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Regulation of Protein Synthesis in Skeletal Muscle

Proteinsyntheseregulation im Muskel

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ZUSAMMENFASSUNG

Ziel: Zusammenfassung relevanter Studienergebnisse zur Regulation der Proteinsynthese in der Skelettmuskulatur. Ergebnisse: Prozesse der Proteinsynthese verändern sich entsprechend unterschiedlicher physiologischen Belastungen. Muskuläre Belastung, Energiebedarf und verfügbare Nährstoffe beeinflussen die Proteinsynthese der Skelettmuskulatur durch die Regulation ribosomaler Aktivität. Die tägliche Variation der Skelettmuskelmasse beträgt etwa 0,5 % und hängt von der Proteinsyntheserate ab. Die Proteinsynthese ist in der arbeitenden Muskulatur während der Belastung inhibiert und steigt während der Nachbelastungsphase an. Krafttraining steigert speziell die myofibrilläre Proteinsynthese. Im Gegensatz dazu werden mitochondriale Proteine bevorzugt durch Ausdauertraining synthetisiert. Diese Ergebnisse zeigen, dass Prozesse der Proteinsynthese den Umbauprozess der Skelettmuskulatur durch Training fördern. Unterschiedliche Signalwege kontrollieren die ribosomale Aktivität der Muskelzelle in Abhängigkeit von externen Signalen. Die Phosphorylierung von Proteinen durch den mTOR/p70S6K Signalweg und die Modulation des Energiesensors AMPK während muskulärer Arbeit sind wichtige Faktoren für die ribosomale Regulation der Proteinsynthese. Zusätzlich ist auch die transkriptionelle Regulation ein entscheidender Faktor, der die Synthese bestimmter Proteine nach Training reguliert. Schlussfolgerung: Die Proteinsynthese in der Skelettmuskulatur ist ein dynamischer Prozess, der unter anderem durch energetische Faktoren beeinflusst wird. Der Einfluss der Trainingsintensität auf Prozesse des Proteinabbaus sollte in zukünftigen Studien genauer untersucht werden um zu einem tieferen Verständnis der Plastizität der Skelettmuskulatur beizutragen.

Schlüsselwörter: Muskelplastizität, Belastung, Aminosäure, Ribosom, Hypertrophie.

SUMMARY

Purpose: Overview of relevant studies of the regulation of protein synthesis in human skeletal muscle. Findings: Muscle protein synthesis is altered in a number of physiological situations. Muscle loading, energetic requirements and nutrients exert a pronounced effect on protein synthesis in skeletal muscle by regulating ribosomal activity. Skeletal muscle mass varies daily by about 0.5 %, depending on the rate of protein synthesis. Protein synthesis is specifically suppressed in working muscle but shows a sustained increase post-exercise. This response reflects the protein pool demonstrating adaptation after the repeated impact of the exercise stimulus. There is a specific increase in myofibrillar protein synthesis with strength training. By contrast mitochondrial proteins are predominantly synthesized after endurance training. The findings support the view that cumulative increases in myocellular protein synthesis allow the gradual remodelling of muscle ultrastructure with training. Distinct signalling pathways emerge which control ribosomal activity in function of environmental cues. Phosphorylation of accessory factors by the mTOR/p70S6K pathway and its modulation by the intracellular energy sensor, AMPK, during muscle work evolve as core elements of ribosomal regulation. In humans, additional phenomena such as changes in transcript expression are suggested to specify the spectrum of proteins that are synthesized post-exercise. Conclusion: Protein synthesis is a dynamic read-out of anabolic stimuli to skeletal muscle that is dictated by energetic requirements. The intensity-dependent contribution of protein breakdown remains to be addressed to reinforce the current understanding of the control of muscle plasticity.

Key Words: Muscle plasticity, load, amino acid, ribosome hypertrophy.

PREAMBLE

Skeletal muscle tissue is subjected to considerable metabolic demand with physical activity (19,34). It has been presumed for more than a century that such elevations in energy expenditure pronouncedly affect muscle mass and composition (41). The biological processes that govern anabolic and catabolic reactions in skeletal muscle have been described only more recently. The present data document that skeletal muscle demonstrates important changes in protein synthesis and breakdown in response to changes in demand.

