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Review

Dietary Protein and Muscle in Aging People: The Potential Role of the Gut Microbiome

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Abstract: Muscle mass, strength and physical function are known to decline with age. This is associated with the development of geriatric syndromes including sarcopenia and frailty. These conditions are associated with disability, falls, longer hospital stay, higher readmission rates, institutionalisation, osteoporosis, and death. Moreover, they are associated with reduced quality of life, as well as substantial costs to health services around the world. Dietary protein is essential for skeletal muscle function. Older adults have shown evidence of anabolic resistance, where greater amounts of protein are required to stimulate muscle protein synthesis and therefore require higher daily amounts of dietary protein. Research shows that resistance exercise has the most beneficial effect on preserving skeletal muscle. A synergistic effect has been noted when this is combined with dietary protein, yet studies in this area lack consistency. This is due, in part, to the variation that exists within dietary protein, in terms of dose, quality, source, amino acid composition and timing. Research has targeted participants that are replete in dietary protein with negative results. Inconsistent measures of muscle mass, muscle function, physical activity and diet are used. This review attempts to summarise these issues, as well as introduce the possible role of the gut microbiome and its metabolome in this area.

Keywords: protein; skeletal muscle; sarcopenia, gut microbiome, metabolome, diet, supplementation

1. Introduction

Skeletal muscle has several important functions beyond locomotion, including insulin-stimulated glucose uptake, regulation of extracellular potassium, influence on bone density via mechanical force on bones, and whole-body protein metabolism (1). Age associated loss of muscle mass starts as early as age thirty, and is a gradual process (1). Typically there is a greater loss of type II fibres; those which are useful for short bursts of speed and power, and the main ones involved in preventing a fall (1). Older people also lose more skeletal muscle with bedrest than their younger counterparts (2). Sarcopenia is a geriatric syndrome defined as the age-related loss of skeletal mass and function, which is quantified by specific objective measures of muscle mass, strength and physical function (3). Sarcopenia is distinct from frailty although the two conditions may overlap. Frailty is defined as increased vulnerability after a stressor event, with increased risk of adverse outcomes (4). A summary of the consequences of loss of skeletal muscle and sarcopenia in older adults is illustrated in Figure 1 (5–9). In terms of cost, it has been estimated that reducing the prevalence of sarcopenia by 10% in the United States would save $1.1 billion in healthcare costs annually (10).

One major risk factor for the development of sarcopenia is protein-energy malnutrition (11). Indeed the Women's Health Initiative, an American study on over 24,000 women age 65-79 years, reported a 12% lower risk of frailty in those with a 20% increase in protein intake over a three year period (12). High protein intake is

associated with increased bone mineral density, reduced rehabilitation time after acute illness, better cardiovascular function, improved mortality in ventilated patients, healing of pressure ulcers, and reduced risk of surgical complications (11,13–15). As life expectancy worldwide has more than doubled over the past two centuries, the importance of understanding and optimising muscle function in older age is paramount.

Among the twenty-one amino acids necessary for protein synthesis in humans, nine are referred to as 'essential amino acids' (EAAs). These are nutritionally essential as they cannot be synthesised in the body (16). Leucine is an EAA that is considered the key regulator of muscle protein anabolism via its activity in activation of the mTOR pathway and inhibition of the proteasome (11). Animal studies also suggest it may suppress muscle protein breakdown (MPB) (17). Optimisation of dietary protein and EAA intake in older adults has been suggested to prevent the development of sarcopenia and skeletal muscle loss.

The role of the gut microbiome in healthy as well as disease states is an ever-growing area of interest to researchers. The gut microbiome has a collective genome size that is 150-fold that of the human host (18), and it has been argued that the metabolic activity and size of our gut microbiome is sufficient to warrant its consideration as one of the organs of the human body, with its own intrinsic functions and metabolic needs (19). Over the age of 65, the resilience of the gut microbiome is reduced, as it becomes more vulnerable to medications, disease and changes in lifestyle, with changed species richness and increased inter-individual variability (20). This review aims to summarise the available literature on dietary protein and skeletal muscle in older adults, with a focus on the potential role of the gut microbiome and metabolome.

