

Human muscle protein synthesis and breakdown during and after exercise

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Abstract

Skeletal muscle demonstrates extraordinary mutability in its responses to exercise of different modes, intensity and duration, which must involve alterations of muscle protein turnover, both acutely and chronically. Here we bring together information on the alterations in the rates of synthesis and degradation of human muscle protein by different types of exercise and the influences of nutrition, age and sexual dimorphism. Where possible we summarize the likely changes in activity of signalling proteins associated with control of protein turnover. Exercise of both the resistance and non-resistance types appears to depress muscle protein synthesis (MPS) whereas muscle protein breakdown (MPB) probably remains unchanged during exercise, however both MPS and MPB are elevated after exercise in the fasted state, when net muscle protein balance remains negative. Positive net balance is achieved only when amino acid availability is increased, thereby raising MPS markedly. However, post-exercise increased amino acid availability is less important for inhibiting MPB than insulin, the secretion of which is stimulated most by glucose availability, without itself stimulating MPS. Exercise training appears to increase basal muscle protein turnover, with differential responses of the myofibrillar and mitochondrial protein fractions to acute exercise in the trained state. Ageing reduces the responses of myofibrillar protein and anabolic signalling to resistance exercise. There appear to be few if any differences in the response of young women and young men to acute exercise, although there are indications that in older women the responses may be blunted more than in older men.

Keywords: Protein turnover; signalling; contractile activity; training.

Introduction

Skeletal muscles show remarkable plasticity in response to changes in the mode, temporal pattern and intensity of loading, which can cause hypertrophy or atrophy, limited hyperplasia, and differential expression of a variety of proteins and even whole organelles such as mitochondria with resultant changes in fuel and protein metabolism. Traditionally, exercise has been categorized, for want of better descriptors as being of either “endurance/ aerobic” versus “resistance” types, the main operative distinction being that repeated endurance exercise (i.e., repeated low intensity contractions that can be performed for prolonged periods of time) results in a phenotypic shift toward a population of fibres with greater oxidative capacity whereas repeated resistance exercise (consisting of much higher intensity contractions) induces fibre hypertrophy (and possibly some hyperplasia involving satellite cell activation). In reality, there is substantial overlap between the patterns of response, but it is becoming increasingly apparent that muscles sense and distinguish specific signals produced by the imposed activity to produce adaptations over time that are specific to the nature, intensity and duration of exercise. For the purpose of this review, exercise will be classified either as resistance or non-resistance because endurance or aerobic as adjectives are so loosely defined as not to take into account high intensity dynamic exercise, such as repeated one-leg knee extension, or possibly interval sprinting.

Anatomical, biochemical, histochemical and metabolic investigations of the end results of muscle adaptation have enriched the literature over the past 100 years. However, only with the application of dynamic methods for the measurement of protein turnover, mainly using stable isotope tracers (12; 107; 130) has much progress been made in describing the nature and regulation in human muscle of the acute and

adaptive alterations to exercise of amino acid and protein metabolism (32; 85; 90; 93; 123). The increase in sensitivity and precision of measurement of labelling of whole classes of proteins and now of individual proteins (60) has borne substantial fruit and will continue to do so with further refinement. Other technical advances in the identification and measurement of alterations of post-translationally modified signalling proteins affecting protein turnover, particularly those influencing protein translation (2; 34; 37; 99; 123) have helped increase descriptions of alterations of the responses of muscle protein turnover to exercise, especially when made in conjunction with dynamic measures of synthesis and breakdown, but progress in understanding the physiological and biochemical regulation of the system have been slower.

The purpose of this review is to describe the regulation of human muscle protein turnover during and after exercise and associated modulation by environmental factors such as nutrient type, composition and rate of supply, sexual dimorphism and ageing as appropriate. The review will be presented as follows: 1) technical approaches to human muscle turnover, 2) exercise and muscle protein net balance, 3) muscle protein synthesis and resistance type exercise (during and after exercise), 4) muscle protein synthesis and non-resistance type exercise (during and after exercise), 5) muscle protein breakdown and exercise (during and after resistance and non-resistance type exercise), 6) the effect of feeding (and synergy with exercise) upon muscle protein metabolism, 7) the effect of chronic training on muscle protein metabolism, 8) gender and exercise and, 9) ageing and exercise.

We have included three tables in this review, in which we have summarized the pertinent data from studies carried out over the past two to three decades in this field,

highlighting specific variables i.e. age and sex, type of exercise performed, nutritional intervention and changes in protein synthesis and breakdown, thereby providing the reader an overview of work in this area. We have also included a figure describing a general scheme of alterations in the major cell signalling pathways involved in regulating protein synthesis in response to exercise and feeding so where possible we will describe only briefly associated changes in the activity of regulatory pathways, as inferred from changes in phosphorylation status.

We will confine ourselves to events on a scale of minutes to hours (rarely days, except for training effects) occurring during or after exercise. Changes during exercise probably mainly reflect altered metabolic priorities towards energy transduction for muscular work with many alterations e.g. inhibition of protein synthesis, increased transamination and oxidation of amino acids being paraprophenomena rather than specific functional, exercise related adaptations. Changes in muscle protein turnover in the post exercise period more likely reflect adaptive remodelling (such as increased synthesis of a group of myofibrillar proteins to support hypertrophy). We will not discuss alterations at the level of gene transcription.

Technical approaches to human muscle protein turnover

Since 1975 when human myofibrillar and sarcoplasmic protein synthesis were first measured (51), advances in techniques have led to a set of methods which are able to reliably measure the effects of physiological changes to muscle protein synthesis over times as short as one hour. Improvements in the sensitivity and precision of gas chromatography mass spectrometry (including combustion mass spectrometry) and more recently the use of proteomic techniques have allowed the measurement of rates

of synthesis of individual proteins (81) over relatively short periods and can now be applied to measure the acute response of MPS to exercise. Generally, this approach will involve a primed, constant infusion of, amongst others, use of [$1-^{13}\text{C}$] leucine (94), [$1-^{13}\text{C}$] α -ketoisocaproate (KIC) (30), d_5 phenylalanine (85; 86) or [ring- $^{13}\text{C}_6$] phenylalanine(47) as tracers, to achieve a steady state of tracer labelling in plasma. An alternative approach is to administer the tracer as a large “flooding” dose, in order to equilibrate the tracer in all the intra- and extracellular amino acid pools, thereby minimizing the uncertainty in the labelling of the immediate precursor for protein synthesis i.e. the amino-acyl tRNA. However, the demonstration that both leucine and phenylalanine stimulate MPS when administered as a large bolus (>3g) has led to the use of this approach being questioned (106). Nevertheless we have recently obtained MPS rates identical to those seen with constant infusion of labelled leucine when using a flooding dose of ^{13}C or ^{15}N labelled proline, probably linked to the fact that proline being a non-essential amino acid (76), and only essential amino acids appear to stimulate MPS in the flooding method.

