Skeletal Muscle Responses to Negative Energy Balance: Effects of Dietary Protein[1,2]

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ABSTRACT

Sustained periods of negative energy balance decrease body mass due to losses of both fat and skeletal muscle mass. Decreases in skeletal muscle mass are associated with a myriad of negative consequences, including suppressed basal metabolic rate, decreased protein turnover, decreased physical performance, and increased risk of injury. Decreases in skeletal muscle mass in response to negative energy balance are due to imbalanced rates of muscle protein synthesis and degradation. However, the underlying physiological mechanisms contributing to the loss of skeletal muscle during energy deprivation are not well described. Recent studies have demonstrated that consuming dietary protein at levels above the current recommended dietary allowance ($0.8 \, g \cdot kg^{-1} \cdot d^{-1}$) may attenuate the loss of skeletal muscle mass by affecting the intracellular regulation of muscle anabolism and proteolysis. However, the specific mechanism by which increased dietary protein spares skeletal muscle through enhanced molecular control of muscle protein metabolism has not been elucidated. This article reviews the available literature related to the effects of negative energy balance on skeletal muscle mass, highlighting investigations that assessed the influence of varying levels of dietary protein on skeletal muscle protein metabolism. Further, the molecular mechanisms that may contribute to the regulation of skeletal muscle mass in response to negative energy balance and alterations in dietary protein level are described. *Adv. Nutr. 3: 119–126, 2012.*

Introduction

The consequences of negative energy balance on total body and skeletal muscle mass are well established. In general, total body mass decreases in response to sustained periods of negative energy balance, and the proportion of body mass loss is ~75% adipose tissue and 25% fat-free mass (FFM)[5] (1). Although the predominant change in body composition is the loss of body fat, which may be beneficial, the concomitant decrease in skeletal muscle mass may negatively affect metabolic processes, muscular function, and physical performance. In overweight and obese individuals attempting to lose weight, decreases in muscle mass may down-regulate metabolic processes, such as protein turnover and basal metabolic rate, thus compromising healthy weight management (2,3). Healthy, normal-weight individuals such as athletes and military personnel may also undergo periods of negative energy balance resulting from dietary energy restriction, increased energy expenditure, or the combined effects of both. Decreased FFM in this population may be of greater concern, decreasing physical performance and increasing susceptibility to injury.

Popular strategies to attenuate muscle loss during negative energy balance include nutritional interventions that provide dietary protein in excess of the current recommended dietary allowance (RDA) ($0.8 \, g \cdot kg^{-1} \cdot d^{-1}$), as several studies have described a potential muscle-sparing effect, consequent to consuming higher protein diets (4–7). Although these benefits of consuming higher protein diets are becoming evident, the physiological mechanisms by which increased protein intake confers protection against the loss of skeletal muscle mass in response to negative energy balance are not well described.

The objective of this article is to provide a contemporary analysis of the available literature regarding the effects of energy restriction on skeletal muscle mass, with an emphasis

on studies assessing the influence of varying levels of dietary protein on skeletal muscle protein metabolism. Experimental models that use a variety of applied and basic molecular biological techniques to characterize the skeletal muscle proteolytic responses to manipulations in energy and dietary protein intake are described. Further, this article reviews molecular mechanisms that may contribute to the regulation of skeletal muscle mass in response to negative energy balance and explores the cellular properties by which dietary protein may conserve skeletal muscle integrity.

Negative energy balance and dietary protein intake: effects on FFM, nitrogen balance, and protein turnover

Energy balance and dietary protein intake are critical factors that contribute to the regulation of skeletal muscle mass by influencing whole-body and skeletal muscle protein metabolism (8–10). In a recent systematic review of publications from 1993 to 2009, Weinheimer et al. (1) reported that in more than half of the studies reviewed, energy restriction induced weight loss of 5–10% of the initial body mass. More than one fourth of this change in total body mass was a result of decreases in FFM. Layman et al. (5) demonstrated a greater retention of FFM and loss of body fat in overweight women adhering to a hypoenergetic diet (7113 kJ/d or 1700 kcal/d) than those consuming higher levels of dietary protein $(1.6 \text{ g·kg}^{-1}\text{·d}^{-1})$ compared with those who consumed the RDA for protein $(0.8 \text{ g·kg}^{-1}\text{·d}^{-1})$.