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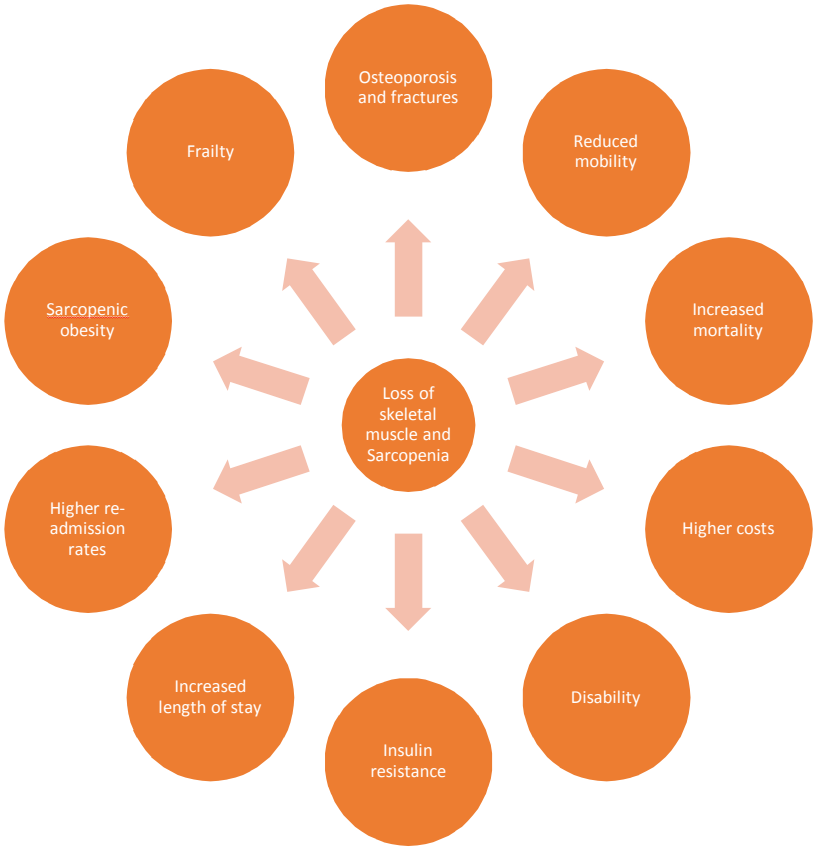

Figure 1: Consequences of loss of skeletal muscle and sarcopenia in older adults

2. Patient Factors

A reduced appetite is common in older adults (21,22). This has been linked to reduced acuity of taste and smell, poor oral health and dentition, reduced chewing efficiency, medications causing reduced saliva production, and changes in the digestive system such as slower gastric emptying and reduced ghrelin levels (21–23). These factors lead to smaller portions being consumed and changes in dietary choices. The prevalence of dysphagia has been estimated at 13% in those over 65 years (24), and is associated with reduced oral intake and malnutrition (25). Pureed or softened diets may lead to reduced intake of meat, which is likely to result in reduced dietary protein intake. Reduced meat consumption in the older population has also been reported elsewhere (22,26).

Chronic disease is common in older adults, with an estimated 40-75% of all people over 65 having a limiting chronic illness (27,28), which can lead to increased catabolism of protein. Many conditions come with dietary restrictions, for example diabetes, chronic kidney disease etc. Rates of polypharmacy are also increased with age, with up to 70% of over 80s taking more than four medications (29). Medication side effects such as dry mouth, nausea, etc. can influence oral intake.

Mobility and access to shopping is a key factor in shaping the dietary habits of older adults (22,30). Falls and fear of falling may reduce mobility (31) and therefore influence the ability of older adults to mobilise for

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shopping, meal preparation and food consumption. Vision is another important factor in shopping and preparing food, with increasing prevalence of poor visual acuity with increasing age (32). A recent qualitative study assessed 30 older adults' food choices and dietary habits and noted that living alone, with associated social isolation and loneliness, had a significant impact on diet. Many showed a lack of motivation for cooking and eating alone (22). Indeed bereavement and living alone have been associated with worse nutrition, while marriage has been linked to better diet quality in older men (22).

