Muscle atrophy in cachexia: can dietary protein tip the balance?

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Current Opinion in Clinical Nutrition and Metabolic Care 2009, 12:611–616

Purpose of review

To review the efficacy of dietary protein supplementation in attenuating muscle atrophy in cachexia.

Recent findings

Only very few recent randomized controlled trials have studied the effects of protein supplementation in clinical cachexia. It appears that supplementation of dietary protein (>1.5 g/kg per day) alone or in combination with other anabolic stimuli such as exercise training maintains or even improves muscle mass, but results on muscle function are controversial and no clinical studies have yet directly linked alterations in cellular signaling or metabolic signatures of protein intake-induced muscle anabolism to muscle weight gain.

Summary

To elucidate the role of dietary protein supplementation in attenuating muscle atrophy in cachectic patients, randomized clinical trials are needed in adequately phenotyped patients using sensitive measures of muscle mass and function.

Keywords

cachexia, dietary amino acids, dietary protein, muscle atrophy

Curr Opin Clin Nutr Metab Care 12:611–616

1363-1950

Introduction

Cachexia is a complex debilitating metabolic syndrome associated with underlying illness [1••] that may not yet be cured but benefit from a multidimensional therapeutic approach consisting of nutritional support, exercise training and/or pharmacological treatment. Although muscle protein wasting is the most distinctive feature of cachexia, the relative influence on muscle protein turnover of common denominators of chronic disease progression, that is, adaptive low physical activity level, compulsory inactivity during acute hospitalization or advancing age versus disease-specific factors such as inflammation and hypoxia, remains unclear. In addition, the relative contribution of dietary protein intake in maintenance or accretion of muscle mass in the different stages of chronic wasting diseases remains unidentified and receives scarce attention in current clinical practice. Efficacy of dietary protein supplementation may not only depend on protein quantity and the specific amino acid formulation but also on the underlying disease and clinical condition, as well as on presence of other intervention strategies targeted at muscle maintenance. These issues can only be addressed by a translational research approach including relevant in-vitro and in-vivo experimental models and controlled clinical trials with adequately phenotyped patients and appropriate outcome measures. In this review, we will elaborate the efficacy of dietary protein supplementation in counteracting muscle atrophy in cachexia by discussing recent findings of intervention studies in muscle wasting patients and by reviewing the putative molecular mechanisms by which dietary protein regulates muscle protein turnover.

Optimal protein intake in cachexia

Maintenance of muscle is determined by the balance between muscle protein synthesis and breakdown, and therefore, to accomplish maintenance or even restoration of muscle mass in cachexia, maximal stimulation of protein synthesis and inhibition of degradation is desired. This requires optimal dietary protein intake, which may very well exceed the Recommended Dietary Allowance (RDA) of 0.8 grams (g) of protein per kilogram (kg) of body weight per day, the amount of protein that adequately maintains nitrogen balance in healthy individuals, including the elderly [2•]. Current estimations of protein requirements are mainly derived from studies on non-cachectic individuals, although some study populations shared characteristics of cachexia such as disuse. For instance, 45 g of essential amino acids per day, in addition to the RDA for protein, preserved muscle strength outcome during compulsory bed rest in elderly volunteers [3]. Optimal protein requirements are difficult

to estimate as they may depend on the protocol, that is, higher values are obtained in short-term studies and estimates may be 40–50% higher using tracer methodology than using nitrogen balance studies [4]. Despite these difficulties and the possible variations in protein requirements in different diseases and clinical conditions, a minimum of 1.5 g/kg of body weight per day or 15–20% of total caloric intake appears justified for cachexia, considering that this amount was determined as optimal protein intake in sarcopenia [5•], which is also characterized by muscle depletion. Furthermore, experimental evidence and recent clinical studies indicate that in contrast to sarcopenia, in which a decreased muscle protein synthetic response has been identified, active cachexia is also characterized by increased muscle protein degradation [6,7].