The catabolic nature of negative energy balance and the protective effect of dietary protein were also demonstrated in postmenopausal obese women who consumed diets ranging from 0.5 to 1.5 g protein·$\text{kg}^{-1}\text{·d}^{-1}$ for 20 wk (11). In that study, the extent of muscle loss in response to negative energy balance was proportional to dietary protein intake. Specifically, all volunteers lost FFM (−1.4 kg and −4.3 kg in the high- and low-protein groups, respectively). However, the percentage of total weight loss due to decreases in FFM was significantly lower for those women consuming high- (17.3%) versus low- (37.5%) protein diets. Others have also demonstrated benefits of consuming higher protein diets during prolonged periods of negative energy balance, with consistent reports documenting the attenuation of the loss of FFM after weight loss (4,6). Taken together, these investigations indicate that a certain degree of lean mass protection is gleaned from the consumption of a higher protein diet during prolonged periods of energy deficit.

Nitrogen balance methodology is widely used as a holistic assessment of protein balance, allowing one to gain valuable insight regarding the relationship between energy status, dietary protein, and skeletal muscle mass. In general, when energy intake is sufficient to meet energy demand, increasing the protein content of the diet imparts no added influence on nitrogen retention (12). However, increasing dietary protein intake may offset the increase in nitrogen excretion and negative nitrogen balance that generally occurs during periods of energy deficiency (13,14). For example, nitrogen balance and basal metabolic rate were preserved in premenopausal women who consumed a higher protein diet $(1.4 \text{ g·kg}^{-1}\text{·d}^{-1})$ during a 10-wk period of negative energy balance induced by dietary restriction coupled with a modest increase in physical activity (15). In a second study, Pikosky et al. (16) demonstrated negative nitrogen balance in healthy young volunteers in response to a 7-d period of negative energy balance (−4184 kJ/d or −1000 kcal/d) elicited solely by an increase in aerobic-type physical activity when protein was consumed at levels similar to the current RDA $(0.9 \text{ g·kg}^{-1}\text{·d}^{-1})$. However, doubling dietary protein intake $(1.8 \text{ g·kg}^{-1}\text{·d}^{-1})$ abrogated the increased nitrogen excretion and resultant negative nitrogen balance that occurred after the 7-d energy deficit. Again, these results indicate that lean body mass may be defended in response to negative energy balance by consuming a diet that provides protein at levels above the RDA, regardless of whether the energy deficit is caused by diet or physical activity.

Although these studies demonstrate a protective effect of consuming high levels of dietary protein during periods of negative energy balance, they fail to elucidate the physiological mechanisms for that effect. Because changes in skeletal muscle mass are likely due to imbalanced rates of protein synthesis and breakdown, amino acid tracer techniques have been used to assess the whole-body and skeletal muscle protein metabolic responses to varying levels of dietary protein and energy intakes (3,14,17–20).

In general, acute periods of negative energy balance associated with fasting result in increased whole-body proteolysis, amino acid oxidation, and nitrogen excretion, which become less pronounced and plateau over an extended period of time as the body adapts to conserve energy and protein reserves (e.g., muscle protein) (3,17,21,22). For example, Nair et al. (17) reported a significant up-regulation of whole-body proteolysis and oxidation after a 72-h fast; however, longer duration studies observed a reversal of this response, as whole-body proteolysis and protein synthesis were decreased by 20% after a 4-wk period of negative energy balance in overweight adults consuming the RDA for dietary protein (3). The down-regulation of protein turnover was proportional to the loss of FFM, which accounted for nearly 25% of the total body mass lost. Recent experimental evidence from Campbell et al. (19) confirms these findings because whole-body protein synthesis and proteolysis were decreased in postmenopausal overweight women who consumed 1.0 g protein·$\text{kg}^{-1}\text{·d}^{-1}$ during a 13-wk period of moderate negative energy balance (−2092 kJ/d or −500 kcal/d). Together, these data suggest that whole-body protein turnover, an energy-requiring process, is down-regulated in response to sustained energy deficit, perhaps to conserve endogenous protein stores when dietary protein intake is equivalent to the current RDA.