Methods for discerning dilution of free intracellular amino acids as measures of fractional protein breakdown (FBR) offer the possibility of measuring both arms of mixed muscle turnover, i.e. synthesis and breakdown in a single study, but they are technically demanding and so far have been applied successfully only in a few studies (84; 132). The arterio-venous (A-V) tracer dilution method (28) and its later modifications, produces values of limb (including skin fat and bone) rather than muscle protein turnover, and if a carbon tracer of a branched chain amino acid is used, amino acid oxidation; it has proved to be very useful (13; 14; 112), but it should, in our view be used selectively, i.e. only when there is confidence of the existence of

steady states of blood flow, unlabelled and labelled amino acid and hormone concentrations. This is mostly due to the fact that changes in blood flow, as a result of exercise, would change the transit time of tracers (and, as a consequence, their uptake and release from muscle tissue) that are not co-temporal and could not be accounted for without exact knowledge of arterio-venous transit times. Studies which violate these conditions produce less than ideal results, and are often only qualitatively indicative. Further explanation of the methodologies involved in measuring protein turnover is outside the remit of this review and we direct the interested reader to the cited review articles (92; 120).

3-methyl histidine, is produced by post translational methylation of histidine residues on actin and myosin and is not subject to reincorporation into protein. Therefore its appearance has been suggested as as an index of the rate of myofibrillar proteolysis when assayed in either biopsied muscle or in muscle dialysate. However, the method in our opinion is unreliable without coincident measures of tracer dilution, muscle blood flow, and particularly muscle microvascular blood flow. A good example of the unreliability of this approach is demonstrated when against almost all other findings in the literature (13; 85; 86), it delivered results of no change in muscle proteolysis after intense exercise (55).

Single measurements of concentrations of analytes such as mRNA or muscle proteins (even when post-translationally modified) are static measures and give no kinetic information about muscle turnover during and after exercise. Moreover, it is also difficult to quantitatively relate expression of mRNA to that of protein expression making it difficult to interpret physiologically unless serial measures.

By and large, we have chosen to focus on cited articles which we view as being the results of measurements likely to be reliable unless otherwise stated.

Exercise and muscle protein net balance

Muscle mass is maintained through the regulated balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). A net gain of muscle mass is only possible if MPS exceeds MPB i.e. protein net balance is positive, whereas the converse occurs when MPB exceeds MPS. In the resting, fasted state (more accurately known as the postabsorptive condition), muscle protein net balance is negative and positive balance is usually achieved only via feeding, with the result that muscle protein lost between meals is replaced, thereby maintaining a stable muscle mass. After exercise in the fasting state, despite the rise in MPS (see below), net muscle protein balance, though becoming less negative, does not achieve a positive value, because the rate of MPB, which exceeded that of MPS before exercise, also rises (13). However, when amino acids or protein are ingested after exercise the net muscle protein balance becomes positive as the rate of MPS surpasses the rate of MPB, which itself may be suppressed (111).

Muscle protein synthesis (MPS) and resistance type exercise (Table 1, 2).

[Table 1 near here](#)

During exercise

Measures of human MPS made during resistance exercise are uncommon as most studies involve exercise of a duration which is shorter than the minimum time period

(~1 h) current methods require to achieve robust measurements with stable isotope tracers. Also, as the exercise is discontinuous, using sets of contraction repetitions with rest periods between, the muscle is not in a steady state and this complicates the interpretation of data obtained, especially with techniques relying on arterio-venous sampling and blood flow. Data from studies in both rodent muscle (24) and human muscle (37; 43) confirm that muscle protein synthesis is depressed during resistance type exercise. In contrast, other work using the arterio-venous tracer dilution method suggests no alteration of the rate of uptake of tracer i.e. leg protein synthesis is unchanged (39). The contradiction between findings from earlier studies (37) and latter report (39) may be the result of methodological differences (i.e. the use of direct incorporation method vs. arterio-venous tracer dilution method) or may possibly be the result of difference in volume of work (see Table 1). This fall in MPS has been shown to be mediated by a decrease in mRNA translation initiation and elongation steps (63) via reduced phosphorylation of 4E binding protein 1 (4EBP-1), a downstream effector of mammalian target of rapamycin (mTOR), and a tendency for a rise in phosphorylation of eukaryotic elongation factor 2 (eEF2), a negative regulator of peptide-chain elongation (37) (Figure 1).

Figure 1 near here

Bylund-Fellenius and colleagues (24) attributed the contraction-induced fall in MPS in perfused electrically stimulated rat muscle to an increase in the AMP:ATP ratio as a result of myosin ATPase activity - which might indeed have possible stimulatory effects on AMP-activated protein kinase (AMPK) activity (52) leading to inhibition of the signalling effect of Tuberous sclerosis complex 2 (TSC2) on mTOR and reduced

4E-BP1 phosphorylation (18). Indeed AMPK- α activity rises by more than 30% as a result of resistance exercise (37) but the importance of the intra-exercise inhibition of mTOR is uncertain as protein synthesis proceeded to rise in the post exercise period despite continued elevation of AMPK phosphorylation (37).

Recent work has also highlighted the efficacy of prior amino acid feeding on MPS during exercise. In overnight fasted subjects, fed with 0.35g.kg fat free mass⁻¹ of essential amino acids and 0.5 g.kg fat free mass⁻¹ of sucrose 1 h prior to exercise (10 x 10 at 70% 1RM), exercise still suppressed MPS during exercise but not below basal rates as seen with the controlled fasted group (43). Similarly protein feeding prior to and during a 2 h intermittent whole body resistance exercise session improved whole body net protein balance and increased muscle protein synthesis during the exercise (7).

Post-Exercise

It is generally agreed that resistance exercise results in increased MPS in the post-exercise recovery period (29; 71; 131). Indeed, an acute bout of resistance exercise can increase the rate of MPS about 2-5 fold after exercise and this effect can persist for up to 48 h in fed subjects (85). Reports differ (37; 100) as to whether there is inhibition of MPS immediately after strenuous contractile activity. In our lab we routinely observe no change above basal in measured incorporation of tracer into protein for about 1-1.5 h (66) but others do not (37; 100); nevertheless most workers who have examined it suggest that any post exercise rise is usually small initially and is maximized later (38; 66). The stimulation of protein synthesis after resistance exercise occurs in both myofibrillar and mitochondrial pools in untrained subjects

(123). Moreover, we have recently investigated the effect of an acute bout of resistance exercise over a wide range of exercise intensities, from 20-90% of 1 RM, on MPS with matched total work output (1620-1800 units) in postabsorptive, healthy, young (24 ± 6 y) and old (70 ± 5 y) men during 4 h post exercise period (66). Our results indicate that the magnitude of the myofibrillar protein synthesis response is intensity dependent at low intensities, with a plateau at intensities between 60-90% of 1RM. The effect of volume of work and adaptive responses of muscle protein turnover to resistance training at different intensities remain to be investigated.

The underlying molecular mechanisms associated with stimulatory effect of resistance exercise have been extensively studied in recent years, initially using rodents (2; 4; 19) and then transferring the techniques for analysis to human muscle (1; 34; 36; 37; 61; 66). Sufficient reports have now emerged to provide what is likely to be a reliable description of the extent and time course of signalling during and immediately after resistance exercise in the fed and fasted states (figure1). A detailed description of alterations of phosphorylation or activity of the cell signaling molecules regulating MPS in response to resistance exercise is beyond the scope of this review. However briefly, the activation of signaling molecules regulating translation initiation and protein synthesis such as Akt (protein kinase B), MAPK (mitogen-activated protein kinase), mTOR and its downstream effectors such as eIF4E-BP1 (eukaryotic initiation factor 4E binding protein 1), p70S6k1 (70 kDa S6 protein kinase) and rps6 (ribosomal protein s6 kinase) have been shown to be associated with increased MPS in the post exercise period (1; 36; 37; 61; 66). Nevertheless, the temporal relationship and longevity of these responses, and the dose response characteristics remain to be elucidated, as does any potential role in the adaptive response of muscle to both acute and chronic exercise.

