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RESEARCH PAPER

Endurance exercise plus overload induces fatigue resistance and similar hypertrophy in mice irrespective of muscle mass

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Abstract

Previous studies have demonstrated that fibre cross-sectional area (FCSA) is inversely related to oxidative capacity, which is thought to be determined by diffusion limitations of oxygen, ADP and ATP. Consequently, it is hypothesised that (1) when endurance training is combined with a hypertrophic stimulus the response to each will be blunted, and (2) muscles with a smaller FCSA will show a larger hypertrophic response than those with a large FCSA. To investigate this, we combined overload with endurance exercise in 12-month-old male mice from three different strains with different FCSA: Berlin High (BEH) (large fibres), C57BL/6J (C57) (normal-sized fibres) and Berlin Low (BEL) (small fibres). The right plantaris muscle was subjected to overload through denervation of synergists with the left muscle acting as an internal control. Half the animals trained 30 min per day for 6 weeks. The overload-induced hypertrophy was not blunted by endurance exercise, and the exercise-induced increase in fatigue resistance was not impaired by overload. All strains demonstrated similar absolute increases in FCSA, although the BEH mice with more fibres than the C57 mice demonstrated the largest increase in muscle mass and BEL mice with fewer fibres the smallest increase in muscle mass. This study suggests that endurance exercise and hypertrophic stimuli can be combined without attenuating adaptations to either modality, and that increases in FCSA are independent of baseline fibre size.

KEYWORDS

capillarisation, fibre cross-sectional area, hypertrophy, oxidative capacity, physical activity

1 | INTRODUCTION

In isolated muscle fibres an inverse relationship between fibre cross-sectional area (FCSA) and maximal oxygen uptake or succinate dehydrogenase activity – dubbed the ‘size principle of striated muscle’ – has been observed and was explicable by oxygen diffusion limitations (van der Laarse, Des Tombe, Groot, & Diegenbach, 1998).

Such potential limitations to fibre size may be limited not only by oxygen diffusion, but also by the diffusion of ATP and ADP, and the size of the myonuclear domain (Degens, 2012; Kinsey, Hardy, & Locke, 2007; van Wessel, de Haan, van der Laarse, & Jaspers, 2010).

Corresponding with the concept that diffusion constraints limit fibre size is the positive relationship between the size and capillary

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supply to a fibre (Bosutti et al., 2015). During overload, the similar time course of angiogenesis and increases in FCSA (Egginton et al., 2011; Plyley, Olmstead, & Noble, 1998) may well serve to mitigate diffusion limitations associated with hypertrophy. Nevertheless, capillaries are only found at the perimeter of muscle fibres and the distance from the capillary to the centre of the fibre increases with hypertrophy and may thus impose a limit on increment in FCSA, no matter the amount of angiogenesis (Degens, 2012). This limit may be alleviated by a reduction in oxidative capacity (van Wessel et al., 2010).

While concurrent training is commonly used to gain the benefits of both resistance and endurance exercise, the combination of both training modalities is thought to lead to diminished responses to each stimulus (van Wessel et al., 2010). For instance, a reduction in oxidative capacity to alleviate potential diffusion limitations of fibre growth during overload runs counter to adaptations from endurance training and vice versa (van Wessel et al., 2010). It is therefore expected that overload-induced increases in FCSA are attenuated by endurance exercise, and increases in oxidative capacity in response to endurance exercise are dampened when accompanied by a hypertrophic stimulus.

Based on the 'size principle' one might expect that muscles with smaller fibres will show a larger hypertrophic response, particularly when combined with endurance exercise, than muscles with larger fibres. In line with this, in human training studies individuals with lower muscle thickness and smaller FCSA experienced greater hypertrophy with resistance training than those with greater muscle thickness and larger FCSA (Haun et al., 2019; Mobley et al., 2018). While many studies of concurrent training have been completed in both humans and rodents (Castoldi et al., 2013; Hickson, 1980; Methenitis, 2018), the effect of baseline muscle mass/FCSA on the response to combined hypertrophic and endurance stimuli is yet to be studied. Therefore, the aim of the present study was to determine the effects of combining a hypertrophic stimulus with endurance exercise on FCSA, muscle mass, oxidative capacity, capillarisation and contractile properties in three mouse strains with a 5-fold difference in plantaris muscle mass: Berlin High (BEH), Berlin Low (BEL) and C57BL/6J (C57) (Kilikevicius, Bunker, & Lionikas, 2016). The BEH mouse strain has a large muscle mass and FCSA, due to myostatin dysfunction and long-term selection for a large muscle mass (Lionikas et al., 2013b), while the BEL strain has a small muscle mass and small fibres (Lionikas et al., 2013b). The muscle mass and FCSA of the C57 mouse strain is in between those of the BEL and BEH mice (Lionikas et al., 2013b). Additionally, mice homozygous for the mutated myostatin *compact* allele, such as the BEH mice, have been reported to have a lower number of capillaries per fibre and capillary density (CD) than wild-type mice, which may further attenuate the hypertrophic response in these mice (Rehfeldt et al., 2005).

We hypothesise that (1) muscles from BEH mice will demonstrate the smallest increase in FCSA of the three strains, whereas muscles from BEL mice will experience the largest increase; and particularly in BEH mice (2) endurance training will lead to an attenuated hypertrophic response and (3) overload will blunt improvements in fatigue resistance.

New Findings

• What is the central question of this study?

Does combining endurance and hypertrophic stimuli blunt the adaptations to both modalities and is this effect greater in muscles with larger baseline fibre cross sectional area?

• What is the main finding and its importance?

Endurance exercise and hypertrophic stimuli can be combined to increase fatigue resistance and fibre size without blunting either adaptation regardless of baseline fibre size.

2 | METHODS

2.1 | Animals

Thirty-six 12-month-old male mice (14 C57, 10 BEH and 12 BEL) were kept in individual cages at 22°C, on a reversed 12 h light-dark cycle with *ad libitum* access to standard chow (LabDiet5001: protein 28.7%, carbohydrate 57.9%, fat 13.4% Lab Supply, TX, USA) and water at the Lithuanian Sports University. All animal procedures were approved by the Lithuanian State Food and Veterinary Service Ref. No. 0230.

2.2 | Surgery to overload the plantaris muscle

To induce compensatory hypertrophy of the plantaris muscle of the right leg, its synergists (the gastrocnemius and soleus) were denervated by cutting the medial and lateral branches of the tibial nerve to the gastrocnemius and soleus, as detailed previously (Degens & Alway, 2003). Sections of these nerve branches were removed to prevent reinnervation. Surgical procedures were performed under aseptic conditions and mice were anaesthetised with an intraperitoneal injection of a mix of ketamine (100 mg kg⁻¹) (Ketamidol, Richter Pharma AG, Wels, Austria) and xylazine (16 mg kg⁻¹) (Sedaxylan, Eurovet, Bladel, Netherlands) in saline. After surgery, the wound was closed with suture and the pain killer ketoprofen (2.5 mg kg⁻¹) (Rifen, Eurovet, Bladel, Netherlands) was administered immediately and again 24 h later. The plantaris muscle of the intact contralateral limb served as the internal control as Baldwin, Cheadle, Martinez, and Cooke (1977) found no differences in enzyme activity and muscle mass between the hindlimb muscles of sham operated animals and contralateral limbs in animals subject to overload surgery.