For optimal dietary supplementation in cachexia, protein source and meal composition also need to be considered, as in the elderly, muscle protein synthesis was hypothesized to be blunted when protein and carbohydrate are coingested or when the quantity of protein is less than 20 g/meal [8]. Finally, timing of protein intake may be important in cachectic patients to avoid adverse effects of high protein intake on overall dietary intake in view of recently described dose-dependent satiating effects of protein in healthy volunteers [9].

Effects of protein and amino acids on muscle mass

There are few recent randomized controlled trials (RCTs) investigating the effect of protein in muscle wasted patients, but these trials do provide new insights into the positioning of nutritional intervention in attenuating muscle atrophy. A recent RCT, which included 59 outpatients with advanced chronic obstructive pulmonary disorder (COPD), reported a positive effect of dietary counseling and food fortification (using milk powder) on muscle mass [10••]. The intervention group consumed more energy and protein (±1.47 g/kg body weight; mean difference 11.8 g/day), resulting in weight gain and maintenance of muscle mass during 6 months of intervention period and weight maintenance during 6 months of follow-up. Although control COPD patients lost weight and muscle mass throughout the study, significant differences were observed between the groups in quality of life, but not in skeletal muscle function. Campbell *et al.* [11••] performed a RCT of individualized nutritional counseling in 56 patients with chronic kidney disease. Intervention aimed at a protein and energy intake of 0.8–1.0 g/kg and at least 125 kJ/kg, respectively, per day. During 12 weeks, the decrease in body cell mass was reduced (3.5%) by the intervention, with a greater increase in energy intake, but no difference in measurable overall protein intake showing that a protein intake around the RDA may attenuate muscle atrophy, but not stimulate muscle regrowth. The effect of increasing protein intake was also investigated in 38 muscle wasted patients with stable chronic heart failure [12••]. Oral supplementation of essential amino acids (8 g/day for 8 weeks in addition to habitual dietary intake ≥125 kJ/kg and protein intake >1.1 g/kg) resulted in a greater increase in body weight compared with nonsupplemented patients (80% of supplemented patients versus 30% of controls >1 kg of body weight gain). Changes in arm muscle size and nitrogen balance were similar but only supplemented patients improved exercise output, peak oxygen consumption and walking distance, illustrating an apparent dissociation between effects of nutritional supplementation on muscle mass versus muscle endurance. These studies confirm the argumentation that a protein intake of at least 1.5 g/kg body weight is needed to induce slight muscle hypertrophy.

Limited studies have yet compared muscle anabolic effects of selective proteins or focused on specific amino acids in patients at risk or suffering from cachexia. In a short-term tracer experiment, Engelen *et al.* [13] reported that supplementation of branched-chain amino acids (BCAAs) to a soy protein meal resulted in a significant acute increase in whole body protein synthesis in weight stable COPD patients with borderline muscle mass but not in age-matched healthy controls. In a recent RCT, the effect of supplementation of a combination of β-hydroxyl β-methyl butyrate (a metabolite of the essential amino acid leucine), glutamine and arginine was studied in weight losing cancer patients with stage III or IV solid tumors or currently metastatic cancer of any initial stage. Administration of this supplement for 8 weeks did not result in significant changes in lean body mass [14••]. Because the experimental conditions in both studies were different and no information on protein balance was available in both groups, no definite conclusions can be drawn and further research should be conducted to clarify the role of specific amino acid supplementation in maintaining muscle mass or preventing muscle wasting during acute weight loss.

Because the cause of cachexia is considered multifactorial, numerous studies have indicated that next to isolated nutritional supplementation, other intervention strategies could be beneficial in attenuating muscle atrophy in cachexia. The beneficial effects of resistance training on maintenance of muscle mass and function in sarcopenia have recently been highlighted [15], but limited studies have specifically addressed the additive effects of nutritional supplementation to (resistance) exercise in cachexia. Several studies on patients with COPD showed that protein/carbohydrate-rich supplements as integrated part of a pulmonary rehabilitation program were effective in inducing (muscle) weight gain and improving physical