With regards to cell signalling, we believe that currently we lack a good understanding of the precise relationship between the extent of the changes in signalling and consequent changes in MPS and MPB. In particular it is not clear if the molecules in the signalling pathways act as simple on-off switches or if they act as amplifiers to modulate the resulting metabolic action. Results from studies of insulin action on human muscle suggest that it is too simplistic to assume a particular physiological effect on protein synthesis or breakdown from alterations in the degree of phosphorylation of any given molecule (50). This information may be most elegantly obtained by time course and dose response data of the kind we have been endeavoring to collect (16; 17; 66).

Muscle protein synthesis and non-resistance type exercise (Table 1, 2)

During exercise

There is not much doubt that during running exercise in rodents muscle protein synthesis is depressed (35). This has been confirmed by more recent studies in which a fall of 26% in MPS was observed during a 2 h treadmill run by rats (46). This type of exercise also increased activity of AMPK and suppressed both mTOR signalling and the overall rate of mRNA translation in mice during running on a treadmill for 30 min which might underlie the changes in MPS (125). In human subjects a fall in whole body protein synthesis was described during walking uphill at 40 % of $VO_2\text{max}$ (93) and similar changes were observed during 2 h of walking at 60 % of $VO_2\text{max}$ (22). As MPS comprises a significant problem of whole body protein synthesis and it is known that the ATP:ADP ratio falls markedly during non-resistance type exercise(24), it is reasonable to assume that, during walking or running

exercise, human MPS falls but there is little documented evidence for this. In fact, during treadmill walking at 40 % of $\text{VO}_{2\text{max}}$ (27), no significant change in MPS was detected from the resting period; however, the basal values may have been uncharacteristically low compared to those on the non-exercise day so this may be a false negative result. It may also be that an insufficiently intense rate of exercise was chosen: cycle ergometer exercise for 1 h at 70% of $\text{VO}_{2\text{max}}$, in young healthy human subjects, increased activation of muscle AMPK α 2 (42) measured in quadriceps biopsies taken immediately after exercise; in comparable studies of exercise for 90 min there was marked Ca^{2+} -induced activation of the calmodulin-dependent protein kinase eukaryotic elongation factor 2 kinase (eEF2K), with accompanying (and probably resultant) inhibition of eEF2 activity (and by inference) protein chain elongation in healthy postabsorptive men (97). These results are consonant with the hypothesis that there is a fall in MPS during cycling and running; however, it is difficult technically to design a study in which the subjects exercise for sufficient time at a high load to satisfactorily observe the effects on protein turnover during exercise - but it should not be impossible. This is a gap waiting to be filled.

Post-exercise

After treadmill walking at 40% of $\text{VO}_{2\text{max}}$ in the post-absorptive state there was an increase in mixed muscle MPS of ~45% (26); a similar change was reported by Sheffield Moore (101) after treadmill walking. Even larger increases in the myofibrillar fractional synthetic rate can be produced by more intense exercise; in fed young men, 1 h of one legged kicking exercise at ~70 % of 1 RM doubled the quadriceps myofibrillar protein synthetic rate by 24 h post exercise, an effect lasting for up to 72 h. (76) .

These results might have been considered surprising before they started to accumulate beyond any doubt because it was generally assumed that exercise of this type (which would be likely to increase mitochondrial biogenesis) would not result in hypertrophy and thus would be unlikely to stimulate myofibrillar protein synthesis. In fact, as we have recently shown in untrained subjects, bouts of either resistance or bicycling exercise stimulate both myofibrillar and mitochondrial protein synthesis - possibly the results of a general post-exercise anabolic signal, whereas in the trained state no increase of myofibrillar synthesis is occasioned by bicycling exercise and no increase of mitochondrial protein synthesis by acute resistance exercise(123).

The degree of change in MPS in response to exercise may depend upon whether or not the exercise produces significant impact force, as it has been observed that there were no significant changes in MPS in healthy subjects after high-intensity swimming (113). However, it is important to note that these measurements were made under fasting conditions after a prolonged training session; the subjects were also highly trained and chronic training has been shown to increase the basal MPS rate and diminish MPS responses to acute bouts of exercise (86; 87; 102).

We now have many descriptions of the alterations of phosphorylation of signalling which might underlie possible changes in MPS after an acute bout of non-resistance type exercise as for changes in MPS itself (e.g. increases in mTOR signalling, decreases in eEF2, mitogen-activated protein kinase (MAPK) etc (1; 11; 74; 98). However, quantitatively, the changes observed are similar to those reported for resistance type exercise and indeed there is little difference in the extent of the

responses after acute exercise in muscles of legs working in different modes i.e. “resistance” and “endurance” in the same individual in the untrained state (123). This suggests that any major increase in contractile activity or possibly fuel utilization in untrained muscle will result in the same global anabolic response. However, after training, the acute anabolic response of MPS becomes more sensitive to the specific mode of exercise, resulting in synthesis of specific subcellular muscle protein fraction (mitochondrial or myofibrillar) subsequently leading to the phenotypic changes seen with the different training modes (123). In addition phenotypic changes probably only result from repeated bouts of either resistance or dynamic types of exercise. We remain puzzled about the significance to the training effect of alterations in signalling protein phosphorylation as only a limited data exists to date, certainly not enough to be able to predict alterations in protein turnover from the phosphorylation changes.

Muscle protein breakdown (MPB) and exercise (Tables 1, 2)

During resistance exercise

The only feasible techniques for measuring protein breakdown during exercise are those based upon arterio venous dilution of tracer amino acids, though as discussed previously the reliability of this approach during non-steady state conditions is questionable. So far as we have been able to discover there are only two studies in which the rate of dilution of a tracer has been measured “during” exercise (actually during rest periods between sets) in the postabsorptive state; in these studies phenylalanine rate of appearance (Ra), indicative of protein breakdown, was not elevated above rest (39; 114). However, it may be that if the major process of muscle proteolysis is via the ATP dependent ubiquitin proteasome system (3) and, as discussed previously AMP:ATP ratio increases during resistance exercise, then it too might be depressed during exercise as for protein synthesis (see above).

After resistance exercise

Whatever the uncertainty regarding the exercise period itself, there is no doubt that in the post absorptive state after exercise human muscle proteolysis is elevated, as shown both by tracer leg dilution (13; 15) and the fractional breakdown rate (FBR) method (85). Before exercise muscle is in net negative amino acid balance and this situation is only marginally improved by strenuous resistance exercise alone, because although MPS increases about two fold, the FBR, which is significantly higher than FSR (a measure of MPS) in the post absorptive state, also increases by 30-50 % by 3 h afterwards, thereby maintaining the negative balance (13; 85; 86). However, the elevation in muscle breakdown appears to be more short-lived than that of FSR (24 rather than 48 h) (85).