This article will review the evidence for control of muscle protein synthesis with particular regard to its regulation by exercise and amino acids. Due to restrictions in space the reviewed material does not include all relevant literature and may be considered as a selective view of the author. The interested reader is referred to further reviews on the larger topic (27,43,52).

PHYSIOLOGICAL REGULATION OF MUSCLE MASS

The effects of exercise on the muscle phenotype (22) emphasize that mechanical factors exert a main influence on muscle anabolism (Fig. 1). For instance, the systematic increase of muscle loading with resistance type training leads to a significant enlargement of muscle fibre cross-section after a few training sessions (45). The consequent anatomical changes and associated alterations of the muscle-tendon unit improve muscle strength (37). On the other hand, exposure to real or simulated microgravity with spaceflight or immobilisation pronouncedly reduces muscle mass within a

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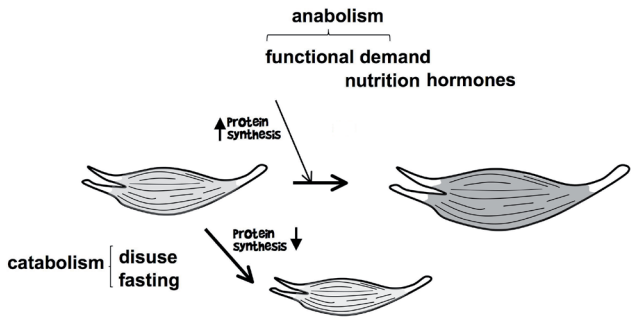

Figure 1: Main factors regulating muscle mass. Drawing summarizing the global regulation of muscle size by muscle use and nutritional interventions and the concomitant control of protein synthesis.

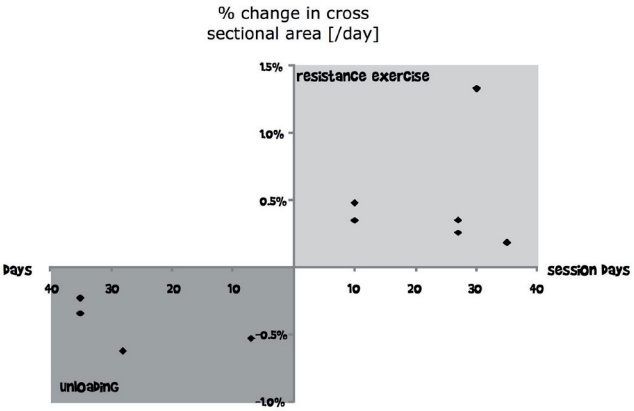

Figure 2: Fractional rate of changes of quadriceps muscle mass per day/session with resistance type training and unloading as calculated from changes in cross sectional area. Right, percentage changes per day of exercise session. Left, percentage changes per day displayed on an inverse time scale. Data stem from human investigations with different models (8, 13, 14, 15, 50, 55, 56).

dozen days (3). The counter effect of stretch and resistive forms of exercise (7,38) indicates that primary mechanical strain of skeletal muscle and not gravity governs muscle mass (38).

The time course of adjustments in muscle mass with altered muscle loading illustrates the important regulation of protein synthesis by mechanical cues. Heavy resistance training sessions involving 10 knee extensions at 80% of 1RM over 10 sessions in 3 weeks can produce a net gain in cross sectional area of vastus lateralis muscle of approximately 4% (8). Hypertrophy continues to manifest at a similar rate up to 27 sessions when a net gain in protein mass between 0.4-0.5% per exercise session is calculated (Fig. 2) (46). This hypertrophy is related to a doubling of protein synthesis in the first 4 hours of recovery from resistance exercise. Fractional synthesis rate has reported to increase to 0.1% of total myofibrillar protein per hour after comparable resistance type exercise (54). The response in protein synthesis is maintained with repetition of the resistance stimulus in the trained state. These observations imply that a rapid up-regulation of protein synthesis post exercise contributes to the accretion of muscle mass with regimens increasing muscle loading.

