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

Tallis, J., James, R., Eyre, E., Cox, V. & Hurst, J.

Original citation & hyperlink:
Tallis, J, James, R, Eyre, E, Cox, V & Hurst, J 2022, 'High-fat diet affects measures of skeletal muscle contractile performance in a temperature specific manner but does not influence regional thermal sensitivity', Journal of Experimental Biology, vol. 225, no. 9, jeb244178. https://doi.org/10.1242/jeb.244178

DOI 10.1242/jeb.244178
ISSN 0022-0949
ESSN 1477-9145

Publisher: The Company of Biologists

HIGH-FAT DIET AFFECTS MEASURES OF SKELETAL MUSCLE CONTRACTILE PERFORMANCE IN A TEMPERATURE SPECIFIC MANNER BUT DOES NOT INFLUENCE REGIONAL THERMAL SENSITIVITY

Jason Tallis[1]*, Rob S. James[1], Emma, L. J. Eyre[1], Val M. Cox[1], Josh Hurst[1]

Research Centre for Sport, Exercise and Life Sciences, Coventry University, Coventry, UK

*Corresponding author: ab0289@coventry.ac.uk

RUNNING TITLE: TEMPERATURE AND HFD AFFECT MUSCLE FUNCTION

ABSTRACT

The present study examined if 20-weeks high-fat diet (HFD) consumption had a temperature specific effect on the contractile performance and regional thermal sensitivity of isolated mouse soleus (SOL) and diaphragm (DIA) 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 SOL and core DIA were isolated from each animal and maximal isometric force and work loop power were assessed at 20˚C, 28˚C, 35˚C 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 SOL. Conversely, isometric force of the DIA 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 SOL and reduced isometric stress of the DIA, 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 SOL or DIA. 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.

KEYWORDS: Obesity, Work Loop, Isometric, Force, Power

SUMMARY STATEMENT: The present work examines the interaction between high-fat diet consumption and acute changes in temperature on the contractile function of isolated mouse skeletal muscle.

INTRODUCTION

Obesity is a global epidemic (WHO, 2000), associated with poor health, reduced quality of life and increased mortality (Abdelaal et al., 2017, Vasquez 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, Tallis et al., 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^{o}C (Ducharme et al., 1991, Ranatunga et al., 1987). Furthermore, exercise can increase muscle temperature by 2-5^{o}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 likely impacts 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, James et al., 2012, Olberding and Deban, 2017, Rall and Woledge, 1990, Frueh et al., 1994, Lannergren 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, James et al., 2012, Lannergren 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 if 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 weight 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 has resulted in inconsistent findings (Tallis et al., 2018). More specifically, HFD feeding duration, the nutritional composition of the diet, contractile modality assessed, and temperature at which the experiments were performed likely influence the result. For example, previous work has examined HFD effects on isolated muscle function at temperatures ranging between 20 and 37ºC (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 likely influences the outcomes of such studies. Assessing the interaction between temperature and HFD on muscle function will allow improved interpretation and comparison between previous work 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 due to 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 likely 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 likely 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 to the same muscle in leaner individuals (James et al., 2015, Donley et al., 2012).

As such, the present work examined if the effects of 20-weeks HFD consumption on the contractile function of isolated skeletal muscle are temperature specific and determined if 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.

METHOD

Animal & Muscle Preparation

Following ethics approval from the Coventry University Ethics Committee, four-week-old CD1 female mice (n=17; Charles River, UK) were randomly assigned (using Microsoft Excel, Windows v. 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-hour light: 12-hour 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, 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 sacrificed 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, Fisher Scientific, 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 weighted 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, Hill et al., 2019, Tallis et al., 2017b, James et al., 2015, Tallis et al., 2014a, Vanhooydonck et al., 2014, James et al., 1995). Whole soleus (SOL) 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 ([mM] NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; $CaCl_2$ 2.54; pH 7.55 at room temperature prior to oxygenation). SOL and DIA represent a peripheral and core muscle respectively and were chosen to allow comparison to 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 SOL, 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 DIA, 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, 101U/R, Falmouth, Cornwall). 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 work loop power. The position of the motor arm was detected via a Linear Variable Differential Transformer (LVDT, DFG5.0, Solartron Metrology, UK). 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 programme in Tespoint (Testpoint, CEC, Massachusetts, USA), via a digital-to-analog board (KPCI3108, Keithley Instruments, Cleveland, OH). Data were sampled at 10kHz.

Maximal isometric force and work loop power were measured at four temperatures (20˚C, 28˚C, 35˚C 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, James et al., 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 temperature 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 utilising assessments of 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 degrees of the target temperature. Prior to the assessment of contractile function, each muscle was allowed to acclimate to any new test temperature for 10 minutes. 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 SOL (James et al., 1995). Given that no such estimates of mean fibre length exist for DIA, the physical length was used (Hill et al., 2020, Tallis et al., 2017a, Tallis et al., 2014b). Using a fixed burst duration (350 ms for SOL; 250 ms DIA), 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 minutes to allow sufficient recovery.

