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Board Invited Review

BOARD INVITED REVIEW

Practical applications of whey protein in supporting skeletal muscle maintenance, recovery, and reconditioning

Emily J. Arentson-Lantz[†,1] and Sean Kilroe[†,‡]

[†]Department of Nutrition and Metabolism, Center for Recovery, Physical Activity and Nutrition, University of Texas Medical Branch, Galveston, TX 77555-1028, USA, [‡]Department of Sports and Health Sciences, College of Life and Environmental Science, University of Exeter, Exeter, UK

[1]Corresponding author: ejlantz@utmb.edu

ORCiD number: 0000-0001-7303-8067 (E. J. Arentson-Lantz).

Abstract

Like humans, many companion animals experience a gradual decline in skeletal muscle mass and function during later years of life. This process, analogous to sarcopenia in humans, increases risk for morbidity and mortality. Periods of reduced activity due to injury or illness, followed by an incomplete recovery, can accelerate the loss of muscle mass and function. Emerging research from human studies suggests that moderate amounts of high-quality protein may attenuate the loss of muscle, while preventing accumulation of fat during periods of disuse. Whey protein is a consumer-friendly and readily available source of high-quality protein. It supports skeletal muscle maintenance during normal aging and may also provide anabolic support during periods of illness, injury, and recovery. Ongoing research efforts continue to refine our understanding of how protein quality, quantity, and meal timing can be optimized to support retention of muscle mass and function during aging. Priority research areas include supplementation with high-quality protein during illness/injury to stimulate anabolism by targeting molecular mechanisms that regulate skeletal muscle metabolism.

Key words: aging, atrophy, disuse, humans, whey protein skeletal muscle.

Introduction

In humans adults, age-related skeletal muscle loss occurs at a rate of 1% to 2% per year beginning around 50 yr of age (Keller and Engelhardt, 2013). This insidious loss of muscle is termed "sarcopenia" and is clinically defined 3 ways: a loss of skeletal muscle mass, low muscle strength, and poor physical performance (Santilli et al., 2014; Cruz-Jentoft et al., 2019). Sarcopenia is associated with an increased risk of physical disability, poor quality of life and mortality (Santilli et al., 2014), and is not limited to humans. Sarcopenia has also been reported in companion animals, such as senior cats and dogs (Cupp et al., 2007). Over an 8-yr period, aging cats lost 34% of their lean body mass (LBM) at a rate of 4.3%/yr (Cupp et al., 2007). Similarly, in aging dogs, the rate of LBM loss over a 4 yr period was 2.4%/yr (Adams et al., 2015).

In contrast to the slow progression of sarcopenia, as little as 3 to 4 d of reduced physical activity due to injury or illness accelerates muscle mass loss. Older adults (> 65 yr) tend to experience a greater loss of muscle during disuse compared with their younger counterparts (Paddon-Jones et al., 2004; Arentson-Lantz et al., 2020). However, recent data from our group show that even healthy, middle-aged adults (~50

Abbreviations

Akt	protein kinase B
DIAAS	digestible indispensable amino acid score
IAA	indispensable amino acid
LBM	lean body mass
MPB	muscle protein breakdown
MPS	muscle protein synthesis
mTORC1	mammalian target of rapamycin complex 1
PGC1α	peroxisome proliferator-activated receptor γ coactivator 1-α
RDA	recommended dietary allowance
WPC	whey protein concentrate
WPI	whey protein isolate

yr), with a largely youthful phenotype, also experience an accelerated loss of muscle during periods of inactivity (English et al., 2016). For context, in older adults, 7 to 10 d of bed rest can result in >1 kg loss of lean mass, primarily from postural muscle in the legs (Drummond et al., 2012; Dirks et al., 2016; Arentson-Lantz et al., 2019a; Kilroe et al., 2020). The duration of disuse matters, although the rate of skeletal muscle deconditioning is thought to be greatest during the first few days of muscle inactivity (i.e., first 7 d). In many instances, a reduction in lean mass can be detected after only 2 d of disuse (Kilroe et al., 2020).