The importance of regulated protein synthesis is supported by studies on muscle atrophy with unloading. In this situation the loss of muscle mass per day (-0.5%) mirrors the values calculated for resistance training. This is paralleled by a fall in fractional synthesis rate of myofibrillar protein from ~0.45 to 0.2% h-1 (11). Protein degradation probably plays an important part to this muscle remodelling as proteolytic processes are known to be affected by changes in muscle activity. The quantitative (i.e. absolute) contribution of this regulation does not appear to be reported (51).

A main observation regarding muscle's adjustment to work is that exercise also alters muscle composition. Thereby important differences exist between the specific remodelling of the cellular components constituting the muscle motor, myofibrils, and the main powerhouse of the cell, mitochondria (26). Whereas 'high load-low repetition' types exercise, increase volume content of myofibrils, 'low load-high repetition' regimens are known to elevate mitochondria content of muscle fibres (26). These opposite adjustments invoke the existence of control mechanisms which specifically regulate muscle makeup in function of mechanical and energetic demand. A main observation in this regard is that peak mechanical load and not work dictates the response of myofibrillar protein synthesis (8).

REGULATION OF PROTEIN SYNTHESIS

Studies with amino acid tracers explain the effect of mechanical and metabolic events resulting from neuromuscular activity in terms of regulatory influences on protein metabolism (8,16). This becomes manifest in an increase in mixed muscle protein synthesis (i.e. the weighted average change of all proteins in muscle) following different forms of exercise (54). This response of muscle protein metabolism can last for 24-48 hours as shown for resistance type exercise (42,53). Depending on the training state the adjustments in protein synthesis may concern both the mitochondrial and myofibrillar protein pools of muscle (54). For instance myofibrillar protein synthesis in untrained subjects is elevated after resistance type but not endurance type of exercise. Conversely, synthesis of mitochondrial proteins is seen after endurance type exercise in both untrained and trained subjects. Cumulative effects of this transient elevation in protein synthesis following each bout of exercise seem likely to induce the above-mentioned muscle adaptation with training (17,26).

In order to understand the control of protein synthesis it is best to describe it from the angle of the organelle which is responsible for the synthesis of protein molecules: the ribosome. Ribosomes are large protein factories which mediate the translation of genetic information encoded on messenger ribonucleic (mRNA) templates in a corresponding peptide strand (Fig. 3A). Ribosomes are composed of two subunits each of which contains a complex of ribonucleic acids and basic proteins. The smaller subunit binds the mRNA, whereas the larger subunit dynamically incorporates specific transfer RNAs with their bound amino acid. After initial binding to mRNA the ribosome scans the mRNA for triplets of nucleotides that encode amino acids and synthesizes a corresponding peptide strand. This is achieved through joining amino acids to the carboxyl end of the growing chain as they derive from the transfer RNAs. The genetic code is based on 64 combinations of triplets that arise from the four nucleobases adenine (A), guanine (G), uracil (U) or cytosine (C). Translation typically starts at an 'ATG' sequence and stops at one of three stop sequences (i.e. UAG, UAA or UGA). The

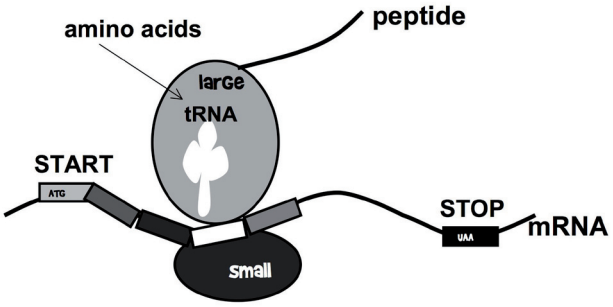

Figure 3A

Figure 3A and 3B: Composition and regulation of the ribosomal translation machinery. A) Sketch visualizing the small and large subunit of a ribosome, with the incorporated transfer RNA (tRNA, clover-like structure) with its bound amino acid. The translation of genetic information encoded in a messenger ribonucleic acid (mRNA) into a corresponding peptide strand is schematically depicted. See text fro more details. B) Drawing visualizing the main pathways integrating physiological cues into regulation of ribosomal activity. Mammalian target of rapamycin (mTOR) evolves a central hub for the integration of mechanical loading, essential amino acids and insulin. Equally mTOR is target to inhibition via an AMPK mediated mechanism. Downstream phosphorylation of accessory factors such as p70S6K mediated phosphorylation of the activator protein S6, and phosphorylation of the inhibitor 4EBP1, initiate protein translation.