Assessment of Work Loop Power Output

Using the previously determined, unique set of muscle length and stimulation parameters gained from twitch and tetanus assessments, power output (PO) was determined using the work loop (WL) technique. The work loop 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 to assessments of isometric force and other methods of determining the power output of isolated skeletal muscle (Josephson, 1985, Josephson, 1993, James et al., 1995, James et al., 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 work-loop, 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 2-6 Hz for SOL and 3-8 Hz for the DIA, 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 ms and -5 ms for SOL and DIA respectively (Tallis et al., 2012, Hill et al., 2020). The burst duration (initially 65 ms for SOL and 55 ms for DIA at 5 Hz and 7 Hz cycle frequency respectively; (Tallis et al., 2012, Hill et al., 2020)) and strain (range between 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 due to the development of an anoxic core (Barclay, 2005), as with similar experimental approaches (James et al., 2015, James et al., 2011, James et al., 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, bones tendon and foil T-clip removed and the muscle blotted on absorbent paper to remove excess Kreb's 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 1,060 kg/m^3 (Mendez and Keys, 1960). Isometric stress was calculated as force ÷ 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 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 & HFD) as the between-subjects factor, and temperature (20, 28, 35 & 40^{o}C) as the within-subject factor, were used to assess data obtained from the isometric and work loop 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 (ηp^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 Hedge's *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 work loop power at each temperature were calculated as a percentage of the performance at 20^{o}C. This approach was taken to control for potential effects of HFD on contractile function and to compare between muscles. Data were logarithmically (log10) transformed to increase linearity and compared using least square linear regression. Data are presented as mean ± 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 was performed using GraphPad Prism (Version 8.3.1, San Diego, California). Statistical significance was *a priori* set at an alpha level of P<0.05.

RESULTS

Animal & Skeletal muscle morphology

Body mass, FPM, body circumference and body length were all significantly greater in the HFD group compared to controls (Table 1. P<0.001; *g*>2.70 in all cases). SOL 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).

Table 1 here

Isometric Tetanus

For peak absolute isometric force of the SOL, there was a significant Treatment*Temperature interaction (Fig 1A. P=0.01; ηp^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 35ºC and 40ºC (Fig 1A. P=1.00). At 20ºC, peak absolute isometric force of the SOL 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 compared to controls (Fig 1A. P<0.033; *g*>1.08 in all cases).

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

For THPT and LSHR measured for the SOL there was no significant Treatment*Temperature interaction (Fig 1C & D. P>0.604; ηp^2<0.042 in each case), or main effect of treatment (Fig 1C & D. P>0.891; ηp^2<0.002 in each case). However, both THPT and LSHR were affected by temperature (Fig 1C & D. P<0.001; ηp^2>0.839). Similarly, an increase in temperature reduced THPT at a level that reached significance between 20ºC and 35ºC (Fig 1C. P<0.001 in each case) and was approaching significance between 35ºC and 40ºC (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 35ºC and 40ºC (Fig 1D. P=0.124).

Figure 1 here

For peak isometric tetanus stress of the DIA, there was a significant Treatment*Temperature interaction (Fig 2A. P=0.038; ηp^2=0.162). Tetanus stress of the HFD group was lower than the control group at 20ºC (Fig 2A. P=0.083; *g*=0.82), 28ºC (Fig 2A. P=0.100; *g*=0.77), 35ºC (Fig 2A. P=0.045; *g*=0.96) and 40ºC (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 20ºC to 35ºC resulted in a significant increase in peak isometric tetanus stress (Fig 2A. P<0.001 in all cases), which then decreased between 35 and 40ºC (Fig 2A. P<0.006).

For THPT and LSHR measured for the DIA, there was no significant Treatment*Temperature interaction (Fig 2B & C. P<0.853; ηp^2<0.020 in each case) or main effect of treatment (Fig 2B & C. P<0.758; ηp^2<0.025 in each case). However, both THPT and LSHR were affected by temperature (Fig 2B & C. P<0.001; ηp^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).

Figure 2 here

Work Loop Power

For absolute net WL power output of the SOL, there was a significant Treatment*Temperature interaction (Fig 3A. P=0.014; ηp^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 SOL at 35ºC was greater in the HFD group compared to control (Fig 3A. P=0.041; g=1.03), with this trend still present 40ºC (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 SOL, there was no significant Treatment*Temperature interaction (Fig 3B, C & D. P>0.180; ηp^2<0.103 in all cases), nor a significant effect of treatment (Fig 3B, C & D. P>0.318; ηp^2<0.067 in all cases). In all cases, there was however a significant effect of temperature (Fig 3B, C & D. P<0.001; ηp^2>0.739 in all cases). Pairwise comparisons indicated 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 data, where an increase in temperature between 20ºC and 28ºC increased the cycle frequency used to elicit peak power (Fig 3D. P<0.001), as did the temperature increase to 40ºC (Fig 3D. P<0.049 in all cases).

Figure 3 here

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

For DIA net WL power output normalised to body mass, there was a significant Treatment*Temperature interaction (Fig 4B. P=0.004; ηp^2=0.250). At 20ºC (Fig 4B. P=0.056; g=0.95), 28ºC (Fig 4B. P=0.056; g=0.97), 35ºC (Fig 4B. P=0.030; g=1.10) and 40ºC (Fig 4B. P=0.041; g=1.03) net WL PO output normalised to body mass was significantly lower in HFD, or approaching significance, with a moderate effect size in each case. Irrespective of treatment, an increase in temperature between 20ºC and 35ºC increased net WL power output

normalised to body mass (Fig 4B. P<0.010 in all cases), but there was no difference between 35^{o}C and 40^{o}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 SOL compared to the control DIA (Table 2. P<0.004 in both cases). Slopes indicating a temperature-induced reduction in THPT and LSHR were not different between the control SOL and control DIA (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 SOL or DIA muscle (Table 2. P>0.172 in all cases).