The cumulative impact of repeated episodes of muscle loss followed by incomplete recovery occurs in parallel with sarcopenia and unless managed, may hasten the onset of frailty (English and Paddon-Jones, 2010). While the majority of research on modifiable behaviors and dietary practices that support healthy aging has been conducted in humans, some findings have been confirmed in companion animals (Laflamme, 2018). It is intuitively desirable to identify interventions for both humans and companion animals of all ages that can protect muscle mass and function during disuse and accelerate recovery (Paddon-Jones and Rasmussen, 2009; English and Paddon-Jones, 2010). Dietary protein is a well-established anabolic stimulus and source of amino acids for building and repairing muscle protein. In this narrative review, will briefly discuss how high-quality dietary protein, such as whey, can be used to support skeletal muscle mass during aging and bouts of disuse as well promote recovery and reconditioning of muscle.

Regulation of Muscle Protein Synthesis

At the broadest level, skeletal muscle mass is regulated by muscle protein synthesis (MPS) and muscle protein breakdown (MPB; Rennie et al., 2004). Although there are a myriad of stimuli that alter the balance of MPS and MPB, two of the primary, modifiable drivers are physical activity (Bodine, 2006; Kumar et al., 2009; Wall et al., 2016) and dietary protein ingestion (Moore et al., 2009; Witard et al., 2014). In the presence of adequate physical activity and nutrition, the rates of MPS and MPB are cyclically balanced and muscle mass is maintained or increased in cases of growth or purposeful physical training (Kumar et al., 2009; Brook et al., 2015). Conversely, with increasing age (Mitchell et al., 2012), periods of physical inactivity, inadequate nutrition, illness or injury (Breen et al., 2013), MPB can chronically exceed MPS resulting in a loss of muscle mass, strength and physical performance (Mitchell et al., 2012; Santilli et al., 2014).

The underlying molecular etiology of muscle loss is complex and multifactorial. Although muscle atrophy is generally characterized by the rate of MPS being persistently lower than the rate of MPB, this is achieved through several pathway-specific signaling cascades. Catabolic diseases and other myopathies like chronic obstructive pulmonary disease, and symptoms of catabolic conditions, e.g., cancer cachexia, increase MPB rates primarily through the activation of certain proteolytic pathways, including the ubiquitin–proteasome pathway (Johns et al., 2013). In contrast, age-related muscle loss, as well as disuse-atrophy, is primarily a result of transiently reduced MPS rates, particularly in response to dietary protein ingestion (termed anabolic resistance), which is characterized by a blunted response in the activity of skeletal muscle anabolic pathways, such as the protein kinase B (Akt)/mammalian target of rapamycin complex 1 (mTORC1) signaling pathway (Cuthbertson et al., 2005; Ferrando et al., 2010; English et al., 2016). The skeletal muscle of older adults exhibits a reduced anabolic response to a protein rich meal after a short 5-d period of bed rest, whereas younger adults maintain their postprandial MPS rates (Tanner et al., 2015).

Leucine is a branch chain amino acid that directly and indirectly activates mTORC1. This activation in turn coordinates a signaling cascade which leads to the assembly of initiation factor E4 complex and activation of ribosomal protein S6, ultimately resulting in an upregulation in MPS (Drummond and Rasmussen, 2008; Sandri et al., 2013). This leucine-activated pathway is considered to be widely conserved across species (Yang and Guan, 2007; Suryawan et al., 2008; Norton et al., 2012). In adult humans, a small amount of leucine (~ 3 g/meal) is considered an "anabolic trigger" to maximally stimulate MPS, if sufficient quantities of the other indispensable amino acids (IAAs) are also present. Indeed, leucine appears to be a critical anabolic signal that is the primary determinant of muscle anabolism in response to a meal (Katsanos et al., 2006; Devries et al., 2018a, 2018b).

