SHORT REPORT

ABSTRACT: We measured titin and nebulin content in muscle biopsies from the vastus lateralis before and 24 h after one bout of high-intensity eccentric knee extensor resistance exercise in seven men (26 ± 3 years). Titin and nebulin content were significantly ($P < 0.05$) reduced after exercise by 30 and 15%, respectively. These results suggest that the structural components of the myofibrillar apparatus are degraded following high-intensity eccentric resistance exercise in humans. Loss of these proteins may have important implications for the mechanisms regulating the adaptive response of skeletal muscle to resistance exercise.

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TITIN AND NEBULIN CONTENT IN HUMAN SKELETAL MUSCLE FOLLOWING ECCENTRIC RESISTANCE EXERCISE

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Historically, myosin and actin have been the primary proteins studied with regard to skeletal muscle structure and function. However, recent studies of the myofibrillar proteins titin (also known as connectin) and nebulin have shown that these two proteins play a significant role in the structure and function of the sarcomere and ultimately the whole muscle. The megadalton protein titin anchors the myosin filament to the Z-disk, contributing to the microscopically observed thick filament.[13,18] Nebulin, along with actin, forms the thin filament.[13,17] In addition to their structural role as a scaffolding for the myofilament, titin and nebulin are involved in the development of the sarcomere, and in the production of active and passive tension in the muscle.[8,9,13,18,19]

Exercise involving an eccentric component typically induces muscle damage and ultrastructural disorganization, as observed by light and electron microscopy.[2,5,10,12,16] Various subcellular components of the muscle are damaged during eccentric loading, including the sarcolemma, cytoskeleton, and myofibril. The most common observation of myofibril disruption is Z-disk streaming and smearing.[2,5,12] Given that titin and nebulin anchor myosin and actin, respectively, to the Z-disk, these microscopic findings suggest that titin and nebulin may be damaged following exercise that induces muscle damage. In support of this notion, qualitative immunohistochemical assessments of the muscle ultrastructure following muscle damage show that in areas of the sarcomere which were greatly disorganized, titin labeling was absent or irregular.[2] The absence of titin labeling in areas of sarcomeric disorganization is also seen in vastus lateralis muscle from individuals with Duchenne muscular dystrophy.[3]

If titin and nebulin are damaged following eccentric exercise, these proteins would be subject to removal from the sarcomere via proteolysis. Therefore, we measured the titin and nebulin content of the vastus lateralis before, and 24 h after, eccentric resistance exercise in humans. We hypothesized that

Abbreviations: TEMED, tetramethylethylenediamine
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a single bout of high-intensity eccentric resistance exercise would induce a reduction in the contents of titin and nebulin.

MATERIALS AND METHODS

Subjects. Seven recreationally active men (26 ± 3 years, 180 ± 5 cm, 87 ± 24 kg, and 17 ± 5% body fat; mean ± SD) were accepted into the study after giving informed consent and following a screening for any metabolic abnormalities via a blood and urine analysis, and medical history questionnaire. The investigation was approved by the Institutional Review Board of the University of Arkansas for Medical Sciences.

Eccentric Exercise Protocol. Each subject completed a bout of unilateral high-intensity eccentric exercise with each leg. The maximal load that each subject could lift concentrically with their knee extensors was first determined and the eccentric workload was set to 120% of the concentric maximum. The eccentric exercise consisted of 10–13 sets of 10 repetitions with 60 s rest between sets of knee extensor exercise on a muscle dynamometer in the isotonic mode (Cybex Norm, Lumenex, Ronkonkoma, New York). The range of sets occurred as a result of the variation in fatigue of the muscles of each subject. When the weight was lowered in less than 0.5 s, the subject completed that set, was deemed fatigued, and the protocol was stopped.

Muscle Biopsy. Muscle biopsies were taken from the vastus lateralis of the dominant leg before the eccentric exercise protocol and from the nondominant leg ~24 h after the eccentric exercise protocol.[1] The muscle was cleansed of excess blood, connective tissue, and fat, and immediately frozen and then stored in liquid nitrogen (−190°C) until analysis.

Titin and Nebulin Measurement. Titin and nebulin quantification was completed using methods adapted from Granzier and Wang.[7] Frozen tissue samples weighing approximately 10 mg were powdered using a stainless steel tissue pulverizer that was pre-cooled in liquid nitrogen. Powdered tissue was transferred directly into 30 volumes (w/v) of hot (60°C) homogenizing buffer consisting of 10 mM Tris-Cl, 1 mM EDTA, 1% SDS, 10% glycerol, 40 mM DTT, and 30 μg/ml pyronin Y. The sample was homogenized at 60°C by hand using a 1-ml ground-glass homogenizer. The homogenate was transferred to a 1.5-ml microcentrifuge tube and centrifuged at room temperature at 2,000*g* for 1 min. The homogenous supernatant was then aliquoted, frozen in liquid nitrogen, and stored at −80°C. Protein concentrations were determined using the BioRad (Hercules, California) protein assay with bovine serum albumin standards.