MPB and non-resistance type exercise

There is an uncertainty regarding the changes in MPB during non-resistance type exercise period. In many studies of cycling exercise the increase in net amino acid efflux from the leg is reported to be large (72; 73) and it has been assumed that this was due to an increase in proteolysis. However, the efflux of amino acids during exercise could easily arise from a greater inhibition of protein synthesis relative to a slowed rate of breakdown, the result of which would still be an expansion of the free amino acid pool and a greater net efflux of amino acids.

However, there is no doubt that in the post absorptive state after non-resistance type exercise that human muscle proteolysis is elevated, as shown in both untrained fasted young and older men after 45 min of treadmill walking at 40% of VO_{2max} . Leg

proteolysis was increased 10 min post exercise but the increase disappeared by 60 min in the young but not the older men. (101).

In contrast, results obtained using the microdialysis technique, suggested an unchanged concentration of 3 methyl histidine (3 MeH) in dialysis fluid from 6- 72 after 1 h of one legged kicking exercise at ~70% of 1RM (55). There is a possibility that the major part of post exercise proteolysis is of non-myofibrillar protein which would not show up as an increase in 3 MeH, but it seems more likely that this result probably speaks more for the unreliability for the method used than a lack of any muscle proteolysis (13; 85; 95).

Signalling and MPB

The signalling pathways controlling MPB and the proteolytic pathways involved in human muscle remain poorly defined, especially during exercise. The different proteolytic pathways (including lysosomal, the calcium-activated and the ubiquitin-proteasome dependent systems, caspases and metalloproteinases as well as non-specific di and tripeptidases) must be involved in the remodelling of skeletal muscle in response to exercise but the part played by each is not clear.

In rat muscle, an elevated activity of calcium activated proteases and metalloproteinases has been reported during / after treadmill running (9; 25). However there are few reports of measurement of acute changes in capacity or control of human muscle proteolytic pathways. Two muscle-specific ubiquitin ligases, muscle atrophy F-box (MAFbx) and muscle-RING-finger protein 1 (MuRF-1), have been shown to stimulate muscle proteolysis (3) rodent muscle. In human muscle, studies of

proteolytic gene expression, specifically ubiquitin proteasome related gene expression, in response to resistance exercise showed upregulation of MAFbx and MuRF-1 messenger RNA (mRNA) but with no significant changes in forkhead box (FOXO) 3A mRNA, a transcription factor involved in protein degradation and apoptosis, in young subjects 4h after resistance exercise (91). Paradoxically, studies carried out by our group showed a downregulation of MAFbx mRNA up to 24h after resistance exercise, which was unexpected considering resistance exercise increases MPB (65) in the post exercise state. This may be related to the volume of exercise carried out in the latter study since subjects performed exercise involving stepping up and down, carrying 25% of the body weight, to complete exhaustion and also the timing of the measurement. Alternatively, there may not be a direct relationship between MAFbx expression and MPB as we have previously observed (50). In all likelihood, multiple pathways are activated and a considerable exists; however, it is intriguing to imagine how intact myofibrillar proteins might be 'dismantled' or remodelled so as to make room for newly synthesized proteins, which according to several (101; 113) reports are made within hours of an exercise stimulus.

Effects of feeding on muscle protein synthesis and exercise (Table 2)

Feeding a mixed meal doubles mixed muscle protein synthesis (94); the effect seems according to the evidence in our hands to be mostly due to the actions of amino acids alone (10; 106) and particularly leucine (106) without much influence of insulin (16; 32). Amino acids increase the synthesis of myofibrillar, sarcoplasmic as well as mitochondrial proteins in skeletal muscle (17) probably in a dose dependent manner (16; 32).

Feeding and resistance exercise act synergistically to increase MPS and lead to positive net muscle protein balance after exercise, greater than that achieved by food alone (14). Several groups have reported that protein or amino acid ingestion, with or without ingested or infused carbohydrate, after an acute bout of resistance (14; 21; 33; 64; 77; 79; 90; 108; 112) or non-resistance type exercises (76) further enhances MPS. For example, a 145% rise in MPS above baseline occurred when a leucine-enriched essential amino acid solution with carbohydrate was taken after a single bout of resistance exercise, whereas without the provision of nutrition only a 41% rise in MPS occurred (36). This increase in the rate of MPS remains persists for a longer period (72h) (76) than with feeding (17) or exercise alone (66). This enhanced effect of feeding post exercise seems to be due to the presence of increased amounts of amino acids and not glucose in the blood (20; 77).

The dose response of MPS to exercise and increasing amounts of protein (80) appears to be similar in shape to that obtained at rest (17), albeit shifted upwards and to the left somewhat as the result of exercise. Although the work demonstrates the synergy between exercise and feeding it also suggests that there is no benefit of ingesting large amounts of protein (>20g, which is actually a relatively small amount) in an attempt to increase protein accretion in muscle - the maximum effective dose is probably 15-20 g of high quality protein such as beef, egg or soy.

Timing of feeding

There is disagreement as to whether amino acid feeding prior to or after resistance exercise promotes MPS to a greater extent. It has been reported (114) that ingestion of essential amino acids taken with a carbohydrate supplement immediately before

resistance exercise, resulted in greater leg uptake of amino acids but the results are quantitatively difficult to believe given that they were made under non steady state conditions and the increases in uptake were physiologically unlikely to represent increases in MPS given their size (20 fold!) but more likely some artefact such as pooling of amino acids within muscle. Furthermore, it has been recently shown by direct measurement of FSR in humans that feeding 1h before an acute bout of high intensity resistance exercise did not further enhance MPS during the 2 h post exercise period (43). Thus, once again, the leg tracer dilution method appears to yield qualitatively and quantitatively different results to those obtained by incorporation of tracer amino acids.

While there is still a disagreement with regard to the appropriate timing of protein feeding required to maximise the muscle protein synthetic response to an acute bout of exercise, there are some reports with respect to chronic exercise training showing that the stimulation in MPS, indicated by indirect measures such as muscle fibre hypertrophy, lean mass accretion and muscle strength gain in young and old men, is enhanced when protein is consumed immediately after the exercise rather than some hours later (40; 53; 67).

Protein quality

There has been considerable interest in the proposition that proteins of different biological quality and digestibility might be more or less efficient at supplying amino acids to muscle after exercise. Recent work by Phillips et al (53; 124) seem to show that whey proteins are superior to casein and soy and that whole milk supplies all that

is required for net muscle protein accretion. Although not yet proven, it seems likely to us that any high quality protein source such as beef, egg or soy will be as good as milk for muscle protein accretion(78).

Anabolic signalling

The underlying molecular mechanisms associated with this enhanced stimulatory effect of feeding after exercise appear to be associated with the enhanced phosphorylation of mTOR, p70S6K1 (p70S6 kinase), and 4E-BP1, greater than that achieved by exercise alone (36; 61; 64) (see Figure 1)

Effects of exercise and feeding on MPB (Table 2)

Amino acids *per se* have at most a small (50) inhibitory effect on human limb protein breakdown, especially in the presence of insulin but the effects are less than seen in animals. Suppression of protein breakdown in human forearm occurs after infusions of mixed or branched chain amino acids (68; 69). Much of the physiological effect of amino acids on MPB at rest is likely to be mediated through increased insulin secretion. However, several workers have reported that increased availability of amino acids after exercise does not significantly inhibit humans MPB (14; 21; 70; 90; 112).