For humans, a food first approach (vs. supplementation) is broadly recommended for overall heath as well as maintaining muscle mass. Meals that include a moderate amount (25 to 30 g) of mixed high-quality plant and animal protein will likely contain sufficient leucine to robustly stimulate MPS in both young and older adults (Symons et al., 2007; English and Paddon-Jones, 2010). However, in some circumstances dietary supplementation may be desirable and/or preferable. Whey is a rapidly digested protein that has repeatedly been shown to effectively and efficiently stimulate MPS in older adults (Burd et al., 2012) and during periods of disuse (Antonione et al., 2008).

Whey protein has long been favored by researchers, clinicians and many consumers because of its full complement of EAAs and rich leucine content (~3.0 g leucine per 25 g of whey protein isolate (WPI); Table 1; USDA, 2019). Whey protein is commercially available as WPI (WPI: >90% protein), whey protein concentrate (WPC35 and WPC80, with 35% and 80% protein-dry basis, respectively) and demineralized whey protein, which has a 13% protein content and high lactose content (Demin 90; Kelly, 2019). Both WPI and WPC80 both have excellent digestible indispensable amino acid scores (DIAAS) of 1.09 and 0.973, respectively (Table 1). As a note, DIAAS is a scientifically validated means of evaluating protein quality of a single ingredient or individual foods in the human diet (FAO, 2011), but the rate-limiting amino acid needs of target populations should be taken into consideration when incorporating whey protein in dietary formulation.

Table 1. Characteristics of commonly available protein sources[1,2]

	WPI	WPC	Casein	SPI	RPC	PPC
∑ IAA, g/25 g protein	12.4	11.7	11.0	9.0	6.7	5.9
∑ BCAA, g/25 g protein	5.6	5.4	4.9	3.4	3.3	2.6
Leucine, g/25 protein	3.0	2.5	2.3	1.5	1.5	1.4
DIAAS score	1.09	0.97	ND	0.90	0.37	0.82

[1]WPI, whey protein isolate; WPC, whey protein concentrate; SPI, soy protein isolate; RPI rice protein concentrate; PPC, pea protein concentrate; IAA, indispensable amino acid; BCAA, branched-chain amino acid; ND, not determined.
[2]amino acid content can be found in the USDA National Nutrient Database for Standard Reference—http://ndb.nal.usda.gov/ (United States Department of Agriculture). DIAAS scores from Rutherfurd et al. (2015).

Current Protein Recommendations

Recommendations from research councils providing population-level guidance for dietary protein adequacy in human and animals are often not nuanced to differentiate between the younger and older adults. Additionally, they are often inaccurately interpreted as either the average or upper limit for protein intake (Wolfe and Miller, 2008). For example, the recommended dietary allowance (RDA) for protein for humans is 0.80 g protein/kg/d and was formulated as an estimate of the *minimum* daily average dietary protein required to meet the needs of 97% of the healthy adult population (Meyers et al., 2006). It should also be recognized that current recommendations for both humans and companion animals do not take into account any additional catabolic burden (e.g., disease and disuse) that may increase protein needs.

Nutritional Strategies to Maintain Muscle Mass

As noted previously, dietary protein in particular is of interest because of its role as an anabolic stimulus and source of amino acids for MPS. However, strategies to optimize protein intake for muscle health in both a healthy, aging population as well as during periods of catabolism and recovery continue to be refined. Several review articles, including a recent systematic review, highlight the diverse approaches researchers have utilized to understand best practices for protein intake during aging and periods of catabolism and recovery in adults (Hanach et al., 2019; Howard et al., 2020; Phillips et al., 2020). While the variability in the duration, timing, and frequency of protein-based nutrition interventions, and age of participants make it difficult to research a consensus, it is likely that current protein recommendations (e.g., *RDA for protein intake in humans: 0.8 g protein/kg body weight/day*) are sufficient for younger individuals, but are not adequate to meet the protein needs of aging and clinical populations. As this field of research moves forward, greater emphasis should be given to the conceptual difference and realistic expectations of nutrition-based strategies that are designed to *preserve* muscle mass and function during aging/catabolic conditions when compared with interventions designed to *build* muscle in a younger/active population.