Consuming a high-protein diet may also contribute to the regulation of muscle mass by maintaining whole-body protein turnover in response to either acute or prolonged periods of negative energy balance (16,23–25). Pikosky et al. (16) assessed whole-body protein turnover in response to a 7-d physical activity–induced energy deficit in young healthy volunteers consuming dietary protein at either 0.9 g·$\text{kg}^{-1}\text{·d}^{-1}$ or 1.8 g·$\text{kg}^{-1}\text{·d}^{-1}$. Although nitrogen balance was maintained

in volunteers consuming protein at levels more than twice the RDA, no differences in whole-body protein turnover were observed between groups. Friedlander et al. (23) also reported the maintenance of whole-body protein turnover in healthy young men consuming 1.2 g protein·kg^{-1}·d^{-1} after a 3-wk 40% energy deficit. Although whole-body protein turnover measurements suggested that consuming dietary protein at RDA levels was adequate, nitrogen balance and resting metabolic rate were lower in response to energy deficiency, which corresponded to a significant decrease in FFM. It is important to note that the whole-body protein turnover assessments were performed in subjects under fasted conditions in these studies; it is possible that the positive effects of dietary protein on whole-body protein turnover may be observed only during the fed state (26). Further, it is important to recognize that although skeletal muscle accounts for ~50–75% of total body protein stores, it only contributes to 30–45% of whole-body protein turnover (27). As such, extrapolating findings from whole-body protein turnover studies to represent the skeletal muscle protein metabolic response to negative energy balance may not be appropriate. Studies directly assessing skeletal muscle protein turnover in response to negative energy balance and varying levels of protein intake are limited.

Only 3 studies investigated the direct skeletal muscle protein metabolic response to negative energy balance (18–20). Pasiakos et al. (18) demonstrated that a 10-d moderate energy deficit (−2092 kJ/d or −500 kcal/d) resulted in a 19% decrease in fasting skeletal muscle protein synthesis (weight maintenance: 0.074 ± 0.01%/h vs. energy deficit: 0.06 ± 0.01%/h) in physically active adults who consumed dietary protein at 1.5 g·kg^{-1}·d^{-1}. Other studies failed to confirm an energy deficit–induced impairment of skeletal muscle protein synthesis (19,20). Campbell et al. (19) reported increased fasting muscle protein synthesis (weight maintenance: 0.04 ± 0.01%/h vs. energy deficit: 0.11 ± 0.01%/h) after a 13-wk modest energy deficit (−2092 kJ/d or −500 kcal/d) in overweight postmenopausal women consuming 1.0 g protein·kg^{-1}·d^{-1}. In contrast, Villareal et al. (20) observed no change in fasting muscle protein synthesis, although postprandial muscle protein synthesis increased (increase in muscle protein synthesis above fasting values: 0.033 ± 0.01%/h) in older adults (60–85 y) after a 3-mo period of negative energy balance (−2092–3138 kJ/d or −500–750 kcal/d). A combination of factors likely contributed to the discordant skeletal muscle protein turnover data observed across studies, including variations in experimental design, dietary interventions, and study populations. For example, Campbell et al. (19) assessed muscle protein synthesis after the 13-wk energy deficit 3 d after reestablishing energy balance (+2092 kJ/d or +500 kcal/d), which may have directly contributed to the dramatic increase in muscle protein synthesis. Further, Villareal et al. (20) assessed muscle kinetics in older obese adults using [5,5,5-^{2}H$_5$]-leucine, whereas Pasiakos et al. (18) characterized muscle protein synthesis in young, physically active adults using [^{2}H$_5$]-phenylalanine. Despite the apparent discrepancies in study populations and methodologies, these data suggest that energy restriction may elicit a down-regulation in muscle protein synthesis in the early stages of negative energy balance, perhaps representing an adaptive mechanism to conserve energy and protein reserves.

