on contractile function and to compare between muscles. Data were logarithmically ($\log_{10}$) transformed to increase linearity and compared using least-squares linear regression. Data are presented as means±s.e.m. ANOVA and *t*-tests were performed using SPSS 26.0 (Chicago, IL, USA), whilst regression analysis, statistical comparison of slopes determined via regression analysis and graphical presentation of data were performed using GraphPad Prism (Version 8.3.1, San Diego, CA, USA). Statistical significance was *a priori* set at an alpha level of P<0.05.

RESULTS

Animal and skeletal muscle morphology

Body mass, fat pad mass, body circumference and body length were all significantly greater in the HFD group than in controls (Table 1; P<0.001; g>2.70 in all cases). Soleus muscle mass was significantly higher in the HFD group (Table 1; P=0.004; g=1.59), but the estimated fibre length found to evoke a maximal isometric twitch response was unchanged (Table 1; P=0.281; g=0.52).

Isometric tetanus

For peak absolute isometric force of the soleus, there was a significant treatment×temperature interaction (Fig. 1A; P=0.01; η_p^2=0.320). Pairwise comparisons indicated that irrespective of group, an increase in temperature resulted in greater force (Fig. 1A; P<0.001 in all cases) other than between 35 and 40°C (Fig. 1A;

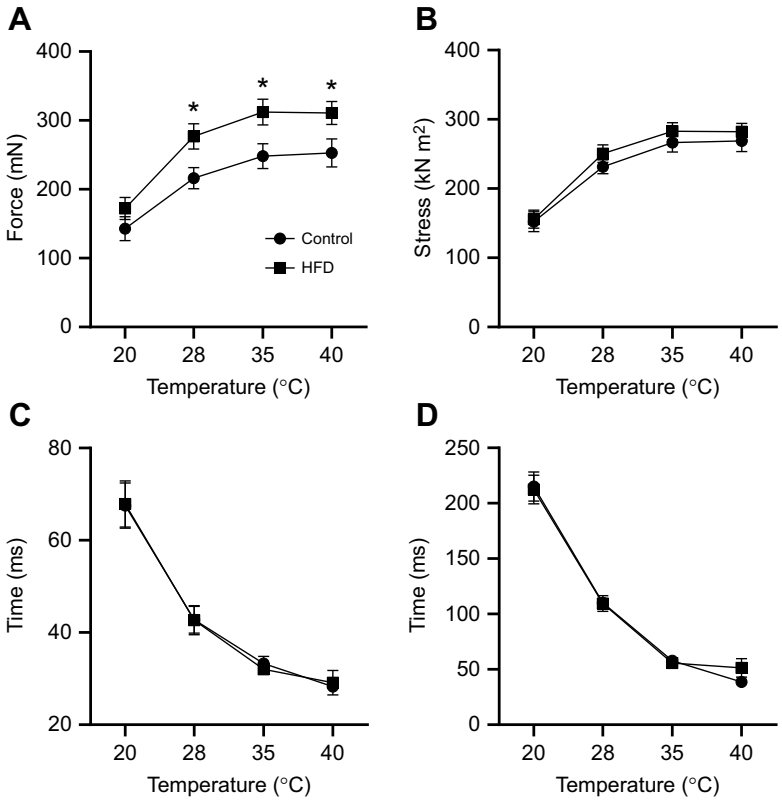

Fig. 1. The effects of temperature and high-fat diet (HFD) on isolated mouse soleus tetanus. (A) Peak absolute isometric tetanus force. (B) Peak isometric tetanus stress. (C) Time to half-peak tetanus force (THPT). (D) Time from last stimulus to half tetanus force relaxation (LSHR). Data are means±s.e.m.; n=8 for control, n=9 for HFD. *Significant difference (P<0.05) between control and HFD groups evaluated using mixed model ANOVA.

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P=1.00). At 20°C, peak absolute isometric force of the soleus did not differ between treatments (Fig. 1A; P=0.237; g=0.57). However, at all other temperatures, the peak force of the HFD group was significantly higher than that of controls (Fig. 1A; P<0.033; g>1.08 in all cases).

