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Effect of acute muscle contusion injury, with and without dietary fish oil, on adult and aged male rats: contractile and biochemical responses

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ABSTRACT

Aim: Contusion injury in aging muscle has not been studied in detail, but older adults are at risk for such injuries due to increased risk of falls. As falls in older populations are unlikely to be eliminated, interventions to minimize the negative impact of falls, including contusion injury should be pursued. Dietary fish oil (FO) is a common often supplement in older adults, which is associated with factors that might reduce or worsen the negative impact of contusion.
Methods: Here, we investigate whether 8 weeks of FO can blunt the impact of contusion injury in adult ($n = 14$) and aged ($n = 12$) rats. We assessed contractility and several biochemical markers in adult and aged gastrocnemius muscles 48 h post-contusion injury, using the uninjured muscles as controls.
Results: Injury reduced force production ~40% ($P < 0.001$), sarcoplasmic reticulum calcium release by ~20% ($P = 0.003$) and significantly increased several markers of muscle damage (i.e., protein carbonyls, Grp78 abundance ($P = 0.022$, 0.006, respectively)), and these injury-related changes were not affected by aging. The effects of FO were limited. A main effect ($P = 0.018$) for FO to increase the myogenic factor Myf5 was observed. In addition FO reduced the injury-associated decline in the mitophagy factor DRP1 ($P = 0.027$).
Conclusion: Although age-related differences in certain protein markers differed, aged muscles exhibited no greater acute functional deficits following injury. Similarly, while FO did not reduce functional deficits, it did not worsen them. However, changes in Myf5 and DRP1 with dietary FO suggest the potential to improve recovery from contusion injury, which should be investigated in future studies.

1. Introduction

Skeletal muscles are frequently subject to mechanical injury that results in loss of tissue function. Contusion injury occurs from a rapid, strong compressive force, typically from a direct blow or a fall (Souza and Gottfried, 2013), and can induce marked impairment of muscle function (Delos et al., 2014). While older adults are less likely to participate in the exercise and sporting activities typically associated with muscle contusions, they are at increased risk for falls. Much of the concern over falls in older adults relates to the likelihood of fracture, especially the hip fracture (Stevens and Sogolow, 2005). This concern is totally appropriate, given the poor outcomes following such fractures, but muscle injury, especially contusion, is an underappreciated consequence of falls (Rubenstein, 2006; Stevens and Sogolow, 2005). Some data indicate that older muscles are both more vulnerable to injury (i.e., show greater loss of strength) and recover from injury more slowly

Abbreviations: ANOVA, analysis of variance; Atg4b, autophagy-related protein 4 homolog B; DHA, docosahexaenoic acid; DRP1, dynamin-related protein 1; E-C, excitation-contraction; EPA, eicosapentaenoic acid; Grp78, 78-kDa glucose regulated protein, aka heat shock protein family A, member 5; HSP25, 25 kDa heat shock protein; LC3B, microtubule-associated protein 1 light chain 3 beta; MANOVA, multivariate analysis of variance; M-cadherin, muscle calcium-dependent cell adhesion protein, aka cadherin 15; MG, medial gastrocnemius muscle; MHC, myosin heavy chain; Myf5, myogenic factor 5; MyoD, myoblast determination protein; p62, ubiquitin-binding protein p62/sequestosome1; Parkin, E3 ubiquitin-protein ligase parkin; S-D, Sprague-Dawley; SOD1, cytoplasmic Cu/Zn superoxide dismutase; SOD2, mitochondrial manganese-dependent superoxide dismutase; SR, sarcoplasmic reticulum; TBS, tris-buffered saline; TBS-T, tris-buffered saline plus Tween20; TFAM, mitochondrial transcription factor A

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(Alway et al., 2013; Baehr et al., 2016; Morris et al., 2004). Although there is a dearth of quantitative muscle function data related to contusion injury in humans, these factors are of major importance to older individuals who are likely to show greater loss and slower recovery of physical function for a given period of inactivity than younger adults, even if the degree of injury is equal (Hui and Rubenstein, 2006). Thus, contusion injury following a fall by an older adult risks a double impairment of muscle function: the injury itself, and the disuse during recovery. Poor recovery of muscle function following a fall-related contusion injury could contribute to the well-established increased risk of falling in patients who have already had a history of at least one clinically-significant fall (Marrero et al., 2017; Rubenstein, 2006).

To date, few studies of muscle contusion injury in aging are available (Ghaly and Marsh, 2010; Jarvinen et al., 1983; Pertille et al., 2012), and none of these studies include any functional (i.e., contractile) measures. One purpose of this study was therefore to report the first data, to our knowledge, on the response of aged muscle to contusion injury. Standard treatment of contusion injuries involves anti-inflammatory measures such as ice and non-steroidal anti-inflammatory drugs (NSAIDS), though the benefits of NSAIDs remain unclear, particularly after the acute phase (Mackey et al., 2012). In aging muscles, standard NSAID treatments could also be less effective, as age-associated increases in oxidative stress have been found to increase the inflammatory response following contusion injury (Ghaly and Marsh, 2010). Dietary fish oil (FO), which contains high levels of DHA, could be an effective intervention to reduce the impact of contusion injury in aging muscles, given that DHA has been found to reduce some markers of inflammation and damage in muscle following eccentric exercise (Dilorenzo et al., 2014). Moreover, multiple health benefits in aging have been ascribed to dietary FO, though not increased longevity (de Magalhaes et al., 2016). This offers the potential to address several age-related risks with one intervention. However, FO is also associated with significant anti-coagulant and pro-oxidant effects (Feillet-Coudray et al., 2013; Feng et al., 2012; Stanger et al., 2012) that could potentially worsen a contusion injury. Accordingly, another purpose of this study was to provide a more detailed assessment of the effects of dietary FO on contusion injury. Because of concerns that FO might exacerbate the injury, we chose to examine acutely injured (48 h post injury) muscles, rather than a longer course of recovery.