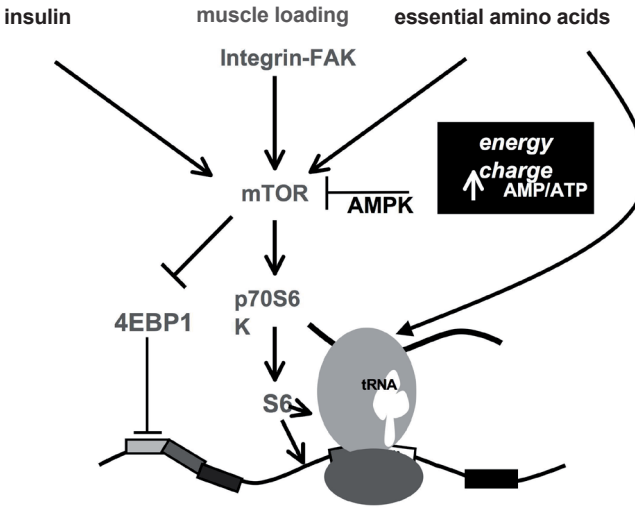

Figure 3B

activity of the ribosome is subtly regulated by a number of accessory factors that promote or inhibit translation initiation (Fig. 3B).

NUTRITIONAL REQUIREMENTS

While resistance exercise enhances muscle protein synthesis, the net protein balance remains negative (i.e. catabolic) in the absence of food intake (50). In fact, skeletal muscle protein synthesis is regulated by a number of dietary factors, including essential amino acids (31,40). Thereby amino acids and the branched-chain amino acid leucine in particular, constitute the main active ingredient (4,55). In this regard, it is important to emphasize that the availability of amino acids up-regulates muscle protein synthesis (11). This effect - for which anatomical endpoints are not defined - has been related to the presence of essential amino acids valine, leucine, and phenylalanine but not nonessential amino acids serine, alanine, or proline.

In consequence nutritional measures are increasingly important to avoid a negative nitrogen balance in clinical situation such as inactivity, injury, aging and disease which enhance catabolic drive (2,6,10).

Based on culture studies deprivation of even a single essential amino acid is expected to causes a decrease in the synthesis of essentially all cellular proteins through an inhibition of the initiation phase of mRNA translation (29). The explicit influence of the lack in a single amino acid for protein synthesis in exercised skeletal muscle has to the best of our knowledge not been assessed. Based on supplementation studies, it is known however, that the ingestion of a high-quality protein meal (25-30 g of protein per meal) maximally stimulates muscle protein synthesis in both young and older individuals (39). This points out that amino acid intake and physical activity act synergistically to counteract the decline in muscle protein synthesis with age (36).

MOLECULAR PATHWAYS OF PROTEIN SYNTHESIS

In the past 15 years molecular mechanisms have been identified which explain mechano- and amino acid-regulated protein synthesis in mammalian cells (27). A central theme of these cellular responses is that they integrate extra-cellular stimuli through coupled biochemical processes which perpetuate the post-translational modification of protein phosphorylation (16). Regarding the control of ribosomal activity by these signalling cascades a key role has been assigned to phosphorylation of the ribosomal S6 protein and the translational repressor, eukaryotic initiation factor 4E binding protein 1 (4EBP1) (1,31). S6 phosphorylation is carried out by the 70-kDa ribosomal protein S6 kinase (p70S6K). This modification is particularly important for the translation of mRNAs containing a 5'-terminal oligopyrimidine motif, many of which encode proteins involved in mRNA translation (29). By contrast, phosphorylation of 4EBP-1 relieves the translation factor eIF4E from inhibition allowing the activation of the ribosome (1). Together, p70S6K and 4EBP1 coordinate the behaviour of both eukaryotic initiation factors and the ribosome (47). For instance the fall in muscle protein synthesis during muscle work is probably been related to reduced phosphorylation of 4EBP1 (31). Accordingly, p70S6K activity is robustly induced after phosphorylation of regulatory sites Thr389, pS411 and Thr421/Ser424 during the acute response to stressful resistance exercise in men (13,30) in relation to training volume (49) and gains in muscle mass (48). Thus the measurement of p70S6K phosphorylation allows conclusions about the degree of activation of protein synthesis.