Table 2 here

DISCUSSION

The present study examined if 20-weeks HFD consumption had a temperature specific effect on the contractile performance and regional thermal sensitivity of isolated mouse SOL and DIA muscle. An increase in temperature to 35˚C improved the contractile function of the SOL and DIA across all of the measured outcomes. A further increase in temperature to 40˚C caused a reduction in the maximal isometric stress of the DIA, but maintenance in work loop power. Conversely, for the SOL an increase in temperature to 40˚C had limited effects on maximal isometric stress but increased work loop power. Collectively, these data infer that maximal contractile function is temperature, muscle and contractile mode-specific. When compared to controls, SOL of the HFD-fed mice had greater maximal isometric tetanus force and absolute WL power, whilst isometric stress of the DIA was reduced, indicating a HFD induced reduction in DIA muscle quality. Whilst HFD consumption did not affect the thermal sensitivity of either the SOL or the DIA 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 is temperature specific.

Effect of Temperature on Contractile Function

Results from the present study indicate that temperature significantly influenced the contractile performance of skeletal muscle. In both the SOL and DIA, the maximal isometric force, activation and relaxation time, WL power output and CF needed to elicit peak power (an indication of shortening velocity) improved with increasing temperature, with the magnitude of 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, James et al., 2012, Olberding and Deban, 2017, Rall and Woledge, 1990, Frueh et al., 1994, Lannergren 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 have directly compared muscle-specific responses and have measured both isometric function and power output. In the present study, an increase in temperature between 20-35^{o}C improved both isometric function and WL power output for both the SOL and the DIA. Our previous work suggests that the thermal optima for maximal WL power output for the SOL may exceed 40^{o}C (James et al., 2015), which is confirmed in the present study. However, the present data provide new insight into temperature effects between 35-40^{o}C, the range where optimal contractile performance occurs. At 40^{o}C, isometric force and activation and relaxation times of the SOL were not different to that achieved at 35^{o}C, however, WL power output and the cycle frequency used to elicit maximal power continued to increase throughout the temperature range studied. In the DIA an increase in temperature from 35 to 40^{o}C resulted in reduced isometric force and decreased activation and relaxation 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 DIA and the SOL respectively between 35-40^{o}C. Furthermore, the variation in performance in our previous work was greater at the higher

484 temperatures which likely further contributes to disparity between the current study and
485 previous findings (James et al., 2015).

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

501 *Temperature Specific Effects of HFD on Contractile Function*

502 A growing body of work has used rodent models to assess the effect of HFD consumption on
503 the contractile performance of isolated skeletal muscle (Ciapaite et al., 2015, Tallis et al.,
504 2017b, Hill et al., 2019, Hurst et al., 2018, Eshima et al., 2017). In many cases, such models
505 evoke substantial changes in fat mass, and as such, provide insight into the direct effects of
506 obesity on skeletal muscle performance. Our previous work has indicated a muscle and
507 contractile mode-specific effect of HFD (Tallis et al., 2017b), however direct comparisons
508 between previously published work is challenging given discrepancies in methodological
509 approaches. Differences in the age, HFD-feeding duration, the nutritional composition of the
510 diet, mode of contractile function assessed and the temperature at which the assessments
511 are performed have been suggested to impact the outcome of these studies (Tallis et al.,
512 2018). Despite some ambiguity in the evidence base, some trends are becoming evident,
513 many of which the current data support. Our data indicate that both maximal isometric force
514 and WL power of the SOL were significantly increased in the HFD group. An increase in the
515 absolute force-producing capacity of postural muscles is something that has been reported
516 previously in both isolated muscle studies (Tallis et al., 2017b) and those that assess human
517 skeletal muscle function *in vivo* (Rolland et al., 2004, Miyatake et al., 2000, Abdelmoula et al.,
518 2012, Maffiuletti et al., 2007). Such effects have been attributed to adaptations in the muscle
519 caused by an increased demand on the postural muscles given the elevated body weight
520 (Lafortuna et al., 2005, Hulens et al., 2001).

521 There is also growing evidence to support a HFD induced reduction in muscle quality (muscle
522 function normalised to muscle size) (Tallis et al., 2017b, Hurst et al., 2018, Hill et al., 2018,
523 Eshima et al., 2020, Eshima et al., 2017) in some muscles. Data in the present study support
524 this concept, where the maximal isometric stress of the DIA was reduced in the HFD group,
525 providing further indication that HFD consumption likely impacts the intrinsic force-producing
526 capacity of some skeletal muscles. As per our previous work (Tallis et al., 2017b), there was
527 no effect of HFD consumption on the muscle quality of the SOL indicating that the HFD effects
528 on muscle function are not uniform. Such responses can likely be attributed to muscle-specific
529 mechanical function and fibre type composition, where muscles with a greater quantity of slow-
530 twitch fibres may be subject to less pronounced effects due to greater oxidative capacity and
531 a 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 to type II predominant fibered muscle (Hua et al., 2017). IMCL has been shown to cause insulin resistance, reduced muscle protein synthesis, mitochondrial dysfunction and a slower myofiber contraction (Masgrau et al., 2012, Coen and Goodpaster, 2012, Golla et al., 2017, Choi et al., 2016), which likely mechanistically accounts for differences in response between DIA and SOL. Interestingly, an increase in the absolute isometric force and WL power of the SOL 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 CF 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 SOL or DIA. Whilst previous work has demonstrated this effect by assessing WL power over a range of CFs (Hurst et al., 2018, Hill et al., 2019), previous work has reported the average WL power of each treatment group at specific CFs which may fail to accurately reflect the muscle-specific CF needed to elicit peak power. By assessing and reporting muscle-specific peak power and the CF at which this occurred, the current approach overcomes this issue and provides a further indication that HFD consumption likely 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, this work makes a novel contribution to the evidence base by examining if the effects of HFD on skeletal muscle function are temperature specific. The HFD induced increase in the maximal isometric force of the SOL was specific to 28^{o}C, 35^{o}C and 40^{o}C, where at 20^{o}C performance was comparable between the HFD and control groups. Whilst HFD had a moderate negative effect on the isometric stress of the DIA across the temperature range, this only reached critical alpha at 35^{o}C. Similarly, the increased absolute WL power output of the SOL only reached alpha at 35^{o}C, however, unlike the isometric stress of the DIA, the effect of HFD was only prevalent at 35^{o}C and 40^{o}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 function have done so using temperatures ranging between 20 and 37ºC (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 that 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 35-40ºC, 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 DIA was more thermally specialised than the more peripheral SOL (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 DIA is more thermally specialised than the SOL, given that for the SOL the temperature-induced increase in isometric force and power was greater than for the DIA.

