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The adaptation and changes of titin system following exercise training

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ABSTRACT

Titin is a large structural protein in muscle that has the ability to store elastic energy. The specific structure of titin and its elastic nature has recently become better understood. In addition, the utilization of stored elastic energy in human movement and the significant contribution of not only tendon but muscle tissue itself to this process has been re-evaluated. In this study, we reviewed the effects of exercise training on titin system (titin expression and titin-complex proteins).

Keywords: Titin, titin-complex proteins, exercise training

The giant sarcomeric protein titin is 3-4 MDa and spans the sarcomere from Z-line to M-line. Titin was first discovered in 1979 by Wang et. al. (1), who initially thought that titin was a collection of polypeptides that formed one large protein. Although titin's physical identification eluded researchers for many years, probably due to its susceptibility to proteolytic cleavage, many scientists, including Earnest Starling and A.F. Huxley, posited its existence (2). Starling and Huxley modeled their theories on the premise that something within striated muscle was regulating passive properties. This is now known to be the role of titin, which is one peptide, encoded by a single gene, TTN (2). Titin is believed to bind actin in the thin filament and the proteins of the thick filament (3). Titin contains extensible and non-extensible regions. The nonextensible regions provide a scaffold for associated proteins and serve as anchors for titin to the Z- and M-lines within the sarcomere (4). The extensible regions span the I-band segment of the sarcomere and have been shown to play a role in the passive properties of striated muscle (5). Titin plays a pivotal role in the Frank-Starling mechanism of the heart by regulating the extent of ventricular filling. Titin regulates passive stretch of the ventricles during filling and resulting recoil following filling (6). The extensible regions of titin contain both a PEVK-rich region (rich in proline, glutamate, valine and lysine) and tandem immunoglobulin (Ig) repeats (7).

These two regions form the molecular spring region in cardiac and skeletal titin. Straightening of the Ig domains and extension of the PEVK region during muscle stretch creates a passive force, which realigns the thick and thin filaments and helps the sarcomere back to its original length after active shortening or after a passive load is removed (8). Extension is the uncoiling movement of titin's elastic regions (as opposed to unfolding, which is changing of the protein's tertiary structure). Cardiac muscle contains an additional spring element, N2B, which further contributes to passive tension development. The contribution of titin to passive force is determined by the size, extension and unfolding of I-band regions. The progression of straightening, extension, and unfolding have been extensively studied using atomic force microscopy (AFM) and immunoelectron microscopy (IEM). These techniques have shown titin behaves as two worm-like chains in series (9). During single molecule AFM experiments, both the Ig and PEVK regions extend and unfold as predicted by the WLC model (10). However, under physiological conditions it is entropically unfavorable for the Ig regions to unfold and refold during each muscle contraction-relaxation cycle. In contrast, the PEVK regions extend and recoil during contraction/relaxation. It is important to define the terms lengthening and recoiling in this context.

Lengthening is not the same as unfolding. Lengthening consists of partial uncoiling of regions of the titin molecule but not complete protein unfolding. Recoiling is the return of titin's PEVK or N2B to their shapes prior to lengthening. IEM, AFM and WLC modeling have uncovered the progression of titin extension and lengthening within the physiologic sarcomere length range. Following initial stretch, Ig regions straighten, producing a small amount of passive force which initiates PEVK extension. In cardiac muscle the N2B region lengthens aspredicted by the WLC model following PEVK straightening. The energetically favorable PEVK recoiling after extension contributes to the bulk of titin's passive force production in striated muscle. The WLC model provides a quantitative assessment of flexible proteins such as titin. Since WLC is a mathematical model it does not fully explain the properties of titin, which is why I have chosen the further investigate the properties of titin(11-13).

mRNA expression of titin-complex proteins and exercise

physical exercise induces remodeling process in skeletal muscle tissue. One of the triggers of adaptation is mechanical loading. Recent studies have shown titin and titin-interacting proteins to participate in the sensing of mechanical stress and strain during muscle action (14). Titin is the only molecule that extends over half a sarcomere, and during muscle action it maintains temporal and spatial assembly of the contractile filaments (15). At Z disk, titin interacts with titin cap (T-cap), which is a linking protein for several signaling and structural proteins, e.g., muscle LIM protein (MLP; also known as CSRP3) and myostatin (16). Interaction of MLP with myogenic regulatory factors (MRFs) in the nucleus and its effect on expression of brain natriuretic peptide (BNP) and atrial natriuretic factor (ANF) make MLP a possible stretch regulatory protein (17). The central I band of titin interacts with calpain 3 and with three muscle ankyrin repeat proteins (MARPs): cardiac ankyrin repeat protein (CARP; also known as Ankrd1), ankyrin repeat domain protein 2 (Ankrd2), and diabetes-related ankyrin repeat protein (DARP; also known as Ankrd23). MARPs are induced under different stress conditions (18). Titin-interacting calpains (3 and 1) as well as calpain 2 are calcium-activated proteinases. The ubiquitous calpains (1 and 2) are thought to be responsible for the release of myofibrillar proteins (e.g., actin and myosin) from sarcomeres, which is followed by ubiquitination and degradation by proteasome. In contrast to ubiquitous calpains, calpain 3 activity correlates negatively with muscle degradation. Calpain 3 is thought to have a role in sarcomere maintenance in mature muscle cells (18).