p70S6K and 4EBP-1 situate distal to the phosphotransferase mammalian target of rapamycin (mTOR) (1,31). This enzyme is central for the integration of various effects, including those of exercise, hormones and nutritional strategies (14). The activity of mTOR is acutely blunted during exercise by a mechanism that in the heart involves the sensor of energy charge, AMP-dependent

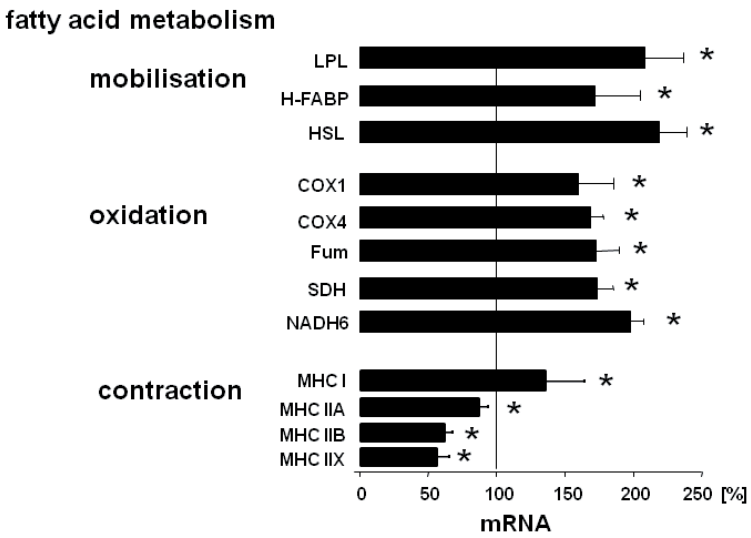

Figure 4: Endurance exercise shifts gene expression towards a slow oxidative phenotype. Bar graph visualizing the mean fold differences between in mRNA levels between untrained and endurance trained subjects for main factors of the mobilization and oxidation of fatty acids and the regulation of muscle contractile structure (myogenesis). The line of identity is given. Asterisk indicates a significant training effect. Data were calculated from Schmitt et al. (2003) and Schmutz et al. (2010). Thus endurance exercise alters the 'blueprint' for protein translation. Abbreviations: LPL, lipoprotein lipase; H-FABP, heart fatty acid binding protein; HSL, hormone sensitive lipase; COX1, subunit 1 of mitochondrial cytochrome c oxidase; COX4, subunit 4 of mitochondrial cytochrome c oxidase; Fu, fumarase; SDH, succinate dehydrogenase; NADH6, NADH dehydrogenase 6; MHCI, type I (i.e. slow) slow myosin heavy chain; MHCIIA, MHCIIB, MHCIIX, different fast types myosin heavy chain.

protein kinase (AMPK) (56). Correspondingly, protein synthesis is inhibited thus enhancing amino acid availability for energy metabolism (14). During recovery from exercise this inhibition is suppressed. In this regard it is important to note that insulin signalling via AKT to mTOR and downstream muscle protein synthesis, as identified in small animals, is not necessarily associated with muscle protein synthesis during feeding, exercise, and immobilization in humans (32). The study of the activation of the mTOR/p70S6K signalling pathway alone provides insight into the specificity and temporal sequence of muscle plasticity in different species.

Regarding the regulation of muscle hypertrophy in response to resistance training the integrin-associated focal adhesion kinase (FAK) meet the requirements of mechano-dependent ribosome regulation. FAK is part of sarcolemmal focal adhesion complexes (costameres) involved in force transmission between muscle fibres and the initiation of mechanical signal transduction towards activation of the ribosomal p70S6K by muscle loading (23,30). The connection of FAK to acute regulation of ribosomal activity is established through the FAK-dependent promotion of p70S6K activation after muscle loading (30) and increased FAK activation status after resistance training (54). In this regard it is important to note that the phosphorylation of the downstream target of FAK, c-jun N-terminal kinase (JNK) (18,28) in skeletal muscle is quantitatively related to muscle tension (33).

