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Theme: *Dietary muscle proteins for stage of life*

Dietary meat and protection against sarcopenia

Gordon S. Lynch and René Koopman

Centre for Muscle Research

Department of Physiology, School of Biomedical Sciences, The University of Melbourne

Correspondence:

Gordon S. Lynch, Ph.D.
Centre for Muscle Research
Department of Physiology
School of Biomedical Sciences
Faculty of Medicine, Dentistry and Health Sciences
The University of Melbourne
Victoria, 3010, AUSTRALIA
tel: 613 8344 0065
email: gsl@unimelb.edu.au

Abstract

Sarcopenia describes the age-related loss of skeletal muscle mass and associated muscle weakness. Sarcopenia is a major global health problem given that the number and proportion of older people in the population is escalating worldwide and represent the fastest growing segment of society. The loss of muscle mass compromises physical capacity, increases susceptibility to falls, and impacts on an individual's functional independence and quality of life. Tackling sarcopenia sensibly and effectively will identify strategies that will enable older adults to age well and age productively. The underlying causes of sarcopenia are complex and multifactorial and will likely require combinatorial therapies to address its symptoms. Nutrition, particularly protein intake, is a more easily modifiable factor, especially when combined with structured (resistance) exercise programs. The relative success of protein feeding strategies for sarcopenia, is limited by a so-called anabolic resistance in older people. Meat contains essential amino acids and nutritive compounds of high quality, and even a moderate intake can increase muscle protein synthesis in older men and women. However, health risks have been identified with the consumption of different meats, with high intake of processed meats increasing the risk for cardiovascular disease and different cancers. Risks for fresh white and red meat are considerably less and modest consumption is encouraged as part of a healthy eating plan for many older adults to ensure adequate protein intake. Other nutritive strategies of relevance for sarcopenia involve fortifying the nutrient value of different meats. Studies on muscle cells and animal models of muscle wasting, have identified the therapeutic potential of the amino acid, glycine, to reduce inflammation, attenuate muscle atrophy, and re-sensitize muscle to anabolic stimuli. Glycine supplementation or feeding animal products with a high glycine content (e.g. gelatin), could represent simple and effective nutritional strategies as part of a suite of therapies to attenuate sarcopenia.

Sarcopenia – a global public health problem

Skeletal muscle is essential for life – not just as an organ for locomotion or breathing, but as an endocrine organ that contracts and releases different factors ('myokines') that signal between other organs and tissues (Whitham et al. 2018). Many muscle wasting diseases and conditions are associated with increased morbidity and mortality and therefore maintaining muscle mass can be critical for survival. Muscle mass is maintained through a balance between protein synthesis and protein degradation. Different factors can influence this balance ultimately to determine whether muscle mass is gained (muscle hypertrophy) or lost (muscle atrophy). These different intrinsic and extrinsic factors include nutrition, genetics, innervation, hormones, metabolism, inflammation, oxidative stress, exercise (physical activity) and diseases. These factors are implicated to some extent with the loss of muscle homeostasis with aging. 'Sarcopenia' describes this age-related muscle wasting mass and weakness. Although precise definitions continue to be controversial, the criteria for the clinical diagnosis of sarcopenia include the presence of low muscle mass accompanied by low muscle strength and/or low physical performance (Evans 2010; Reijnierse et al. 2016). Aging compromises skeletal muscle structure, metabolism and function, although generally there is a greater loss of muscle strength than mass and weakness appears more closely associated with the risk of disability and mortality (Evans 2015). It has been suggested that a more comprehensive definition of sarcopenia should also include measures of insulin resistance and basal metabolic rate and these measurements together with accurate assessments of muscle mass and strength would provide an index of the actual contribution of skeletal muscle to the age-associated risk of morbidity and mortality (Evans 2015).