Whey and other dairy-based proteins have been widely utilized to evaluate the efficacy of protein supplementation in attenuating the age-related decline of muscle mass and function (Dominique et al., 2018; Hidayat et al., 2018; Hanach et al., 2019). A recent systematic review meta-analysis of over 36 randomized-controlled trials showed no meaningful effect of protein supplementation on LBM, muscle strength, or physical performance in community-dwelling middle-aged and older adults (>50 yr), even when in combination with resistance exercise (Dominique et al., 2018). While this may be initially perceived as a negative finding, the authors point out that participants were considered to be a healthy population who regularly consumed protein in excess of the RDA. Therefore, we should consider that consuming moderate portions of high-quality protein in a mixed meal is largely sufficient to support muscle health in healthy, older adults.

The insidious nature of sarcopenia (~1% LBM lost each year or 400 to 500 g of LBM in 70 kg man; Kyle et al., 2001) presents challenges for detecting meaningful changes in LBM in response to nutrition-based interventions. Disuse-based models (bed rest or limb immobilization) are useful for studying aging muscle, because of the accelerated loss of LBM and function that occurs in a relatively short amount of time (days vs. years). A limitation of bed rest/immobilization studies is that they represent "best case scenario" for aging or a period of physical inactivity related to illness/injury without the underlying catabolic effects of a disease that often occur in clinical populations. However, these models remain powerful tools to understand the fundamental pathways driving muscle loss and assess protein-based interventions to protect aging muscle health.

Our group recently examined how improving dietary protein quality with whey protein counters the negative impact of disuse on body composition and function of healthy older adults (~70 yr old) in the context of a bed rest model (Arentson-Lantz et al., 2019b). Subjects were randomized to either a control group and provided a diet with a mix of plant and animal-based proteins or the experimental group where whey protein replaced some of the whole food sources of protein. In both groups, protein intake was held to ~ 0.9 g protein/kg d (~16% of total kcal), which moderately exceeds the RDA for protein. Subjects in the whey group lost roughly 35% less leg lean mass than the control group following 7 d of strict bed rest (–1,035 vs. –680 g leg lean mass in control vs. whey group); however, preservation of LBM did not translate into to the preservation of strength. Whey protein also promoted a modest loss in fat mass (~300 g) during disuse (Arentson-Lantz et al., 2019b). Others groups have shown that supplementing 20 g of additional leucine-enriched whey protein twice daily does not confer any additional benefit in older men habitually consuming protein well above the RDA (1.0 g protein/kg/d) during a 5-d period of leg immobilization (Dirks et al., 2014). Together, these data suggest that improving protein quality rather than increasing total energy intake or altering macronutrient profiles may offer a protective effect for LBM during disuse. Improving protein quality rather than increasing protein quantity is a pragmatic, potentially cost-saving mechanism to optimize muscle metabolism without sacrificing other nutrients in the diet that are important for overall metabolic health (vitamin D, fiber, etc.).

The mechanisms underlying the beneficial metabolic effect of whey protein on muscle mass during disuse are not yet fully understood. While the leucine-rich content of whey protein is important for maintaining skeletal muscle anabolic sensitivity, there may be additional benefits for muscle health.

In a longer duration bed rest study (19 d) with young men, regular consumption of a high protein diet enriched with whey (1.8 g protein/kg/d + potassium bicarbonate vs. control group, 1.2 g protein/kg/d) protected against disuse-induced reductions in skeletal muscle oxidative capacity (Bosutti et al., 2016). Preliminary work in cell-based studies of may provide a potential mechanistic basis for these findings. In C_2C_{12} myocytes, the addition of leucine stimulates NAD-dependent deacetylase sirtuin-1 activity via peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1 α) resulting in an increase in oxygen consumption (Sun and Zemel, 2009; Vaughan et al., 2013). Further work is needed to understand how improving dietary protein quality contributes to maintaining skeletal muscle bioenergetics during aging and disuse.

