Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

The effect of caloric restriction on the forelimb skeletal muscle fibers of the hypertrophic myostatin null mice

Mohamed I. Elashry[c,d,*], Antonios Matsakas[b], Sabine Wenisch[e], Stefan Arnhold[d], Ketan Patel[a]

[a] School of Biological Sciences, Hopkins Building, Whiteknights, University of Reading, RG6 6UB, United Kingdom
[b] Hull York Medical School, University of Hull, United Kingdom
[c] Anatomy and Embryology Department, Faculty of Veterinary Medicine, University of Mansoura, 35516, Egypt
[d] Institute of Veterinary Anatomy, Histology and Embryology, Justus Liebig University of Giessen, 35392, Germany
[e] Clinic of Small Animals, c/o Institute of Veterinary Anatomy, Histology and Embryology, Justus Liebig University of Giessen, 35392, Germany

ARTICLE INFO

Keywords:
Skeletal muscle
Myostatin
Food restriction
Myosin heavy chain

ABSTRACT

Skeletal muscle mass loss has a broad impact on body performance and physical activity. Muscle wasting occurs due to genetic mutation as in muscular dystrophy, age-related muscle loss (sarcopenia) as well as in chronic wasting disorders as in cancer cachexia. Food restriction reduces muscle mass underpinned by increased muscle protein break down. However the influence of dietary restriction on the morphometry and phenotype of forelimb muscles in a genetically modified *myostatin* null mice are not fully characterized. The effect of a five week dietary limitation on five anatomically and structurally different forelimb muscles was examined. C57/BL6 wild type ($Mstn^{+/+}$) and *myostatin* null ($Mstn^{-/-}$) mice were either given a standard rodent normal daily diet *ad libitum* (ND) or 60% food restriction (FR) for a 5 week period. *M. triceps brachii Caput laterale* (T.lateral), *M. triceps brachii Caput longum* (T.long), *M. triceps brachii Caput mediale* (T.medial), *M. extensor carpi ulnaris* (ECU) and *M. flexor carpi ulnaris* (FCU) were dissected, weighted and processed for immunohistochemistry. Muscle mass, fibers cross sectional areas (CSA) and myosin heavy chain types IIB, IIX, IIA and type I were analyzed. We provide evidence that caloric restriction results in muscle specific weight reduction with the fast myofibers being more prone to atrophy. We show that slow fibers are less liable to dietary restriction induced muscle atrophy. The effect of dietary restriction was more pronounced in $Mstn^{-/-}$ muscles to implicate the oxidative fibers compared to $Mstn^{+/+}$. Furthermore, peripherally located myofibers are more susceptible to dietary induced reduction compared to deep fibers. We additionally report that dietary restriction alters the glycolytic phenotype of the $Mstn^{-/-}$ into the oxidative form in a muscle dependent manner. In summary our study shows that calorie restriction alters muscle fiber profile of forelimb muscles of Myostatin null mice.

1. Introduction

Skeletal muscle the main protein reservoir in the body, is a highly adaptable tissue that changes its physical as well as composition based on physiological demands. Mechanical and nutritional stimuli cause an increase in muscle mass. In contrast undernutrition, aging and diseases as cancer cachexia reduces the muscle mass. Unbalanced nutrient intake with increased energy requirements enhances the muscle catabolism resulting in fiber atrophy and muscle mass loss (Koskelo et al., 1990). Muscle loss also occurring in progressive wasting diseases e.g. cancer cachexia (Bruggeman et al., 2016) and age-related sarcopenia leads to a decrease in muscle mass and strength (Thomas, 2007). Additionally the role of physiological and metabolic stimuli e.g. physical activity, disuse and immobilization, food restriction, drugs and diseases cause molecular and cellular dysregulation which results in loss of muscle mass (Carmeli and Reznick, 1994). It has been reported that ageing induces muscle mass loss of 16%, 18%, 37% and 38% for soleus, extensor digitorum longus, plantaris and gastrocnemius muscles respectively with greater fiber area loss compared to the decrease in body mass (Brown and Hasser, 1996). Similarly it was reported that dietary restriction causes a decrease in muscle fiber size but not fiber number in chicken and rabbits (Tanaka et al., 1992; Timson et al., 1983). In addition the glycolytic fibers were selectively decreased in the cross sectional area lead to a proportional increase in the area of oxidative fibers in bovine muscle (Greenwood et al., 2009). Muscle phenotype also changed according to the dietary challenges, It has been reported that dietary deprivation for 48 h upregulates the expression of fast myosin heavy chain 2b mRNA with no change in fiber type composition for EDL