2.3 | Endurance exercise

One week after the overload surgery, mice were allocated either to a sedentary group or to an endurance exercise group. The endurance exercise protocol consisted of running at a speed of 12 m min⁻¹, 30 min day⁻¹, 5 days per week on a treadmill (Savage & McPherron, 2010) for 6 weeks. A shock-grid with a current of 1 mA was used to encourage running. Four mice refused to run during the first five exercise sessions and were removed from the exercise group and replaced with an animal from the sedentary group. The training adaptation of the mice starting later did not differ from those that underwent six full weeks of training. Exercise was undertaken under the supervision of a researcher in the dark with a red light.

2.4 | *In situ* m. plantaris contractile properties

Seven weeks after the initial surgery, the mice were anaesthetised as detailed above. Since the terminal experiment required a longer period of anaesthesia than the denervation surgery, the pedal withdrawal reflex was tested every 10 min and 20% of the original dose was administered to the animal when needed. The gastrocnemius of one hindlimb was excised to expose the sciatic nerve and weighed. The animal was then moved to the *in situ* force measurement apparatus where its body temperature was kept at 36.5°C. The distal plantaris tendon was cut and attached to a force transducer (Dual-Mode Muscle Lever Systems 305C-LR, Aurora Scientific, Aurora, Ontario, Canada) using 3-0 silk suture.

The sciatic nerve was cut proximally and then stimulated with 0.2 ms pulses incrementally every 30 s until maximal twitch force was achieved. After this, the stimulating current was increased by 10% to ensure supramaximal stimulation. The muscle was then stimulated every 30 s to determine optimal length, defined as the muscle length at which maximal active twitch force was produced.

The maximal tetanic force was then determined by stimulating the muscle twice, 5 min apart, for 300 ms at 200 Hz. Five minutes after the final tetanus, the muscle was stimulated with a series of 330 ms, 40 Hz trains once every second for 4 min to assess fatigue resistance. A fatigue index was then calculated as the force of the last contraction divided by the force of the strongest contraction of the series.

The same process was repeated on the contralateral limb. The overloaded and control plantaris muscles were stimulated in random order. After the contractile properties were determined for both plantaris muscles, the animal was killed with carbon dioxide. The plantaris muscles were then removed, weighed and mounted in OCT embedding medium (Thermo Scientific, Loughborough, UK) in liver, frozen in liquid nitrogen and stored at -80°C.

2.5 | Muscle morphology

A cryostat was used to cut serial 10 µm sections from the mid-belly of each plantaris muscle at -20°C and three sections were mounted per slide.

Two slides were blocked with 10% goat serum (Vector Laboratories, Burlingame, CA, USA) in phosphate-buffered saline (PBS) for 60 min. Then one slide was incubated for 2 h in an antibody cocktail containing monoclonal antibodies BAD-5 (1:600), SC-71 (1:600) and 6H1 (1:50) in blocking solution (Developmental Studies Hybridoma Bank, Iowa City, IA, USA) to identify types I, IIa and IIx fibres, respectively. The second slide was incubated for 2 h with BF-F3 (1:100) and biotinylated *Griffonia simplicifolia* lectin I (5 µl ml⁻¹; Vector Laboratories) in blocking solution to identify type IIb fibres and capillaries, respectively. After three 5 min PBS washes the first slide was incubated in secondary antibodies Alexa Fluor 350IgG2b for type I (1:500), Alexa Fluor 488 IgG1 for type IIa (1:500) and Alexa Fluor 555 IgM for type IIx (1:500) (Thermo Fisher Scientific, Waltham, MA, USA). The second slide was incubated in Alexa Fluor 555 IgM for type IIb (1:500) and streptavidin, Alexa Fluor 350 conjugate (1:200) (Thermo Fisher Scientific) for capillaries. After three further 5 min washes the slides were mounted with ProLong Diamond Antifade mountant (Thermo Fisher Scientific).

Digital images of both the oxidative and the glycolytic regions (deep and superficial, respectively) of the plantaris muscle were taken using a fluorescence microscope (Zeiss Axio Imager Z1, Carl Zeiss, Oberkochen, Germany) at ×20 magnification. For each digital image, BTablet (BaLoH Software, Ooij, Netherlands) was used to manually trace and type fibres and record capillary coordinates. The researcher was not blinded to the experimental conditions. Using these data, AnaTis (BaLoH Software) was used to calculate FCSA, capillary to fibre ratio (C:F), capillary domain size, local capillary to fibre ratio (LCFR), capillary fibre density (CFD) and an index of the heterogeneity of capillary spacing (log_RSD).

2.6 | Succinate dehydrogenase optical density

A third serial slide from each plantaris muscle was stained for succinate dehydrogenase (SDH) activity as described previously (Ballak et al., 2016). The optical density (OD) of the stain has been shown to give a quantitative indication of oxidative capacity (van der Laarse, Diegenbach, & Elzinga, 1989). Photomicrographs of stained sections were taken on a light microscope with an AxioCam ICMI camera (Carl Zeiss) and a 660 nm filter. For each sample, a series of filters with known OD were used to create a calibration curve to adjust for variation in background staining and lighting between sections. ImageJ (NIH, Bethesda, MD, USA, <http://imagej.net/Downloads>) was used to determine the OD of the stain for each fibre. Integrated SDH activity (SDH-INT) was calculated as the SDH-OD multiplied by the FCSA.

2.7 | Statistics

Statistical analyses were completed with IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). Data were tested for normal distribution with the Shapiro-Wilk test with strain, overload and exercise as factors, and where applicable fibre type and region.

Non-normally distributed data were transformed logarithmically. To study differences in responses to overload and exercise, a repeated-measures ANOVA was performed with muscle region and overload as within factors, and strain (3 levels: C57, BEH and BEL), exercise and fibre type (5 levels: IIa, IIb, IIx, IIa/IIx, IIb/IIx) as between factors. In addition to main effects, two-way interactions were considered. To determine the nature of two-way interactions with strain, the analysis was performed in each group separately. Effects and interactions were considered significant at $P < 0.05$. A Bonferroni correction was applied to correct for the effect of multiple testing.

3 | RESULTS

Data on body mass, muscle mass, contractile properties, FCSA, capillarisation and SDH-OD for C57 mice have been presented before (Hendrickse, Krusnauskas, Hodson-Tole, Venckunas, & Degens, 2020). These data are required to make a comparison between strains.

3.1 | Body mass and muscle masses

The BEH mice had the highest body mass, followed by C57 and then BEL mice ($P < 0.001$; Table 1). The strain \times time interaction ($P = 0.047$) was reflected by a significant decrease in the body mass of C57 mice during the period of overload ($P = 0.003$), but not in the other strains (Table 1). Like the differences in body mass, the BEH mice had the highest m. gastrocnemius mass, followed by C57 and then BEL mice ($P < 0.001$; Table 1). A similar pattern was seen in the severity of atrophy of the denervated m. gastrocnemius mass in the limb subjected to surgery to overload the m. plantaris (strain \times overload interaction $P < 0.001$). The muscle mass: body mass ratio for the m. gastrocnemius mass was smaller in BEH mice when compared to C57 (Bonferroni *post hoc* $P < 0.001$; Table 1) and BEL ($P = 0.007$) mice, and was decreased after denervation (to induce overload in the plantaris muscle) in all strains ($P < 0.001$), while exercise led to an increase in m. gastrocnemius mass relative to body mass ($P = 0.045$).