In the context of exercise, titin and titin-complex proteins have been little studied. The response of these proteins to the exercise is interesting already because of their location in the center of muscle action and their regulative possibilities related to that. It has been shown that in animal muscles mRNA levels of MLP, CARP, Ankrd2, and DARP are already elevated a few hours after exercise (19), and knockout studies of these proteins suggest their involvement in structural and regulatory roles in skeletal muscle (19). However, little is known about the effect of physical exercise on these proteins in human muscle. Titin staining has been found to disappear from some myofibers within 3 days after eccentrically biased exercise and protein content to be reduced by 30% in human skeletal muscle 24 h postexercise (20). Titin is located in between the contractile filaments in the muscle sarcomere. During muscle contractions, it maintains the order of thin and thick filaments and is subjected to high stretching forces during eccentric muscle action. Thus it is not surprising that after eccentric muscle action remodeling of titin structures is observed in animal and human skeletal muscle (21). After lengthening contractions, abnormal staining of titin was observed consistently with unorganized contractile filaments in animal studies. In human skeletal muscle, titin staining disappears from some myofibers within 3 days after eccentrically biased exercise and protein content falls by 30% 24 h postexercise (21). In a study, did not see any changes in the mRNA expression of titin. In a recent study, however, decrease in titin mRNA expression was observed 1 h after mild eccentric exercise (22). The present results are well in line with previous observation from an animal study where titin mRNA expression did not change between 3 and 96 h after a single downhill running session but was increased 3 h after repeated exercise (22). The results together suggest that titin mRNA level is first decreased shortly after the eccentric exercise and possibly increased much later. Reappearance of titin can be clearly observed by microscopy after 7–8 days postexercise. Titin isoform composition is interesting because it determines the passive mechanical properties of the sarcomere. In the present as in another study, it observed that one of the eight subjects had a different titin isoform composition (represented by 2 bands on SDS-PAGE). The subject with a different titin isoform composition showed the largest change in titin mRNA expression (1.4 times the control value) as well as CARP mRNA level at 30 min and Ankrd2 mRNA level at 2 days. This result is interesting in the light of a recent suggestion that MARPs may alter titin isoform expression (22).

titin is the probable backbone of striated muscle stress and stretch-sensing structures. One member of stretch-sensing structures is CARP, which interacts with titin at the I band. only study show increased CARP mRNA expression after physical exercise in human skeletal muscle. In rodents, increased mRNA expression CARP has been observed 6 h after lengthening muscle actions (22) and 3 h after prolonged concentric contractions (23). It observed increased CARP mRNA expression as early as 30 min postexercise, which can be regarded as an extremely rapid response. Increase in CARP mRNA expression is as fast as that of the immediate early genes *c-jun* and *c-fos* (38) or that of some ubiquitin ligases (23). This suggests a role for CARP early in adaptation after physical exercise. The

upregulation of CARP is known to activate angiogenesis and muscle hypertrophy is known to be accompanied by capillary growth. previous observations of increased angiogenic and extracellular matrix remodeling proteins cysteine-rich angiogenic protein 61 and connective tissue growth factor in the same muscle samples from the same experiment (23) supports the idea that the angiogenesis is activated.

MLP interacts with titin through T-cap. In rodents, increased mRNA expression of MLP has been observed 6 h after lengthening muscle actions and 3 h after prolonged concentric contractions. In one study, the increase in the mRNA levels of MLP was observed 2 days postexercise. This was the first study to show increased MLP mRNA expression after physical exercise in human skeletal muscle. A recent study additionally suggests that MLP promotes myosin heavy chain expression, and especially slow myosin heavy chain is upregulated if MLP is coexpressed with calsineurin. Because MLP is also located in the nucleus, it may regulate the transcriptional processes involved in regeneration and/or adaptation to increased physical loading. In the nucleus, MLP enhances the binding of MRFs to DNA. Interestingly, a few hours after exercise, the transcription of MRFs is activated. MRFs such as myogenic factor-5 and myogenic differentiation factor D are early markers of myogenesis (21). Thus the increase in MLP mRNA levels may be a step toward myogenesis after physical exercise.