* Corresponding author at: Institute of Veterinary Anatomy, Histology and Embryology, Justus Liebig University of Gießen, Frankfurter Str. 98, 35392, Gießen, Germany.
E-mail address: mohammed.elashry@vetmed.uni-giessen.de (M.I. Elashry).

http://dx.doi.org/10.1016/j.acthis.2017.06.002
Received 12 April 2017; Received in revised form 2 June 2017; Accepted 5 June 2017

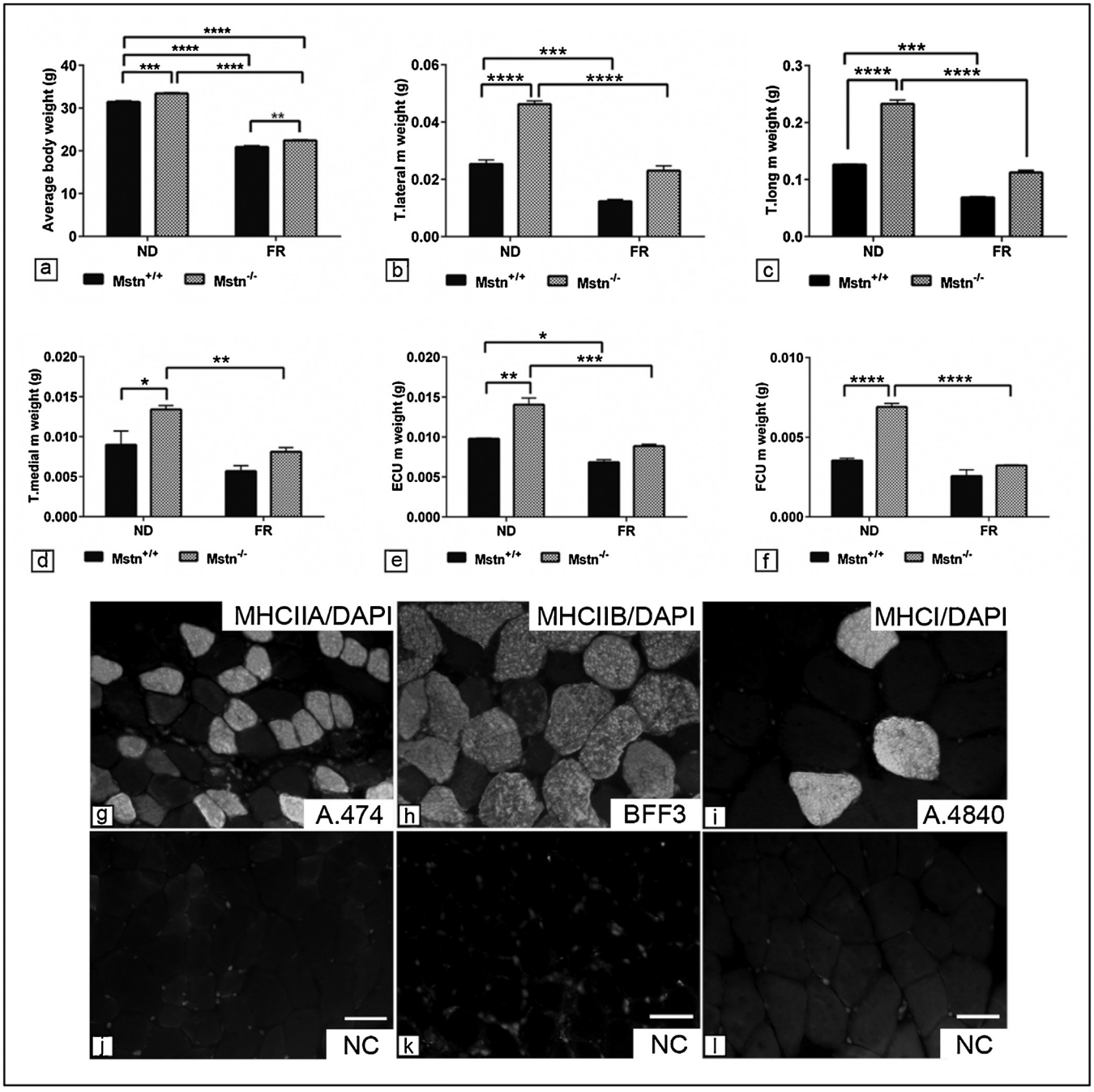

Fig. 1. Dietary restriction induces body weight and muscle mass specific reduction.
a Average body weight (g) for normal diet (ND) and food restricted (FR) *Mstn*$^{+/+}$ and *Mstn*$^{-/-}$ mice (N = 4 for all groups). Significant increase in the *Mstn*$^{-/-}$ ($P < 0.001$) compared to the *Mstn*$^{+/+}$ on normal diet. FR decreases the body weight for *Mstn*$^{+/+}$ and *Mstn*$^{-/-}$ compared to genotype-matched ND mice. **b-f** Average muscle weight (g) for **b** T.lateral, **c** T.long, **d** T.medial, **e** ECU, and **f** FCU muscles. Specific muscle mass increases in *Mstn*$^{-/-}$ ND compared to *Mstn*$^{+/+}$ ND. FR induces more muscle mass reduction in T.lateral and T.long muscles of *Mstn*$^{-/-}$ (diet and genotype interaction P < 001 and 0.0001) respectively compared to genotype matched control. *Mstn*$^{-/-}$ display more muscle loss in T.medial, ECU and FCU muscles compared to *Mstn*$^{+/+}$ following FR. **g-l** Immunofluorescent images for T.long muscle of *Mstn*$^{+/+}$ND against A.474, BFF3 and A.4840 specific MHC antibodies show **g** type IIA^{+}, **h** type IIB^{+} and **i** type I^{+} fibers compared to **j**, **k** and **l** the corresponding negative control (NC) respectively. DAPI was used to visualize the myonuclei. All values displayed as mean ± SEM. * = P < 0.05, ** = P < 0.01, *** = P < 0.001 and **** = P < 0.0001). Scale bar in **g-l** = 20 μm.

and soleus muscles in rat (Mizunoya et al., 2013b). However 4 weeks fatty diet administration induced reduction in the fast MHC2b and improved the oxidative metabolism in the EDL of rat (Matsakas et al., 2013; Mizunoya et al., 2013a). Previously, it was shown that fasting induces muscle proteolysis via glucocorticoid activation (Wing and Goldberg, 1993) as well as causing an increase in the ATP-dependent proteolysis and upregulation of ubiquitin conjugates and polyubiquitins in rat skeletal muscle (Medina et al., 1991).

Myostatin is a TGF-β family member of secreted proteins that negatively regulates skeletal muscle proliferation (Sharma et al., 2001). *Myostatin* protein interference via knock out results in significant myofiber hyperplasia and hypertrophy (McPherron et al., 1997). Furthermore, systemic administration of *Myostatin* induced muscle atrophy in mice (Zimmers et al., 2002). Increased myostatin expression was also associated with muscle wasting in cancer cachexia, ageing, after HIV infection and in the course of chronic obstructive pulmonary disease (COPD) (Costelli et al., 2008; Gonzalez-Cadavid et al., 1998; Plant et al., 2010; Yarasheski et al., 2002). *Myostatin* knock out mice showed