The mass of the sedentary control m. plantaris was largest in the BEH and smallest in the BEL mice, with that of the C57 mice in between (one-way ANOVA $P < 0.001$; Figure 1a). These differences were also found when all plantaris masses were included in a repeated measures ANOVA ($P < 0.001$). In each strain, overload induced an increase in m. plantaris mass ($P < 0.001$), which was most pronounced in the BEH and least in the BEL mice, with the increase in muscle mass in C57 mice in between (strain \times overload interaction $P < 0.001$, Bonferroni *post hoc* $P \leq 0.014$; Figure 1a). However, the percentage change in plantaris mass with overload (relative to contralateral limb) was similar across all strains and was not significantly affected by endurance exercise (Figure 1e). The plantaris muscle mass: body mass ratio in BEL mice was smaller than in BEH (Bonferroni *post hoc* $P = 0.025$; Table 1) and C57 ($P = 0.015$) mice, and increased with overload in all strains ($P < 0.001$; Table 1).

TABLE 1 Body mass and gastrocnemius muscle mass in young adult C57BL/6J (C57), Berlin High (BEH) and Berlin Low (BEL) mice exposed to overload with or without exercise

	C57				BEH				BEL				Effects and interactions
	Sed pre	Sed post	Ex pre	Ex post	Sed pre	Sed post	Ex pre	Ex post	Sed pre	Sed post	Ex pre	Ex post	
Body mass (g)	38.1 \pm 3.2	37.5 \pm 4.8	41.5 \pm 7.3	34.8 \pm 5.8	66.0 \pm 10.4	63.6 \pm 6.7	57.8 \pm 5.7	58.7 \pm 2.2	24.6 \pm 3.9	23.9 \pm 3.8	21.2 \pm 3.7	21.4 \pm 2.6	S*** T* ST*
	SC (n = 7)	SO (n = 7)	EC (n = 7)	EO (n = 7)	SC (n = 5)	SO (n = 5)	EC (n = 5)	EO (n = 5)	SC (n = 5)	SO (n = 5)	EC (n = 7)	EO (n = 7)	
Gastrocnemius mass (mg)	129 \pm 11	74 \pm 11	133 \pm 15	74 \pm 10	181 \pm 13	88 \pm 7	185 \pm 13	97 \pm 25	68 \pm 10	41 \pm 8	67 \pm 14	40 \pm 11	S*** O*** SO***
Gastrocnemius mass (relative to body mass) (%)	0.350 \pm 0.050	0.200 \pm 0.033	0.385 \pm 0.034	0.218 \pm 0.043	0.287 \pm 0.032	0.141 \pm 0.024	0.315 \pm 0.016	0.164 \pm 0.037	0.287 \pm 0.028	0.175 \pm 0.046	0.312 \pm 0.053	0.192 \pm 0.065	S*** O*** E*
Plantaris mass (relative to body mass) (%)	0.057 \pm 0.011	0.085 \pm 0.016	0.061 \pm 0.010	0.088 \pm 0.011	0.061 \pm 0.010	0.082 \pm 0.011	0.063 \pm 0.006	0.086 \pm 0.004	0.047 \pm 0.008	0.066 \pm 0.008	0.050 \pm 0.013	0.070 \pm 0.024	S*** O***

S: effect of strain; T: effect of time; O: effect of overload surgery; ST: strain \times time interaction; SO: strain \times overload interaction; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. EC, exercise control; EO, exercise overload; Ex, exercise; SC, sedentary control; Sed, sedentary; SO, sedentary overload.