The studies reviewed thus far highlight the catabolic nature of negative energy balance and the associated muscle-sparing effects of consuming a high-protein diet. Regardless of whether energy deficit is induced by energy restriction, increased energy expenditure, or a combination, varied measures of whole-body and skeletal muscle protein metabolism indicate that consuming dietary protein in excess of the RDA confers a level of protection for skeletal muscle integrity. The remainder of this review focuses on recent studies assessing the molecular mechanisms regulating protein synthesis and breakdown and the potential influence of amino acids as signaling molecules.

Intracellular regulation of skeletal muscle mass: effects of negative energy balance and dietary protein

A series of intracellular networks that influence the molecular regulation of muscle protein turnover likely contribute to the loss of skeletal muscle mass in response to negative energy balance. Although certain elements of these intricate signaling pathways independently modulate critical steps involved in the cellular control of skeletal muscle anabolism and proteolysis, commonality between pathways does exist. However, the finite mechanisms by which cellular signaling molecules function in concert to regulate skeletal muscle mass in response to nutritional manipulation remain to be elucidated.

The cellular regulation of skeletal muscle protein synthesis has been well described (28,29). Briefly, a cascade of intracellular signaling events influenced by energy status, growth factors, and nutrient availability regulate muscle protein synthesis by modifying mRNA translation initiation and elongation (18,30). Perhaps the most important nutritionally regulated signaling component affecting mRNA translation is a multiunit protein complex termed mammalian target of rapamycin complex 1 (mTORC1), which includes the mammalian target of rapamycin (mTOR) kinase. mTORC1 is the central component of the insulin-signaling cascade [insulin/insulin-like growth factor (IGF)–phosphatidylinositol 3-kinase (PI3K) pathway) (**Fig. 1**) that regulates protein synthesis and mRNA translation through 2 primary mechanisms: 1) inactivation of the repressor of mRNA translation, eukaryotic translation initiation factor 4E-binding protein 1 (eIF4E-BP1), and 2) the activation of 70-kDa ribosomal protein S6 kinase. Together, changes in the phosphorylation state of these critical intracellular signaling proteins affect mRNA translation initiation and elongation, which, in turn, may directly influence the rate of protein synthesis.

A key intracellular signaling protein that may serve an important role in regulating the skeletal muscle response to negative energy balance is AMP–activated protein kinase (AMPK), which functions as a fuel sensor in many tissues, including skeletal muscle. It inhibits anabolic signaling pathways when cellular ATP levels are decreased and AMP levels increase in response to limited energy availability (31). More specifically, upstream mTORC1 signaling via the tuberous sclerosis complex 2