The effect of exercise training on muscle protein metabolism (Table 3)

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Chronic resistance exercise increases mean muscle fiber cross-sectional area and induces muscle hypertrophy. Although we are largely ignorant of the time course of

the changes, and the exact mechanism involved, they must involve alterations in both MPS and, for remodelling and to achieve destruction of obsolete proteins, MPB. Several workers have reported that resistance training increases the basal rate of muscle protein synthesis (5; 86). It has also been reported that even short-term (2 weeks) resistance exercise training increases resting MPS but the data is difficult to interpret since MPS was measured shortly (between 3 h to 18 h) after the last bout of exercise and MPS may have been increased due to the acute effect of the exercise and not the training *per se* (54; 129; 131). In support of an increase in the rates of resting MPS after training, phosphorylation of Akt-mTOR-p70S6k is reportedly elevated when compared to pre-training (123). However, a study from the same laboratory failed to confirm this increase in basal MPS in response to chronic training (109). Nevertheless, with colleagues we investigated the effects of acute resistance or non-resistance (cycling) exercise in legs of the same individual before and after 10 weeks training on the synthesis of myofibrillar and mitochondrial proteins: in the resistance trained leg there was an increase in the basal synthesis rate of myofibrillar protein, whereas the non-resistance type exercise increased only basal mitochondrial protein synthesis (123). These results point to the likelihood that repeated bouts of one particular mode of exercise induces increases in the synthesis of different subcellular fractions, not as a result of short term modulation of translational activity but the activation of specific programs of gene transcription (65; 91). It has also been reported that chronic resistance training inhibited the muscle protein synthetic response to an acute bout of resistance exercise (86). However, the same laboratory recently reported a ~48% increase in MPS in response to an acute bout of resistance exercise following 12 weeks of chronic resistance exercise training (62). This difference was suggested to be due to the relatively lower stimulus in the trained state,

since resistance exercise was performed at the same absolute intensity before and after training in the previous study (62). However, with colleagues we have also observed a reduced acute myofibrillar synthetic response (~30%) to an acute bout of resistance exercise in the resistance trained leg at the same relative intensity (123).

It has also become evident that not only does the magnitude of response change, but also the temporal response of MPS to acute resistance exercise is mutable; chronic resistance exercise has been shown to cause a more rapid but more short lived rise in MPS than an acute bout in the untrained individuals (109). Therefore it appears that training status is an important variable when assessing the response of muscle to acute resistance exercise.

Regarding the effects of non-resistance type exercise training, an elevated resting MPS of vastus lateralis by 22% has been reported after a 16 week of bicycle training (45 min at 80% peak heart rate, 3-4 days/wk)(102). It is likely that the modest increase in mixed muscle FSR was the result of much higher increase in the mitochondrial and/or sarcoplasmic protein fractions. Even four weeks following a running/walking programme, exercising at 65-85% of maximum heart rate modestly elevates basal mixed muscle FSR (~17%); however basal FBR was, somewhat paradoxically, also reportedly increased (~40%) resulting in a more negative protein balance (87).

If there are increases in MPS after non-resistance type exercise training then why do muscles not hypertrophy? The increase in MPS after dynamic exercise training may be partially related to an increase in synthesis of proteins that are responsible for

bringing about adaptations associated with this type of exercise i.e. increased mitochondrial volume, mitochondrial enzyme activity and mitochondrial protein synthesis (48; 57). In support of this Short and colleagues reported increased synthesis of glucose transport (GLUT) proteins, mitochondrial proteins, mitochondrial enzymes levels and a 22% increase in resting mixed MPS following a 16-week “aerobic” exercise training program (102; 103). Recently, Wilkinson et al reported that chronic dynamic exercise over 10 weeks enhances only mitochondrial protein synthesis and has no effect on myofibrillar protein synthesis or on the basal phosphorylation of Akt-mTOR-p70S6k in young healthy men (123). On a transcriptional level, non-hypertrophic exercise (30 min of treadmill running at 75% of maximal O₂ uptake) increased the mRNA abundance and transcription of a variety of myogenic and metabolic genes (for myogenic differentiation, hexokinase II and pyruvate dehydrogenase kinase 4) after exercise, peaking 4-8 h post exercise and returning to basal within 24 h (126). The cumulative effects of this transient elevation following repeated dynamic training seem likely to induce above mentioned muscle adaptation associated with non-resistance type exercise (31).

Adaptive changes to dynamic training has recently been shown: with down regulation of AMPK, extracellular signal-regulated kinase (ERK) 1/2 and mTOR signalling activity following 10 daily intense cycling bouts for 45min to 60 min at 75% - 90% in healthy men (11). Increased expression of the muscle specific transcriptional coactivator, peroxisome proliferator-activated receptor- γ co-activator (PGC) - 1 α suggests it may also be associated with the adaptive responses of muscle to regular dynamic exercise; leading to mitochondrial biogenesis and increased oxidative

capacity (88; 89). However, the physiological role of muscle PGC-1 α in adaptive responses to exercise training still needs to be explored fully.

Sex differences in muscle protein metabolism and exercise

Unfortunately, little is known about the mechanisms that lead to sexual dimorphism in body composition, with men having greater muscle mass than women. Testosterone is well known to have an anabolic effect on muscle (41; 58) and testosterone secretion during puberty is highly likely to be responsible for the increase in muscle mass during early adulthood. Testosterone also increases the basal rate of MPS in both young and old men (23; 116) but this effect is unlikely to be due to acute changes in protein synthesis but instead to gene-dependent changes driven by nuclear androgen receptors. Female sex hormones may inhibit MPS and muscle growth in rats (115) but there are no detectable differences between young men and women in basal mixed muscle FSR or the response to intravenous amino acid feeding at moderate insulin availability (Smith, G, Mittendorfer B, Atherton, P and Rennie MJ, unpublished observation). Similarly, there have been no reported differences in the basal or post exercise rates of MPS or MPB between young adult men and women (44; 59; 75; 82). However, we and others have recently reported that post menopausal women have about 20-30% higher basal rates of MPS than men (56; 105) and a smaller response to feeding (105), so sex differences in muscle protein metabolism do appear to occur with age and probably as a result of changes in hormonal status. These differences appear to occur irrespective of body composition i.e. our subjects were obese (body mass index (BMI) 36-38) (105) compared to the subjects studied by Nair's group (56) who reported the similar sex differences in basal MPS. It is known that older women have a lower hypertrophic response than men (~33% less) following a resistance

exercise training programme (three days per week, 26 weeks) (6), possibly as a result of their inability to maintain adaptive responses to chronic resistance training, since elderly men increased the basal rate of MPS by ~50% after 3 months training, whereas in the the elderly women the increase was only ~15% (104).