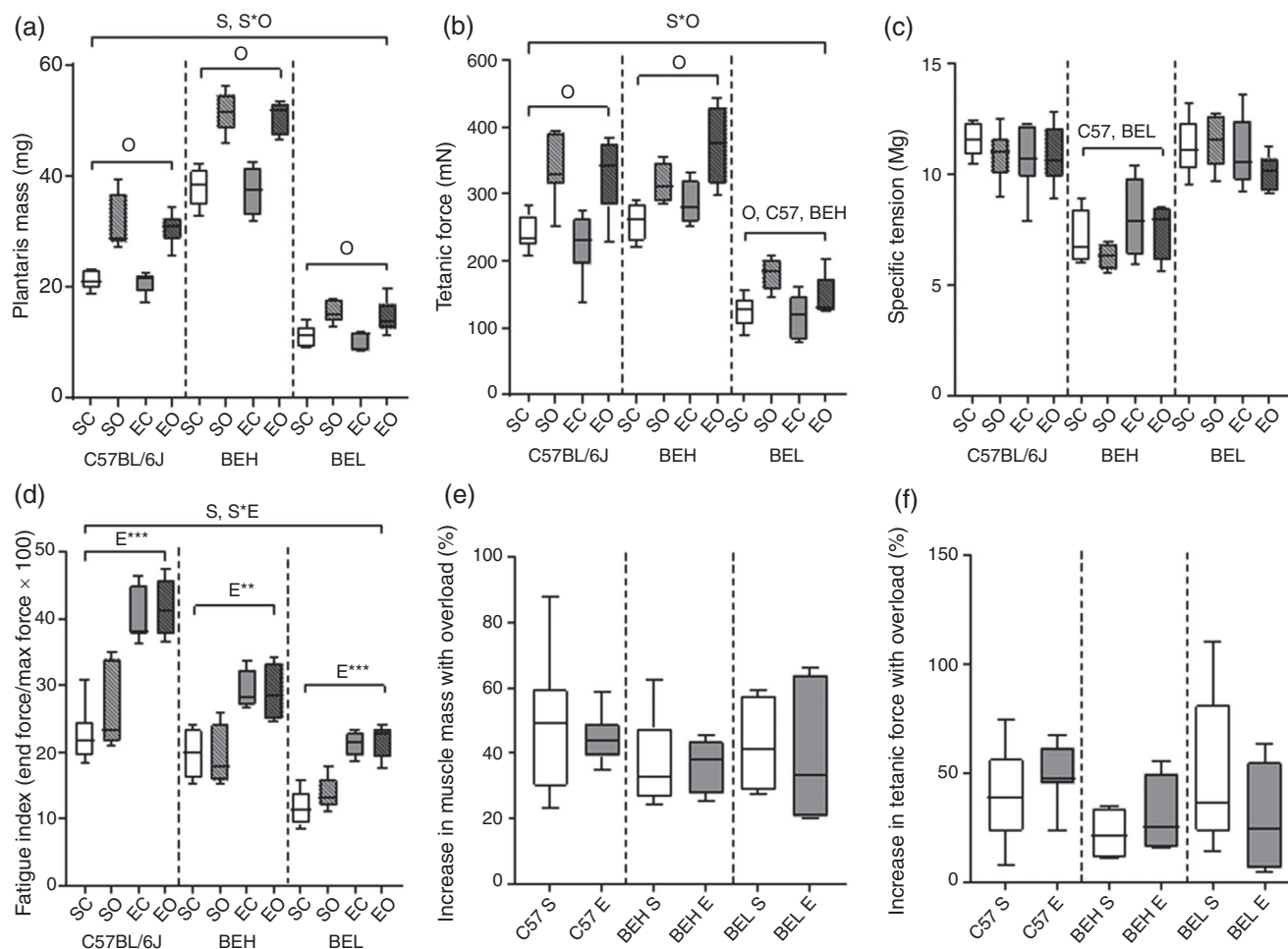


FIGURE 1 Effects of overload and exercise on plantaris muscle mass and contractile properties in C57BL/6J (C57), Berlin High (BEH) and Berlin Low (BEL) mice. (a) Plantaris muscle mass; (b) maximal tetanic force; (c) specific tension as tetanic force/muscle mass; (d) fatigue index; (e) percentage increase in muscle mass with overload relative to contralateral control limb; (f) percentage increase in tetanic force with overload relative to contralateral control limb. S: effect of strain ($P < 0.001$); O: effect of overload ($P < 0.001$); E*: effect of exercise ($P < 0.01$); E***: effect of exercise ($P < 0.001$). S*O: strain × overload interaction ($P \leq 0.046$); S*E: strain × exercise interaction ($P = 0.05$); C57: significantly different from C57 ($P < 0.001$); BEH: significantly different from BEH ($P < 0.001$); BEL: significantly different from BEL ($P < 0.001$). E, exercise; EC, exercise control; EO, exercise overload; S, sedentary; SC, sedentary control; SO, sedentary overload

3.2 | Contractile properties and fatigue resistance

The maximal isometric tetanic force was lower in BEL mice compared to that of the other strains ($P < 0.001$; Figure 1b). Overload induced a significant increase in maximal tetanic force ($P < 0.001$), but the strain × overload interaction ($P = 0.046$) indicates that this response differed between strains; in Figure 1b it can be seen that BEH mice had a greater increase in tetanic force than BEL mice with overload (Bonferroni *post hoc* $P = 0.013$). However, the percentage increase in tetanic force with overload relative to the contralateral limb did not differ significantly between strains and was not significantly affected by endurance exercise (Figure 1f). Specific tension (force per g muscle mass) was lower in BEH mice than in the other two strains ($P < 0.001$; Figure 1c) and did not change significantly with overload in any of the strains.

The fatigue resistance, reflected by the fatigue index, was highest in the C57 and lowest in the BEL mice, with that of the BEH mice in between ($P < 0.001$; Figure 1d). Exercised muscles had greater fatigue resistance compared to those of sedentary animals in all strains, but the strain × exercise interaction ($P = 0.005$) was reflected by a smaller effect of exercise in BEH (48.4% increase) mice when compared to C57 and BEL mice (64.2% and 68.5% increases, respectively) (Bonferroni *post hoc* $P < 0.05$).

3.3 | Fibre composition and fibre cross-sectional area

BEH mice had a greater proportion of type IIb fibres compared to BEL mice ($P = 0.001$). The glycolytic region of m. plantaris contained a lower

proportion of type IIa fibres than the oxidative regions ($P < 0.001$) and this difference was most pronounced in C57 mice (strain \times region interaction $P = 0.043$; Table 2).

The proportion of type I and type IIa fibres was higher ($P < 0.001$), while the percentages of type IIb and IIx fibres were lower in overloaded muscles ($P < 0.001$) regardless of exercise status. BEL mice demonstrated a greater increase in the percentage of type I fibres (strain \times overload interaction $P = 0.003$) and BEH mice demonstrated a greater increase in type IIa fibre proportion with overload (strain \times overload interaction $P = 0.021$). Since fewer than 2.5% of the fibres in all regions were type I/IIa (Table 2), they were omitted from further analysis.

The FCSA in the sedentary control m. plantaris was greater in BEH compared to C57 mice, which in turn was greater than that of BEL mice ($P < 0.05$). When all muscles were included in a repeated measures ANOVA, FCSA was larger in the plantaris muscle of BEH than BEL mice (Bonferroni *post hoc* $P = 0.004$) with no significant difference between C57 and the other two strains (Figure 2a–c). Overload induced similar increases in FCSA in all three strains ($P < 0.001$; Figure 2a–c). Percentage changes in FCSA with overload relative to the contralateral control limb as performed by (Fisher et al., 2017) was similar across all strains and was not significantly affected by endurance exercise (Figure 2d). As there was a region \times strain interaction for FCSA ($P = 0.014$; Figure 2b,c), repeated measures ANOVAs were completed for each strain separately to determine the nature of this interaction. In the C57 mice there was a region effect ($P = 0.014$) and a region \times exercise interaction ($P = 0.007$) for FCSA that was reflected by an endurance exercise-induced reduction in the FCSA in the glycolytic, but not in the oxidative region of the muscle (Figure 2b,c). In the BEH mice a region \times overload interaction was found ($P = 0.037$), which was evident as a lower hypertrophic response in the oxidative than in the glycolytic region of the muscle (Figure 2b,c).

3.4 | Overall capillarisation

BEL mice had a higher C:F in the plantaris muscle than C57 mice ($P = 0.045$; Figure 3a–c). The oxidative region of the plantaris muscles had a greater C:F than glycolytic region in C57 and BEH mice, but not in the BEL mice (strain \times region interaction $P = 0.017$). Overload led to an increase in C:F ($P < 0.001$) in all strains. An overload \times exercise interaction was also found, which was reflected by a larger increase in C:F in exercised overloaded than in non-exercised overloaded muscles, and a lower C:F in exercised than in non-exercised control muscle ($P = 0.024$).

BEL mice had greater CD compared to C57 and BEH mice ($P \leq 0.025$; Figure 3d–f). In C57 mice only, the CD was lower in the glycolytic than in the oxidative region of the plantaris muscle (strain \times region interaction $P = 0.030$).

The heterogeneity of capillary spacing index, $\log_{10}SD$, was higher in the glycolytic than in the oxidative region of the plantaris muscle in the BEL mice only (strain \times region interaction $P = 0.027$; Figure 3g–i). An overload \times exercise interaction in all strains ($P = 0.011$) was reflected

by an increased in $\log_{10}SD$ with overload in non-exercised muscles and a decrease in exercised muscles (Figure 3g–i).

3.5 | Oxidative capacity and oxygen supply to demand

3.5.1 | Succinate dehydrogenase optical density

The fibres in the oxidative region of the muscle had greater SDH-OD than those in the glycolytic region ($P < 0.001$ Figure 4b,c).

3.5.2 | Supply: demand ratio

To assess differences in the matching of oxygen supply (LCFR) to oxygen demand (SDH-INT) for a fibre, the LCFR/SDH-INT was calculated (Figure 4d–f). An overload effect was found ($P = 0.020$), but there was also a region \times overload interaction ($P = 0.010$). *Post hoc* analyses revealed that only in the oxidative region of BEL mice did overload induce a significant decrease in this ratio ($P = 0.001$).

4 | DISCUSSION

The main observation of the present study is that in mouse strains with a 3-fold difference in muscle mass, the response to regular endurance exercise with or without concomitant overload is similar. In addition, in none of the strains did the combination of overload and endurance training attenuate the hypertrophic response to overload or blunt the exercise-induced increases in fatigue resistance.