Maximal isometric stress of the soleus did not differ between treatment groups (Fig. 1B; P=0.436; η_p^2=0.036) but was significantly affected by temperature (Fig. 1B; P<0.001; η_p^2=0.967). An increase in temperature resulted in greater peak stress (Fig. 1B; P<0.001 in all cases) other than between 35 and 40°C (Fig. 1B; P=1.00). There was no significant treatment×temperature interaction (Fig. 1B; P=0.222; η_p^2=0.093), indicating that the effect of temperature on stress was not affected by treatment.

For THPT and LSHR measured for the soleus, there was no significant treatment×temperature interaction (Fig. 1C,D; P>0.604; η_p^2<0.042 in each case), or main effect of treatment (Fig. 1C,D; P>0.891; η_p^2<0.002 in each case). However, both THPT and LSHR were affected by temperature (Fig. 1C,D; P<0.001; η_p^2>0.839). Similarly, an increase in temperature reduced THPT at a level that reached significance between 20 and 35°C (Fig. 1C; P<0.001 in each case) and was approaching significance between 35 and 40°C (Fig. 1C; P=0.057). An increase in temperature resulted in a reduced LSHR (Fig. 1D; P<0.001 in all cases) other than between 35 and 40°C (Fig. 1D; P=0.124).

For peak isometric tetanus stress of the diaphragm, there was a significant treatment×temperature interaction (Fig. 2A; P=0.038; η_p^2=0.162). Tetanus stress of the HFD group was lower than that of the control group at 20°C (Fig. 2A; P=0.083; g=0.82), 28°C (Fig. 2A; P=0.100; g=0.77), 35°C (Fig. 2A; P=0.045; g=0.96) and 40°C (Fig. 2A; P=0.051; g=0.93); in each case, the statistical data indicated this was at a level that was statistically different or approaching significance, but in all cases with a moderate effect size. Irrespective of group, each increase in temperature between 20 and 35°C resulted in a significant increase in peak isometric tetanus stress (Fig. 2A; P<0.001 in all cases), which then decreased between 35 and 40°C (Fig. 2A; P<0.006).

For THPT and LSHR measured for the diaphragm, there was no significant treatment×temperature interaction (Fig. 2B,C; P<0.853; η_p^2<0.020 in each case) or main effect of treatment (Fig. 2B,C; P<0.758; η_p^2<0.025 in each case). However, both THPT and LSHR were affected by temperature (Fig. 2B,C; P<0.001; η_p^2>0.842 in each case), with each temperature increase resulting in reduced THPT and LSHR (Fig. 2B,C; P<0.024 in each case).

WL power

For absolute net WL power output of the soleus, there was a significant treatment×temperature interaction (Fig. 3A; P=0.014; η_p^2=0.209). Pairwise comparisons indicated that irrespective of group, an increase in temperature resulted in an increased absolute WL power output (Fig. 3A; P<0.001 in all cases). Furthermore, absolute power output of the soleus at 35°C was greater in the HFD group than in the control (Fig. 3A; P=0.041; g=1.03), with this trend still present 40°C (Fig. 3A; P=0.081; g=0.86).

For net WL power output relative to muscle mass, net WL power output relative to body mass and the cycle frequency used to elicit peak WL power in the soleus, there was no significant treatment×temperature interaction (Fig. 3B–D; P>0.180; η_p^2<0.103 in all cases), nor a significant effect of treatment (Fig. 3B–D; P>0.318; η_p^2<0.067 in all cases). In all cases, there was, however, a significant effect of temperature (Fig. 3B–D; P<0.001; η_p^2>0.739 in all cases). Pairwise comparisons indicated that an increase in temperature resulted in an increased WL power relative to muscle mass and WL power relative to body mass (Fig. 3B,C; P<0.001). Temperature effects were not as uniform across the cycle frequency

