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Oswald Biological Sciences Second Place: RNA Degradation is Elevated with Age-, but not Disuse-Associated Skeletal Muscle Atrophy

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RNA Degradation is Elevated with Age-, but not Disuse-Associated Skeletal Muscle Atrophy

Abstract

Aging and inactivity are both associated with decreased muscle size and protein content. The possible role of RNA degradation in the loss of protein has not yet been investigated. Therefore, we hypothesized that RNA degradation was elevated with muscle atrophy in aging and disuse. Brown Norway/Fisher344 male rats at 6 and 32 months were hindlimb suspended (HS) for 14 days to induce muscle atrophy or remained weight bearing (WB). Cytosolic extracts from gastrocnemius muscles were prepared for Western analysis of DCP-2 protein (marker of p-bodies) and RNA degradation assay. In vitro total RNA decay assay was performed using 30ug of total RNA (from tibialis anterior) incubated with 20ug of S15 extracts from gastrocnemius. RNA integrity was determined using the Agilent Technologies algorithm to calculate the RNA Integrity Number (RIN); decay rate and half-life were calculated for each sample. Results indicated an increase in DCP-2 protein at 32 months of age in both HS and WB groups. In addition, an almost 2-fold increase in decay rate and 48% decrease in half-life of total RNA was observed in muscle from 32 month old rats. However, no significant difference in decay rate and half-life was observed with disuse at either 6 or 32 months. We conclude that muscle atrophy associated with aging, but not disuse, may be due to a decrease in total RNA because of increased RNA degradation.

Introduction

Skeletal muscle atrophy occurs with disuse and during aging and the loss of muscle protein is the main determinant of this decrease in muscle size. The relative contribution of decreased protein synthesis or increased degradation in the loss of muscle size are under active investigation. It is currently unclear whether RNA degradation plays a role in the regulation of muscle protein homeostasis and if it is changed under muscle atrophy conditions. Therefore, the goal of this study was to determine if changes in RNA degradation occur in muscles of different aged rats undergoing acute disuse atrophy. We hypothesize that RNA degradation is elevated with age- and disuse-induced skeletal muscle atrophy.

Materials and Methods

Animals: Brown Norway/Fisher 344 male rats of 6 and 32 months of age were used for all experiments. Rats were hindlimb suspended (HS) for 2 weeks or were allowed to remain weight bearing (WB). At the end of the experiment rats were humanely euthanized and gastrocnemius and tibialis anterior muscles were dissected and frozen at -80ºC until further use.

Total RNA isolation: RNA was isolated from tibialis anterior using Totally RNA (Ambion) and stored at -80ºC until analysis.

Cytosolic (S15) Extract Preparation: 100mg gastrocnemius was homogenized and centrifuged to obtain a post-mitochondrial (S15) cytosolic fraction .

In-Vitro RNA Decay Reaction: In-vitro RNA decay was measured by incubating 30mg of total RNA with 20mg of S15 extracts at 37^0C for five time points (0, 3, 6, 9, and 12 minutes). RNA decay was stopped by adding phenol after each time point. Remaining RNA was extracted using phenol/chloroform, resuspended in DEPC water, and stored at -80ºC until analysis.

Decay Rate and Half-Life Determination: RNA integrity was determined at each time point using the Agilent Technologies algorithm to calculate the RNA Integrity Number (RIN). The decay rate and half-life were calculated for each sample using the RIN value and the logarithmic decay rate calculation.

Western Analysis: Protein extracts from gastrocnemius muscles were prepared in RIPA buffer and 30mg of total protein was loaded and run on 4-15% gradient Tris-HCl gels. Proteins were transferred to PVDF-FL membranes and incubated with DCP2 antibody overnight at room temperature (1:1,000; Abcam) followed by secondary antibody incubation at room temperature for 30 minutes (1:30,000, LI-COR Biosciences). Actin antibody (1:1,000; Santa Cruz) was used as a loading control. Protein abundance of DCP2 was measured using image-based Odyssey software and expressed as arbitrary density units normalized to Actin.

Statistical Analysis: Values are depicted as means ± SE. Two-way ANOVA followed by Holm-Sidak post-hoc test was used to determine differences between samples. Statistical significance was assumed at $p < 0.05$. * indicates statistical significance.

Results

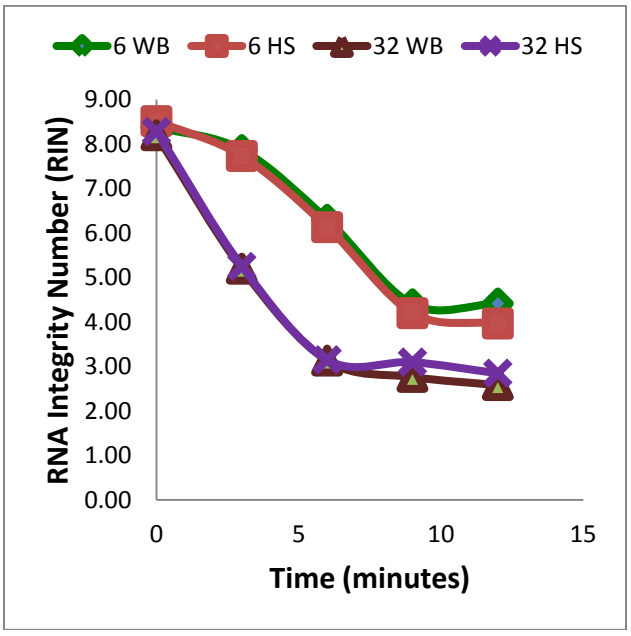

Figure 1: Change in RIN over time with age and disuse. Change in RIN over five time points of the WB and HS rats is depicted.