Effects of age on muscle protein metabolism and exercise

There is some controversy regarding the rates of basal muscle protein synthesis in the elderly, with some earlier studies reporting reduced basal muscle protein synthetic rate in the elderly compared to young subjects (96; 121; 127). However, if the magnitude of this fall is correct then the rate of muscle wasting in the elderly would be expected to be much greater than commonly seen, and most workers now agree that in healthy men aging has no effect on the basal rate of MPS and net protein balance is not reduced in healthy elderly people (32; 117; 119). A moderate increase in physical activity has shown to prevent the age-associated loss of muscle strength and also the age associated increase in the muscle fat infiltration in the elderly people (49). Moreover, it has been shown that in older people MPS can be stimulated by both resistance exercise and nutrition (32; 38; 131). However, we have recently demonstrated that older men show anabolic resistance of MPS to an acute bout of resistance exercise over a wide range of exercise intensities, with a ~30 % lower response in older men than in young men (66).

Although others have reported that the response to an acute bout of resistance exercise in older people is delayed (38) we found no such effect (66). This discrepancy may be due to the higher volume of exercise used in this study and the fact that these subjects were studied under overnight fasted conditions (66). A similar “anabolic blunting” effect has been observed in the elderly with feeding, revealing a reduced sensitivity

and capacity of response to the anabolic effects of amino acids alone (32), or with amino acids plus glucose mixture (118).

The poorer anabolic response of MPS to exercise in the older muscle seems to be related to a reduced activation of upstream of mTOR signalling pathways and elevated AMPK activity compared to young muscle after resistance exercise (38). Studies in rodents have revealed that the activation of mTOR signaling after resistance exercise is reduced and the activity of AMPK is elevated in old rats compared to young rats (45; 83; 111).

There is a paucity of data regarding the measurement of muscle protein breakdown in response to exercise in the elderly. Using the arterio-venous tracer dilution method resting leg protein breakdown is suggested to be increased slightly in older men (119). However, we have data that reveals no difference in basal muscle protein breakdown but that the normal inhibition of MPB by insulin is significantly less in elderly (122). It would appear that “anabolic blunting” is a widespread feature of ageing muscle. Clearly the goal in ageing is to minimise muscle wasting and attempt to maintain muscle mass and function, for that to be achievable we need to understand the synergy between exercise and feeding and develop appropriate exercise and feeding strategies for the elderly.

Summary and conclusions

In summary, skeletal muscle shows extraordinary plasticity in response to exercise. An acute bout of resistance or non-resistance type exercise depresses MPS during the exercise period whereas MPS is elevated after exercise in both fasted and fed state;

this stimulation appears to be dose and threshold dependent, however the role of workload remain to be investigated. Despite different loading patterns as a result of undertaking resistance or non-resistance type exercise, contractile activity results in similar acute anabolic responses in untrained muscle. However, following a period of training the acute muscle response is diminished and is dependant upon mode of exercise i.e. resistance vs. non-resistance resulting in stimulation of either myofibrillar (resistance) or mitochondrial (non-resistance) protein synthesis, most likely reflecting adaptive changes to the mode of exercise. Whether or not there are alterations in MPB during an acute bout of resistance or non-resistance type exercise still remains unclear. However there is enough data to suggest that MPB is elevated after both types of exercise. A net gain in muscle mass (MPS - MPB) after exercise is achieved only when amino acid availability is increased during post-exercise period. Approximately 20g of high quality protein such as milk protein is sufficient to elicit the maximum synthetic response and consequently net accretion of muscle mass. Ageing reduces the response of myofibrillar protein synthesis to exercise and feeding, and recent reports have suggested that gender differences also exist in muscle protein turnover, specifically a diminished response to exercise in elderly women.

AMPK activation in response to cellular energy depletion during exercise appears to play an important role in the inhibition of protein synthesis. Increased protein synthesis after exercise is mediated through alterations in signal transduction involving activation of mTOR and sequential downstream effectors. The temporal and dose-response relationship between the exercise and phosphorylation of cell signalling pathways involved in the control of protein synthesis and degradation is only beginning to delineated. Although these phosphorylation events are in general

qualitatively of a kind expected to occur as a result of an anabolic stimulus like exercise, much more work is required to uncover the signals that switch them on and off and ultimately control the adaptive response i.e. muscle hypertrophy or increased mitochondrial biogenesis. Certainly it is currently impossible to directly relate the sizes of alterations in muscle protein turnover with those of phosphorylation of signalling molecules. When we can do this we will be much closer to our goal of understanding of the regulation of muscle mass and function and to develop strategies to maximise the maintenance of muscle in health and disease.

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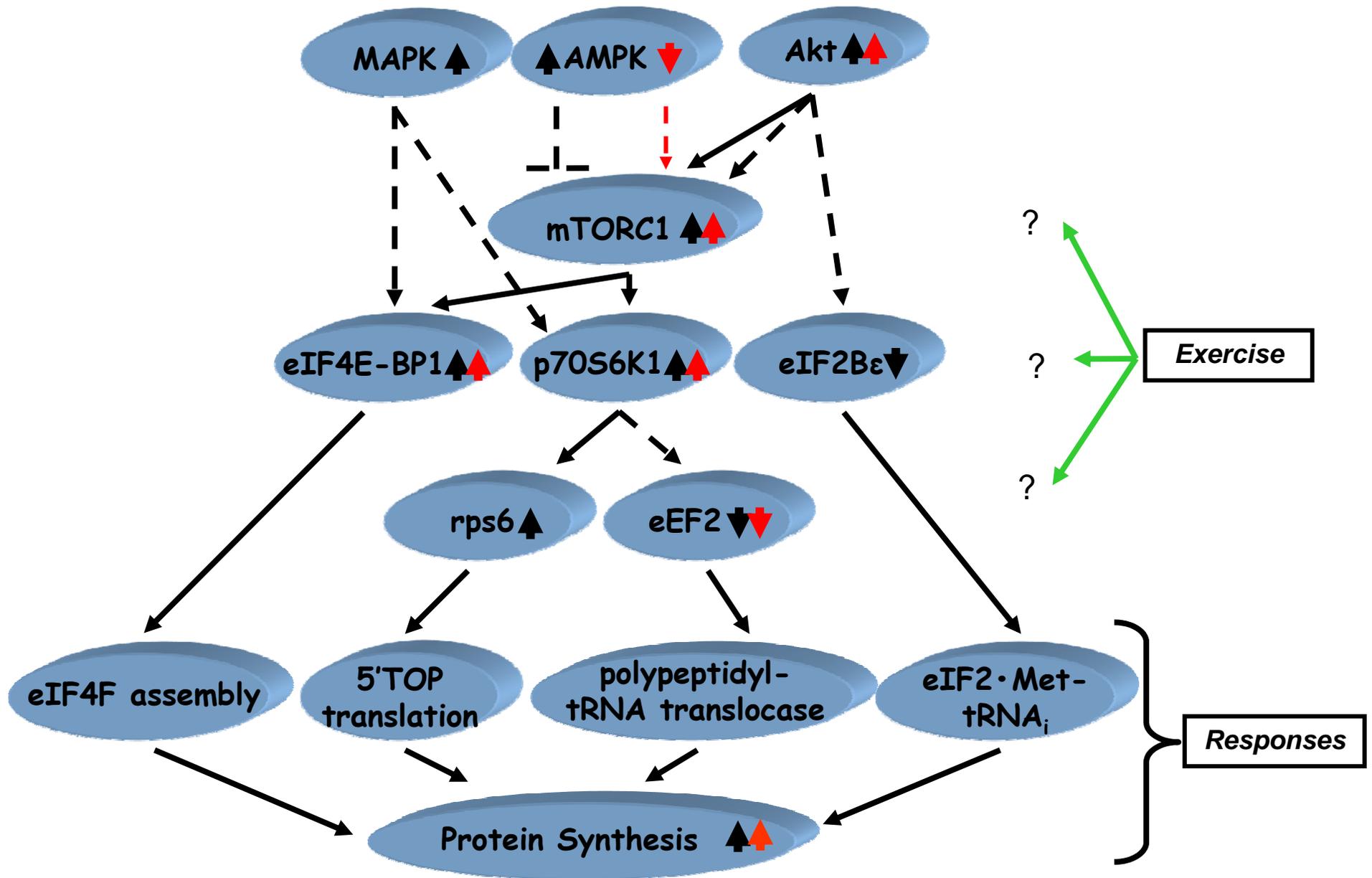
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▲/▼ = phosphorylation with feeding
 ▲/▼ = phosphorylation with exercise