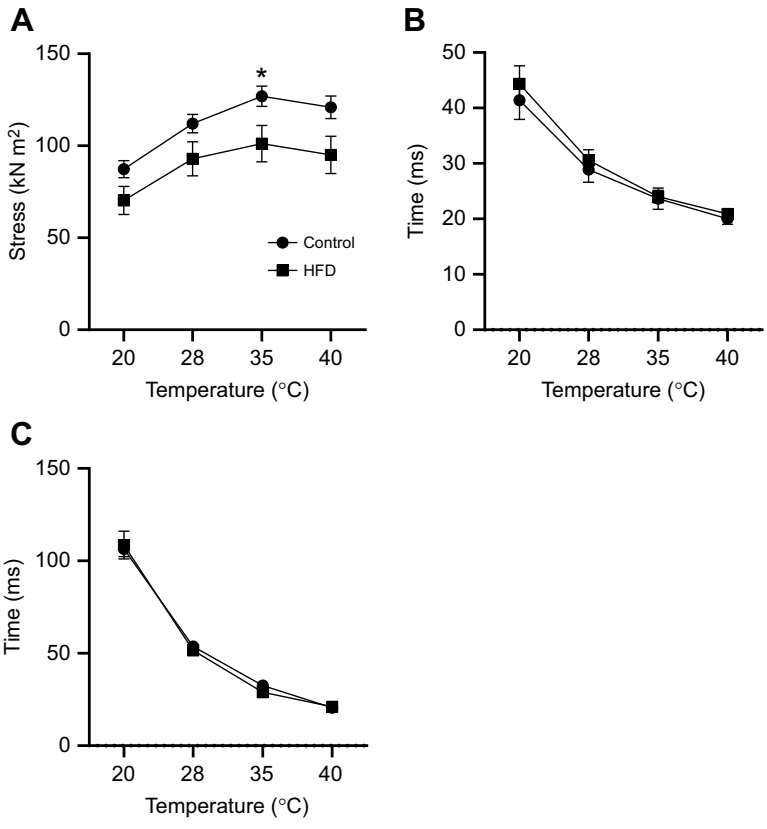

Fig. 2. The effects of temperature and HFD on isolated mouse diaphragm tetanus. (A) Peak isometric tetanus stress. (B) THPT. (C) LSHR. Data are means±s.e.m.; n=8 for control, n=9 for HFD. *Significant difference (P<0.05) between control and HFD groups evaluated using mixed model ANOVA.

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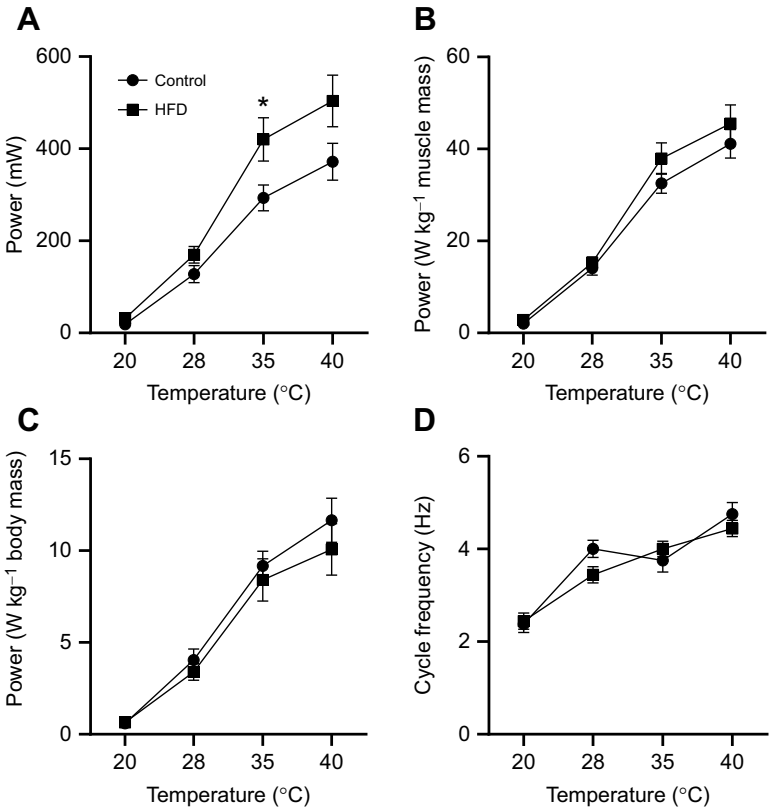

Fig. 3. The effects of temperature and HFD on isolated mouse soleus work loop power. (A) Net absolute work loop power output. (B) Net work loop power output normalised to muscle mass. (C) Net work loop power output normalised to body mass. (D) Cycle frequency to elicit maximal power. Data are means±s.e.m.; n=8 for control, n=9 for HFD. *Significant difference (P<0.05) between control and HFD groups evaluated using mixed model ANOVA.

data, where an increase in temperature between 20 and 28°C increased the cycle frequency used to elicit peak power (Fig. 3D; P<0.001), as did the temperature increase to 40°C (Fig. 3D; P<0.049 in all cases).