The present data make a novel contribution to the evidence base, demonstrating that the thermal sensitivity of both the DIA and SOL were 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 DIA.

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) provides 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 SOL 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 seperately, the influence of temperature and HFD on isolated skeletal muscle function (Rummel et al., 2021, James et al., 2015), the physiological relevance, of in particular the lower temperatures, may be questioned. Whilst in the extremes, peripheral muscle may undergo fluctuations in temperature of as much as 15^{o}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^{o}C lower than core body temperature (Rummel et al., 2019), this is less likely for core muscle such as the DIA. At present, the typical operating temperatures of specific skeletal muscle has not been thoroughly investigated, which can likely be attributed to challenges in obtaining this information. However, the temperatures used in the present study allowed for comparison to previous work and 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 likely

coincides with typical operating temperatures. Data from the present study advocates 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 SOL compared to the DIA 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 SOL were increased, whereas maximal isometric stress of the DIA were reduced in the HFD-fed mice when compared to 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 due to 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.

667 **TABLES**

668

Table 1. Animal and Skeletal Muscle Morphology

	Body Mass (g)	FPM (g)	Circumference (cm)	Body Length (cm)	SOL M.Mass (mg)	SOL 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
Hedges *g*=	4.27	4.57	4.19	2.71	1.59	0.52

Control N=8; HFD N=9; FPM is fat pad mass; SOL is soleus; HFD is high fat diet [Data represented as Mean±S.E.M]

669

670

Table 2. Thermal Sensitivity Regression Analysis

	Isometric Force		THPT		LSHR		WL PO	
	Control	HFD	Control	HFD	Control	HFD	Control	HFD
				SOL				
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	
				DIA				
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	

SOL is soleus; DIA is diaphragm; HFD is high fat diet; THPT is time to half peak tetanus; LSHR is time from last stimulus to half relaxation; WL is work loop

671

672

673

674

675

676

677

678

679

680

681

682

683

684

685 **FIGURE TITLES**

686 Figure 1. The effects of temperature and HFD on isolated mouse SOL peak absolute isometric
687 tetanus force (A), peak isometric tetanus stress (B), Time to half peak tetanus force (C) and
688 time from Last stimulus to half tetanus force relaxation (D). [Data presented as mean±S.E.M;
689 n=8 for control; n=9 for obese; * indicates statistical difference (P<0.05) between control and
690 HFD groups evaluated using mixed model ANOVA]

691 Figure 2. The effects of temperature and HFD on isolated mouse DIA peak isometric tetanus
692 stress (A), Time to half peak tetanus force (B) and time from last stimulus to half tetanus force
693 relaxation (C). [Data presented as mean±S.E.M; n=8 for control; n=9 for obese; * indicates
694 statistical difference (P<0.05) between control and HFD groups evaluated using mixed model
695 ANOVA]

696 Figure 3. The effects of temperature and HFD on isolated mouse SOL net absolute work loop
697 power output (A), net work loop power output normalised to muscle mass (B), net work loop
698 power output normalised to body mass (C) and cycle frequency to elicit maximal power (D)
699 [Data presented as mean±S.E.M; n=8 for control; n=9 for obese; * indicates statistical
700 difference (P<0.05) between control and HFD groups evaluated using mixed model ANOVA]

701 Figure 4. The effects of temperature and HFD on isolated mouse DIA net work loop power
702 output normalised to muscle mass (A), net work loop power output normalised to body mass
703 (B) and cycle frequency to elicit maximal power (C) [Data presented as mean±S.E.M; n=8 for
704 control; n=9 for obese; * indicates statistical difference (P<0.05) between control and HFD
705 groups evaluated using mixed model ANOVA]

706

707

708

709

710

711

712

713

714

715

716

717

718

719

720

721

722

REFERENCES

ABDELAAL, M., LE ROUX, C. W. & DOCHERTY, N. G. 2017. Morbidity and mortality associated with obesity. *Annals of translational medicine,* 5.

ABDELMOULA, A., MARTIN, V., BOUCHANT, A., WALRAND, S., LAVET, C., TAILLARDAT, M., MAFFIULETTI, N. A., BOISSEAU, N., DUCHE, P. & RATEL, S. 2012. Knee extension strength in obese and nonobese male adolescents. *Appl Physiol Nutr Metab,* 37**,** 269-75.

ALTRINGHAM, J. D. & BLOCK, B. A. 1997. Why do tuna maintain elevated slow muscle temperatures? Power output of muscle isolated from endothermic and ectothermic fish. *J Exp Biol,* 200**,** 2617-27.

ASKEW, G. N., YOUNG, I. S. & ALTRINGHAM, J. D. 1997. Fatigue of mouse soleus muscle, using the work loop technique. *J Exp Biol,* 200**,** 2907-12.

