Effects of Single 48 H Fasting on Soleus Muscle Mass and Contractile Properties in Old and Young Mice

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ABSTRACT

Background. Both fasting and ageing processes lead to muscle wasting. We studied the effect of single 48 h fasting on soleus skeletal muscle mass and force in young and old mice.

Methods. The study involved 18 young and 13 old mice. Body and soleus (SOL) muscle mass were measured followed by assessment of peak and specific SOL force under ex vivo conditions. SOL muscle half-relaxation time and 20 Hz to 100 Hz force ratio were also measured.

Results. After fasting, weight loss was greater (p < .05) in young mice compared to old mice (17.0 ± 2.6 versus 12.0 ± 1.7%, respectively). Fasted young, but not old mice showed reduction (p < .05) in SOL mass compared to the control values. On the other hand, specific SOL force was reduced (p < .05) only in old mice, while 20 Hz to 100 Hz force ratio decreased (p < .05) only in young mice after fasting.

Conclusions. Our results showed that 48 h fasting caused lower SOL muscle peak and specific force in old but not young mice.

Keywords: starvation, peak force, specific force, ageing.

INTRODUCTION

Periodic fasting or restriction of the exogenous nutrient intake is a popular strategy to limit energy intake and improve health (Longo & Mattson, 2014). Skeletal muscles provide a significant energy reserve as they represent 30–40% of the body mass and 60% of total body protein (Janssen, Heymsfield, Wang, & Ross, 2000; Lee et al., 2000). It is thus not surprising that skeletal muscle is highly sensitive to fasting which is reflected in activation of FoxO transcription factors leading to increased capacity of proteolysis pathways even after relatively short periods of fasting (Egerman & Glass, 2014). These changes are associated with imbalance between protein breakdown and synthesis, which are key determinants of muscle mass (Bowen, Schuler, & Adams, 2015). Malnutrition is considered to be a major contributing factor to ageing-related loss of muscle mass or sarcopenia (Bowen et al., 2015). Indeed, food intake of older persons in their seventies decreases by nearly 30% compared to the intake of younger persons in their twenties (Morley, 2017). Both malnutrition and ageing processes act synergistically to promote muscle wasting and thus further compound elderly to the poorest quality of life and prognosis (Morley, 2017).

Extent of muscle wasting varies between different skeletal muscles (Sandri et al., 2006). Slow-twitch SOL muscle tends to show a smaller degree of wasting compared to fast-twitch extensor digitorum longus (EDL) during caloric restriction or fasting (Sandri et al., 2006). This is probably associated with a fact that SOL shows particularly high levels of involvement in locomotion and posture in mice as well as humans (Roy, Hutchison, Pierotti, Hodgson, & Edgerton, 1991). Preservation of SOL might be particularly important in old mice showing ageing-related decrease in muscle mass and greater body mass (Lin et al., 2018). Thus we studied changes on mass and contractile properties...
of SOL muscle in young and old mice after 48 h fasting. We hypothesized that SOL muscle of old mice would show a greater degree of muscle mass and force loss after this intervention.

METHODS

Animals and experiments. The study was carried out at the Lithuanian Sports University with approval of all the procedures by the Lithuanian Republic Alimentary and Veterinary Public Office (No. G2-45, 2016). Males of C57BL/6J (B6) mouse strain were housed in standard cages (cage dimensions: 267 x 207 x 140 mm) at a temperature of ~20°C and 40–60% humidity with the normal 12/12-h light/dark cycle reversed. Animals were fed standard chow diet (56.7 kcal% carbohydrate, 29.8 kcal% protein, 13.4 kcal% fat; LabDiet 5001, USA) and received tap water ad libitum. Before the experiment mice were randomly subdivided into the two control groups for young (n = 9, age – 6 months) and old (n = 6, age – 24 months) mice and two fasting groups for young (n = 9, age – 6 months) and old mice (n = 7, age – 24 months). Both control groups were provided with ad libitum access to food and water. The fasting mice had ad libitum access to water, but did not receive any food for 48 h. Mice were weighed at 0 h, 24 h and 48 h of the intervention. At the end of the intervention mice were sacrificed by the exposure to CO₂. Immediately afterwards, as in our previous study, SOL muscle was dissected and weighed (Kvedaras et al., 2017).

Muscle contractile properties. Force output of SOL muscle was assessed in vitro using muscle test system (1200A-LR Muscle Test System, Aurora Scientific Inc., Canada) as in our previous study (Kvedaras et al., 2017). For these measurements, muscles were placed between two platinum electrodes in Tyrode solution (121 mM NaCl, 5 mM KCl, 0.5 mM MgCl₂, 1.8 mM CaCl₂, 0.4 mM NaH₂PO₄, 0.1 mM NaEDTA, 24 mM NaHCO₃, 5.5 mM glucose) which was bubbled with a gas mixture of 95% O₂ and 5% CO₂ at pH 7.4 and ~22°C. Firstly, optimal muscle length was established evoking repeated single twitch contractions. Afterwards peak tetanic force was evaluated using 900 ms electrical stimulation at 20, 50, 80, 100, 150 and 200 Hz. Specific muscle force was calculated as peak tetanic force divided by the muscle mass.