For net WL power output relative to muscle mass and the cycle frequency used to elicit peak WL power output in the diaphragm, there was no significant treatment×temperature interaction (Fig. 4A,C; P>0.525; η_p^2<0.049 in all cases), nor a significant effect of treatment (Fig. 4A,C; P>0.481; η_p^2<0.035 in all cases). In both cases, there was, however, a significant effect of temperature (Fig. 4A,C; P<0.001; η_p^2>0.115 in both cases). For both measures, an increase in temperature between 20 and 35°C increased WL power relative to muscle mass and the cycle frequency used to elicit peak WL power (Fig. 4A,C; P<0.001 in both cases); however, there was no difference between 35 and 40°C (Fig. 4A,C; P>0.114 in both cases).

For diaphragm net WL power output normalised to body mass, there was a significant treatment×temperature interaction (Fig. 4B; P=0.004; η_p^2=0.250). At 20°C (Fig. 4B; P=0.056; g=0.95), 28°C (Fig. 4B; P=0.056; g=0.97), 35°C (Fig. 4B; P=0.030; g=1.10) and 40°C (Fig. 4B; P=0.041; g=1.03), net WL power output normalised to body mass was significantly lower in the HFD group, or approaching significance, with a moderate effect size in each case. Irrespective of treatment, an increase in temperature between 20 and 35°C increased net WL power output normalised to body mass (Fig. 4B; P<0.010 in all cases), but there was no difference between 35 and 40°C (Fig. 4B; P>0.342 in both cases).

Thermal sensitivity

Regression analysis demonstrated that the slope indicating the increase in isometric force and WL power with temperature was greater in the control soleus than in the control diaphragm (Table 2; P<0.001 in both cases). Slopes indicating a temperature-induced reduction in THPT and LSHR were not different between the control soleus and control diaphragm (Table 2; P>0.24 in both cases). The temperature-induced increase in isometric force and WL power, and reduction in THPT and LSHR were not significantly different between the control and HFD groups for either soleus or diaphragm muscle (Table 2; P>0.172 in all cases).

DISCUSSION

The present study examined whether 20 weeks of HFD consumption had a temperature-specific effect on the contractile performance and regional thermal sensitivity of isolated mouse soleus and diaphragm muscle. An increase in temperature to 35°C improved the contractile function of the soleus and diaphragm across all of the measured outcomes. A further increase in temperature to 40°C caused a reduction in the maximal isometric stress of the diaphragm, but maintenance of WL power. Conversely, for the soleus, an increase in temperature to 40°C had limited effects on maximal isometric stress but increased WL power. Collectively, these data infer that maximal contractile function is temperature, muscle and contractile mode specific. When compared with controls, the soleus of the HFD-fed mice had greater maximal isometric tetanus force and absolute WL power, whilst isometric stress of the diaphragm was reduced, indicating a HFD-induced reduction in diaphragm muscle quality. Whilst HFD consumption did not affect the thermal sensitivity of either the soleus or the diaphragm muscles, these data show for the first time that HFD-induced effects on contractile function are less apparent at lower temperatures, indicating that direct effects of HFD on skeletal muscle function are temperature specific.

Effect of temperature on contractile function

The results from the present study indicate that temperature significantly influenced the contractile performance of skeletal