Amino acid supplementation is a practical alternative for individuals experiencing difficulties consuming a sufficient quantity or quality of protein at each meal. Indeed, regularly providing moderate doses of leucine (~3 to 4 g leucine, similar to the leucine content of a serving of whey protein) has a protect effective on LBM during disuse. Consuming 3 to 4 g of leucine at each meal reduced lean leg mass loss following 7 d of inactivity by almost 50% in middle-aged and older adults (English et al., 2016; Arentson-Lantz et al., 2020). The protection of LBM by leucine supplementation is primarily through preventing the decrease in basal MPS that typically occurs during disuse (–10% ± 10% reduction vs. 30% ± 9% reduction, leucine supplemented vs. control group; English et al., 2016). Others have reported that supplementing young men with leucine during 7 d of leg immobilization does not attenuate the loss of LBM and function (Backx et al., 2018). These discrepancies in the efficacy of leucine to prevent the loss of muscle mass may be related to the age of the participants (older vs. young) or mode of disuse (single-leg immobilization vs. bed rest).

While leucine content of whey protein provide anabolic benefits to muscle health during disuse in adults, further work is need to understand how long the benefits can be maintained and how they are translated into phenotypic and functional outcomes. In a recent 14-d bed rest study supplementing middle-aged adults with leucine (3 to 4 g/meal) middle-aged adults had a robust protective effect on LBM during the initial 7 d of inactivity. However, the rate of lean leg mass loss in leucine-supplemented subjects was the same as the control condition during subsequent 7 d of bed rest (English et al., 2016). It is likely that the modest nutritional benefits of leucine/whey protein are eventually overcome by the pro-inflammatory environment associated with longer-term periods of inactivity (Karlsen et al., 2020). These and other time-course or saturation effects (Verhoeven et al., 2009; Leenders et al., 2011) as well as the inherent heterogeneity and variability in study populations are important factors when implementing and interpreting nutrition-based interventions.

Protein Supplementation and Restoration of Muscle Mass During Recovery

There is a clear need to optimize nutrition support for recovery of muscle mass and function following disuse. An estimated 70% of hospitalized adults are discharged below their preadmission level of function and many experience long-lasting physical and metabolic impairment (Boyd et al., 2008). Current rehabilitation approaches focus on increasing physical activity to promote activation of muscle and improve mobilization. However, following inactivity/injury older adults are often unable to perform resistance exercise at workloads necessary to stimulate MPS (Howard et al., 2020). The inclusion of protein-based nutrition interventions may provide an additive anabolic boost for activity-based rehabilitation programs. Supplementing orthopedic patients with 20 g of EAA twice daily before and after elective surgery preserved LBM at 2 wk postsurgery (–3.4% ± 2.2% vs. –14.3 ± 3.6% quadricep muscle volume; 20 g EAA vs. placebo) and accelerated recover of functional mobility (Dreyer et al., 2013). However, subjects in the placebo group reported consuming less than the RDA for protein (0.65 g protein/kg/d) and it is unclear if the additional EAAs conferred any additional benefit on muscle health or if the difference in the groups was driven by the suboptimal protein intake in the placebo group. A "preloading" supplementation strategy is not feasible for unanticipated hospitalization/illness and other groups have considered protein supplementation immediately following inactivity. Deer et al. (2019) recently completed a pilot study where older adults were supplemented with whey protein following acute hospitalization. While the study was not powered to detect differences between placebo and the whey protein intervention, the findings from this study found that whey protein supplementation was feasible and well tolerated in this clinical population at high risk for loss of muscle mass and function (Deer et al., 2018). Further work is needed to understand the unique anabolic needs during restoration of muscle mass and function following disuse and how protein intake can be accelerate recovery.

Practical Considerations

Whey protein is a consumer-friendly and readily available source of high-quality protein to support skeletal muscle maintenance during the aging process as well as during periods of disuse and recovery. Moving forward, there are several priority areas where we should continue to develop and challenge our understanding of how protein intake can be formulated to promote muscle health.

- Optimize protein quality to support retention of muscle mass and function during aging.
- Supplement with high-quality protein, as appropriate, during illness/disuse.
- Identify how protein intake/supplementation can be tailored to complement current rehabilitation strategies to accelerate restoration of muscle mass and function following inactivity.

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Authors' Contributions

E.A.L. and S.K. shared writing responsibilities with EAL serving as the primary author. Both authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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