Figure 1 In response to negative energy balance, mRNA translation and muscle protein synthesis may be down-regulated as a result of decreased nutrient and growth factor availability, causing reduced mTORC1 activation. Decreased mTORC1 activation and subsequent decreases in muscle protein synthesis, coupled with increased FOXO nuclear localization, increased transcription of atrophy-related genes, with up-regulated caspase 3 activation and muscle protein ubiquitylation provide a possible mechanism contributing to skeletal muscle loss in response to periods of negative energy balance. Synthetic stimulators are depicted in gray, whereas inhibitors of synthesis are shown in black. Akt, protein kinase B; AMPK, AMP-activated protein kinase; eEF2, eukaryotic elongation factor 2; eEF2K, eukaryotic elongation factor 2 kinase; eIF4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; FOXO, forkhead box O; IRS-1, insulin receptor substrate 1; MAFbx, muscle atrogin F-box protein; mTORC1, mammalian target of rapamycin complex 1; MuRF1, muscle RING-finger protein 1; p70S6K, 70-kDa S6 kinase; PI3K, phosphatidylinositol 3-kinase; Rheb, ras homolog enriched in brain; rpS6, ribosomal protein S6; TSC, tuberous sclerosis complex; Ub, ubiquitin.
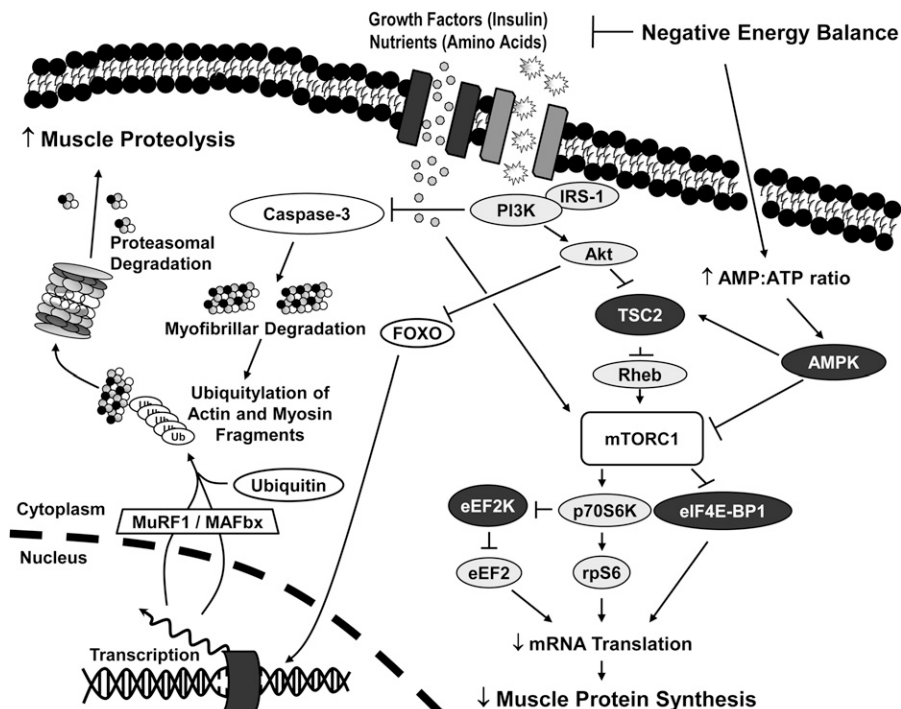
is sensitive to cellular energy status mediated through AMPK (32,33). Inhibition of mTORC1 activity and its subsequent downstream events has been demonstrated in rat skeletal muscle in response to increased AMPK activity. This occurs primarily by AMPK-dependent phosphorylation of the tuberous sclerosis complex 2 complex and direct phosphorylation of the mTOR kinase (31,34). To the best of our knowledge, there have been limited in vivo human studies assessing intracellular regulation of skeletal muscle protein metabolism in response to negative energy balance. In 1 study, decreased protein kinase B (Akt) and eIF4E-BP1 phosphorylation were observed after a 10-d period of modest negative energy balance (−2092 kJ/d or −500 kcal/d) in physically active adults (18). Although no changes in AMPK activity were observed, decreased intracellular signaling occurred with a concomitant decrease in skeletal muscle protein synthesis.

Although these findings are intriguing, the cellular regulation of skeletal muscle proteolysis in response to negative energy balance and manipulations in dietary protein is not well described. The 4 primary intracellular pathways that contribute to the regulation of skeletal muscle proteolysis are the autophagy/lysosomal, calpain-dependent, caspase-mediated, and ubiquitin proteasome (UP) systems. However, the available literature suggests that only 2 of these systems (caspase-mediated and UP) are major regulators of muscle mass during periods of negative energy balance. Although inflammation-induced muscle degradation resulting from autophagy/lysosomal muscle proteolysis has been well established (35), the contribution of the lysosomal system to

increased muscle loss consequent to energy deficiency is only now being elucidated (36–38). Increased lysosomal activity has been demonstrated in response to short-term fasting in transgenic mice expressing the autophagy gene, microtubule-associated protein 1A/1B-light chain 3, fused with green fluorescent protein (38). However, data from other animal models demonstrate diminished lysosomal enzyme activity during extended periods of energy restriction (39). Furthermore, calcium-dependent proteolysis indicative of calpain activity has also been postulated to function in many intramuscular processes, including myogenesis, the regulation of muscle protein turnover, and the initial cleavage of the myofibril (40). However, there is limited evidence that negative energy balance sufficiently modulates calpain expression or activation to meaningfully contribute to muscle loss because a 14-d 25% energy restriction failed to promote calpain activation in physically active young women (41). With extremely limited and often conflicting data, it is not evident at this point that the autophagy/lysosomal and calpain-dependent systems significantly contribute to the degradation of human skeletal muscle during negative energy balance and, more importantly, in response to alterations in dietary protein intake.