Lastly, socioeconomic status has an influence on dietary choices amongst older people. Lower socioeconomic scores have been associated with lower diet quality, and the price of food is a factor in food decisions (22). The estimated cost of malnutrition in England is £19.6 billion per year, with approximately half of this being attributed to people over 65 (27). In addition to the huge costs associated with frailty and sarcopenia, poor nutrition is extremely costly to our healthcare systems. As the population ages, we can expect these costs to increase accordingly. Please refer to Figure 2 for a summary of the factors leading to lower protein intake in older adults.

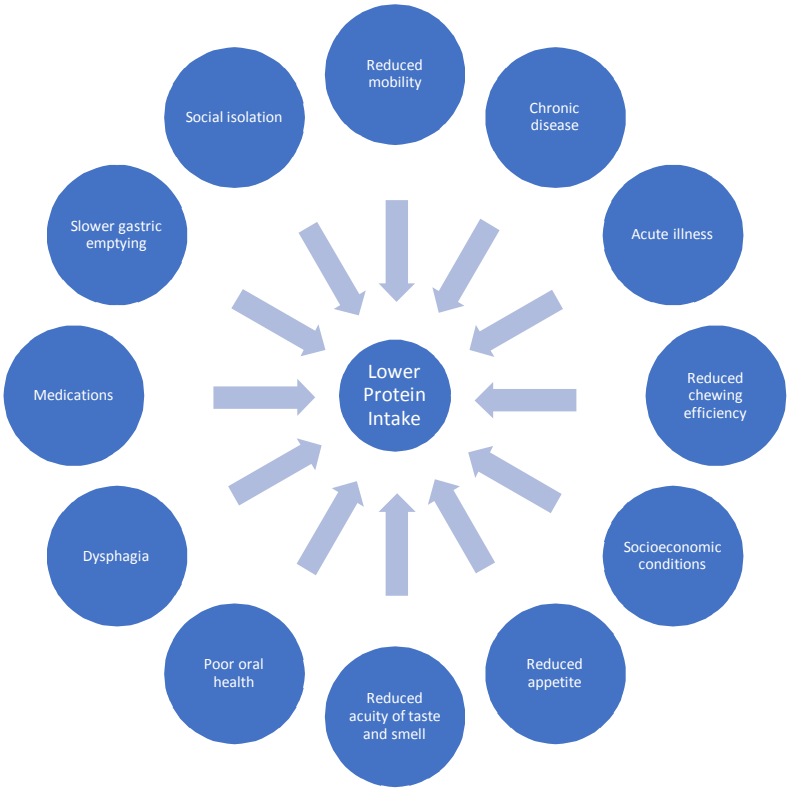

Figure 2: Factors leading to lower protein intake in older adults

3. Anabolic Resistance

Skeletal muscle mass is regulated by the processes of muscle protein synthesis (MPS) and MPB. MPS rates are largely controlled by responsiveness to anabolic stimuli, such as consumption of food, and physical activity. Catabolic stressors include illness, physical inactivity and inflammation, of which the older population tend to

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have higher rates. Ageing does not seem to influence MPB to the same degree as MPS, hence MPS is typically considered the more appropriate target for intervention.

Older adults have shown evidence of 'anabolic resistance', whereby a higher dose of protein is required to achieve the same MPS response as a younger person (1,16,33–35).). The aetiology of these impairments may lie within the aging process, chronic disease or others such as physical inactivity (see Table 1). There are multiple mechanisms postulated and may involve impairments at some, if not all, levels of protein metabolism (see Table 2).

The concept of anabolic resistance is still questioned by some however, with a systematic review by Shad et al. (2016) finding 18 papers with sufficient evidence of age-related muscle anabolic resistance, and 30 papers which did not (6). It is our view that these negative results are possibly due to some of the following methodology and study design limitations; a recurrent these in this area of research. The review only included studies of healthy individuals, 15 of which had only male participants. Discrepancies among the studies included were substantial, including the dose, source and leucine-content of the supplementation, the intensity and volume of exercise, and the use of exercise or protein in isolation or in combination (6). There may also be a sex-difference in anabolic resistance (36,37), which has received almost no attention in the literature.

