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Examinations of optimal meal protein intake and frequency for athletes

Layne E. Norton

LAYNE E. NORTON[1]*, GABRIEL J. WILSON[2]
*Corresponding author
1. Division of Nutritional Sciences, University of Illinois, Urbana-Champaign
Bevier Hall Room 430, 905 S. Goodwin Ave, Urbana, IL 61801, USA
2. Animal Sciences Room 42, 1207 W. Gregory Drive, Urbana, IL 61801, USA

ABSTRACT: The RDA for protein is 0.8 g/kg bodyweight, however this may not be sufficient for athletes looking to maximize skeletal muscle protein synthesis (MPS) and muscle mass. The amount of protein source required to maximize MPS is likely dependent upon its leucine content. Leucine is responsible for most of the anabolic effects of a meal and current research suggests that 3g (~0.05g/kg bodyweight) of leucine is required to maximize this response. The MPS response to a mixed meal is only 3 hours long despite producing elevations in amino acids for 5 hours, thus athletes maximizing MPS will require inducing this response multiple times throughout the day. Consuming multiple meals per day containing 3g of leucine may be beneficial in maximizing MPS.

PROTEIN "NEEDS" VERSUS OPTIMAL PROTEIN INTAKE FOR ATHLETES

Dietary protein needs are defined as the interaction between the amount and quality of protein to meet metabolic requirements. Current health guidelines for dietary protein use the RDA which provides only the minimum amount of protein to maintain short-term nitrogen balance (0.8g/kg) based largely on data from young adults in ideal health and energy balance (1). There is some evidence that the RDA for protein may not be sufficient for certain populations (2). Many athletes consume protein in excess of the RDA and in some anecdotal reports, over 4g/kg (3). Interestingly, several reports have demonstrated that protein needs are only moderately increased by exercise (4). The current consensus is that meeting the minimum requirements for the most limiting amino acids in protein will lead to a plateau of nitrogen retention and any further increase in plasma amino acids would stimulate increased oxidation and elimination of the "excess" amino acids, implying that protein intake above requirements does not matter (5). This assumption fails to account for the metabolic actions of "excess" amino acids on stimulating MPS. The mere fact that 'excess' amino acids are oxidized does not mean that these amino acids do not initiate signalling in skeletal muscle prior to their oxidation. These metabolic actions of amino acids are more relevant to the athlete looking to maximize muscle mass, and performance. For these individuals the concern is not limited to the minimum protein intake to meet requirements, but rather the *optimal* intake of protein to maximize the anabolic effects of amino acids for maximal muscle mass and function in high force requiring activities. Therefore, the purpose of this review is to examine the current literature on the anabolic effects of amino acids to determine optimal levels of protein intake to maximize MPS and skeletal muscle mass.

This is clear evidence that amount of protein consumed in addition to the source of protein is crucial for maximizing skeletal muscle protein synthesis in response to a meal

Defining optimal protein intake for athletes

Current recommendations for protein intakes are evaluated on a gram per day basis. This approach assumes that protein distribution throughout the day is not important so long as the overall protein goals are met. Current research however suggests protein distribution does matter as changes in protein turnover in muscle are regulated on a meal to meal basis (6, 7). The amount of protein at a meal necessary to maximize protein synthesis will likely define the optimal protein intake at a meal to maximize the anabolic benefits of protein intake. Determining the correct meal frequency will provide the other crucial component for optimizing MPS and skeletal muscle growth. Optimal intake will then equal optimal meal intake multiplied by optimal meal frequency. Therefore, the remainder of this paper will focus on these two variables.

LEUCINE AS A MARKER OF MUSCLE PROTEIN SYNTHESIS IN RESPONSE TO A MEAL

The branched chain amino acid leucine has been proposed as the key amino acid for stimulation of MPS (8-11). It has been previously demonstrated that leucine accounts for the majority of the anabolic effect of a meal and administration of amino acid mixtures lacking leucine or with only small amounts of it do not stimulate MPS in adults (10, 12). Furthermore, our lab has shown that complete meals containing isonitrogenous amounts of either wheat gluten or whey protein differentially stimulate MPS based on their leucine contents (13). Interestingly the post prandial plasma leucine profiles closely mimicked the leucine content of the respective diets. This is likely due in part to the relative absence of the branched chain amino transferase (BCAT) enzyme in the liver which allows dietary leucine to reach the plasma in

