MUSCLE STIFFNESS AND RESPONSE TO EXERCISE IN CALORIC RESTRICTED AND AD LIBITUM-FED ELDERLY RATS

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INTRODUCTION

It is well known that the strength and size of skeletal muscle decrease significantly with age. This may be partly a result of elderly muscle having an impaired response to exercise, indicated by reduced activation of biochemical pathways responsible for muscle growth after resistance training compared to younger muscle [1,2]. Furthermore, it is known that advanced glycation end products accumulate in the muscle extracellular matrix of aged skeletal muscle [3] increasing tissue stiffness. It has been hypothesized that mechanotransduction mechanisms required for myocytes to sense mechanical stimuli may be compromised by this increased stiffness and this impairment may explain the reduced response to exercise in elderly muscle. Calorie restriction has been shown to lower levels of glycation compared to ad libitum-fed rats [4]. The purpose of this study was to compare caloric restricted rats to ad libitum-fed rats in order to test whether the activation of pathways responsible for muscle growth in response to exercise is increased in caloric restricted rats compared to age-matched controls and to determine if this response is related to muscle stiffness.

METHODS

This study was a pilot study involving five elderly male F344 rats age-matched to a 40% survival rate. Three rats were fed a lifelong caloric restricted diet, and two rats were fed ad libitum diets. All procedures were approved by the Texas Tech Institutional Animal Care and Use Committee. To perform resistance exercise, rats were first anesthetized and their left foot placed in the footplate of a rat dynamometer (Figure 1). Electrodes were placed subcutaneously to span the peroneal nerve to stimulate contraction of the dorsiflexor muscles. Rats performed three sets of ten maximum eccentric contractions of the plantar flexors and were immediately euthanized for muscle harvesting.

Material stiffness measurements of the right extensor digitorum longus (EDL) muscle were taken within one hour of sacrifice using a custom-built tensile tester. Muscle samples were positioned with approximately 25% of the muscle belly length in each clamp. The specimen was lengthened until a resistive force was developed, and the gauge length in this position was used as $l_0$. Force measurements were recorded as the specimen was lengthened until midbelly failure. CSA of the muscle was estimated as an ellipse using width and thickness measurements of the muscle midbelly when laid flat. Force was normalized to CSA, and length was normalized to $l_0$ to create a stress-strain curve. The Young’s Modulus was calculated as the slope of a linear trend line fit to the roughly linear region of this curve (Figure 2).
The right and left tibialis anterior (TA) muscles were isolated immediately after sacrifice and frozen in liquid nitrogen. A measure of response to exercise was obtained by quantifying the activation of focal adhesion kinase (FAK), a membrane protein critical in mediating cellular responses to mechanical loading, including cell growth [5]. Activation of FAK was quantified by measuring the percentage of FAK that was phosphorylated using immunoprecipitation, western blotting, and immunodetection analysis.

**RESULTS AND DISCUSSION**

The EDLs of the caloric restricted rats appear to be less stiff than those of the ad libitum-fed rats (Table 1). Because caloric restriction is known to lower levels of glycation compared to an ad libitum diet, the reduced muscle stiffness in the caloric restricted rats may be due to less glycation and crosslinking of the extracellular matrix.

Analysis of FAK phosphorylation in the tibialis anterior shows that ad libitum-fed rats displayed little far less FAK activation directly following resistance exercise compared to caloric restricted rats. Additionally, the phosphorylation percentage seems to decrease as stiffness increases.

**CONCLUSIONS**

Caloric restricted rats displayed decreased skeletal muscle stiffness as well as substantially increased activation of FAK following resistance exercise compared to ad libitum-fed rats. The reduced muscle stiffness in caloric restricted rats may be a result of decreased extracellular matrix glycation, and this decreased stiffness may explain their increased sensitivity to mechanical loading of the muscle. Although this pilot study cannot offer any firm conclusions, it provides support for further investigation into the effects that muscle stiffness may have on mechanotransduction and elderly muscle’s decreased ability to respond to exercise as well as support for caloric restriction as a means to modulate muscle stiffness.

**REFERENCES**


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