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Appendix

Titin Molecular Weight Determination

Titin molecular weight was quantified with a previously developed method utilizing SDS-VAGE[26]. Protein homogenates were prepared from frozen samples and loaded into 1% agarose gels. A small 12.8% acrylamide plug was placed at the bottom of the gel apparatus to hold the agarose gel in place. Human soleus and rat cardiac muscles were used as titin standards. Gels were run two at a time at 25 mA for 4.5 hours at 4°C. Gels were stained according to the Bio-Rad Silver Stain Plus kit protocol, and bands were identified and quantified with densitometry (Quantity One; Bio-Rad).

Collagen Content

Hydroxyproline content was used to determine the collagen content (μg collagen/mg wet weight tissue) of the supraspinatus and infraspinatus muscles with use of a modification of a previously published protocol[27]. Tissue samples were hydrolyzed in 6-mol/L HCl at 110°C overnight and neutralized with NaOH to pH 6.98 to 7.04. Samples were then incubated with a chloramine-T solution for twenty minutes at room temperature, followed by addition of a *p*-dimethylaminobenzaldehyde solution and incubation at 60°C for thirty minutes. The hydroxyproline concentration was determined with spectrophotometry at 550 nm and was normalized to the wet mass of the original tissue sample. Standard solutions provided a calibration curve for spectrophotometry. The measured hydroxyproline content was used to calculate the collagen amount by using the constant 7.46, corresponding to the average number of hydroxyproline residues in a collagen molecule[28]. ■