AUNE, D., SEN, A., PRASAD, M., NORAT, T., JANSZKY, I., TONSTAD, S., ROMUNDSTAD, P. & VATTEN, L. J. 2016. BMI and all cause mortality: systematic review and non-linear dose-response meta-analysis of 230 cohort studies with 3.74 million deaths among 30.3 million participants. *BMJ,* 353**,** i2156.

BARCLAY, C. J. 2005. Modelling diffusive O(2) supply to isolated preparations of mammalian skeletal and cardiac muscle. *J Muscle Res Cell Motil,* 26**,** 225-35.

BASTARDOT, F., MARQUES-VIDAL, P. & VOLLENWEIDER, P. 2019. Association of body temperature with obesity. The CoLaus study. *Int J Obes (Lond),* 43**,** 1026-1033.

BLANCA, M. J., ALARCON, R., ARNAU, J., BONO, R. & BENDAYAN, R. 2017. Non-normal data: Is ANOVA still a valid option? *Psicothema,* 29**,** 552-557.

BOTT, K. N., GITTINGS, W., FAJARDO, V. A., BARANOWSKI, B. J., VANDENBOOM, R., LEBLANC, P. J., WARD, W. E. & PETERS, S. J. 2017. Musculoskeletal structure and function in response to the combined effect of an obesogenic diet and age in male C57BL/6J mice. *Mol Nutr Food Res*.

BRENNER, B. & EISENBERG, E. 1986. Rate of force generation in muscle: correlation with actomyosin ATPase activity in solution. *Proc Natl Acad Sci U S A,* 83**,** 3542-6.

CANDAU, R., IORGA, B., TRAVERS, F., BARMAN, T. & LIONNE, C. 2003. At physiological temperatures the ATPase rates of shortening soleus and psoas myofibrils are similar. *Biophys J,* 85**,** 3132-41.

CHOI, S. J., FILES, D. C., ZHANG, T., WANG, Z. M., MESSI, M. L., GREGORY, H., STONE, J., LYLES, M. F., DHAR, S., MARSH, A. P., NICKLAS, B. J. & DELBONO, O. 2016. Intramyocellular Lipid and Impaired Myofiber Contraction in Normal Weight and Obese Older Adults. *J Gerontol A Biol Sci Med Sci,* 71**,** 557-64.

CIAPAITE, J., VAN DEN BERG, S. A., HOUTEN, S. M., NICOLAY, K., VAN DIJK, K. W. & JENESON, J. A. 2015. Fiber-type-specific sensitivities and phenotypic adaptations to dietary fat overload differentially impact fast- versus slow-twitch muscle contractile function in C57BL/6J mice. *J Nutr Biochem,* 26**,** 155-64.

COEN, P. M. & GOODPASTER, B. H. 2012. Role of intramyocelluar lipids in human health. *Trends Endocrinol Metab,* 23**,** 391-8.

DONLEY, J. M., SEPULVEDA, C. A., AALBERS, S. A., MCGILLIVRAY, D. G., SYME, D. A. & BERNAL, D. 2012. Effects of temperature on power output and contraction kinetics in the locomotor muscle of the regionally endothermic common thresher shark (Alopias vulpinus). *Fish Physiol Biochem,* 38**,** 1507-19.

DUCHARME, M. B., VANHELDER, W. P. & RADOMSKI, M. W. 1991. Tissue temperature profile in the human forearm during thermal stress at thermal stability. *J Appl Physiol (1985),* 71**,** 1973-8.

EDWARDS, R. H., HARRIS, R. C., HULTMAN, E., KAIJSER, L., KOH, D. & NORDESJO, L. O. 1972. Effect of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps muscle in man. *J Physiol,* 220**,** 335-52.

ERIKSSON, H., SVARDSUDD, K., LARSSON, B., WELIN, L., OHLSON, L. O. & WILHELMSEN, L. 1985. Body temperature in general population samples. The study of men born in 1913 and 1923. *Acta Med Scand,* 217**,** 347-52.

ESHIMA, H., TAMURA, Y., KAKEHI, S., KAKIGI, R., HASHIMOTO, R., FUNAI, K., KAWAMORI, R. & WATADA, H. 2020. A chronic high-fat diet exacerbates contractile dysfunction with impaired intracellular Ca(2+) release capacity in the skeletal muscle of aged mice. *J Appl Physiol (1985),* 128**,** 1153-1162.

ESHIMA, H., TAMURA, Y., KAKEHI, S., KUREBAYASHI, N., MURAYAMA, T., NAKAMURA, K., KAKIGI, R., OKADA, T., SAKURAI, T., KAWAMORI, R. & WATADA, H. 2017. Long-term, but not short-term high-fat diet induces fiber composition changes and impaired contractile force in mouse fast-twitch skeletal muscle. *Physiol Rep,* 5.

FLEGAL, K. M., KIT, B. K., ORPANA, H. & GRAUBARD, B. I. 2013. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA,* 309**,** 71-82.

FRUEH, B. R., HAYES, A., LYNCH, G. S. & WILLIAMS, D. A. 1994. Contractile properties and temperature sensitivity of the extraocular muscles, the levator and superior rectus, of the rabbit. *J Physiol,* 475**,** 327-36.

GOLLA, S., REN, J., MALLOY, C. R. & PASCUAL, J. M. 2017. Intramyocellular lipid excess in the mitochondrial disorder MELAS: MRS determination at 7T. *Neurol Genet,* 3**,** e160.

HARRISON, S. M. & BERS, D. M. 1989. Influence of temperature on the calcium sensitivity of the myofilaments of skinned ventricular muscle from the rabbit. *J Gen Physiol,* 93**,** 411-28.