Sarcopenia is an important global public health problem affecting especially the developed world where the number and proportion of older adults in the populations is escalating (Ortman et al. 2014). The significance of the problem is clear when one considers

that the proportion of adults aged over 60 years is expected to increase to 27% by 2050 and that 5-13% of older adults have low muscle mass (as high as 50% in those aged 80 years and older). In normal aging there is a 1% loss of muscle mass after 30 years of age, which tends to accelerate after 70 years of age (Morley et al. 2014; Baugreet et al. 2017). Frail elders who have lost significant muscle mass and strength often require assistance for accomplishing even the most basic tasks of independent living, and they are also at increased risk of serious injury from sudden falls and fractures. This imposes a significant but modifiable economic burden on healthcare services in most industrialized nations (Lynch 2011). There is clearly an urgent and unmet clinical need to better understand the mechanisms responsible for sarcopenia and to develop interventions that can attenuate, prevent, and possibly reverse sarcopenia.

Many of the contributing cellular and molecular mechanisms underlying age-related muscle wasting and weakness have been described in detail elsewhere (Lynch 2011; Clark & Fielding 2012; Argiles et al. 2015; Cohen et al. 2015; Hepple & Rice 2016; Sousa-Victor & Muñoz-Cánoves 2016). Sarcopenia is characterized by a slow, progressive decline in muscle quantity and quality attributed to the progressive atrophy and loss of individual muscle fibers associated with the loss of some motor units, and a reduction in muscle 'quality' because of alterations in muscle metabolism. Age-related changes in skeletal muscle structure and function are associated with a complex interaction of factors affecting neuromuscular transmission, motor unit remodeling and shifts in muscle fiber composition. There are age-related alterations in excitation-contraction coupling, increased generation of reactive oxygen species and myonuclear apoptosis, changes in muscle architecture including loss of muscle fibers and infiltration of fibrotic and other non-contractile material. There are also decreases in circulating levels of anabolic factors and hormones such as testosterone, growth hormone, and insulin-like growth factor-I, and higher systemic and local (muscle) levels of

inflammatory markers including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and C reactive protein (CRP) (Ryall et al. 2008; Schaap et al. 2009; Tintignac et al. 2015; Ogawa et al. 2016; Piasecki et al. 2016; Welch et al. 2018).

Anabolic resistance – implications for sarcopenia

The decrease in skeletal muscle mass with aging can be attributed to a chronic disturbance in the regulation of skeletal muscle protein turnover (Figure 1), leading to an imbalance between protein synthesis and protein breakdown (Koopman & van Loon 2009). Although it was originally assumed that healthy older people had decreased rates of basal muscle protein synthesis (Short et al. 2004), consideration of factors such as health status, habitual physical activity and dietary habits, led to the consensus that there is little or no difference in basal muscle protein synthesis rates between healthy young and old individuals (Cuthbertson et al. 2005; Katsanos et al. 2005; Symons et al. 2007). Since the rate of muscle loss with aging is less than two per cent per year, there is support for the hypothesis that basal fasting protein synthesis and/or breakdown rates are not substantially impaired with aging (Volpi et al. 2001, Rennie 2009, Phillips et al. 2017). In contrast, the protein synthetic response to anabolic stimuli such as protein ingestion and physical exercise is impaired in older individuals (Wall et al. 2015). As humans are under some degree of feeding- and exercise-induced stimulation of protein synthesis during most of the day, this so-called 'anabolic resistance' is primarily responsible for skeletal muscle atrophy during aging (Moore 2014; Murton 2015; Morton et al. 2018). The exact mechanisms responsible for anabolic resistance have yet to be established, but inactivity and chronic low-grade inflammation are strongly associated with physical decline in older humans (Fougere et al. 2017) and considered probable instigators of anabolic resistance (Ham et al. 2014a). Older adults are more likely to experience periods of inactivity through lifestyle changes or acute or chronic illnesses and progress from a state of healthy aging to frailty and loss of functional independence. Thus, the importance of

structured exercise (especially resistance training) and incidental activity during aging cannot be underestimated as an essential self-directed behavior that can help retain the capacity for a robust anabolic response to dietary protein (Symons et al. 2011; Morris & Jacques 2013; Moore 2014). In addition to age-related changes in physical activity contributing to nutritional deficiencies in older adults, other factors also need to be considered, including changes in cognitive function, alterations in taste and chemosensory acuity, impaired swallowing with difficulties in chewing because of associated problems with teeth and gums, and potential issues arising from the need to take medications for various health conditions (Baugreet et al. 2017).