4.1 | Differences between strains

Although smaller than the difference found in the soleus muscle (Lionikas et al., 2013b) we found a 3-fold range in body mass, gastrocnemius mass and plantaris mass between the BEL (smallest) and BEH (largest) mice. In a sedentary non-overloaded state, FCSA of m. plantaris was largest in BEH mice and smallest in BEL mice with C57 mice in between. The size of the differences in FCSA, however, evidenced that contrasted muscle mass between these strains was also partly due to differences in number of fibres, which is line with previous findings of both larger fibre number and FCSA in animals with myostatin dysfunction when compared to wild-type mice (Omairi et al., 2016).

The maximal isometric tetanic force of the plantaris muscle was lower in the BEL than C57 and BEH mice, which is explained by their smaller muscle size as the specific tension was similar to that of C57 mice. As a consequence of the lower specific tension in BEH mice, seen before in mice with myostatin dysfunction (Amthor et al., 2007; Mendias, Kayupov, Bradley, Brooks, & Clafin, 2011; Omairi et al.,

TABLE 2 Fibre type composition (%) for different fibre types in the oxidative and glycolytic regions of plantaris muscles exposed to overload with or without exercise in young adult C57BL/6J, BEH and BEL mice

Fibre type	C57				BEH				BEL				Effects and interactions	
	SC	SO	EC	EO	SC	SO	EC	EO	SC	SO	EC	EO		
Oxidative region														
Type I	0.1 ± 0.2	6.7 ± 4.8	0.2 ± 0.5	3.2 ± 4.4	—	0.4 ± 0.8	—	2.3 ± 1.6	0.3 ± 0.4	7.5 ± 9.0	0.7 ± 1.2	13.1 ± 11.3	S* O*** SO**	
Type I/Ia	—	0.9 ± 1.1	—	0.1 ± 0.3	—	0.4 ± 1.0	—	—	—	0.3 ± 0.7	—	2.1 ± 5.1	—	
Type IIa	30.5 ± 9.3	32.4 ± 15.3	22.2 ± 17.8	40.1 ± 10.4	15.7 ± 5.0	35.4 ± 17.6	10.8 ± 1.5	57.7 ± 8.0	19.9 ± 15.9	36.7 ± 20.7	30.1 ± 23.6	29.3 ± 12.3	R*** O*** SR* SO*	
Type IIa/IIx	8.1 ± 6.0	10.0 ± 6.8	2.1 ± 1.8	12.3 ± 5.7	2.3 ± 4.4	5.4 ± 5.5	4.7 ± 4.0	18.7 ± 10.4	15.8 ± 15.5	14.2 ± 2.2	5.3 ± 2.8	12.2 ± 4.3	S* R* O*** SE* OE**	
Type IIx	22.1 ± 7.2	18.0 ± 6.5	20.8 ± 7.3	14.6 ± 4.2	31.2 ± 11.1	19.5 ± 6.0	20.6 ± 3.9	13.9 ± 4.1	32.3 ± 3.3	21.1 ± 10.5	28.8 ± 7.9	21.6 ± 7.2	S* O**	
Type IIb/IIx	3.8 ± 2.7	7.4 ± 6.5	2.6 ± 1.5	6.4 ± 5.5	3.2 ± 1.5	5.4 ± 4.9	3.2 ± 1.5	—	5.1 ± 2.2	4.4 ± 3.6	3.6 ± 3.0	5.0 ± 4.9	SO*	
Type IIb	35.4 ± 9.4	24.7 ± 12.0	52.2 ± 22.8	23.4 ± 6.8	47.6 ± 9.7	33.5 ± 23.4	57.6 ± 6.9	7.4 ± 3.4	26.6 ± 26.6	15.7 ± 16.1	31.4 ± 20.2	16.7 ± 14.9	S* R* O***	
Glycolytic region														
Type I	—	3.3 ± 3.7	0.5 ± 1.0	0.5 ± 0.9	0.4 ± 0.9	2.0 ± 2.3	0.2 ± 0.4	3.1 ± 3.6	—	7.1 ± 8.0	0.6 ± 1.3	13.1 ± 14.1	S* O*** SO**	
Type I/Ia	—	1.3 ± 1.1	—	—	—	—	—	—	—	1.2 ± 2.2	—	2.3 ± 5.4	—	
Type IIa	7.4 ± 3.7	23.9 ± 14.1	12.9 ± 16.2	26.2 ± 7.1	7.6 ± 4.0	28.8 ± 17.8	10.6 ± 4.6	37.3 ± 15.1	20.9 ± 20.9	33.0 ± 17.8	16.4 ± 12.2	23.2 ± 10.6	R*** O*** SR* SO*	
Type IIa/IIx	6.0 ± 4.1	8.3 ± 5.4	2.2 ± 2.0	8.8 ± 8.7	0.8 ± 1.3	4.6 ± 5.2	3.7 ± 2.6	10.1 ± 5.8	11.8 ± 13.9	11.8 ± 3.3	4.3 ± 3.9	13.9 ± 7.3	S* R* O*** SE* OE**	
Type IIx	14.3 ± 5.7	20.7 ± 6.2	16.6 ± 9.6	16.2 ± 10.4	16.0 ± 7.1	19.6 ± 10.0	18.3 ± 3.3	20.8 ± 9.0	26.2 ± 12.4	24.6 ± 10.9	26.8 ± 4.0	21.5 ± 6.5	S* O**	
Type IIb/IIx	5.4 ± 3.8	10.5 ± 5.8	3.0 ± 3.2	7.0 ± 7.4	4.5 ± 4.2	4.9 ± 3.9	5.8 ± 4.8	5.7 ± 4.4	3.2 ± 2.9	4.2 ± 2.3	5.4 ± 2.9	5.8 ± 4.8	SO*	
Type IIb	67.0 ± 10.8	32.0 ± 12.5	64.8 ± 25.8	41.3 ± 9.9	70.7 ± 13.9	40.1 ± 29.0	61.4 ± 8.8	22.9 ± 14.5	37.9 ± 38.8	18.2 ± 14.0	46.4 ± 17.6	20.1 ± 13.0	S* R* O***	

S: strain effect; R: region effect; O: overload effect; SR: strain × region interaction; SO: strain × overload interaction; SE: strain × exercise interaction; OE: overload × exercise interaction; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. EC, exercise control; EO, exercise overload; SC, sedentary control; SO, sedentary overload.