Table 1. Factors influencing anabolic resistance

Anabolic Resistance Aetiology	References
Declining activity levels	(1,11,38–40)
Protracted disuse events	(11,41–44)
Chronic inflammation	(39,45–48)
Insulin resistance	(1,46,48–51)
Higher circulating oxidative and inflammatory stressors	(1,39,49)
Obesity	(46,52)
Reduced oestrogen/testosterone	(1,54)
Increased production of catabolic hormones such as cortisol	(49)
Alcohol	(53)
Smoking	(1)
Poor vitamin D status	(39)
Reduced food intake	(39)
Metabolic acidosis	(1)
More chronic & acute disease in older adults (increased catabolic conditions)	(15)

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Anabolic Resistance Mechanisms	References
Differences in gene expression of proteins involved in MPS	(55–59)
Dysregulation of key signalling proteins in the mTOR pathway	(1,48,56,57,60–62)
Decreased phosphorylation of mTORC1	(48,60,63–65)
Impaired transport of amino acids into muscle/peripheral tissues	(39,61,66,67)
Diminished mRNA translational signalling	(60,65,68,69)
Inflammation (raised TNFα/ IL-6/ hs-CRP/NFkB)	(1,48,60,70,71)
Decreased phosphorylation of transcription factors (e.g. p70S6K, S6K1)	(48,60,61,68)
Dysregulation of nutritive blood flow to skeletal muscle	(39,51,72)
Attenuated protein digestion & absorption	(39,73,74)
Mitochondrial dysfunction	(1,20,58)
Autophagy/mitophagy dysfunction	(1,58)
Denervation of muscle fibres	(39,75)
Higher splanchnic extraction of protein	(15,74)
Lipid-induced muscle insulin resistance	(20,76)
Increased AMPKα phosphorylation (leads to increased MPB)	(56)
Increased cortisol generation within muscle by 11bHSD1	(77)
Loss of skeletal muscle stem cells	(78)
Insufficient protein dose given in the trial	(6)

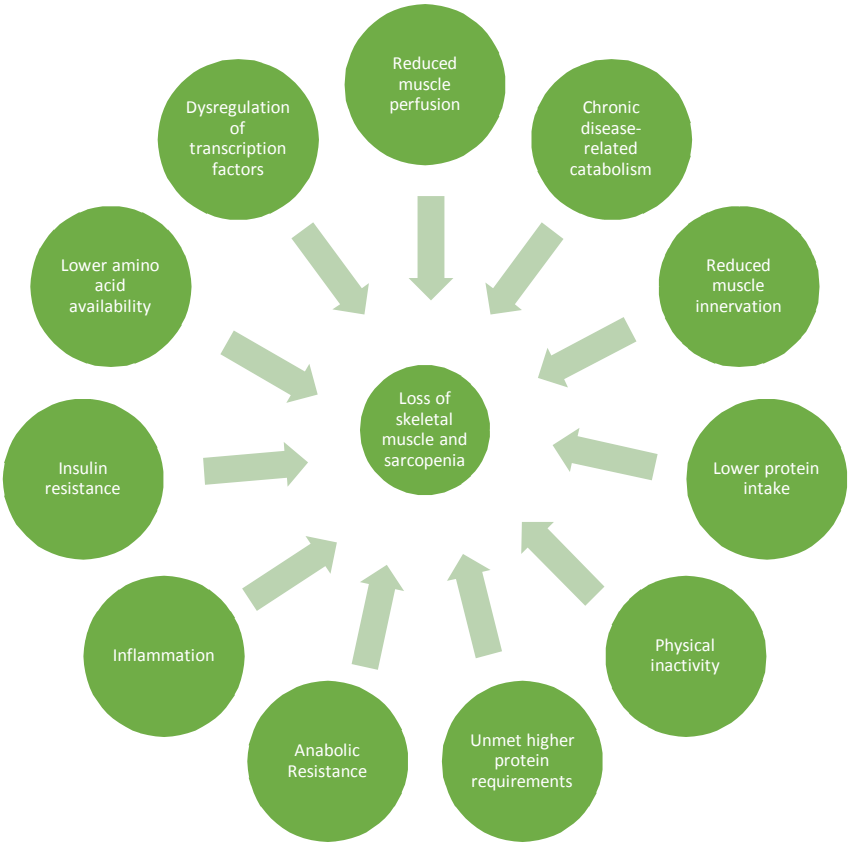

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Figure 3: Factors leading to loss of skeletal muscle and sarcopenia in older adults

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4. Dietary Protein

4.1. Quantity of Protein

It is now considered consensus that a higher RDA of 1-1.3g/kg/day should be consumed by older adults (1,79–82), to offset catabolic conditions. Indeed the PROT-AGE Study Group advise that those with severe illness or injury or with marked malnutrition may need as much as 2.0 g/kg/day (15). There is significant variability in the protein quantity administered in studies, which is highly relevant considering the MPS response to protein is believed to be dose-dependent.