We have demonstrated that during acute inflammation, the anabolic response to leucine is impaired (Ham et al. 2016). The failure of leucine to stimulate skeletal muscle protein synthesis, even at higher doses, has been also shown in rodent models of sepsis and endotoxemia (Lang & Frost 2004; 2005). These observations highlight the impact of inflammation on amino acid sensing and stimulation of protein synthesis and challenges the hypothesis that anabolic resistance can be overcome simply by providing more substrate (i.e. dietary protein or leucine) (Ham et al. 2014a). In addition to providing adequate amounts of protein, we propose that other nutrients or treatments that sensitize skeletal muscle to leucine have therapeutic potential for muscle wasting conditions.

Nutritional considerations for sarcopenia – importance of dietary protein

Protein intake is among the factors that regulate protein turnover. The amount and quality of protein is well-known to determine the protein synthetic response to meal ingestion. A dose-response study in healthy older men using whey proteins found that ingestion of 30-40 grams of protein stimulated protein synthesis more than smaller amounts (Pennings et al. 2011). Overall, these acute studies suggest that ensuring each meal contains this amount of protein would provide health benefits for this population. However, most older individuals consume

158 a breakfast containing relatively low protein content (Tieland et al. 2015) or skip breakfast
159 altogether (Deshmukh-Taskar et al. 2013). Including a high-quality protein source at
160 breakfast may be the easiest way to increase daily protein intake. Data from large cohort
161 studies suggest that the loss of muscle mass in older men and women is attenuated in those
162 that consume considerably more protein than the current Recommended Dietary Allowance
163 (RDA, 0.8 g/kg/d) (Baum et al. 2016; Courtney-Martin et al. 2016). In fact,
164 recommendations from the ESPEN Expert Group for healthy older people is that the diet
165 should provide at least 1.0-1.2 g protein/kg body weight/day (Deutz et al. 2014).

166 In addition to the amount of protein, digestibility and quality also determine the anabolic
167 response. Generally, ingestion of faster digestible proteins results in a more rapid increase in
168 plasma amino acid concentration and a greater muscle protein synthetic response. Indeed,
169 ingestion of a casein protein hydrolysate (i.e. predigested casein), accelerates protein
170 digestion and absorption, enhances postprandial amino acid availability compared to its intact
171 protein, and increases the skeletal muscle protein synthesis (Koopman et al. 2009). Whey
172 protein stimulates protein synthesis to a greater extent than casein (Pennings et al. 2011).
173 Digestibility of protein is not the only factor that differentiates whey and casein. The
174 anabolic response to casein hydrolysate is markedly less than that of whey, whereas the
175 digestion and absorption kinetics are similar (Pennings et al. 2011). The difference in protein
176 synthesis following intake of whey and casein hydrolysate is mainly attributed to the
177 difference in leucine content. Clearly, amino acid composition of dietary proteins plays an
178 important regulatory role in postprandial muscle protein synthesis.

179 The quality of protein is mainly determined by its amino acid profile as different amino acids
180 have different anabolic properties (Landi et al. 2016). For example, L-arginine and its
181 metabolite L-citrulline are known to protect muscle cells from wasting by modulating protein
182 synthesis (Ham et al. 2014c). Generally, essential amino acid availability is a key driver of

skeletal muscle protein synthesis and the branched-chain amino acid leucine plays a unique role in the modulation of skeletal muscle metabolism (Ham et al. 2014). Unlike any other amino acid, leucine directly modulates the mechanistic Target of Rapamycin Complex 1 (mTORC1), one on the main anabolic signaling pathways in skeletal muscle. Indeed, leucine administration has been demonstrated to increase protein synthesis in cell culture systems and mammals. In addition, studies in humans have clearly demonstrated that the acute anabolic response to a suboptimal dose of protein can be enhanced when the leucine content of the protein bolus is increased (Wall et al. 2013). As a result, studies have been performed to assess whether longer-term leucine supplementation can enhance muscle mass. Initial studies using modest doses of leucine (2.5 g leucine/meal) did not show any improvements in muscle mass (Verhoeven et al. 2009; Leenders et al. 2011).More recent work from the Phillips laboratory suggests that higher doses of leucine (5g leucine/meal) can chronically increase protein synthesis in older males and females potentially reducing muscle wasting in that population (Murphy et al. 2016; Devries et al. 2018).