SPECIFICITY OF REGULATION

The assessment of the acute effects of exercise reveals important information on the specific control of protein synthesis in skeletal muscle. Firstly, it is striking that protein synthesis is depressed during intense exercise before it rises again during the recovery phase from a workout (31,42). This possibly reflects the energetic requirements of protein synthesis which are not met during intense muscle work. In rodent muscle this is supported by the implication of AMPK in the inhibition of mTOR and downstream protein synthesis with endurance exercise (1) (Fig. 3B). The degree to which such a mechanism is involved in the modulation of muscle's response to endurance and resistance type training in non idealized situation in men where type and duration of exercise, and diet are not specifically controlled, is not established.

In this regard it is important to consider that that the spectrum of proteins being synthesized after exercise is refined after a repeated impact with training. For instance, in untrained subjects the synthesis rate of mitochondrial proteins is elevated after both resistance and endurance type exercise (54). After training for resistance or endurance, however, up-regulated synthesis of mitochondrial proteins is confined to endurance exercise. A definite explanation of this observation is not readily available. Probably it is related to increased amounts of mitochondrial gene transcripts after endurance training (44) because these serve as templates for protein translation. Gene profiling studies point out that altered transcript expression with endurance training on a bicycle involves the down-regulation of messages for the main myofibrillar components of fast type muscle fibres (Fig. 4). Transcript profiling also identifies a marked reduction in the expression of mitochondrial factors in quadriceps muscle during recovery from eccentric vs. concentric type bicycle exercise at matched power output (20). The lengthening of contracting muscle during the eccentric but not concentric type bicycle exercise implies the important role of mechanical factors for muscle's expression response to exercise. This relates to the well understood influence of 'muscle stretch' for myofibrillar protein deposition (34). These findings highlight a potentially important relation of mRNA expression and the protein synthetic response of exercised muscle (5). The findings support the hypothesis that quantitative changes in expressed genes define the set of transcripts that can be translated by the ribosome.

In line with this suggestion, exercise-induced myogenesis is now implied in the recovery of muscle mass during rehabilitation (32). This possibly reflects the replenishment of synthetic capacity for the production of encoded proteins.

PROTEIN TURNOVER

A common observation with endurance type training on a bicycle is the maintained increase in protein turnover of mitochondria despite a flattening of the net response in muscle mass or com-

position (17). The underlying mechanisms are not understood but likely reflect a concomitant increase in protein synthesis and breakdown in frequently recruited muscle fibres. This possibly reflects wear-and-tear of molecules that necessitates replacement to rebuild destroyed biological structures (9). The liberated amino acid could therefore also serve metabolic purposes such as suspected from the historic experiments of Liebig (14,41,42). Balanced increases in anabolic and catabolic processes could explain why muscle mass remains unchanged after endurance training despite the increased synthesis of myofibrillar and mitochondrial proteins. Similar relationships are expected for load bearing exercise from the observation that hypertrophy of muscle fibres with downhill skiing is inversely related to fibre cross sectional area the before the intervention (21).

A mechanistic understanding of the underlying processes is not available. In this regard, observations on the effects of muscle loading on mechano-sensitive FAK are of interest. FAK has been shown to amplify the effects of muscle loading on gene expression (15). Under normal loading increased amount of FAK has been shown to promote the expression of both, protein translation and degradation factors (15). This role relates to the enhanced presence of FAK with the sarcolemma of muscle fibre types which demonstrate elevated protein turnover and expression of ribosomal subunits due to frequent recruitment for contraction (25,35). This notion is in line with reduced activation of FAK in the situation of muscle unloading (24). Thus quantitative changes in this upstream signal regulator would offer an explanation for elevated protein turnover with sustained increases in muscle recruitment.

CONCLUSIONS

The review of the literature underscores that protein synthesis is pronouncedly regulated by mechanical load, energy charge and supplementation of essential amino acids. Differential sensitivity of the ribosomal translation capacity for contractile and mitochondrial factors allows controlling macroscopic effects by tailored exercise interventions. A number of molecular concepts offer to maximize the therapeutic effects of exercise interventions although relevant aspects of molecular fine tuning are insufficiently understood.

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