performance [16–18], but only one study [19] was yet able to disentangle the effect of nutritional support as adjunct to exercise training. In this study, cachectic patients receiving exercise only did not gain weight or increase muscle mass in contrast to those receiving exercise, nutritional supplementation and anabolic steroids. Agin *et al.* [20] studied in a RCT the effect of 14 weeks of intervention consisting of whey protein (1 g/kg body weight) in addition to consumption of energy and protein above RDA. Treatment with whey protein promoted weight and fat gain with little effect on physical function and quality of life, whereas resistance training increased muscle mass, muscle strength and quality of life, leading to functional improvement. Combining whey protein and resistance exercise did not further promote accretion of muscle mass achieved by exercise alone. Potential enhancing effect of anabolic pharmacological agents as single therapy or as precursor or cotreatment of the muscle growth response to rehabilitation is currently a hot topic. The efficacy of anabolics is affected by protein intake, but no clinical data are available regarding the response to anabolics in cachexia during normal and high protein intake (0.8–1.0 versus >1.5 g/kg body weight, respectively).

Molecular regulation of muscle protein metabolism by dietary protein in cachexia

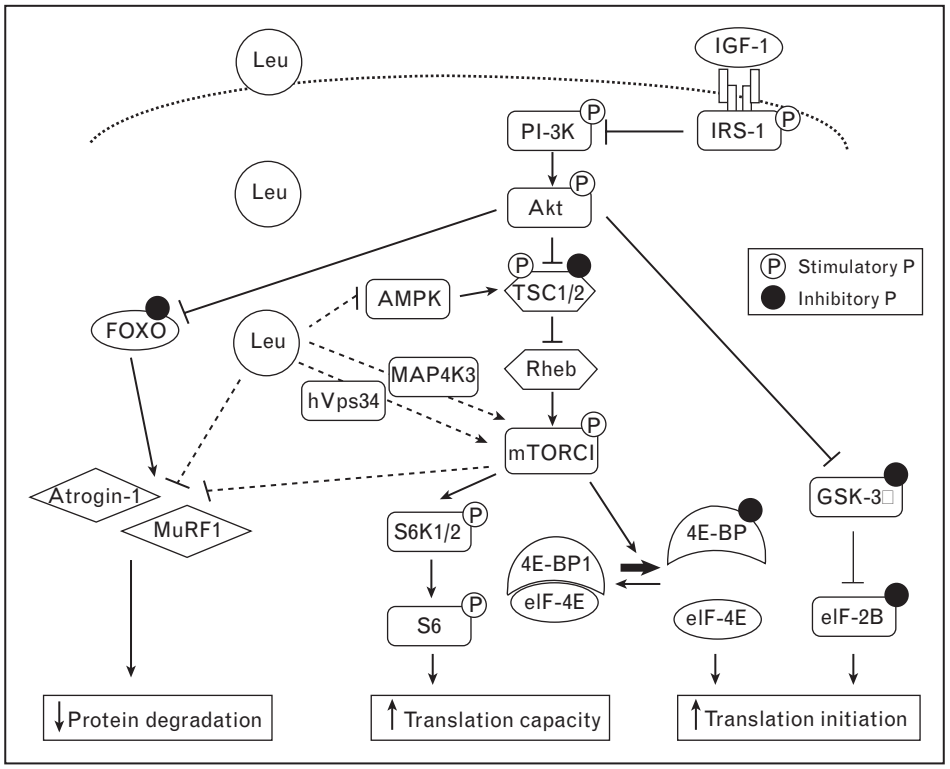
Despite the potential for dietary protein in attenuating muscle atrophy in cachexia, no clinical studies are available in which the putative molecular mechanisms by which dietary protein influences muscle protein turnover have been addressed. This could, however, be instrumental in optimizing and fine-tuning the dose, composition and timing of dietary protein as well as potential combined treatments. To this end, the following paragraphs will discuss the molecular mechanisms of protein synthesis and breakdown, which could be involved in beneficial effects of dietary protein in attenuating muscle atrophy.