Data analysis. The statistical analysis was performed using Prism 7.0 and IBM SPSS Statistics (v20) software. Data normality was verified using Smirnov–Kolmogorov test. Two-way analysis of variance (ANOVA) with Bonferroni’s post hoc test was applied to assess the effects of age (OLD versus YOUNG) and fasting (FASTING versus CONTROL). The level of significance was set at p < .05. All data are presented as means ± SD.

RESULTS

Body and muscle mass. Old mice were heavier than young mice (Figure 1A). After fasting, body weight loss was greater (p < .05) in young mice compared to old mice (17.0 ± 2.6 versus 12.0 ± 1.7%, respectively, Figure 1A). Fasted young group had a lower (p < .05) SOL muscle mass than control group (Figure 1B).

Figure 1. Body mass (A) and soleus (SOL) muscle mass (B) in young and old mice before and after 48h fasting (A)

Notes. Data are shown as mean ± SD. *p < .05 for differences between young groups and differences between old groups, respectively.
Muscle contractile properties. Data on force generating capacity of SOL muscle are presented in Figure 2. SOL peak force was significantly lower ($p < .05$) only in fasted old group (Figure 2A). SOL specific force was also lower ($p < .05$) only in fasted old group (Figure 2B). There were no significant differences ($p > .05$) in half-relaxation time between control and fasting groups in both young and old mice (Figure 2C). 20 Hz to 100 Hz force ratio was lower ($p < .05$) only in fasted young group compared to control group (Figure 2D).

DISCUSSION

We examined if susceptibility of SOL muscle to fasting-induced atrophy and functional decline increases with age in mice. In contradiction to our expectations, after 48 h fasting young mice lost more SOL muscle mass compared to old mice though muscle weight normalized force deceased only in old muscles. This resulted in greater functional decline of SOL muscle in old mice compared to young mice.

Food deprivation for prolonged period of time promotes protein breakdown and inhibits protein synthesis (Jagoe, Lecker, Gomes, & Goldberg, 2002). Fasting leads to an increase in plasma levels of glucocorticoids and catabolic cytokines (Crossland, Constantin-Teodosiu, Gardiner, & Greenhaff, 2017). As a consequence, there is up-regulation of muscle E3 ubiquitin ligases, MuRF-1 and atrogin-1, which in turn promote proteasome-mediated protein degradation in skeletal muscle (Egerman & Glass, 2014). The intensity of skeletal muscle wasting in catabolic conditions varies with the muscle fiber. During fasting, type I oxidative red muscle fibers are more resistant to atrophy than type II glycolytic white muscle fibers (Holeček & Mičuda, 2017; Sandri et al., 2006). It appears that SOL muscle is more resistant to 24-h-fasting-induced fiber CSA.
atrophy than EDL (Sandri et al., 2006). Our results show that fasting-induced loss of SOL muscle mass was significant only in young mice. Thus, SOL muscle of old mice shows a particular high resistance to fasting-induced atrophy.

The reduction in skeletal muscle mass often leads to reduction in muscle strength and athletic performance (Berkovich, Stark, Eliakim, Nemet, & Sinai, 2019). Interestingly, we observed a large fasting-induced decrease in SOL peak and specific force only in old mice. Our previous study has shown that fasting leads to reduction of SOL specific force in BEH+/+ and BEH mouse (Fokin et al., 2019). SOL shows high involvement in postural activities and locomotion and its impairment could have significant functional implications in elderly (i.e. increased risk of falls) (Roy et al., 1991). It appears that loss of muscle mass cannot be used as an indicator of functional decline in old mice subjected to fasting.

20 Hz to 100 Hz force ratio was decreased only in young mice after fasting. This ratio is sensitive to impairments in excitation-contraction coupling as it decreases when less calcium is released from sarcoplasmic reticulum per pulse of electrical stimuli (Westerblad, Duty, & Allen, 1993). Fasting is associated with glycogen depletion which has been linked to impairment in calcium release from sarcoplasmic reticulum (Nielsen, Cheng, Ørtenblad, & Westerblad, 2014). Collins-Hooper et al. reported that 24-h fasting was associated with a decrease in accumulation of non-contractile proteins in fast twitch muscle fibers (Collins-Hooper et al., 2015). It appears that detailed studies of calcium transients and force generation by contractile filaments are needed to explore mechanisms for a decline in the low-to-high frequency force ratio in SOL muscle of young mice and loss of specific SOL force in old mice after 48-hour fasting.

**CONCLUSION**

In summary, SOL muscle mass is more affected by 48 h fasting in young than in old mice. However, the most important finding was that fasting caused lower SOL muscle peak and specific force in old but not young mice.

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