It is well documented, however, that the UP system serves a major role in modulating skeletal muscle proteolysis (42). Additionally, the proteasome regulates cell-cycle, antigen processing through class I major histocompatibility complex molecules, gene expression, and cell signaling (43). In addition to these primary functions, there is evidence suggesting that the proteasome is involved in noncatalytic activities,

including transcription regulation, DNA repair, and chromatin remodeling (44). As it relates to skeletal muscle degradation, a number of choreographed steps must occur before muscle proteins may be degraded by the proteasome itself. Myofibrils, the functional units of myocytes, composed of actin and myosin filaments spanning the length of the muscle cell, do not serve as substrate for the proteasome in their native state (45). They must first be broken down into actin and myosin monomers before they can enter the proteasome's 20S catalytic core.

Calpains may potentially contribute to myofibrillar cleavage (46), although skeletal muscle proteolysis initiation is most likely instigated by caspase 3, a cysteine protease most notably involved in apoptosis (47). Inactive procaspase 3 is first converted to active caspase 3 via caspase 9–mediated cleavage (47). Active caspase 3 then breaks down targeted myofibrillar proteins, producing a characteristic 14-kDa actin fragment and other polypeptide segments. These cleavage products may then be tagged with ubiquitin, mediated by the ATP-dependent E1 class of ubiquitin-activating enzymes (48). Once activated, ubiquitin is conjugated to a member of the E2 class of ubiquitin-conjugating enzymes. The final step of ubiquitin ligation to the target protein is mediated by the E3 class of ubiquitin ligases. This tagged protein is then recognized by the 26S proteasome, denatured, and degraded into peptide fragments, typically 2–25 amino acids long (48).

The activities of caspase 3 and the UP system are regulated in part by the insulin/IGF-PI3K pathway (**Fig. 1**). In tissue culture experiments with rat myocytes, the addition of insulin to the growth medium significantly suppresses serum deprivation (starvation)–induced actin cleavage (47). However, when insulin was added to cells containing a dominant-negative (defective) PI3K, no changes were observed in actin cleavage. Data from other reports confirm these findings (49), which, taken together, clearly demonstrate the critical role of insulin in regulating caspase 3 activity through PI3K. Further, these data illustrate a potential mechanism by which skeletal muscle proteolysis is increased in response to hypoinsulinemia, a physiological characteristic of negative energy balance (18).

Dephosphorylation/inactivation of Akt may also increase the expression of UP components (50). Akt is responsible for phosphorylation of the forkhead box O (FOXO) family of transcription factors (51). However, in the absence of Akt-mediated phosphorylation, these FOXO transcription factors migrate into the nucleus and increase expression of a number of atrophy-related genes, including the muscle-specific ubiquitin ligases muscle atrogin F-box protein (MAFbx) (atrogin-1) and muscle RING-finger protein 1 (50). More specifically, addition of the PI3K inhibitor LY294002 to the growth medium of mouse muscle cells produces enhanced nuclear localization of FOXO1 and increased MAFbx mRNA transcription (52,53). Overexpression of FOXO3A in mouse myotubes also significantly up-regulates MAFbx expression (49). As such, it appears that the insulin/IGF-PI3K pathway not only regulates myofibrillar cleavage through caspase 3, but also the ubiquitylation of the resulting protein fragments via modification of ubiquitin-ligase expression.