Regulation of muscle protein synthesis by dietary protein

The regulation of muscle protein synthesis is very similar to that of other cell types, whereas some protein degradation routes unique to striated muscle have been identified. In nonpathological conditions, modulation of muscle protein turnover relies on the postprandial availability of nutrients like amino acids, which directly, and indirectly via the actions of or in combination with insulin, stimulate muscle protein synthesis and decrease degradation (Fig. 1). Anabolic effect of amino acids may partly be attributed to increased substrate availability; however, a mixture of the BCAAs or leucine alone stimulates protein synthesis to the same extent as a complete mixture of amino acids, indicative of a signaling role of these particular amino acids [21–23]. In contrast to protein synthesis signaling by insulin [24] or insulin-like growth factor-I (IGF-I) [25], these anabolic actions of BCAAs do not appear to be receptor-mediated [26], or require insulin receptor substrate (IRS-1) phosphatidylinositol-3 kinase (PI3K), and PKB/Akt activation. Subsequent signaling to increase protein synthesis by insulin/IGF-I involves stimulation of mRNA translation via activation of eukaryotic initiation factors (eIFs) by inhibition of glycogen synthase kinase (GSK)-3 and activation of mammalian target of rapamycin (mTOR; reviewed in detail by Glass [25] and Proud [24]). Inhibition of GSK-3 abrogates its suppressive effect on eIF2B. Currently, there is no evidence to support regulation of GSK-3 activity by amino acids in the control of translation initiation. mTOR, on the other hand, is activated indirectly by Akt signaling – as this suppresses TSC1/2-inhibition of mTOR – resulting in increased activity of eukaryotic translation initiation factor 4E (eIF4E) as a consequence of the dissociation of eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) following mTOR-mediated phosphorylation. In addition to stimulatory effects on eIF activity, insulin/IGF-I signaling also promotes protein synthesis by increasing translation capacity and elongation. This occurs via phosphorylation of S6K1/2 and eIF-4E by the mTORC1 complex (mTOR associated with raptor and GbL) [27]. Supplementation of rats, which were starved for 18 h, with dietary protein results in decreased binding of 4E-BP1 to eIF4E [28], whereas long-term amino acid supplementation attenuated aging-induced decrease in muscle sarcomers [29•]. However, dietary protein supplementation does not always result in differences on muscle protein metabolism as is indicated by unchanged ^{13}C-valine enrichment in muscle tissue [30], or muscle mass in tumor-bearing mice [31•].

Leucine alone is sufficient to increase protein synthesis, which is mediated via the phosphorylation of 4E-BP1 and S6K1 as a result of phosphorylation and activation of mTOR in the mTORC1 complex. Of all amino acids, leucine appears the most potent stimulator of mTORC1 phosphorylation [22]. Glutamine even has inhibiting effects on mTORC1 signaling in cultured muscle cells [32,33]. The mechanism by which leucine stimulates mTORC1 phosphorylation is not fully understood, but human vacuolar protein sorting-34 (hVps34) and mitogen-activated protein kinase-3 (MAP4K3) have recently been described to be involved [34,35]. Leucine also stimulates protein synthesis by inhibiting adenosine monophosphate-activated protein kinase (AMPK)-mediated phosphorylation of TSC2, which negatively controls mTORC1, linking energy availability to mTORC1 signaling [23]. Indeed, supplementation of dietary leucine increased phosphorylation of mTOR, S6K1 and 4E-BP1 and

Figure 1 Regulation of protein synthesis and degradation by insulin/insulin-like growth factor-I and leucine signaling



AMPK, adenosine monophosphate-activated protein kinase; eIF, eukaryotic initiation factor; GSK, glycogen synthase kinase; IGF, insulin-like growth factor; IRS, insulin receptor substrate; mTOR, mammalian target of rapamycin.

formation of active eIF4E complex in 7-day-old piglets [36]. In contrast, addition of leucine had no effect on muscle or carcass weight in cachectic mice [31•]. This could be explained by the recent finding that dietary leucine influences peak activation but not duration of skeletal muscle protein synthesis and mTOR activation [37•]. Discrepancies between anabolic signaling signatures and muscle maintenance have also been described in weight stable COPD patients with muscle atrophy, as increased phosphorylation of Akt and downstream mediators (i.e., 4E-BP1, S6K1 and GSK-3β) were explained as a failed attempt to maintain muscle mass [38].