Meat as an excellent source of protein

Meat contains a large quantity of essential amino acids and nutritive factors of high quality and availability, including minerals like iron and zinc, and a variety of vitamins, especially B group vitamins (Rondanelli et al. 2015). Even a moderate intake of lean meat has been shown to increase protein synthesis in both young and old of both sexes (Symons et al. 2007; WHO 2007) and synergistic effects between meat intake and resistance exercise to increase muscle mass in older people have been reported (Daly et al. 2014). Meat also contains other compounds that can influence protein metabolism, including creatine, carnitine, carnosine, and conjugated linoleic acid (Rahman et al. 2009; Tarnopolsky & Safdar 2008; Moon et al. 2013; Young et al. 2013; Artioli et al. 2018). Some authors have even touted a diet for preventing sarcopenia that proposes eating meat 4-5 times a week; including white meat

twice per week, lean red meat less than twice per week, and processed meat less than once per week (Rondanelli et al. 2015).

Some studies have shown high consumption of meat products (especially processed meats) are linked with unfavorable health outcomes and increased risk for cardiovascular diseases, cognitive impairments, and some cancers, particularly colorectal cancer (Kouvari et al. 2016; Tabung et al. 2017). Other studies have disputed these findings (Binnie et al. 2014; McNeill 2014). Processed meat products are typically red meats that have been cured, salted or smoked (e.g. ham or bacon) to improve their durability, color and taste, and often contain high amounts of minced fatty tissue (e.g. sausages) (Rohrmann & Linseisen 2016). High consumption of processed meats may lead to an increased intake of saturated fats, cholesterol, salt, nitrite, and heme iron. Multiple (large cohort) studies have linked processed meat consumption and risk of chronic diseases such as cardiovascular disease, diabetes, and different cancers (Cascella et al. 2018; Quintana Pacheco et al. 2018).

Abete and colleagues (2014) conducted a meta-analysis of thirteen cohort studies to examine the association between consumption of meat (total, red, white and processed) and all-cause, cardiovascular disease and ischemic heart disease mortality. They found those in the highest category of processed meat consumption had 22% and 18% higher risk of mortality from any cause and cardiovascular disease, respectively. Red meat consumption was associated with a 16% higher risk of cardiovascular disease mortality, but there was no similar association for total and white meat consumption. While acknowledging the large heterogeneity in their data analyses they concluded that processed meat consumption could increase the risk of mortality from any cause and cardiovascular disease, and red meat consumption was positively but weakly associated with cardiovascular disease mortality (Abete et al. 2014). More moderate meat consumption within healthy adult populations may have less influence on morbidity or

mortality and some studies have suggested meat intake be reduced to a maximum of 70 grams per day (Millward & Garnett 2010).

In the context of sarcopenia, where reduced overall protein intake has considerable health implications for older people, attention is focused on increasing protein intake through nutrient-rich meats, fish and vegetable sources, and through supplements and fortified foods. The nutrient density of meat proteins has considerable potential to ameliorate sarcopenia (Phillips 2012). Considerations for meat consumption include the method of preparation and cooking since this influences the digestibility of the meat, amino acid absorption kinetics and subsequent stimulation of muscle protein synthesis. This is important for older individuals who generally experience a reduced food-chewing efficiency. Studies have assessed the effect of meat texture on dietary protein digestion rate, amino acid availability, and subsequent postprandial protein balance in older men. Pennings and colleagues (2013) demonstrated that ingestion of minced meat compared with steak resulted in a more rapid absorption of amino acids, leading to increased availability of amino acids in the circulation. Although no differences were observed in postprandial muscle protein synthesis, whole-body net protein accretion was significantly greater after minced meat intake. Similarly, consuming rare meat leads to less pronounced rises in plasma amino acid concentration in older individuals than consuming meat that has been cooked well-done (Buffière et al. 2017). Clearly, meat processing and cooking methods also affect postprandial amino acid availability and subsequent protein turnover.