Titin and nebulin were separated using a polyacrylamide gel electrophoresis apparatus (Hoeffer SE 600, Amersham Pharmacia Biotech, Piscataway, New Jersey) and methods adapted from Fairbanks et al.[4] The buffering system for running gels was 40 mM Trizma acetate, 20 mM sodium acetate, 2 mM EDTA, and 0.1% SDS, pH 7.4. Gels consisted of the same running buffer with 3% polyacrylamide (50:1 acrylamide to bisacrylamide), 0.1% ammonium persulfate, and 0.1% tetramethylethylenediamine (TEMED), and were allowed to polymerize overnight. A total of 30 μg total protein per lane was loaded on the gel. Gels were run at 15°C and 25 mA per gel for 12 h. Separated proteins were then visualized with the gel remaining on the glass plate using a Coomassie-based staining reagent (GelCode Blue, Pierce, Rockford, Illinois), which has been used previously to quantify titin and nebulin.[11,20] Gels were digitally photographed (Eagle Eye II, Stratagene, La Jolla, California), and densitometry was completed using the NIH Image software (Ver. 1.60). Each gel was loaded with four lanes representing pre-exercise state and four lanes representing post-exercise state for each subject. An average of the densities of the appropriate four lanes was taken to represent the pre- and post-exercise titin and nebulin levels, respectively. We were unable to normalize our data to a control protein in the pre-and post-exercise samples because, to our knowledge, there is no protein that is known not to change with the exercise regimen used in this study and that is found in the molecular weight range of titin and nebulin. However, all of the muscle samples were obtained, frozen, stored, and processed identically.

Statistics. Titin and nebulin content and the ratio of titin to nebulin before and after exercise were compared using a paired *t*-test. Pearson product correlation was used to compare the change in titin to the change in nebulin from before to after exercise. Data are presented as mean ± SE, and significance was accepted at a level of $P < 0.05$.

RESULTS

Both titin and nebulin were decreased ($P < 0.05$) following the eccentric exercise bout (Fig. 1). Titin was reduced by 30% (2,080 ± 450 to 1,098 ± 168 density units) and nebulin was reduced by 15% (3,898 ± 517 to 3,264 ± 449 density units). The ratio of titin to nebulin was reduced ($P < 0.05$) from pre-

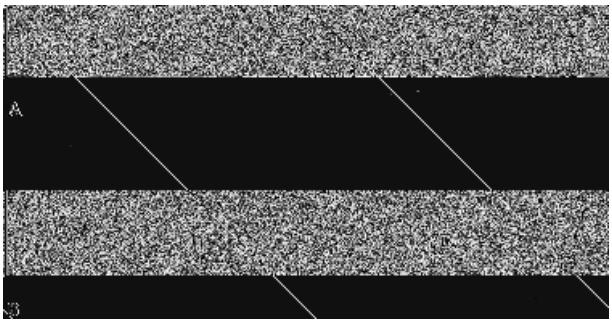

FIGURE 1. Representative Coomassie-stained gel for determining titin **(A)** and nebulin **(B)** protein levels before and after high-intensity eccentric resistance exercise. Each pair of lanes represents pre-exercise (left) and post-exercise (right) states. Each sample was run in quadruplicate and averaged to represent the value of pre- and post-exercise states for each subject.

to post-exercise (0.61 ± 0.16 to 0.33 ± 0.03). However, the loss of titin was not associated with the loss of nebulin ($P > 0.05$).

DISCUSSION

The results of the current study demonstrate that the myofibrillar proteins titin and nebulin are decreased, via either direct damage or degradation, following eccentric resistance exercise in humans. These data are supported by the findings that mixed skeletal muscle protein degradation (i.e., when all of the muscle proteins are considered together) and 3-methylhistidine excretion, a component of actin and myosin that reflects breakdown of these two myofibrillar proteins, are elevated following resistance exercise.[14,15] However, it is also possible that, following the exercise bout, the synthesis rates of titin and nebulin, relative to the synthesis rates of other muscle proteins or relative to the change in degradation rates of titin and nebulin, were decreased. This relative alteration in synthesis rate could then give rise to a reduction in the amount of these two proteins in the muscle.

Fry et al.[6] have shown that human muscle contains two isoforms of titin, titin-1 and titin-2. Not all subjects, however, expressed both isoforms of titin, and the expression of one or two isoforms was independent of the muscle (i.e., vastus lateralis, gastrocnemius, or soleus), and muscular strength and endurance. In the current study, we did not attempt to differentiate which subjects expressed titin-1 and titin-2, nor did we determine which isoform(s) were altered with the eccentric protocol. Implications of alterations in the content or percent distribution of the isoforms of titin in human skeletal muscle are unclear. Studies of cardiac muscle from failing human hearts have shown an additional titin isoform that is not present in normal heart muscle.[11]

In summary, these findings suggest that the myofibrillar damage that is typically induced with eccentric resistance exercise involves the loss of titin and nebulin. Titin isoform-specific changes and the time course in the loss and restoration of titin and nebulin following eccentric exercise in humans would provide important information into the mechanisms of muscle degradation, regeneration, and adaptation to resistance exercise.

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