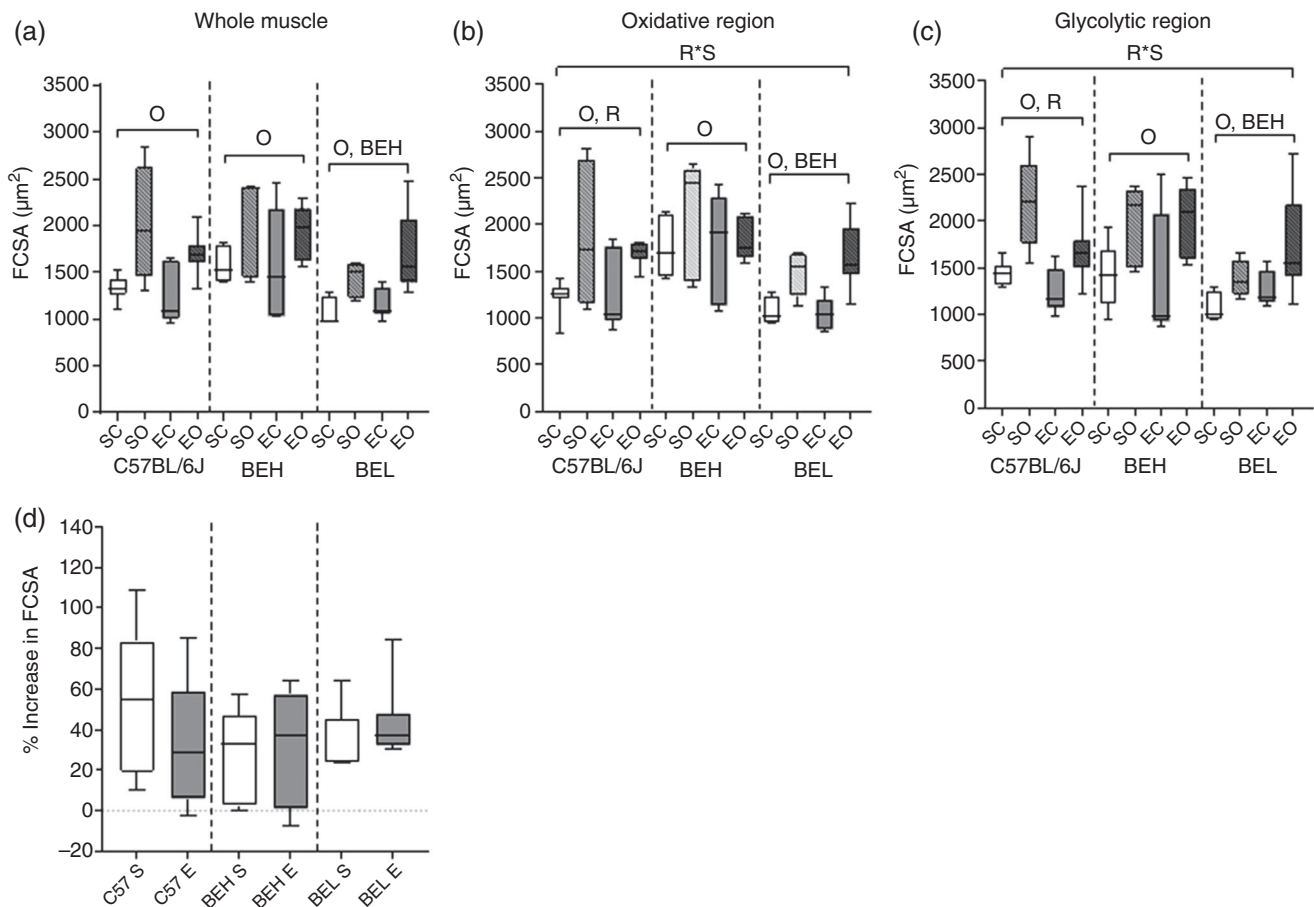


FIGURE 2 Effects of age, overload and exercise on fibre cross-sectional area (FCSA) and percentage increase in FCSA relative to contralateral control limb in C57BL/6J (C57), Berlin High (BEH) and Berlin Low (BEL) mice. (a) Whole plantaris muscle, (b) oxidative region, (c) glycolytic region, (d) percentage increase in FCSA due to overload relative to contralateral control limb. R: effect of region ($P = 0.026$); O: effect of overload ($P < 0.001$); R*S: region \times strain interaction ($P = 0.014$); BEH: significantly different from BEH ($P = 0.004$). E, exercise; EC, exercise control; EO, exercise overload; S, sedentary; SC, sedentary control; SO, sedentary overload

2016), the maximal isometric force was similar to that of C57 mice, despite the larger muscle mass.

Similar to previous observations in the extensor digitorum longus muscle of myostatin knock-out mice (Mouisel et al., 2014; Savage & McPherron, 2010), we observed that the fatigue resistance of the plantaris muscle of BEH mice was lower than that of C57 mice, with that of BEL mice in between. One possible explanation is the larger proportion of supposedly glycolytic type IIb fibres in the plantaris muscle of the BEH mice compared to the other strains. However, we found no significant difference in SDH-OD in the pooled fibre population between strains, and perhaps the lower fatigue resistance in BEH than in control mice is more related to the lower ratio of ATP consumption to isometric tension of type I than type IIb fibres (Stienen, Kiers, Bottinelli, & Reggiani, 1996). The lowest fatigue resistance in BEL mice was rather unexpected given they had the highest CD, which has been found to have a positive relationship with fatigue resistance (Tickle, Hendrickse, Degens, & Egginton, 2020). One explanation is that the mass-specific metabolic cost of repolarisation through Na^+/K^+ -ATPase activity is proportional to the fibre surface area: volume ratio, which is larger in small than in large fibres, as illustrated during

maturational muscle growth in the lobster (Jimenez, Dasika, Locke, & Kinsey, 2011). The larger C:F in the BEH mice relative to the other strains was unexpected given the lower capillarisation found in the rectus femoris (Rehfeldt et al., 2005), but this disparity may be attributed to the different muscles studied here.

4.2 | Response to regular endurance exercise

In contrast to previous observations in myostatin null mice (Matsakas et al., 2012), we did not see an exercise-induced increase in tetanic force or specific tension. In the study by Matsakas et al. (2012) the improvement in specific tension in the myostatin null mice was accompanied by a reduction in fibre size, which we also did not see. The discrepancy between that and our study may be related to the higher training volume and intensity in that study, although an identical training programme to the one we used did induce a reduction in plantaris mass in myostatin null mice (Savage & McPherron, 2010). Another possible explanation is that the other studies used myostatin null mice (Matsakas et al., 2012; Savage & McPherron, 2010), whereas