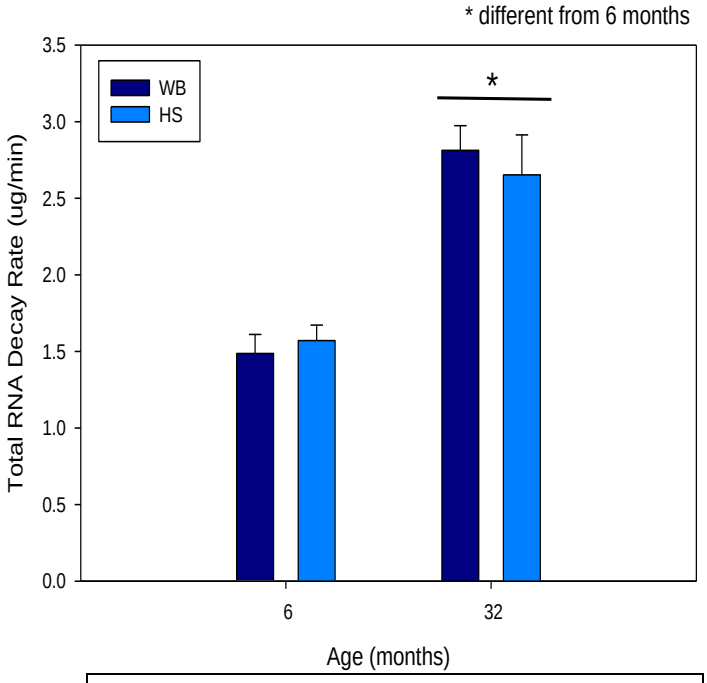

Figure 2: Total RNA decay rate significantly increased with age but not disuse. Representative RNA decay rate of WB and HS rats is depicted. * Indicates main effect for age.

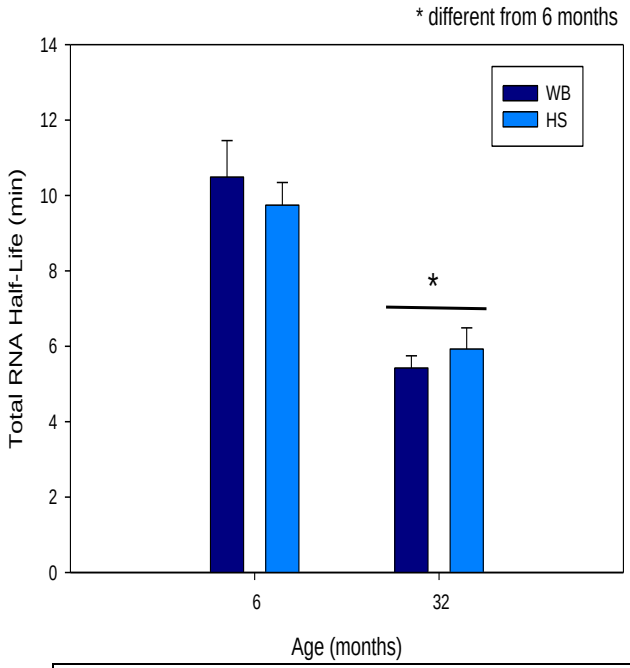

Figure 3: Total RNA half-life significantly decreased with age but not disuse. Calculated half-life of total RNA from WB and HS rats is depicted. * indicates main effect for age.

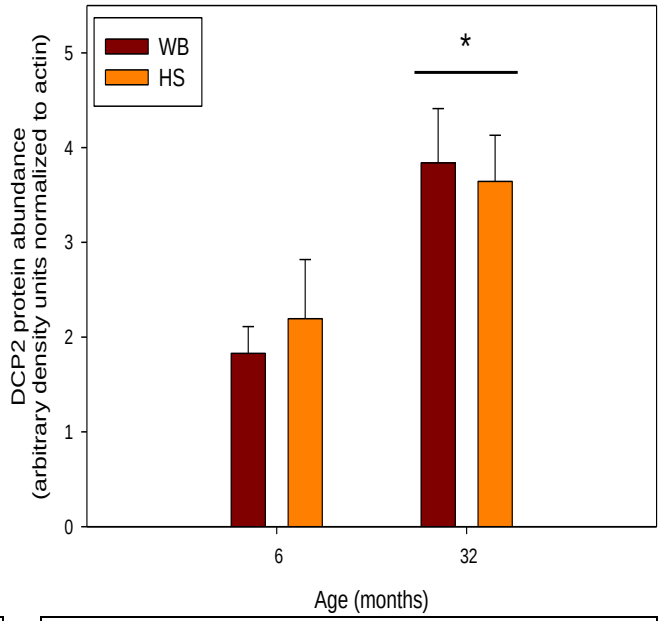

Figure 4: DCP2 protein abundance increased with age but not disuse. Representative DCP2 protein abundance in the WB and HS rats is depicted. * indicates main effect for age.

Below is the summary of the results above:

- RNA Integrity Number (RIN), a measure of RNA quality, decreased over time in gastrocnemius muscle from old rats (32months) at a faster rate, independent of disuse status (HS).
- There is a 2-fold increase in total RNA Decay rate in gastrocnemius muscles from old rats (32 months).
- There is a 48% decrease in half-life of total RNA in the muscle from old rats (32 months).
- The total RNA Decay rate and half-life are not changed with disuse (HS) of the muscle.
- The Decapping Protein-2 (DCP-2), one of proteins associated with RNA degradation, is significantly elevated in the muscle of old rats (32 months), but is not different between weight bearing (WB) and hindlimb suspension (HS) at either age.

Conclusion

The results from this study indicate that muscle atrophy associated with age, but not disuse of the muscle, may result from increased total RNA degradation, which likely leads to decreased RNA available for protein synthesis.

Acknowledgement

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