4.2. Quality of Protein

Some proteins, such as wheat protein, are deemed lower quality, as they lack or are low in one or more EAAs and fail to stimulate MPS to the same degree as higher quality sources (83). Red meat contains a balanced amount of all EAAs (45), however older adults eat less red meat than their younger counterparts. The definition of protein quality has evolved and now typically includes digestibility and absorption, as well as amino acid composition.

4.3. Source of Protein

Worldwide ~60% of protein consumed is from plant sources (84), however animal sources predominate in Europe and the United States. Plant proteins tend to have lower digestibility, and lower leucine content (84,85), while meat contains more EAAs per weight than any other food (11). Gorissen et al. (2016) compared protein infusions given to healthy older men. They reported an increased MPS rate in animal versus plant source, and within animal sources (e.g. whey preferable to casein) (86). Little is known about other protein sources such as mycoprotein, aquatic algae etc. (85). Further research into protein sources is necessary to identify optimal agents for older adults, particularly in the context of growing concerns about the environmental impact of certain foods (87).

4.4. Timing of Protein

Timing of protein consumption both throughout the day (1,16,88), and in relation to exercise (35), has been an issue of debate. Cardon-Thomas et al. (2017) assessed per meal protein intake of older adults, and reported adequate daily totals, but no participants achieved adequate per-meal protein intake (89). Some have argued that evenly spread intake across meals may be beneficial for MPS, as may pre-sleep protein ingestion (90,91). Murphy et al. (2015) compared skewed to evenly distributed protein intake throughout the day in 20 overweight older men. They found greater MPS in those with balanced protein distribution (92). This has been debated by others who reported that total protein intake, irrespective of the pattern, was the most important factor in maximising the anabolic response (13).

Resistance exercise and protein ingestion have a synergistic positive effect on MPS, with highest level of MPS approximately one-hour post exercise (93). In young men, Burd et al. (2011) reported that the sensitivity of the muscle to protein is increased for 24 hours post exercise (94). More research is needed to establish the

importance of protein intake distribution in older adults, whether an anabolic window exists post exercise, and if so whether supplementation of protein during this window elicits a greater response in MPS.

4.5. Speed of Protein Digestion

A fast-digestive protein, such as whey, is one which releases its amino acids relatively quickly in the digestive process. Studies have shown improved MPS with fast digestive proteins (95,96), albeit in healthy participants. Many now recommend that we use fast-digestive proteins in older adults and disease states such as sarcopenia, in order to maximise the benefits on skeletal muscle function (15,97).

5. Protein and Skeletal Muscle

Observational studies give weight to the hypothesis that poor protein intake contributes to sarcopenia and poor clinical outcomes. Three large studies have supported an association between protein intake and muscle strength and mass, although results have conflicted on whether this is confounded by fat mass (98–100).

5.1. Protein Supplementation Studies

Clinical trials of protein supplementation have shown varying results. Multiple trials carried out in healthy replete older adults, without an exercise intervention, have been negative (101–103). Systematic reviews have concluded that the most promising results are for specific EAAs, particularly leucine, but also its metabolite β-hydroxy β-methylbutyric acid (HMB) (7,49,103). However, studies of leucine supplementation have had mixed results, with some promising findings (35,49,104), and some negative trials (49,105). There is currently a large ongoing multi-centre trial assessing the benefits of leucine in patients with sarcopenia (106).

The Deutz study group have done a number of trials showing improvements in muscle mass and nutritional status with HMB supplementation and more work is needed to definitively establish its benefits (107,108). Both leucine and HMB hold promise for those in whom suboptimal quantities of protein are consumed, for example older adults with smaller appetites, poor oral health etc. Supplementation with these more targeted regulators of MPS may be most effective for overcoming anabolic resistance in this cohort.

Some trials have combined protein with other nutritional supplements. For example Bo et al. (2018) assessed combined supplementation of whey protein, vitamin E and D in their cohort of sixty adults with sarcopenia and reported improvements in both muscle mass and strength (109). However this design can make interpretation of where exactly the benefits lie difficult.