HEAD, S. I., GREENAWAY, B. & CHAN, S. 2011. Incubating isolated mouse EDL muscles with creatine improves force production and twitch kinetics in fatigue due to reduction in ionic strength. *PLoS One,* 6**,** e22742.

HILL, C., JAMES, R. S., COX, V. M., SEEBACHER, F. & TALLIS, J. 2020. Age-related changes in isolated mouse skeletal muscle function are dependent on sex, muscle, and contractility mode. *American Journal of Physiology-Regulatory, Integrative Comparative Physiology and Ecology,* 319**,** R296-R314.

HILL, C., JAMES, R. S., COX, V. M. & TALLIS, J. 2018. The Effect of Increasing Age on the Concentric and Eccentric Contractile Properties of Isolated Mouse Soleus and Extensor Digitorum Longus Muscles. *J Gerontol A Biol Sci Med Sci,* 73**,** 579-587.

HILL, C., JAMES, R. S., COX, V. M. & TALLIS, J. 2019. Does Dietary-Induced Obesity in Old Age Impair the Contractile Performance of Isolated Mouse Soleus, Extensor Digitorum Longus and Diaphragm Skeletal Muscles? *Nutrients,* 11.

HOFFMANN, M. E., RODRIGUEZ, S. M., ZEISS, D. M., WACHSBERG, K. N., KUSHNER, R. F., LANDSBERG, L. & LINSENMEIER, R. A. 2012. 24-h core temperature in obese and lean men and women. *Obesity (Silver Spring),* 20**,** 1585-90.

HOFMANN, M., SCHOBER-HALPER, B., OESEN, S., FRANZKE, B., TSCHAN, H., BACHL, N., STRASSER, E. M., QUITTAN, M., WAGNER, K. H. & WESSNER, B. 2016. Effects of elastic band resistance training and nutritional supplementation on muscle quality and circulating muscle growth and degradation factors of institutionalized elderly women: the Vienna Active Ageing Study (VAAS). *Eur J Appl Physiol,* 116**,** 885-97.

HOPKINS, W. G., MARSHALL, S. W., BATTERHAM, A. M. & HANIN, J. 2009. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc,* 41**,** 3-13.

HUA, N., TAKAHASHI, H., YEE, G. M., KITAJIMA, Y., KATAGIRI, S., KOJIMA, M., ANZAI, K., EGUCHI, Y. & HAMILTON, J. A. 2017. Influence of muscle fiber type composition on early fat accumulation under high-fat diet challenge. *PLoS One,* 12**,** e0182430.

HULENS, M., VANSANT, G., LYSENS, R., CLAESSENS, A. L., MULS, E. & BRUMAGNE, S. 2001. Study of differences in peripheral muscle strength of lean versus obese women: an allometric approach. *Int J Obes Relat Metab Disord,* 25**,** 676-81.

HURST, J., JAMES, R. S., COX, V. M., HILL, C. & TALLIS, J. 2018. Investigating a dose-response relationship between high-fat diet consumption and the contractile

performance of isolated mouse soleus, EDL and diaphragm muscles. *Eur J Appl Physiol*.

IVEY, F. M., TRACY, B. L., LEMMER, J. T., NESSAIVER, M., METTER, E. J., FOZARD, J. L. & HURLEY, B. F. 2000. Effects of strength training and detraining on muscle quality: age and gender comparisons. *J Gerontol A Biol Sci Med Sci,* 55**,** B152-7; discussion B158-9.

JAIJEE, S., QUINLAN, M., TOKARCZUK, P., CLEMENCE, M., HOWARD, L., GIBBS, J. S. R. & O'REGAN, D. P. 2018. Exercise cardiac MRI unmasks right ventricular dysfunction in acute hypoxia and chronic pulmonary arterial hypertension. *Am J Physiol Heart Circ Physiol,* 315**,** H950-H957.

JALIL, B., HARTWIG, V., MORONI, D., SALVETTI, O., BENASSI, A., JALIL, Z., PISTOIA, L., MINUTOLI TEGRIMI, T., QUINONES-GALVAN, A., IERVASI, G., L'ABBATE, A. & GUIDUCCI, L. 2019. A Pilot Study of Infrared Thermography Based Assessment of Local Skin Temperature Response in Overweight and Lean Women during Oral Glucose Tolerance Test. *J Clin Med,* 8.

JAMES, R. S. 2013. A review of the thermal sensitivity of the mechanics of vertebrate skeletal muscle. *J Comp Physiol B,* 183**,** 723-33.

JAMES, R. S., ALTRINGHAM, J. D. & GOLDSPINK, D. F. 1995. The mechanical properties of fast and slow skeletal muscles of the mouse in relation to their locomotory function. *J Exp Biol,* 198**,** 491-502.

JAMES, R. S. & TALLIS, J. 2019. The likely effects of thermal climate change on vertebrate skeletal muscle mechanics with possible consequences for animal movement and behaviour. *Conservation physiology,* 7**,** coz066.

JAMES, R. S., TALLIS, J. & ANGILLETTA, M. J., JR. 2015. Regional thermal specialisation in a mammal: temperature affects power output of core muscle more than that of peripheral muscle in adult mice (Mus musculus). *J Comp Physiol B,* 185**,** 135-42.

JAMES, R. S., TALLIS, J., HERREL, A. & BONNEAUD, C. 2012. Warmer is better: thermal sensitivity of both maximal and sustained power output in the iliotibialis muscle isolated from adult Xenopus tropicalis. *J Exp Biol,* 215**,** 552-8.