Taken together, current information suggests that there is an unknown mechanism by which skeletal muscle becomes refractory to the anabolic effects of elevated amino acids that is independent of the mTOR signalling pathway

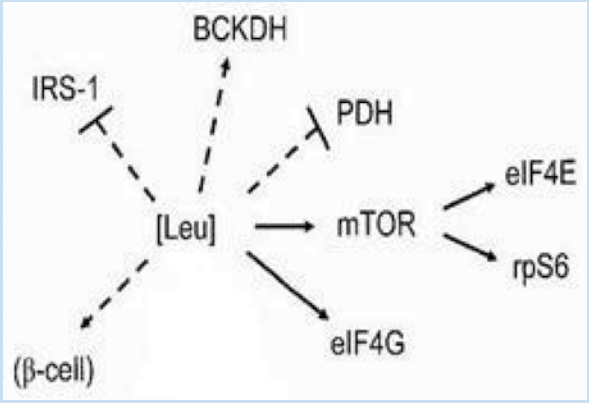

Figure 1. (Used with permission of Norton and Layman (9)). Intracellular leucine concentrations influence protein synthesis (solid lines), including translation initiation factors eIF4E, rpS6, and eIF4G and elongation factor eEF2, and energy metabolism (dashed lines) through BCKDH, pyruvate dehydrogenase (PDH), insulin receptor substrate-1 (IRS-1), and insulin release from the b-cell of the pancreas. Arrows indicate stimulation, and blocked lines indicate inhibition.

levels almost identical to those consumed in a meal (14). These data are in accordance with our lab's previous postulation that maximizing MPS is dependent upon achieving a specific postprandial concentration of leucine in the plasma (8).

Leucine stimulates MPS in skeletal muscle through increased translation

Evidence suggests that leucine increases MPS mostly via stimulation of the mammalian target of rapamaycin (mTOR) signalling pathway (9, 11) (Figure 1). mTOR stimulates translation initiation by increasing the formation of the eukaryotic initation factor 4F complex (eIF4F). eIF4F mediates the binding of the 43S preinitiation complex to an mRNA, the rate limiting step in translation initiation (15-17). This is mediated through increasing the phosphorylation of the inhibitory protein eukaryotic initiation factor 4E binding protein 1 (4E-BP1) which binds eukaryotic initiation factor 4E (eIF4E), preventing it from associating with eukaryotic initiation factor 4G (eIF4G), the rate limiting step in eIF4F complex formation (18). Phosphorylation inhibits 4E-BP1, allowing eIF4E to bind to eIF4G and form the eIF4F complex. Leucine induced activation of mTOR also increases the phosphorylation of the ribosomal protein p70 S6 kinase (S6K) (15, 16). S6K selectively increases the translation of mRNAs that encode components of the MPS mechanism. This increases the cell's total capacity for MPS. Leucine has also been demonstrated to increase MPS independently of mTOR by increasing the phosphorylation of eIF4G (11, 19).

Leucine as a determinant of optimal meal protein intake

Several studies have demonstrated that orally administering an essential amino acid (EAA) solution containing approximately ~3-4g of leucine will maximize post prandial skeletal MPS (20, 21). While administering greater doses of leucine (~8g) has no further stimulatory effect on MPS (21). Therefore, it is likely that 3g (or ~0.05g/kg bodyweight) of leucine is sufficient to maximize the anabolic response of a meal. Interestingly, this indicates that the choice of protein source is crucial in determining how much protein will be required at a meal to maximize MPS. Wheat protein, for example is approximately 7 percent leucine whereas whey protein isolate is approximately 11 percent leucine and maximizing MPS would require a far greater amount of wheat than whey at a meal. Our lab has demonstrated differential stimulation of MPS when feeding isocaloric, isonitrogenous meals containing wheat or whey protein, supporting this contention (13). Furthermore, Anthony et al. has demonstrated that mTOR activation is significantly greater in rats fed meals containing whey protein than those fed soy protein (approximately 8 percent leucine) (22). This is clear evidence that amount of protein consumed in addition to the *source* of protein is crucial for maximizing MPS in response to a meal.