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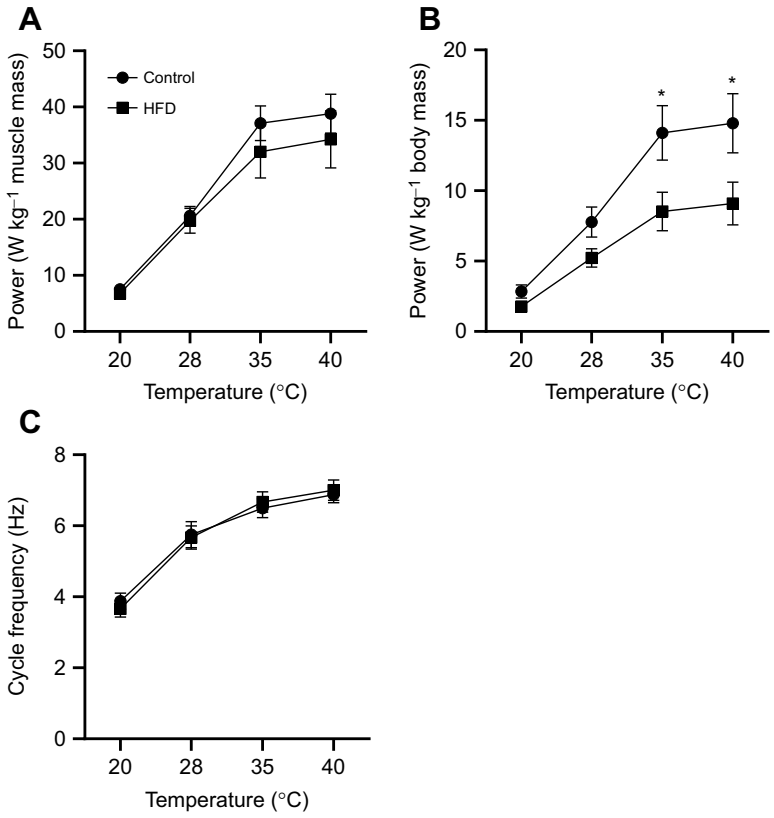

Fig. 4. The effects of temperature and HFD on isolated mouse diaphragm work loop power. (A) Net work loop power output normalised to muscle mass. (B) Net work loop power output normalised to body mass. (C) Cycle frequency to elicit maximal power. Data are means±s.e.m.; n=8 for control, n=9 for HFD. *Significant difference (P<0.05) between control and HFD groups evaluated using mixed model ANOVA.

muscle. In both the soleus and diaphragm, the maximal isometric force, activation and relaxation time, WL power output and cycle frequency needed to elicit peak power (an indication of shortening velocity) improved with increasing temperature, with the magnitude of the improvement decreasing with every increment in temperature. Such effects are consistent with previous studies that have assessed the effect of temperature on the contractile function of isolated mammalian, amphibian, reptilian and fish muscle (James et al., 2015, 2012; Olberding and Deban, 2017; Rall and Woledge, 1990; Frueh et al., 1994; Lännergren and Westerblad, 1987; Prezant et al., 1990; Ranatunga, 1998; Altringham and Block, 1997). Such temperature-induced improvements in contractile function are driven by optimising the activity of enzymes involved with energy metabolism and contractile function as well as a reduction in passive stiffness (Harrison and Bers, 1989; MacIntosh, 2003; Edwards et al., 1972; Brenner and Eisenberg, 1986; Stein et al., 1982; Seebacher et al., 2014).

Despite an abundance of literature examining the effect of temperature on isolated muscle function, few studies have directly compared muscle-specific responses and have measured both isometric function and power output. In the present study, an increase in temperature between 20 and 35°C improved both isometric function and WL power output for both the soleus and the diaphragm. Our previous work suggests that the thermal optima for maximal WL power output for the soleus may exceed 40°C (James et al., 2015), which is confirmed in the present study. However, the present data provide new insight into temperature effects between 35 and 40°C, the range where optimal contractile performance occurs. At 40°C, isometric force and activation and relaxation times of the soleus were not different to that achieved at 35°C; however, WL power output and the cycle frequency used to elicit maximal power continued to increase throughout the temperature range studied. In the diaphragm, an increase in temperature from 35 to 40°C resulted in reduced isometric force and decreased activation and relaxation

Table 2. Thermal sensitivity regression analysis

	Isometric force		THPT		LSHR		WL power output	
	Control	HFD	Control	HFD	Control	HFD	Control	HFD
Soleus								
Slope	0.013	0.013	−0.019	−0.019	−0.038	−0.034	0.067	0.064
R^2	0.704	0.660	0.665	0.740	0.907	0.870	0.896	0.860
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Slope diff. P	0.973		0.990		0.172		0.600	
Diaphragm								
Slope	0.008	0.007	−0.015	−0.016	−0.035	−0.36	0.037	0.035
R^2	0.634	0.512	0.736	0.799	0.971	0.892	0.869	0.880
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Slope diff. P	0.590		0.727		0.810		0.615	

THPT, time to half-peak tetanus; LSHR, time from last stimulus to half relaxation; WL, work loop.

times; however, maximal WL power output did not significantly change. These results indicate that maximal isometric force and power output have different thermal sensitivity, where the optimal range of temperatures to elicit maximal force is lower than that for maximal power. The muscle and contractile mode-specific trends demonstrated in the present study were not evident in our previous work, given the random approach to temperature selection in James et al. (2015) resulting in 2 and 7 observations of contractile function for the diaphragm and the soleus, respectively, between 35 and 40°C. Furthermore, the variation in performance in our previous work was greater at the higher temperatures which probably further contributes to disparity between the current study and previous findings (James et al., 2015).