JAMES, R. S., TALLIS, J. A., SEEBACHER, F. & STOREY, K. 2011. Daily torpor reduces mass and changes stress and power output of soleus and EDL muscles in the Djungarian hamster, Phodopus sungorus. *J Exp Biol,* 214**,** 2896-902.

JAMES, R. S., YOUNG, I. S., COX, V. M., GOLDSPINK, D. F. & ALTRINGHAM, J. D. 1996. Isometric and isotonic muscle properties as determinants of work loop power output. *Pflugers Arch,* 432**,** 767-74.

JOSEPHSON, R. K. 1985. Mechanical power output from striated muscle during cyclical contraction. *Journal of Experimental Biology,* 114**,** 493-512.

JOSEPHSON, R. K. 1993. Contraction dynamics and power output of skeletal muscle. *Annu Rev Physiol,* 55**,** 527-46.

KASAI, T., HIROSE, M., MATSUKAWA, T., TAKAMATA, A. & TANAKA, Y. 2003. The vasoconstriction threshold is increased in obese patients during general anaesthesia. *Acta Anaesthesiol Scand,* 47**,** 588-92.

LAFORTUNA, C., MAFFIULETTI, N., AGOSTI, F. & SARTORIO, A. J. I. J. O. O. 2005. Gender variations of body composition, muscle strength and power output in morbid obesity. 29**,** 833-841.

LAKENS, D. 2013. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol,* 4**,** 863.

LANNERGREN, J. & WESTERBLAD, H. 1987. The temperature dependence of isometric contractions of single, intact fibres dissected from a mouse foot muscle. *J Physiol,* 390**,** 285-93.

MACINTOSH, B. R. 2003. Role of calcium sensitivity modulation in skeletal muscle performance. *News Physiol Sci,* 18**,** 222-5.

MAFFIULETTI, N. A., JUBEAU, M., MUNZINGER, U., BIZZINI, M., AGOSTI, F., DE COL, A., LAFORTUNA, C. L. & SARTORIO, A. 2007. Differences in quadriceps muscle strength and fatigue between lean and obese subjects. *Eur J Appl Physiol,* 101**,** 51-9.

884 MANGUM, J., SIECK, D., ELY, M., LARSON, E., MINSON, C. & HALLIWILL, J. 2018. Effect
885 of Increased Skeletal Muscle Temperature on Intramuscular Histamine
886 Concentrations. *The FASEB Journal,* 32**,** 726.2-726.2.
887 MASGRAU, A., MISHELLANY-DUTOUR, A., MURAKAMI, H., BEAUFRERE, A. M.,
888 WALRAND, S., GIRAUDET, C., MIGNE, C., GERBAIX, M., METZ, L., COURTEIX, D.,
889 GUILLET, C. & BOIRIE, Y. 2012. Time-course changes of muscle protein synthesis
890 associated with obesity-induced lipotoxicity. *J Physiol,* 590**,** 5199-210.
891 MENDEZ, J. & KEYS, A. 1960. Density and composition of mammalian muscle. *Metabolism,*
892 9**,** 184-188.
893 MIYATAKE, N., FUJII, M., NISHIKAWA, H., WADA, J., SHIKATA, K., MAKINO, H. & KIMURA,
894 I. 2000. Clinical evaluation of muscle strength in 20-79-years-old obese Japanese.
895 *Diabetes Res Clin Pract,* 48**,** 15-21.
896 OLBERDING, J. P. & DEBAN, S. M. 2017. Effects of temperature and force requirements on
897 muscle work and power output. *J Exp Biol,* 220**,** 2017-2025.
898 PIMENTA, F. B., BERTRAND, E., MOGRABI, D. C., SHINOHARA, H. & LANDEIRA-
899 FERNANDEZ, J. 2015. The relationship between obesity and quality of life in Brazilian
900 adults. *Frontiers in psychology,* 6**,** 966.
901 PINTO, R. S., CORREA, C. S., RADAELLI, R., CADORE, E. L., BROWN, L. E. & BOTTARO,
902 M. 2014. Short-term strength training improves muscle quality and functional capacity
903 of elderly women. *Age (Dordr),* 36**,** 365-72.
904 PREZANT, D. J., RICHNER, B., VALENTINE, D. E., ALDRICH, T. K., FISHMAN, C. L.,
905 NAGASHIMA, H., CHAUDHRY, I. & CAHILL, J. 1990. Temperature dependence of rat
906 diaphragm muscle contractility and fatigue. *J Appl Physiol (1985),* 69**,** 1740-5.
907 RALL, J. A. & WOLEDGE, R. C. 1990. Influence of temperature on mechanics and energetics
908 of muscle contraction. *Am J Physiol,* 259**,** R197-203.
909 RANATUNGA, K. W. 1998. Temperature dependence of mechanical power output in
910 mammalian (rat) skeletal muscle. *Exp Physiol,* 83**,** 371-6.
911 RANATUNGA, K. W. 2018. Temperature Effects on Force and Actin(-)Myosin Interaction in
912 Muscle: A Look Back on Some Experimental Findings. *Int J Mol Sci,* 19.
913 RANATUNGA, K. W., SHARPE, B. & TURNBULL, B. 1987. Contractions of a human skeletal
914 muscle at different temperatures. *J Physiol,* 390**,** 383-95.
915 RICHARDSON, J. T. 2011. Eta squared and partial eta squared as measures of effect size in
916 educational research. *Educational Research Review,* 6**,** 135-147.
917 ROGERS, P. & WEBB, G. P. 1980. Estimation of body fat in normal and obese mice. *Br J*
918 *Nutr,* 43**,** 83-6.
919 ROLLAND, Y., LAUWERS-CANCES, V., PAHOR, M., FILLAUX, J., GRANDJEAN, H. &
920 VELLAS, B. 2004. Muscle strength in obese elderly women: effect of recreational
921 physical activity in a cross-sectional study. *Am J Clin Nutr,* 79**,** 552-7.
922 ROSSI, R., BOTTINELLI, R., SORRENTINO, V. & REGGIANI, C. 2001. Response to caffeine
923 and ryanodine receptor isoforms in mouse skeletal muscles. *Am J Physiol Cell Physiol,*
924 281**,** C585-94.
925 ROSSI, R., MAFFEI, M., BOTTINELLI, R. & CANEPARI, M. 2005. Temperature dependence
926 of speed of actin filaments propelled by slow and fast skeletal myosin isoforms. *J Appl*
927 *Physiol (1985),* 99**,** 2239-45.
928 RUMMEL, A. D., SWARTZ, S. M. & MARSH, R. L. 2019. Warm bodies, cool wings: regional
929 heterothermy in flying bats. *Biol Lett,* 15**,** 20190530.
930 RUMMEL, A. D., SWARTZ, S. M. & MARSH, R. L. 2021. A proximal-distal difference in bat
931 wing muscle thermal sensitivity parallels a difference in operating temperatures along
932 the wing. *Proc Biol Sci,* 288**,** 20210009.
933 SAVASTANO, D. M., GORBACH, A. M., EDEN, H. S., BRADY, S. M., REYNOLDS, J. C. &
934 YANOVSKI, J. A. 2009. Adiposity and human regional body temperature. *Am J Clin*
935 *Nutr,* 90**,** 1124-31.
936 SEEBACHER, F., TALLIS, J., MCSHEA, K. & JAMES, R. S. 2017. Obesity-induced decreases
937 in muscle performance are not reversed by weight loss. *Int J Obes*.