5.2. Protein Supplementation & Exercise

Exercise is recognised as a potent stimulator of anabolic response in muscle. In all ages protein intake and exercise act synergistically to increase MPS. Multiple trials have reported greater improvements in muscle mass, muscle strength, and physical function when protein intake is combined with exercise, particularly resistance training (35,45,110).

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Cermak et al. (2012) carried out a meta-analysis of trials assessing protein supplementation in the context of resistance training (111). Interestingly, amongst the six studies looking at older adults (>50 years, 215 subjects), none had found significance in fat free mass (FFM) versus placebo. However, when the data were pooled, protein supplementation was found to increase FFM by 38% versus placebo (111).

5.3. Issues with Protein Supplementation Trials

Conflicting results are not uncommon in this area. Issues with trials include short follow up time, small sample sizes with insufficient power, different doses of protein used, different sources of protein, different settings (hospital vs community), inconsistent timing of supplementation, use of fast versus slow digestive proteins, supplementing replete populations, and heterogeneity of populations studied, as well as substantial variation in the measures used to monitor dietary protein intake. Indeed The International Sarcopenia Initiative (2014) carried out a systematic review and concluded that the results of nutritional supplementation trials are equivocal due to low numbers of high quality studies and heterogeneous study design (7).

A key consideration is ensuring participants have adequate baseline energy requirements. Supplementing protein in the context of insufficient energy intake will lead to protein being metabolised for energy, rather than leading to increased MPS (82). This is likely to be especially relevant in older adults with reduced appetites. Furthermore, a large variety of measures are used for estimating muscle mass, muscle strength and physical function (see supplementary tables 1, 2 and 3). The International Working Group on Sarcopenia recommended that Computed Tomography and Magnetic Resonance Imaging (MRI) equally be considered the gold standard imaging techniques and discouraged the use of Bioelectrical Impedance Analysis due to its inaccuracy (112). Dual-energy X-ray absorptiometry (DXA) is the most commonly used measure of muscle mass, however FFM and muscle protein mass can be overestimated due to water retention and/or lipid content of muscle in older adults (82). More high quality, well designed research is needed in this area, to determine the benefits of protein supplementation in older adults, with and without exercise.

5.4. Surrogate Markers of Protein Intake

Studies use multiple ways of estimating dietary protein intake (see supplementary table 5). The validity and reliability of these dietary measures has usually been verified in younger populations and may not be relevant to older people. Indeed reduced reliability coefficients of the Food Frequency Questionnaire have been reported with increasing age (113).

In order to overcome this, researchers have sought objective estimates of dietary intakes. Protein is the major nitrogen-containing substance in the body, and therefore urinary excretion of nitrogen is used as a marker of protein loss (33,101). Urinary (45,114) and blood urea concentration (114), and urinary HMB levels (107) have also been used with the aim of objectively verifying compliance. These methods are not without limitations, as they may not consider subtle changes with protein metabolism that occur with age, such as increased splanchnic uptake (15). The amount of fermentation metabolites detectable in the urine depends on the digestibility of the protein (115), so this too, needs to be considered.

Other novel techniques in this area include the measurement of MPS using oral labelled isotopes such as deuterium oxide or 3-Methylhistidine, which can be measured via a single blood or urine test the following day (116–118). These methods are less invasive and significantly cheaper than intravenous versions. These techniques represent significant advances in an area with challenging methods, and show promise in measuring efficacy of interventions, as well as providing mechanistic insights into the sarcopenic phenotype.

6. The Role of the Gut Microbiome

The composition of the bacterial species in one's gut is dependent on age, diet, health, and geographical location, with significant individual variability (119,120). Multiple cross-sectional studies have found associations between gut microbiome composition and frailty (120–123), while the ELDERMET study showed significant loss of diversity amongst people in a care-home setting versus community dwellers (124). Among older hospitalised patients, polypharmacy has been significantly associated with gut microbiota dysbiosis (122). Evidently, the gut microbiome has been implicated in many aging-associated processes, with recent animal studies even showing that transferring gut microbes of young killifish to older ones extends the lifespan of the older fish (125). It has been hypothesised that a gut-muscle axis exists. More research is warranted to explore this theory (20).