Given that power is a product of force×shortening velocity (or work done×cycle frequency) (Josephson, 1985), the difference in the temperature needed to elicit peak power is likely to be driven by a temperature-induced reduction in passive stiffness (Seebacher et al., 2014) or the temperature-specific sensitivity of shortening velocity where, for example, in the soleus, the isometric force at 40°C was reduced but the cycle frequency to elicit maximal power and, as a consequence, maximal power output increased. It has been suggested that myosin ADP release and ATP-induced actin–myosin dissociation influence shortening velocity and are both sensitive to temperature (Ranatunga, 2018). The present findings support the idea that the temperature to maximise shortening velocity differs from the temperature that optimises physiological processes involved with force production (Ranatunga, 2018). Furthermore, differences between the soleus and diaphragm indicate a muscle-specific temperature range where enzymatic activity is optimised. Although not unanimous (Rossi et al., 2005), previous work indicates that myosin ATPase activity of slow and fast fibres becomes similar as temperature increases (Candau et al., 2003), indicating a difference in temperature sensitivity between fast and slow fibre types.

Temperature-specific effects of HFD on contractile function

A growing body of work has used rodent models to assess the effect of HFD consumption on the contractile performance of isolated skeletal muscle (Ciapaite et al., 2015; Tallis et al., 2017b; Hill et al., 2019; Hurst et al., 2018; Eshima et al., 2017). In many cases, such models evoke substantial changes in fat mass and, as such, provide insight into the direct effects of obesity on skeletal muscle performance. Our previous work has indicated a muscle and contractile mode-specific effect of HFD (Tallis et al., 2017b); however, direct comparison between previously published work is challenging given discrepancies in methodological approaches. Differences in the age, HFD-feeding duration, nutritional composition of the diet, mode of contractile function assessed and temperature at which the assessments are performed have been suggested to impact the outcome of these studies (Tallis et al., 2018). Despite some ambiguity in the evidence base, some trends are becoming evident, many of which the current data support. Our data indicate that both maximal isometric force and WL power of the soleus were significantly increased in the HFD group. An increase in the absolute force-producing capacity of postural muscles is something that has been reported previously in both isolated muscle studies (Tallis et al., 2017b) and those that assess human skeletal muscle function *in vivo* (Rolland et al., 2004; Miyatake et al., 2000; Abdelmoula et al., 2012; Maffiuletti et al., 2007). Such effects have been attributed to adaptations in the muscle caused by an increased demand on the postural muscles given the elevated body mass (Lafortuna et al., 2005; Hulens et al., 2001).

There is also growing evidence to support a HFD-induced reduction in muscle quality (muscle function normalised to muscle size) (Tallis et al., 2017b; Hurst et al., 2018; Hill et al., 2018; Eshima et al., 2020, 2017) in some muscles. Data in the present study support this concept, where the maximal isometric stress of the diaphragm was reduced in the HFD group, providing further indication that HFD consumption probably impacts the intrinsic force-producing capacity of some skeletal muscles. As per our previous work (Tallis et al., 2017b), there was no effect of HFD consumption on the muscle quality of the soleus, indicating that the HFD effects on muscle function are not uniform. Such responses can probably be attributed to muscle-specific mechanical function and fibre-type composition, where muscles with a greater quantity of slow-twitch fibres may be subject to less pronounced effects as a result of the greater oxidative capacity and smaller accumulation of lipid directly in the muscle (Tallis et al., 2017b; Ciapaite et al., 2015). In support of this, type I dominant muscle has been shown to be less susceptible to intra-myocellular lipid (IMCL) accumulation following HFD consumption compared with type II predominant fibred muscle (Hua et al., 2017). IMCL has been shown to cause insulin resistance, reduced muscle protein synthesis, mitochondrial dysfunction and a slower myofibre contraction (Masgrau et al., 2012; Coen and Goodpaster, 2012; Golla et al., 2017; Choi et al., 2016), which probably mechanistically accounts for differences in response between the diaphragm and soleus. Interestingly, an increase in the absolute isometric force and WL power of the soleus without prevalent changes in muscle quality, an adaptation that might be expected following resistance training (Hofmann et al., 2016; Ivey et al., 2000; Pinto et al., 2014), may indicate some degree of impairment in myogenesis in the HFD group.