Titin and exercise training

The different investigations have determined that the pattern of titin protein band expression in skeletal muscle between athletes and non-athletes is different (24). The titin protein band expression unique to the athletes coincided with superior performance in a power activity such as jumping and maximal dynamic strength. Titin analysis through gel electrophoresis often results in two protein bands (25). The first band is identified as either T1 or alpha-connectin. The second band is often identified as T2 or beta-connectin. This second band is assumed to be a proteolytic fragment of T1**.** Mc Guigan et al. (2003) determined changes in titin and myosin MyHC isoforms in vastus lateralis muscle from twenty-four male subjects after explosive jump squat training for 8 weeks and showed that there was no significant difference in the expression of these two isoforms between trained and untrained subjects(26).

In a study carried out by Trappe et al. (2002), titin and nebulin content was measured in muscle biopsies from the vastus lateralis before and 24 h after a bout of high-intensity eccentric knee extensor resistance exercise in seven men .These authors observed that titin titin and nebulin amounts were significantly reduced ($p<0.05$) after exercise by 30 and 15%, respectively, suggesting that the structural components of the myofibrillar apparatus were degraded in this type of exercise in humans(20). These findings were also supported by Komi (2000), who reported that mechanical direct perturbations in the structural proteins of the sarcomere, such as titin, can occur after maximal and brief cyclical exercises. Titin expression was also examined in different athletic populations (5 subjects for each group) with increased levels of strength and power (weightlifters, powerlifters, sprinters) compared with non-athletes. One-repetition maximum in the squat exercise and counter movement vertical jump trials were performed to assess strength and power capabilities, respectively. Gel electrophoresis analyses of muscle samples indicated that non-athlete groups presented lower titin-1 (intact titin) and higher titin-2 (degraded titin) percentages than the weightlifter, powerlifter and sprinter groups (24). This investigation showed that there was a differential expression of titin protein bands in competitive athletes with increased levels of strength and power in comparison to untrained non-athletic individuals. However, it is not known if the two bands were isoforms or proteolytic fragments dependent on the exercise type, because molecular weight standards for titin did not yet exist. Some relationships between titin characteristics and athletic performance were observed; however, no conclusions have been drawn with respect to the contribution of titin to strength or power capabilities (24). The relationship between the modifications in titin molecular structure and neuromuscular performance was assessed on gastrocnemius muscle biopsies from 23 subjects who performed explosive muscular exercises for 15 weeks. A significant increase in strength was observed, but titin and MyHC expression remained unchanged (27).

In one study, 45 days of exercise training but not 15 or 30 days resulted in a variation of titin expression. In particular, it observed a significant increase of titin in the mouse gastrocnemius muscle after 6 weeks of endurance training. The antibody and method that used in these experiments could not discriminate between the different isoforms of titin; thus, the results describe the expression of all titin molecules.The lack of a reduction in titin content suggested that the endurance training protocol did not evoke long-term muscle damage. The intensity and modality of the workload in the endurance training could be stimuli that induce titin expression. In this respect, recent studies have identified a titin kinase domain-associated signaling complex which functions in response to mechanical stretch to regulate muscle gene transcription (28). Titin upregulated expression has been also found in the vastus lateralis muscle of 16 subjects in response to 20 week endurance training, and appears to play an interesting role in the improvement of insulin sensitivity (29). However, the involved signaling pathways are unknown.

The increased expression of titin could be a structural adaptation of the muscles involved in running to maximize the storage and release of elastic recoil energy. In this respect, during running, mammals seem to select stride frequencies that maximize the muscle activity of alternate stretch-shorten patterns, and as a consequence, they reduce the expenditure of the energy for the locomotion. Results from measurements on the long heads of rat triceps brachii muscle indicated that the group exercised by a chronic eccentric training produced significantly more passive and active lengthening force compared with sedentary animals (30). Reich et al. (2000) suggested that the changes in the muscle elastic properties, for which titin may be responsible, may serve as a mechanism protecting the muscle from possible damage due to eccentric training. In addition, analysis of treadmill locomotion in the heterozygous B6-+/mdm mice, which present a mutation in the titin coding region, did not have any apparent muscle pathology, but showed an altered gait including stride, stance and swing time, as compared to B6-+/+ controls. Therefore, titin also plays a functional role in the dynamics of muscle contraction. Further studies are being carried out on different muscle types (fast and slow fibers) to quantify titin expression in response to different training protocols.

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