OPTIMAL DISTRIBUTION OF PROTEIN INTAKE

Examination of post prandial protein synthetic refractoriness

The other component of maximizing MPS is proper distribution of protein intake. Previous research has indicated that the anabolic response to purified amino acid solutions containing leucine alone or in combination with other essential amino acids lasts approximately 2 hours before returning to baseline (23, 24). Our lab has recently demonstrated that consuming a complete meal delays and prolongs the anabolic response of a meal to approximately 3 hours with a peak in MPS from 45-90 minutes (25). Interestingly, while both plasma and intramuscular leucine concentrations were peaked from 45-180 minutes, and remained elevated above baseline for 5 hours, MPS had returned to baseline at 180 minutes. Furthermore, phosphorylation of the mTOR targets 4E-BP1 and S6K were also still peaked at 3 hours and closely followed plasma leucine levels. Thus, increases in plasma leucine were able to trigger mTOR signalling and MPS, but sustained elevations of plasma leucine and mTOR signalling were not sufficient to maintain elevated protein synthesis.

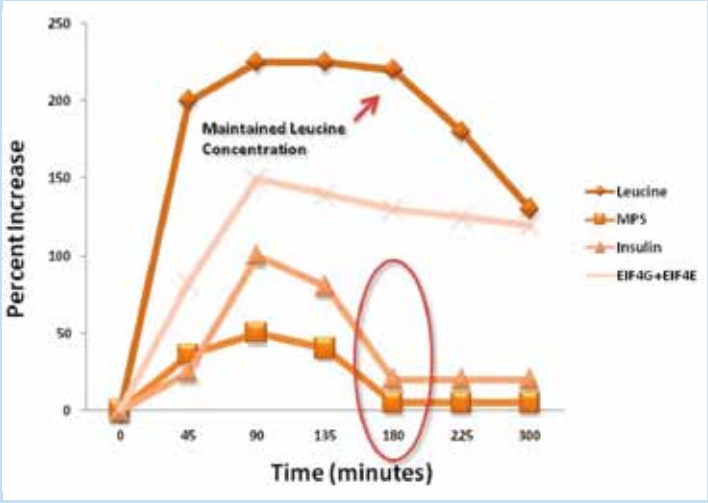

Figure 2. Protein synthetic refractoriness to elevated plasma leucine (Data from Norton et al.(27)).

This suggests a "refractory" response of MPS to prolonged elevations in plasma amino acids. Bohe et al. also documented the refractory nature of skeletal muscle to constant elevations in amino acids during a 6 hour infusion of essential amino acids (23). The infusion produced constant elevations in plasma EAAs; however, MPS lasted only 2 hours and was unable to be further stimulated during the 6 hr period.

MECHANISMS TO EXPLAIN PROTEIN SYNTHETIC REFRACTORINESS IN SKELETAL MUSCLE

Membrane bound protein stat hypothesis

A proposed mechanism for MPS refractoriness is that there is a membrane bound protein stat, either extra cellular, or intracellular, which is sensitive to relative changes in amino acid concentrations, rather than absolute changes.

Evidence for this was provided by Borsheim, et al. (26) who investigated the effects of 6 grams of EAAs and 6 grams of mixed EAAs and NEAAs (approximately 3 grams of each) on MPS in two doses spaced 1 hour apart following resistance exercise. After the first dose of amino acids was given, results showed a rise in plasma amino acid concentrations and an increase in MPS, with double the MPS occurring in response to the 6 grams of EAAs as opposed to the mixed dosage. Further, while the amino acid concentrations remained elevated above basal levels one hour after administration, MPS had returned to baseline. When the second dosage of EAAs was administered a similar anabolic response was seen in comparison to the first administration. These results led Wolfe (27) to suggest that (p. 655) "it appears that an increasing concentration of extra cellular amino acids) activates the synthetic process and the decline in concentration decreases synthesis, irrespective of the absolute value of the concentration". Bohé et al. (23) suggested that if the protein stat hypothesis is true, than some signalling pathways initially stimulated by amino acids must be "turned off" or inhibited, and proposed that the most likely factors would be inhibition of eukaryotic initiation factors.