The contractile force needed to elicit peak WL power at each temperature was not influenced by treatment, indicating that HFD may not have influenced the maximal shortening velocity of either the soleus or diaphragm. Whilst previous work has demonstrated this effect by assessing WL power over a range of contractile forces (Hurst et al., 2018; Hill et al., 2019), this work has reported the average WL power of each treatment group at specific contractile forces, which may fail to accurately reflect the muscle-specific contractile force needed to elicit peak power. By assessing and reporting muscle-specific peak power and the contractile force at which this occurred, the current approach overcomes this issue and provides a further indication that HFD consumption probably has little effect on the maximum muscle shortening velocity.

Whilst these data generally confirm previous findings regarding the effect of HFD on skeletal muscle function, our work makes a novel contribution to the evidence base by examining whether the effects of HFD on skeletal muscle function are temperature specific. The HFD-induced increase in the maximal isometric force of the soleus was specific to 28, 35 and 40°C, where at 20°C performance was comparable between the HFD and control groups. Whilst HFD had a moderate negative effect on the isometric stress of the diaphragm across the temperature range, this only reached critical alpha at 35°C. Similarly, the increased absolute WL power output of the soleus only reached alpha at 35°C; however, unlike the isometric stress of the diaphragm, the effect of the HFD was only prevalent at 35 and 40°C. Whilst these data indicate a need to supplement traditional hypothesis testing with further statistical analysis, such as measures of effect size for more accurate interpretation of data, they also demonstrate for the first time the temperature-specific impact of HFD on contractile function where the detrimental effects are exaggerated at higher temperatures. Previous studies that have used isolated muscle models to assess the effects of HFD on muscle

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function have done so using temperatures ranging between 20 and 37°C (Bott et al., 2017; Tallis et al., 2017b; Ciapaite et al., 2015). Although it has been proposed that temperature may be a source of ambiguity in published findings (Tallis et al., 2018), the present data are the first to provide direct evidence to support this claim. Whilst the effect of HFD on muscle function and the subsequent impact on physical function may be reduced with temperature, in endothermic mammals, typical muscle operating temperatures coincide with those where the greatest HFD impact has been demonstrated (MacIntosh, 2003). As such, future work examining the effect of HFD on isolated skeletal muscle function should make assessments of contractile function between 35 and 40°C, to improve the generalisability of the results to *in vivo* muscle function. Furthermore, future investigations utilising isolated skeletal muscle models of contractile function should consider moving away from fixed sub-optimal temperatures and select a muscle and contractile mode-specific temperature that elicits optimal performance.

Effect of HFD on regional thermal sensitivity

Our previous work documents the regional thermal sensitivity of rodent skeletal muscle, where contractile function of the core diaphragm was more thermally specialised than that of the more peripheral soleus (James et al., 2015). The present data advance this work by directly comparing the temperature effect on contractile performance between muscles using regression analysis, whereas in previous work such effects were determined based on significant muscle by temperature interactions. The approach by James et al. (2015) may therefore not be the most refined for determining regional thermal sensitivity. The data in the present study, however, confirm that the diaphragm is more thermally specialised than the soleus, given that for the soleus, the temperature-induced increase in isometric force and power was greater than for the diaphragm.

The present data make a novel contribution to the evidence base, demonstrating that the thermal sensitivity of both the diaphragm and soleus was not affected by HFD and the subsequent increase in stored adipose tissue. Obesity is associated with high body heat content, related to physiological mechanisms resulting in greater heat production and impaired heat loss (Savastano et al., 2009). Despite this, core body temperature is still tightly regulated and may only be subject to a small increase in obese individuals (Eriksson et al., 1985; Bastardot et al., 2019; Hoffmann et al., 2012). Data from the present study indicate that any potential modest changes in core temperature did not affect the thermal sensitivity of the diaphragm.