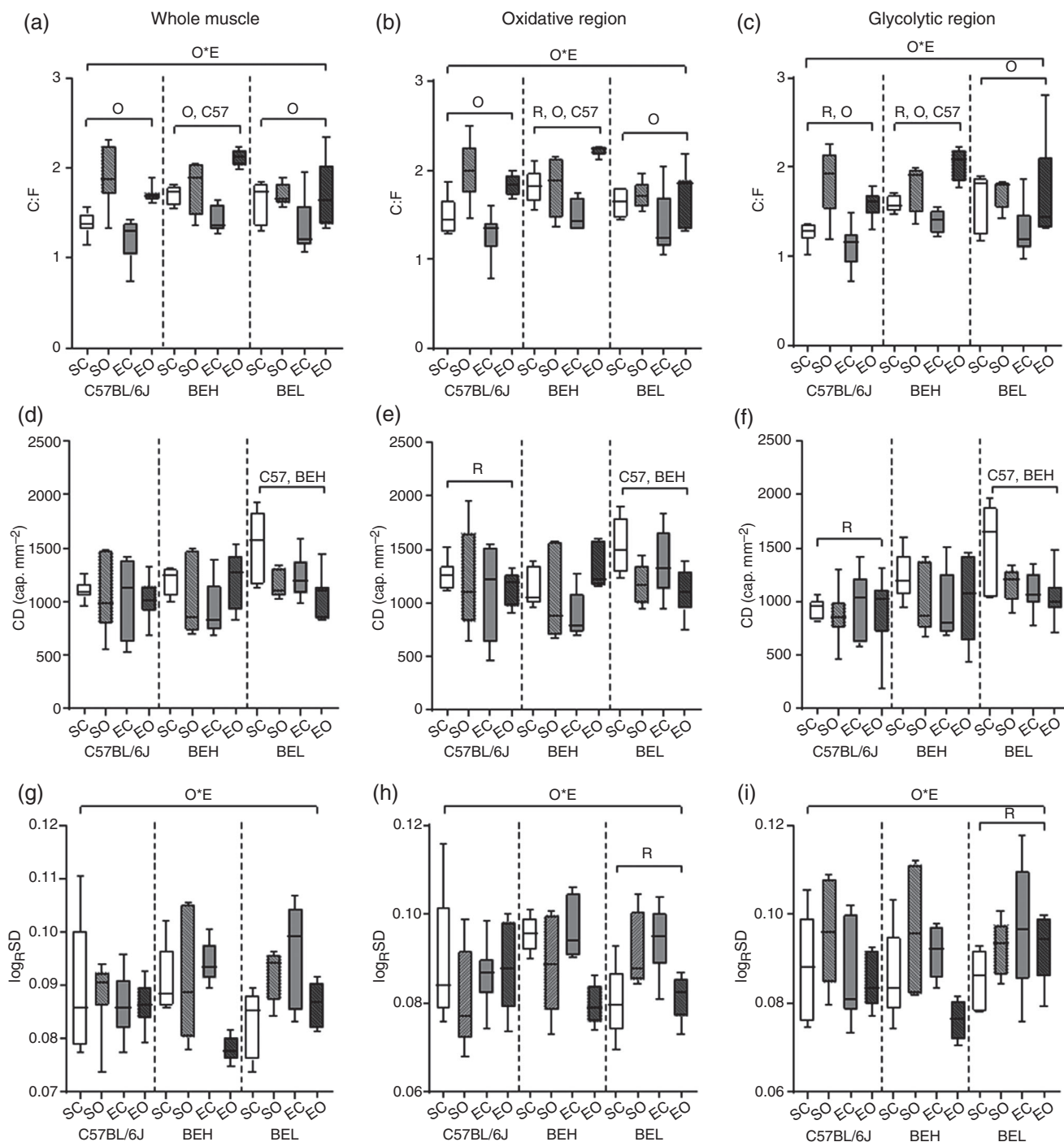


FIGURE 3 Effects of age, overload and exercise on skeletal muscle capillarisation in C57BL/6J (C57), Berlin High (BEH) and Berlin Low (BEL) mice. (a–c) Capillary to fibre ratio (C:F) in the whole plantaris muscle (a), oxidative region (b) and glycolytic region (c). (d–f) Capillary density (CD) in the whole muscle (d), oxidative region (e) and glycolytic region (f). (g–i) Logarithmic standard deviation of the radius of the capillary domains (\log_{RSD}) in the whole muscle (g), oxidative region (h) and glycolytic region (i). R: effect of region ($P \leq 0.013$); O: effect of overload ($P < 0.001$); O*: overload \times exercise interaction ($P \leq 0.024$); C57: significantly different from C57 ($P \leq 0.016$); BEH: significantly different from BEH ($P = 0.025$). EC, exercise control; EO, exercise overload; SC, sedentary control; SO, sedentary overload

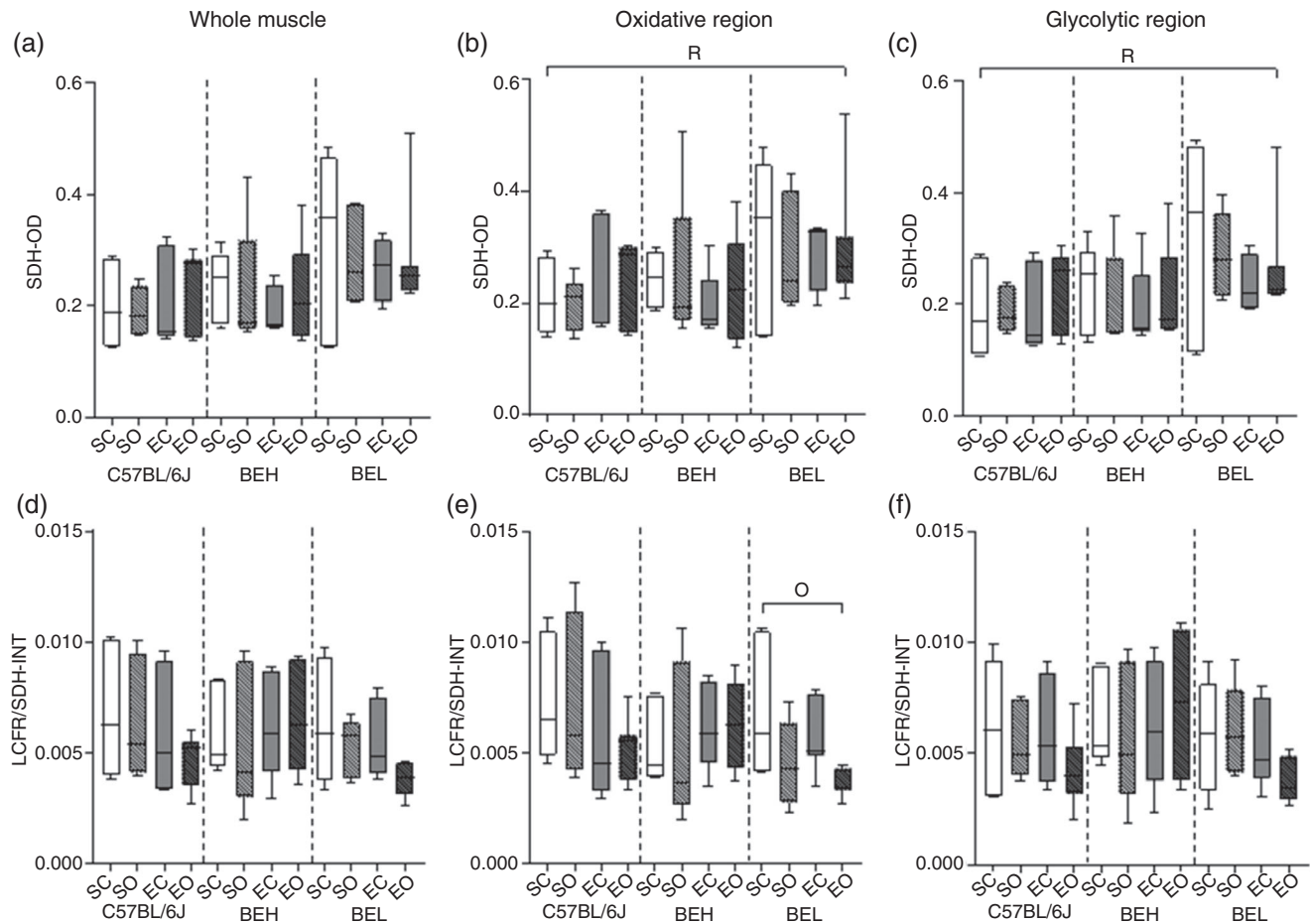


FIGURE 4 Effects of age, overload and exercise on succinate dehydrogenase optical density (SDH-OD) and local capillary to fibre ratio (LCFR)/integrated succinate dehydrogenase activity (SDH-INT) in C57BL/6J (C57), Berlin High (BEH) and Berlin Low (BEL) mice. (a–c) SDH-OD in the whole muscle (a), oxidative region (b) and glycolytic region (c). (d–f) LCFR/SDH-INT in the whole plantaris muscle (d), oxidative region (e) and glycolytic region (f). R: effect of region ($P \leq 0.013$); O: effect of overload-induced hypertrophy ($P < 0.001$). EC, exercise control; EO, exercise overload; SC, sedentary control; SO, sedentary overload

BEH mice are homozygous for the *compact* allele (Lionikas et al., 2013a). Whatever the cause of the discrepancies between studies, the endurance exercise did not induce a significant change in muscle mass, fibre size, force or specific tension in any of the strains studied.