While there has been much focus on strategies for falls prevention, it is unrealistic to believe that falls can be eliminated in older individuals. It is therefore essential to identify interventions in older adults to minimize the negative consequences of falls, including muscle contusion. In this exploratory study, we assessed the effects of dietary FO on muscle contractile function following contusion injury in adult and aged rats. To our knowledge, this is the first study to do so. In addition, we assessed changes in sarcoplasmic reticulum (SR) Ca^{2+} release. Impairment of this key step in excitation-contraction (E-C) coupling had been found to closely follow the time course of muscle force loss and recovery following eccentric muscle injury (Ingalls et al., 1998), though the finding is not universal (Nielsen et al., 2007). As our laboratory (Russ et al., 2011; Russ et al., 2015b; Russ et al., 2014) and others (Gaboardi et al., 2018) have found that SR Ca^{2+} release is reduced with age in uninjured muscles, we hypothesized that Ca^{2+} release might be reduced more by injury in aging muscle. Furthermore, we have reported that age-related changes in the lipid composition of SR membranes are associated with loss of the Ca^{2+} release of the isolated SR (Russ et al., 2015a, 2015b). As dietary FO and injury are known to alter membrane lipid composition (Helge et al., 2001; Henry et al., 2015), it was hypothesized that FO might mitigate post-injury impairment of SR function. Finally, we chose to examine several biochemical and molecular markers of processes related to injury to determine possible mechanisms by which dietary FO could mitigate the negative consequences of muscle contusion. As there is little work in this area, we chose to evaluate markers associated with a broad spectrum of responses to injury, rather than examining one specific pathway or mechanism in great detail.

2. Materials & methods

2.1. Experimental design

2.1.1. Experimental animals

Adult (7 months, $n = 14$) and aged (22 months, $n = 12$) Sprague-Dawley (S-D) rats were assigned to either control (Ctl) or fish oil (FO) supplemented diets ($n = 7$ adult and 6 aged rats per diet). Following arrival, rats had *ad libitum* access to standard rat chow (Harlan no. T8640 Teklab 22/5) and water for a 1 week acclimation period. Body mass and mass of food disappearance (as an estimate of dietary intake) were measured weekly. Animal use and all procedures were approved by the Ohio University Institutional Animal Care and Use Committee, and the Principles of Laboratory Animal Care (National Institutes of Health publication no. 86–23, revised 1985) were followed throughout the study.

2.1.2. Dietary intervention

Following the 1-week acclimation, rats were ranked based on food disappearance, then assigned to 1 of 2 dietary interventions, balanced for food disappearance. Rats in the control group were fed AIN-93 M purified diet, *ad libitum*. Rats in the FO group were fed AIN-93 M diet supplemented with 33.65 g FO/kg diet. Soybean oil comprised the remaining amount of fat for total 40 g fat per 1 kg diet, or 4% of total weight. Previous studies of aged S-D rats from our laboratory indicate average body mass of 550 g and food intake ~20 g/d (Russ et al., 2017). Based on these data, we estimated a FO dose of ~1.22 g/kg body mass/day. Diets were prepared by Research Diets Inc. (New Brunswick, N.J., USA) and were color-coded. Investigators were blinded to the color of the diets. The FO for the diet was provided by Abbott Nutrition (Columbus, Ohio, USA). Food was replaced weekly, at which time the remaining pellets were weighed to measure food disappearance.

2.1.3. Contusion injury protocol

Both adult and aged animals were subjected to an experimental contusion injury protocol at the conclusion of the dietary intervention. Animals anesthetized by isoflurane were injured by dropping a known weight from a fixed height onto a rounded impactor bar contact with the medial gastrocnemius (MG) muscle (Fig. 1) while the hindlimb was held with the knee in full extension. This method of blunt injury is considered more similar to human muscle contusion injuries than other blunt trauma models, such as crush injury (Souza and Gottfried, 2013). The specific protocol used here is derived from previous work (Delos et al., 2014) indicating that the induced injury rapidly and markedly reduces muscle force production, and shows ~85–90% recovery of force after 2 weeks (in young rats). Contractile testing and tissue harvest occurred 48 h after injury. We chose this time for acute injury assessment as mechanically-injured muscles typically exhibit their greatest deficits between 24 and 72 h post injury.

2.2. Dependent variables

2.2.1. Hindlimb muscle contractile testing

In situ contractile testing of the MG was conducted in a manner similar to that we have published previously (Russ et al., 2014). Because of the time involved with testing both the injured and uninjured limbs in each animal, we evaluated responses to twitch and tetanic (100 Hz, 500 ms train) stimulation, rather than a full force-frequency relationship. Contractile forces were recorded and stored using Spike2 software (CED, Cambridge UK). Following contractile testing, muscles were rinsed in ice-cold physiological saline, blotted dry and weighed prior to processing or freezing in liquid nitrogen.