A greater subcutaneous adipose tissue resulting in impaired thermal conductivity and increased heat insulation, a higher vasoconstriction threshold, a reduced surface area to mass ratio and decreased skin blood flow during exercise as an artefact of obesity (Savastano et al., 2009; Kasai et al., 2003; Vroman et al., 1983; Verbraecken et al., 2006) provide the potential for a greater shift in temperature for muscle of the periphery. In support of this, evidence indicates an increase in the peripheral muscle temperature of overweight and obese individuals (Jalil et al., 2019; Savastano et al., 2009). Despite the potential for an upwards shift in the typical operating temperature of peripheral muscle, our data indicate that the thermal sensitivity of the soleus was not affected by HFD consumption.

Limitations and future direction

Future work should focus on assessing the impact of HFD on skeletal muscle function at temperatures that reflect the typical operating temperatures of muscle to more accurately understand the *in vivo* consequences of the findings. This concept is something that should be applied across other areas of research where assessments of isolated muscle function are used in the experimental model.

Although the range of temperatures used in the present study reflects those used in previous work that have examined, albeit separately, the influence of temperature and HFD on isolated skeletal muscle function (Rummel et al., 2021; James et al., 2015), the physiological relevance, in particular, of the lower temperatures, may be questioned. Whilst in the extremes, peripheral muscle may undergo fluctuations in temperature of as much as 15°C (Ducharme et al., 1991; Ranatunga et al., 1987) and recent work in *Carollia perspicillata* indicates that peripheral bat wing muscle may operate up to 12°C lower than core body temperature (Rummel et al., 2019), this is less likely for core muscle such as the diaphragm. At present, the typical operating temperature of specific skeletal muscle has not been thoroughly investigated, which can probably be attributed to challenges in obtaining this information. However, the temperatures used in the present study allowed comparison with previous work and this was important for contextualising HFD effects where such temperatures have been previously used (Bott et al., 2017; Tallis et al., 2017b; Ciapaite et al., 2015). Moreover, studies that assess temperature over a broad range are important in identifying performance optima, which probably coincides with typical operating temperatures. Data from the present study advocate the use of temperatures that enable optimal contractile performance in future work.

Furthermore, animals were housed in groups of 8–10 without access to running wheels, whereas engagement in voluntary exercise or completion of a structured exercise regime may have provided greater thermal stress in the HFD-fed mice and as such a greater stimulus to evoke changes in regional thermal sensitivity.

It is well established that skeletal muscle function will acclimate following chronic exposure to unaccustomed temperatures (James and Tallis, 2019). Given the differences in thermoregulatory responses between obese and non-obese individuals, future work may focus on examining thermal acclimation to temperatures that are warmer and cooler than typical ambient temperatures. From an ecological perspective, successful seasonal acclimation is important for survival in many mammalian species (James and Tallis, 2019).

Conclusion

The present study provides further insight into the effects of temperature on skeletal muscle contractile performance. In line with previous work, an increase in temperature resulted in improved isometric force, reduced activation and relaxation times, and greater WL power. However, these findings demonstrate a muscle and contractile mode-specific response where the thermal optimal was higher for the soleus than for the diaphragm, and the optimal temperature for maximal isometric function was lower than that for maximal WL power. Our results also demonstrate for the first time that HFD consumption does not influence regional thermal sensitivity but does elicit temperature-specific effects on contractile function. Maximal isometric force and absolute WL power of the soleus were increased, whereas maximal isometric stress of the diaphragm was reduced in the HFD-fed mice when compared with controls, with such effects being more pronounced at higher temperatures. Beyond providing further important insight into the effect of temperature on muscle function, findings from the present study are important in the interpretation of previous work, particularly as some differences between studies that have examined the impact of HFD on skeletal muscle function now seem likely

given the differences between studies in test temperature. Furthermore, these data should be considered in the design of future work utilising models of isolated skeletal muscle function to justify selected assessment temperatures.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: J.T., R.J., J.H.; Methodology: J.T., R.J., J.H.; Formal analysis: J.T., J.H.; Investigation: J.T., E.E., J.H.; Data curation: J.T., J.H.; Writing - original draft: J.T., J.H.; Writing - review & editing: J.T., R.J., E.E., V.C., J.H.; Visualization: J.T.; Supervision: J.T., R.J., V.C.; Project administration: J.H.

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