Limited published data detail alterations in the UP system and associated subcomponents during periods of negative energy balance and varying levels of dietary protein. Although there are studies examining these cellular pathways in certain models of protein catabolism, including trauma (54), sepsis (55), cancer (56), and denervation (57), the level of severe muscle loss in those states is not comparable to studies assessing weight loss or the effects of exercise interventions. However, 6 mo of energy restriction (−3138 kJ/d or −750 kcal/d), combined with increased physical activity, was recently demonstrated to induce increased expression of FOXO3A, muscle RING-finger protein 1, and MAFbx mRNA in previously sedentary, obese older women (58). Similarly, muscle Akt phosphorylation was decreased in healthy young adults after a 10-d period of moderate energy deprivation (−2092 kJ/d or −500 kcal/d) (18), further implying that the muscle loss observed during energy restriction may in part be due to increases in caspase 3 and UP activity. In this same study, increased enzymatic activity of the UP β_5 subunit was observed that mirrored increased muscle protein breakdown (J. Carbone, S. Pasiakos, L. Vislocky, N. Rodriguez, unpublished data).

Effects of protein quality and branched-chain amino acids on the skeletal muscle response to negative energy balance

It is well established that the amino acid composition of dietary protein can influence the regulation of skeletal muscle protein turnover. Increasing branched-chain amino acid (BCAA) levels during energy restriction can support gluconeogenesis, maintain whole-body and muscle protein synthesis, and attenuate nitrogen excretion and whole-body and muscle proteolysis (59). Of particular importance is the BCAA leucine, a potent independent stimulator of muscle protein synthesis in cell culture and animal models through enhanced cellular regulation of mRNA translation (29). Human studies have demonstrated stimulation of the mTORC1 pathway, increased muscle protein synthesis (29,60,61), and decreased whole-body proteolysis after consumption of a leucine-containing food product during exercise (61–65). Leucine supplementation also appears to attenuate muscle proteolysis and continues to do so in the presence of the mTOR inhibitor rapamycin, acting independently of mTORC1, although dependent on functional PI3K signaling (64,65).

Although the recommended leucine intake is currently 14 mg·kg^{-1}·d^{-1} (66), the amount required to maximize the stimulation of muscle anabolic intracellular signaling may be at least 40–65 mg·kg^{-1}·d^{-1} (67,68) and even up to 7–12 g·d^{-1} to contribute to the preservation of muscle mass during stressors such as energy restriction (59). Leucine stimulation of anabolic pathways is decreased in the presence of sufficient essential amino acids (EAAs) (69), indicating an increased basal synthetic rate with adequate EAA ingestion, thus highlighting the importance of consuming dietary protein at levels above the current RDA during periods of energy deficit. In neonanatal pigs, supplementing a low-protein diet with leucine significantly increased muscle protein synthesis compared with a low-protein diet without

additional leucine (70). Furthermore, phosphorylation states of intracellular regulators of muscle protein synthesis (mTOR, 70-kDa S6 kinase, and eIF4E-BP1) were similar in pigs that consumed a leucine-supplemented, low-protein diet or a high-protein diet without additional leucine, highlighting the potential synthetic effect resulting from increased leucine intake.

There is a paucity of current reports examining the effects of total protein intake on regulators of human muscle proteolysis, although the past 20 y provided a number of findings supporting an anticatabolic effect of leucine and BCAAs (62,63,71–73). In healthy men, the oral ingestion of a carbohydrate solution containing isonitrogenous amounts of either EAAs (threonine, methionine, histidine) or BCAAs resulted in BCAA suppression of whole-body proteolysis to a greater extent than EAAs (71). These data suggest that leucine, and perhaps the remaining BCAAs, may have the ability to directly influence muscle protein breakdown.

Conclusions

Decreases in FFM after periods of negative energy balance may inhibit healthy weight management and decrease skeletal muscle function and performance. Although increasing dietary protein intake, and perhaps leucine, to more than the RDA has been demonstrated to spare muscle mass, a thorough understanding of the underlying molecular mechanisms is requisite to the development of nutritional countermeasures to mitigate the detrimental effects of negative energy balance.

Clearly, further study is required to assess the combined effects of negative energy balance and dietary protein intake on cellular mechanisms contributing to the regulation of skeletal muscle mass. Systematic, comprehensive studies that address changes in body composition, nitrogen balance, and whole-body and skeletal muscle protein turnover, in combination with expression and activity patterns of intracellular regulators of muscle mass, are required to identify nutritional agents (i.e., amino acids) to counteract decreases in FFM occurring in response to negative energy balance.

Acknowledgments

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