The endurance exercise did increase, however, the fatigue resistance of the plantaris muscle in all three strains. Although fatigue resistance is positively related to CD (Tickle et al., 2020) and oxidative capacity (Burke, Levine, Tsairis, & Zajac, 1973), we did not see improvements in either of these measures with endurance exercise, even though others, using an identical programme, showed an increase in oxidative capacity (citrate synthase activity) in myostatin null mice (Savage & McPherron, 2010). Given the absence of angiogenesis and increased oxidative capacity in our study, the exercise-induced increase in fatigue resistance must be attributable to other factors, such as improvements in vasodilatation and maximal blood flow (Snell, Martin, Buckey, & Blomqvist, 1987). The lesser improvement in fatigue resistance with endurance exercise in BEH than in the other mice may be due to their high proportion of type IIb fibres that are

less economical than slower isoforms during isometric contractions (Stienen et al., 1996).

4.3 | Response to overload by synergist muscle inactivation

In contrast to our prediction that BEH mice would demonstrate the smallest increase in FCSA with overload, the increase in fibre size found in this strain was similar to that of the other strains. BEH mice demonstrated a similar increase in FCSA when compared to BEL, thereby suggesting that baseline FCSA is not predictive of increases in fibre size. This is similar to the findings in mice that baseline muscle mass is not predictive of hypertrophic response (Kilikevicius et al., 2016), but is in contrast to findings in humans (Haun et al., 2019).

The largest increase in muscle mass with overload in BEH and the smaller increase in muscle mass found with overload in BEL mice are

likely due to the greater and lower numbers of fibres found in the muscles of these strains, respectively (Lionikas et al., 2013b), as they demonstrated similar increases in FCSA. The similar growth in FCSA despite the larger diffusion distances in BEH mice may be due to the greater C:F, which would blunt diffusion distances for the delivery of oxygen, preventing an anoxic core of the muscle fibre, and supplying growth factors and amino acids to facilitate hypertrophy (Degens, 2012; Hendrickse & Degens, 2019).

Overload of the plantaris muscle led to a similar angiogenic response in all three strains that was evident in the increased C:F found with hypertrophied muscle. The muscle stretch induced by overload is thought to induce capillary growth independent of changes in blood flow (Deveci & Egginton, 2002; Egginton, Zhou, Brown, & Hudlicka, 2001) and has been demonstrated in multiple studies (Ballak et al., 2016; Tickle et al., 2020). Interestingly, LCFR/SDH-INT was lower after overload in the oxidative region of BEL mice, suggesting that oxygen supply does not match demand after the muscle fibres have hypertrophied in this region.

4.4 | Effects of regular endurance exercise on the hypertrophic response to overload

Here we demonstrate that increases in muscle mass and FCSA with overload were not impaired by endurance exercise. This indicates that there was no trade-off in this adaptation when both stimuli were used and suggests that interference in signalling pathways found in some acute studies that combine hypertrophic stimuli and endurance exercise may not manifest in blunted increases in FCSA when aerobic exercise volume is moderate (Murach & Bagley, 2016). However, it may be that greater endurance exercise volume increases the risk of diminishing the hypertrophy and strength adaptations in response to overload (Methenitis, 2018).

The maintenance of SDH-OD with hypertrophy seems to challenge the concept of an inverse relationship between fibre size and oxidative capacity (van der Laarse et al., 1998; van Wessel et al., 2010). This has also been found elsewhere (Ballak et al., 2016; Campbell, Jasmin, & Michel, 1996) and indicates a proportional increase in fibre size and mitochondrial biogenesis. Perhaps the apparent contradiction of the size principle is overcome by decreases in type IIb and IIx fibres and increases in type I and IIa fibres we observed, the latter of which have greater concentrations of subsarcolemmal mitochondria compared to the homogeneous distribution found in glycolytic fibres (Wust, Gibbings, & Degens, 2009), which would reduce the oxygen diffusion limitation. Such relocation of mitochondria to the periphery of the fibre has indeed been observed during an 8-fold increase in FCSA during maturational growth in the blue crab (Hardy, Dillaman, Locke, & Kinsey, 2009). A potential complication is that it would result in greater diffusion distances for ATP from these mitochondria to the myofibrils in the centre of the fibre (Degens, 2012; Kinsey et al., 2007).

4.5 | Effect of overload on endurance exercise-induced improvements in fatigue resistance

The inverse relationship thought to exist between fibre size and oxidative capacity (van Wessel et al., 2010) would indicate that combining hypertrophic and endurance stimuli would blunt adaptations to endurance training in addition to attenuating the increase in FCSA with overload. Similar to the maintained hypertrophic response in overloaded muscle when endurance exercise was used, the increase in fatigue resistance with endurance exercise was not impaired by overload. This adds further weight to the suggestion that adaptations to hypertrophic and endurance stimuli can be achieved simultaneously. The commensurate increase in C:F which occurs with fibre hypertrophy, evident by the maintenance of CD and CFD, likely prevented the increase in average diffusion distance from capillaries to mitochondria, thus blunting any diffusion limitations associated with increased FCSA (Degens, 2012). The oxidative fibre type shift which occurred with overload may have contributed to the similar fatigue resistance in exercised overloaded muscles when compared to those only subjected to regular endurance exercise as this would make the fibres more resistant to fatigue in spite of increases in FCSA (Stienen et al., 1996).

4.6 | Conclusions

Despite different increases in muscle mass, which can be attributed to differences in fibre number, the three strains studied demonstrated similar increases in FCSA and capillarisation in response to overload. The hypertrophic response to overload was not impaired by concomitant endurance exercise training, and improvement in fatigue resistance after running training was not blunted with simultaneous overloading the muscles. This suggests that adaptations to hypertrophic and endurance stimuli are maintained when combined, regardless of baseline muscle mass and fibre size.

4.7 | Limitations

Although muscle performance was improved by endurance exercise, the volume used was insufficient to induce significant increases in oxidative capacity or capillarisation of skeletal muscle. Regardless, the study demonstrated that adaptations to both hypertrophic and endurance stimuli can still occur to their full-scale when both modalities are used concurrently. Stress associated with shock grid use may have blunted hypertrophy (Conner, Wolden-Hanson, & Quinn, 2014), but this is likely to be limited as the hypertrophic response was not diminished with exercise.

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COMPETING INTERESTS

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

P.H., T.V. and H.D. contributed to the conception and design of the work. All authors contributed to the acquisition, analysis or interpretation of data for the work. All authors also contributed to the drafting of the work or revising it critically for important intellectual content. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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