SEEBACHER, F., TALLIS, J. A. & JAMES, R. S. 2014. The cost of muscle power production: muscle oxygen consumption per unit work increases at low temperatures in Xenopus laevis. *J Exp Biol,* 217**,** 1940-5.

STEIN, R. B., GORDON, T. & SHRIVER, J. 1982. Temperature dependence of mammalian muscle contractions and ATPase activities. *Biophys J,* 40**,** 97-107.

TALLIS, J., HIGGINS, M. F., COX, V. M., DUNCAN, M. J. & JAMES, R. S. 2014a. Does a physiological concentration of taurine increase acute muscle power output, time to fatigue, and recovery in isolated mouse soleus (slow) muscle with or without the presence of caffeine? *Can J Physiol Pharmacol,* 92**,** 42-9.

TALLIS, J., HIGGINS, M. F., SEEBACHER, F., COX, V. M., DUNCAN, M. J. & JAMES, R. S. 2017a. The effects of 8 weeks voluntary wheel running on the contractile performance of isolated locomotory (soleus) and respiratory (diaphragm) skeletal muscle during early ageing. *J Exp Biol,* 220**,** 3733-3741.

TALLIS, J., HILL, C., JAMES, R. S., COX, V. M. & SEEBACHER, F. 2017b. The effect of obesity on the contractile performance of isolated mouse soleus, EDL, and diaphragm muscles. *J Appl Physiol (1985),* 122**,** 170-181.

TALLIS, J., JAMES, R. S., COX, V. M. & DUNCAN, M. J. 2012. The effect of physiological concentrations of caffeine on the power output of maximally and submaximally stimulated mouse EDL (fast) and soleus (slow) muscle. *J Appl Physiol (1985),* 112**,** 64-71.

TALLIS, J., JAMES, R. S., LITTLE, A. G., COX, V. M., DUNCAN, M. J. & SEEBACHER, F. 2014b. Early effects of ageing on the mechanical performance of isolated locomotory (EDL) and respiratory (diaphragm) skeletal muscle using the work-loop technique. *Am J Physiol Regul Integr Comp Physiol,* 307**,** R670-84.

TALLIS, J., JAMES, R. S. & SEEBACHER, F. 2018. The effects of obesity on skeletal muscle contractile function. *J Exp Biol,* 221.

VANHOOYDONCK, B., JAMES, R. S., TALLIS, J., AERTS, P., TADIC, Z., TOLLEY, K. A., MEASEY, G. & HERREL, A. J. P. O. T. R. S. B. B. S. 2014. Is the whole more than the sum of its parts? Evolutionary trade-offs between burst and sustained locomotion in lacertid lizards. 281**,** 20132677.

VASQUEZ, E., BATSIS, J. A., GERMAIN, C. M. & SHAW, B. A. 2014. Impact of obesity and physical activity on functional outcomes in the elderly: data from NHANES 2005-2010. *J Aging Health,* 26**,** 1032-46.

VERBRAECKEN, J., VAN DE HEYNING, P., DE BACKER, W. & VAN GAAL, L. 2006. Body surface area in normal-weight, overweight, and obese adults. A comparison study. *Metabolism,* 55**,** 515-24.

VROMAN, N. B., BUSKIRK, E. R. & HODGSON, J. L. 1983. Cardiac output and skin blood flow in lean and obese individuals during exercise in the heat. *J Appl Physiol Respir Environ Exerc Physiol,* 55**,** 69-74.

WHO. 2000. *Obesity: preventing and managing the global epidemic* [Online]. https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/: World Health Organisation. [Accessed 14/07/2020 2020].

YAICHAROEN, P., WALLMAN, K., MORTON, A. & BISHOP, D. 2012. The effect of warm-up on intermittent sprint performance and selected thermoregulatory parameters. *J Sci Med Sport,* 15**,** 451-6.