→ = direct stimulation

- - - ▶ = indirect stimulation
 - - - ⊥ = indirect inhibition

Table 1. Effect of resistance and non-resistance type exercise on human muscle protein synthesis and breakdown in the postabsorptive state.

Subjects	Fasted/ Fed	Exercise protocol	FSR ^a period	Muscle fraction	Synthesis (FSR or [Rd])		Net change	Breakdown (FBR ^b or [Ra])		Net change	Comments	Ref
					Basal	PEX ^c		Basal	PEX			
6 M ^d	Fasted	4 x 6-12 reps ^e 80% 1RM ^f x 3 types of curl	4 h	Mixed	0.067	0.100	↑				FSR increased at 4 h and 24 h PEX	(29)
5 M	Fasted	5 x 10 reps at 12RM, 4 x 8 at 10RM (x LP ^g , LE ^h & LC ⁱ)	3 h	Mixed	0.044 [32]	0.104 [62]	↑	[48]	[69]	↑	4 h PEX	(13)
4 M, 4 F ^j	Fasted	8 x 8 reps 80% 1RM either LC or SC ^k	3 h	Mixed	0.05	0.12	↑	0.11	0.15	↑	FBR ↑ up to 24 h & FSR ↑ up to 48 h	(85)
6 M, 6 F	Fasted	8 x 10 flexions 120%	3-4 h	Mixed	0.036	0.08	↑	0.075	0.105	↑	FBR immediately PEX	(86)
7 F	Fasted	Leg then arm exercises over 1 h	5 h	Mixed	0.045	0.048	↔				Leg ex ^l performed 1st	(113)
6 M	Fasted	6 x 8 reps at 80%	10 min- 3 h	Mixed	basal	+0.030	↑				Increase at 180 min PEX	(100)
6 EM	Fasted	6 x 8 reps at 80%	10 min- 3 h	Mixed	basal	+0.044	↑				Transient increase over 10 min	(100)
5 M, 2 F	Fasted	8 x 10 reps 75% LP, 8 x 8 reps 80% LE	During Ex	Mixed	[22]	[30]	↔	[43]	[52]	↔	During Ex	(39)
7 M, 4 F	Fasted	10 x 10 reps at 80%	hourly	Mixed	0.06	0.009	↑				1 and 2 h PEX, 0.04 during Ex	(37)
8 M	Fasted	4 x 10 reps 80% LP, 4 x 10 reps 80% LE	4 h	Mixed	0.04	0.094	↑				Difference in mixed FSR, no change in Myofibrillar FSR	(62)
7 F	Fasted	Swim 1.5h	5 h	Mixed	0.045	0.064	↑				Non Resistance type exercise	(113)
7 F	Fasted	Combined RE ^m and Swim over 2.7h	5 h	Mixed	0.045	0.082	↑					(113)
6 M	Fasted	45min at 45% VO ₂ max	10 min- 3 h	Mixed	basal	+0.036	↑	basal	(+80)	↑ at 10 min	FSR increased at 60 min of basal by 180 min	(101)
6 EM ⁿ	Fasted	45min at 45% VO ₂ max	10 min- 3 h	Mixed	basal	+0.083	↑	basal	(+75)	↑ at 10 min only	FSR increased at 10 min basal at 60 and 180 min	(101)

^a FSR, fractional synthetic rate (%·h⁻¹); ^b FBR, fractional breakdown rate (%·h⁻¹); ^c PEX, post exercise; ^d M, male; ^e reps, repetitions; ^f RM, repetition maximum; ^g LP, leg press; ^h LE, leg extension; ⁱ LC, lengthening contractions; ^j F, female; ^k SC, shortening contractions; ^l Ex exercise; ^m RE, resistance exercise; ⁿ EM, elderly male.

Table 2. . Effects of resistance and non-resistance type exercise on human muscle protein synthesis and breakdown in the fed state

Subjects	Fasted/Fed	Exercise protocol	FSR ^a period	Muscle fraction	Synthesis (FSR or [Rd])		Net change	Breakdown (FBR ^b or [Ra])		Net change	Comments	Reference
					Basal	PEX ^c		Basal	PEX			
6 M ^d	I/V AA ^e (10 g AA.h ⁻¹)	5 x 10 reps ^f 12RM ^g , 4 x 8 reps 10RM (x squat, curl & LE ^h)	3 h	Mixed	0.065	0.144	↑	[38]	[50]	↔		(14)
3 M, 3 F ⁱ	Oral 40 g AA	5 x 10 reps 75% 1RM, 4 x 8 reps 75% 1RM (x squat, curl & LE)	4.5 h	Mixed	[50]	[85]	↔	[75]	[74]	↔	With 40 g EAA ^j similar response, only net balance significant	(112)
3 M, 3 F	Oral 6 g EAA	10 x 8 reps 80% LP ^k , 8 x 8 reps 80% LE	3 h	Mixed	[50]	[170]	↑	[60]	[75]	↔	Similar response at 1 and 3 h	(90)
3 M, 3 F	Oral 6 g EAA (PRE- EX) ^l	10 x 8 reps 80% LP, 8 x 8 reps 80% LE	2 h	Mixed	[65]	[190]	↑	[80]	[90]	↔	Increase Rd during exercise and 1h PEX	(114)
3 M, 3 F	Oral 6g EAA	10 x 8 reps 80% LP, 8 x 8 reps 80% LE	2 h	Mixed	[45]	[50]	↔	[60]	[45]	↔	No change throughout Ex ^m and PEX period	(114)
3 M, 3 F	Oral 6 g EAA (at 1 and 2 h PEX)	10 x 8 reps 80% LP, 8 x 8 reps 80% LE	3 h	Mixed	[25]	[120]	↑	[38]	[36]	↔	Ra only elevated at 3 h, Rd return to basal 3 h	(21)
3 M, 3 F	Oral 15 g EAA (x2 1h apart)	8 x 8 reps at 80%	3 h	Mixed	basal	0.188	↑				Exercise alone 0.076 %.h ⁻¹	(111)
8 M	Oral 10 g whey + CHO ⁿ	4 x 10 reps 80% LE, 4 x 10 reps 80% LP	2 h	Mixed	0.05	0.115	↑				CHO alone 0.08 %.h ⁻¹	(108)
10 M	Oral 7g protein.h ⁻¹	6 x 10 reps at 80%	3 h	Mixed	0.045	0.09	↑					(109)
16 M	Leu EAA ^o + CHO	10 x 10 reps at 70%	hourly	Mixed	0.06	0.16	↑				1h PEX, during exercise 0.045 %.h ⁻¹	(36)
6 M, 5 F	Leu EAA +CHO (0.35 g + 0.5 g.kg.FM ⁻¹)	10 x 10 reps at 70%	hourly	Mixed	0.06	0.12	↑				Prior feeding FSR elevated only at 2 h PEX, FSR AUC ^p over 4 h similar to control group	(43)
10 M	Oral 10 g prot. h ⁻¹	RE over 2 h	2 h	Mixed	0.06	0.085	↑				Feeding throughout	(7)
20 M	Oral 10 g prot. h ⁻¹ + 10 g CHO	Resistance & cycle exercise over 2 h	2 h	Mixed	0.056	0.083	↑				Feeding throughout	(8)
10 M	Oral 1.1 g prot.kg ⁻¹	5 x 10 reps at 80%	4 h	Myo ^q	0.06	0.11	↑				Mitochondrial FSR increased from 0.075 to 0.15 %.h ⁻¹	(123)
10 M	Oral 1.1 g prot.kg ⁻¹	45 min 75% VO ₂ max	4 h	Myo	0.055	0.055	↔				Mitochondrial FSR increased from 0.075 to 0.18 %.h ⁻¹	(123)
7 M	Oral 6 g prot.h ⁻¹	20 x 10 reps at 75%	3 h	Myo	0.057	0.164	↑	[29]	[26]	↔	Sarcoplasmic FSR elevated 3 fold to 0.22 %.h ⁻¹	(70)
8 M	Oral 7-8 g prot.h ⁻¹	6 x 10 reps LC ^r , 6 x 10 reps SC ^s	3+4 h	Myo	0.07	0.13	↑				Similar FSR at 4 and 8 h PEX	(79)
8 M	45 g EAA + CHO	Stepping exercise (+25% body wt) till fatigued	3 h	Myo	0.042	0.135	↑				0.05 at 3 h PEX, elevated at 6 and 24 h	(33)