Other meat products to support protein turnover

Meat is clearly a good source of amino acids and effective in stimulating skeletal muscle protein synthesis, and important for the maintenance of skeletal muscle mass. The anabolic resistance in older individuals may, however, necessitate other interventions that can sensitize the muscle for amino acids to support skeletal muscle protein turnover. It has been suggested

that other animal products may be valuable modulators of protein metabolism. Gelatin, a food derived from animal collagen, has been proposed to affect collagen synthesis with implications for injury prevention and tissue repair (Shaw et al. 2017). Gelatin has a very different amino acid profile than meat (Symons et al. 2007) or milk protein (Pennings et al. 2011) and is rich in glycine (23%), proline (13%) and hydroxyproline (11%) (Igase et al. 2018). Indeed, intake of 15 grams of gelatin rapidly increases plasma availability of glycine (2.2-fold) and proline (by 55%) (Shaw et al. 2017). As studies have demonstrated that muscle glycine levels are reduced in mouse models of diabetes (Giesbertz et al. 2015) aging (Houtkooper et al. 2011), and in muscle samples from frail older individuals (Fazelzadeh et al. 2016), we suggest that providing additional glycine could improve muscle metabolism during conditions of stress.

Glycine modulates cellular homeostasis via a receptor-mediated response and via its intracellular metabolism (Koopman et al. 2017). Glycine can directly activate glycine-gated chloride (Cl^-) channels (GlyR) expressed in inflammatory cells such as macrophages, thereby normalizing [Ca^{2+}] and reducing pro-inflammatory cytokine production. In addition, glycine can deliver carbon units to the folate cycle, thereby modulating the production of cellular components (such as nucleotides and phospholipids) and affecting the balance between oxidized (GSSG) and reduced glutathione (GSH), a crucial modulator of cellular redox status. In a series of studies in mice to assess whether glycine administration protected muscles from wasting during inflammatory conditions, we showed glycine administration (1g/kg/day for 3 weeks) attenuated the loss of muscle mass and strength by 50% in a mouse model of cancer cachexia and attenuated the cancer-induced stimulation of muscle inflammation, macrophage infiltration and the production of reactive oxygen species (Ham et al. 2014b). Glycine also prevented superoxide production and maintained the protein synthetic response to leucine in an *in vivo* model of acute inflammation (lipopolysaccharide injections) in mice (Ham et al.

2016). Furthermore, glycine feeding (1g/kg/day) during calorie restriction, which is characterized by systemic inflammation, attenuated muscle atrophy in mice (Caldow et al. 2016; Ham et al. 2016). Combined, our data clearly highlight how glycine can effectively reduce inflammation, attenuate muscle loss during various condition, and re-sensitize skeletal muscle to anabolic stimuli. Therefore, animal products high in glycine, such as gelatin, could be simple and effective dietary supplements with anti-inflammatory actions that attenuate muscle wasting (Figure 1).

Conclusion

Advances in science, medicine and biomedical engineering (among other disciplines) have enabled humans to live longer lives, especially in developed countries. Living longer, however, does not necessarily mean living better, with many more older individuals presenting with diseases and conditions that compromise quality of life. Some of the most serious consequences of aging are its effects on skeletal muscle, with progressive age-related muscle wasting and weakness leading to a reduction in the ability to perform tasks of daily living, decreasing functional independence and having a devastating impact on quality of life. Addressing the underlying causes of sarcopenia and treating its consequences, represents a significant unmet clinical need. Possible interventions include pharmacologic and nutritional strategies and structured physical activity, especially resistance training. The relative success of nutritional (especially protein feeding) strategies for sarcopenia, is limited by the well-described phenomenon of 'anabolic resistance'.