Regulation of protein degradation by dietary protein

Maintenance of muscle mass by insulin/IGF-I signaling also involves suppression of protein degradation. Proteolytic systems known to be suppressed by insulin/IGF-I signaling include the 26S-ubiquitin (Ub) proteasome pathway (UPP) [25] and lysosomal protein degradation (autophagy) [39]. In muscle, increased protein degradation by the UPP has been shown to be of great importance in muscle atrophy in experimental models [25] and humans [40]. Although various components of the UPP have been reported to be increased during active atrophy, the most consistent are the increased expressions of striated muscle-specific E3 Ub-ligases MuRF1 and atrogin-1/MAFbx. The expression of these so-called 'atrogenes' is negatively regulated by insulin/IGF-I signaling [41,42]. The molecular basis for this control of UPP-mediated protein degradation has to a large extent been clarified and relies on the negative regulation of the transcription factors FOXO1/3 [42,43]. In the presence of insulin/IGF-I, Akt-mediated phosphorylation inhibits FOXO1/3 nuclear translocation, suppressing FOXO-dependent transcription of atrogin-1 and MuRF1. Interestingly, the expression of Bnip3 – an essential protein in lysosomal protein degradation – is also regulated by FOXO3, demonstrating how insulin signaling controls multiple proteolytic systems in skeletal muscle [44].

In a recent study, it was shown that dietary leucine supplementation inhibits muscle protein breakdown in rats [45]. In cultured muscle cells, insulin and leucine were found to act additively in downregulating 14kDa E2 Ub-conjugating enzyme expression [46], whereas BCAA reduced atrogin-1 and MuRF1 expression [47]. The signaling route employed by leucine to inhibit atrogin-1 and MuRF1 expression has not been identified. However, as IGF-I-mediated inhibition of atrogin-1 and MuRF1 expression was shown to require mTOR activity

[48], it is tempting to speculate that leucine may decrease expression of the atrogenes by activation of mTOR.

Despite our increasing understanding based on experimental studies of the mechanisms by which protein/amino acids can directly or indirectly stimulate anabolic or anticatabolic signaling, much of this awaits confirmation in cachexia models and clinical cachexia.

Conclusion

Recent in-vitro studies provide convincing evidence for proanabolic and anticatabolic effects of amino acids, but limited data are available in experimental animal models and in patients suffering from cachexia. Recent studies on weight stable patients with muscle atrophy show that enhancing protein intake may result in maintenance or accretion of muscle mass depending on the amount of protein. Efficacy in terms of muscle regain and physical functioning appears enhanced when patients receive an additional anabolic stimulus such as exercise training, but more RCTs are needed to disentangle the effects of dietary protein and resistance exercise on muscle mass and functional improvement in acute and chronic wasting. In contrast to sarcopenia, relatively little attention has been given to potential differential effects of different types of protein or of specific amino acids on muscle wasting in cachectic patients. Surprisingly, the efficacy of protein intake in attenuation of muscle atrophy in cancer (pre)cachexia or as adjunct to cancer specific therapy is still rarely investigated in RCTs. The variable disease course of many chronic wasting conditions may also demand fine tuning of protein supplementation, but no data are yet available on, for example, protein requirements in specific diseases during clinically and weight stable conditions, in comparison to acute exacerbations or in the recovery phase. Therefore, to optimize the application of dietary protein in cachexia management, expansion of supplementation studies in experimental cachexia models is required, as well as increased efforts to conduct proof-of-concept controlled clinical trials, which combine molecular signatures of protein turnover regulation in skeletal muscle biopsies and sensitive measures of muscle mass and function.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 669–670).

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