8 M	1.4 x BMR, 15% Protein	1 leg kicking 67% VO ₂ max for 1h	3.5 h	Myo	0.04	0.1	↑	0.12 at 24 h, 0.08 at 48 h basal by 72 h	(76)
6M	0, 5, 10, 20 or 40 g of whole egg protein	4 x 8 – 10 reps (x LP, curl & LE)	4 h	Mixed/Albumin	0.055/0.21	0.105/0.41	↑	Linear dose response up to 20 g protein	(80)

^a FSR, fractional synthetic rate (%.h⁻¹); ^b FBR, fractional breakdown rate (%.h⁻¹); ^c PEx, post exercise; ^d M, male; ^e AA, amino acids; ^f reps, repetitions; ^g RM, repetition maximum; ^h LE, leg extension; ⁱ F, female; ^j EAA, essential amino acids; ^k LP, leg press; ^l PRE-EX, pre exercise; ^m Ex exercise; ⁿ CHO, carbohydrate; ^o Leu EAA, leucine enriched essential amino acids; ^p AUC, area under the curve; ^q Myo, myofibrillar; ^r LC, lengthening contractions; ^s SC, shortening contractions.

Table 3 Effects of resistance and non-resistance type exercise training on human muscle protein synthesis and breakdown in the postabsorptive or fed states.

Subjects	Fasted/ Fed	Exercise protocol	FSR ^a period	Muscle fraction	Synthesis (FSR or [Rd])		Net change	Breakdown (FBR ^b or [Ra])		Net change	Comments	Ref
					Basal	Post Training		Basal	PEX ^c			
2 M ^d , 4 F ^e	Fasted	2 weeks RE ^f training	4 h	Mixed	0.049	0.075	↑				Studied 3 h after last bout of exercise	(131)
4 EM ^g , 2 EF ^h	Fasted	2 weeks RE training	4 h	Mixed	0.03	0.076	↑				Studied 3 h after last bout of exercise	(131)
4 M, 3 F	Fasted	2 weeks RE training	12-13 h	Mixed/ MHC ⁱ	0.048/ 0.038	0.10/ 0.072	↑				16 h PEX, may be temporal effect not training effect	(54)
3 EM, 4 EF	Fasted	2 weeks RE training	12-13 h	Mixed/ MHC	0.037/ 0.024	0.102/ 0.050	↑					(54)
4 EM	Fasted	12 weeks RE training	12 h	Mixed	105	170	↑				mg/kg/h absolute rate	(129)
8 EF	Fasted	12 weeks RE training	12 h	Mixed	95	150	↑				~17 h PEX	(129)
19 M, 20 EM	Fasted	10 weeks RE training	5 h	Mixed/ MHC	0.041/ 0.028	0.066/ 0.042	↑				Similar effect in Myosin heavy chain, Study 4 days PEX	(5)
16 M	1/12 daily intake/30 min	12 weeks RE training	6 h	Mixed	0.048	0.066	↑				Studied 18-20 h after last bout of ex	(128)
6 M, 6 F	Fasted	8 x 10 flexion 120%	3-4 h	Mixed	0.045	0.067	↑				Acute Ex ^l response only in untrained	(86)
6 M, 6 F	Fasted	8 x 10 flexion 120%, regular RE training	3-4 h	Mixed	0.073	0.082	↔				Basal FSR higher in trained	(86)
8 M	Fasted	4 x 10 reps 80% LP ^k , 4 x 10 reps 80% LE ^l , 8 weeks training	4 h	Mixed/ Myo ^m	0.061	0.075	↑				Difference in mixed muscle fraction not in myofibrillar fraction	(62)
10 M	7g prot.h ⁻¹	6 x 10 reps ⁿ 80% LE, 8 weeks training	3 h	Mixed	0.048	0.123	↑				Basal not high despite feeding and training	(109)
10 M	1.1g prot.kg ⁻¹	5 x 10 reps 80% LE, 10 weeks training	4 h	Myo	0.08	0.12	↑					(123)
10 M	1.1g prot.kg ⁻¹	45min 75% VO ₂ max, 10 weeks cycling	4 h	Myo	0.05	0.075	↔				Mitochondrial FSR increased only in cycle group in response to acute exercise	(123)
4M, 4F	Fasted	Running/walking at 65-85% maximal heart rate, 4 weeks training	5 h	Mixed	0.077	0.089	↑	0.105	0.143	↑	Studied ~40 h after last bout of exercise, NET balance decreased	(87)
38M, 40W	Fasted	Bicycle training at 80% maximal heart rate, 4 months training	10 h	Mixed	0.04	0.05	↑				Studied 5 days after last bout of exercise	(102)

^aFSR, fractional synthetic rate (%.h⁻¹); ^bFBR, fractional breakdown rate (%.h⁻¹); ^cPEX, post exercise; ^dM, male; ^eF, female; ^fRE, resistance exercise; ^gEM, elderly male; ^hEF, elderly female; ⁱMHC, myosin heavy chain; ^jEx, exercise; ^kLP, leg press; ^lLE, leg extension; ^mMyo, myofibrillar; ⁿreps, repetitions.