Meat contains essential amino acids and nutritive vitamins, minerals and other compounds of high quality, and a moderate intake of lean meat can increase protein synthesis in older men and women with synergistic effects when combined with resistance exercise training.

However, health risks have been identified with the consumption of different meats, with high intake of processed meats increasing the risk for cardiovascular disease and different cancers. The risks for fresh white and red meat are considerably less and modest consumption is encouraged for many older adults to ensure adequate protein intake. Fortifying meats to enhance their nutrient value also has relevance for sarcopenia. In studies on muscle cells and animal models of muscle wasting, the amino acid, glycine, reduced inflammation, attenuated muscle atrophy, and re-sensitized skeletal muscle to anabolic stimuli. Glycine feeding or animal products with a high glycine content (such as gelatin), could represent simple and effective nutritional strategies for attenuating age-related muscle wasting and weakness.

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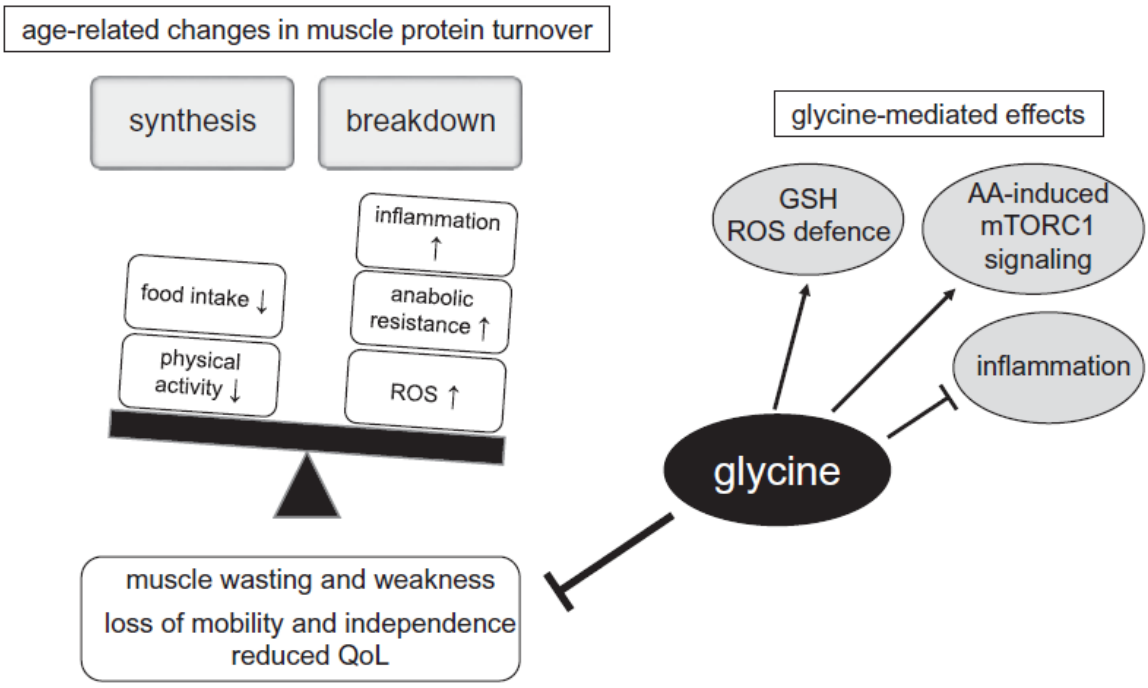

Legend for Figure 1.

Ageing is associated with an increase in inflammation and oxidative stress which impact on protein homeostasis; decreasing protein synthesis and increasing protein breakdown, leading to muscle wasting and weakness and contributing to the loss of independence and reduced quality of life (QoL). Glycine can attenuate muscle wasting by reducing inflammation, increasing defense mechanisms against reactive oxygen species, and increasing mTORC1 signaling. Glycine feeding or animal products with a high glycine content (such as gelatin), could represent simple and effective nutritional strategies for attenuating age-related muscle wasting and weakness.