However, the research in our lab did not support this contention, as phosphorylation of S6K and 4E-BP1 were tightly correlated with plasma Leu concentrations ($r=0.724$ & $r=0.730$ $p<0.0001$), and still MPS became refractory (25). This may suggest that another mechanism is responsible for inhibiting MPS, with a possible candidate being insulin.

Insulin and protein synthetic refractoriness

Insulin may, at least in part, explain the refractory nature of MPS to constant elevations in plasma amino acids. Insulin is not required to stimulate MPS in adults, but it does optimize the MPS response of muscle to amino acids (24, 28). Bohe et al. (23), and Norton et al. (25) showed that the plasma insulin time course somewhat paralleled the decline in MPS. Perhaps sustained elevations in insulin are necessary to sustain the anabolic response of MPS associated with elevated plasma leucine. If this is true, it is likely that the mechanism is independent of insulin's effects on the mTOR signalling pathway since MPS can become refractory to elevations in plasma amino acids even when mTOR signalling remains elevated above baseline (25). While insulin is not necessary to initiate translation, insulin is known to stimulate peptide elongation in skeletal muscle (29). If declining plasma insulin concentrations reduce peptide elongation it may explain the refractory phenomenon of MPS. Taken together, current information suggests that there is an unknown mechanism by which skeletal muscle becomes refractory to the anabolic effects of elevated amino acids that is independent of the mTOR signalling pathway (Figure 2).

SUGGESTIONS FOR OPTIMAL MEAL DISTRIBUTION

The time course of MPS in response to a meal and the refractory nature of MPS in response to constant elevations in amino acids make it seem unlikely that an additional stimulation can be achieved 3 hours post prandially with a second meal of similar composition to the first, as plasma leucine concentrations remain peaked. Thus, in order to avoid refractoriness and maximize MPS it may be best to consume larger doses of protein that contain sufficient leucine to maximize mTOR signalling and MPS while allowing enough time (4-6 hours) for post prandial amino acid levels to fall in between meals in order to re-sensitize the system.

Thus, in order to avoid refractoriness and maximize skeletal muscle protein synthesis it may be best to consume larger doses of protein that contain sufficient leucine to maximize mTOR signalling and protein synthesis and allow enough time (4-6 hours) for post prandial amino acid levels to fall in between meals

According to the protein stat theory, a second nutritional intervention which may overcome refractoriness is to create a supraphysiological rise in plasma amino acid levels between meals. A free form amino acid supplement would likely be rapidly digested and empty into the bloodstream quickly, potentially elevating plasma amino acid levels above their meal induced plateau. Finally, it may also be advantageous to consume a carbohydrate source between high protein meals if insulin does in in fact play a role in MPS becoming refractory. Evidence for this was provided by, Padden-Jones et al. (30) who demonstrated that consuming 30 grams of carbohydrates and 15 grams of free form essential amino acid supplement containing ~3g of leucine

in between meals spaced 5 hours apart enhanced MPS compared to unsupplemented subjects fed the same meals. This suggests that supplemental free form amino acids and carbohydrates may either enhance the anabolic response to a meal or somehow overcome the refractory response. The potential of free form amino acid supplements and carbohydrate ingestion between meals to overcome refractoriness is a future focus of our lab's research.

CONCLUSION

Athletes are more active and have very different goals with regards to nutrition compared to the average person. Athletes looking to maximize muscle mass and strength may benefit from protein levels well above the RDA. While the RDA focuses on minimum needs to achieve nitrogen balance, athletes should focus on consuming sufficient protein to maximize beneficial metabolic outcomes of greater protein intakes on a meal to meal basis. Current research suggests that the amino acid leucine is responsible for much of the anabolic properties of a meal and maximization of MPS in response to a meal is dependent upon consuming sufficient leucine (3g or ~0.05g/kg bodyweight) to saturate the mTOR signalling pathway. The amount of protein required at a meal to achieve this outcome will differ based on the leucine content of the protein source with leucine rich protein sources like dairy, egg, meats and poultry being preferable to leucine poor sources of protein such as wheat. These leucine rich meals should be consumed multiple times per day and consumption of carbohydrate with free form essential amino acids ingested between whole protein meals may further optimize MPS, possibly by overcoming refractoriness.

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