6.1. Animal Models

Many animal models have been used in the study of the gut microbiome (see supplementary table 4). Studies carried out in mice, rats and hamsters have shown higher microbial diversity in those fed soy protein versus animal protein (126,127) and increased abundance of *Bacteroidales* family S24-7 in those fed soy protein versus other diets (128). Li et al. (2017) assessed high protein, low carbohydrate diets in dogs and found increased abundance of *Clostridium hiranonis*, *Clostridium perfringens*, and *Ruminococcus gnavus*, as well as decreased *Bacteroidetes* to *Firmicutes* ratio and an increase in the *Bacteroides* to *Prevotella* ratio (129), the latter of which has been proposed as a biomarker of good health (130).

6.2. Human Data

The digestive system consists of a complex interaction between digestive secretions, intestinal conditions, and the gut microbiome. Nutrients, especially dietary proteins, provide energy sources for the host, as well as substrates for the gut microbiota (115). A significant proportion of undigested peptides and proteins can reach the colon, and this is increased in the context of a high protein diet (114). Consumption of proteins with high digestibility, or a low protein diet, results in less protein reaching the colon, limiting the amount available for protein-fermenting bacteria (115). Work done in this area has shown that a high protein diet does shift the gut microbiome from carbohydrate to protein fermentation, with a diverse metabolic output including branched-chain fatty acids, ammonia, amines and others (114).

It has been reported that protein consumption is correlated positively with gut microbiome diversity (131). This is based on studies carried out on healthy volunteers (132), elite athletes (133), and obese/overweight

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individuals (134). The source of protein is influential, with plant protein associated with more *Bifidofacterium*, *Lactobacillus*, *Roseburia*, *Eubacterium rectale*, and *Ruminococcus bromii;* and less *Bacteroides* and *Clostridium perfringens* (131,132). Meanwhile animal protein is associated with higher levels of *Bacteroides*, *Alistipes*, *Bilophila* and *Ruminococcus*, and lower levels of *Bifidobacterium* (131,132). High levels of *Bacteroides* have also been reported with Western diets, which are high in protein and animal fat (18), although it has been suggested that differences in fat content, rather than protein, is the major influencing factor here (135).

Significant associations have been reported between increased levels of faecal short chain fatty acids (SCFAs), *Prevotella* and some *Firmicutes*, with consumption of a Mediterranean diet (20,136), which is typically lower in protein than animal-based diets, although may contain high levels of plant-source protein. Indeed, certain microbial clusters are associated with long term dietary patterns (18). Clusters can change within 24 hours of controlled feeding, however research shows that microbiome composition is far more influenced by long term diet patterns, rather than acute changes (137). Dietary pattern studies make assessment of the contribution of each macronutrient difficult.

A healthy gut microbiome plays a role in many of the physiological processes implicated in the mechanisms for the development of anabolic resistance (see table 2). These include suppression of chronic inflammation, prevention of insulin resistance, modulation of host gene expression, enhancement of antioxidant activity and maintenance of gut barrier function (20). A reduced rate of dietary protein digestion has also been hypothesised as one of the processes involved (84). Indeed production of SCFAs by the gut microbiome has been associated with anabolism itself (138) and depletion of taxa producing SCFAs may promote anabolic resistance (139). A randomised controlled trial has been carried out exploring the effect of modulating the gut microbiome on muscle function and frailty, where 60 older adults received a prebiotic or placebo for 13 weeks. Promisingly, both exhaustion and handgrip strength were significantly improved in the treatment arm (140), highlighting the potential role for the gut microbiome in future interventions, but the study remains to be replicated.

There is increasing evidence for the association between exercise and the gut microbiome, which may be secondary to both host health and diet (141,142). Claesson et al. (2012) showed that gut microbiota diversity is inversely correlated with physical function in frail older adults (120), suggesting a potential role for the gut microbiome in the development of, and therefore potentially prevention of, sarcopenia (20). The gut microbiomes of critically ill patients on average display enrichment of virulent pathogens, and loss of health-promoting microbes (143). Protein supplementation has shown some benefits for muscle parameters in this population (144,145), but whether this effect is modulated by the gut microbiome is not known.

The hypothesis that the dysbiotic gut plays a role in the loss of skeletal muscle and response to protein is yet to be tested. If supported, the gut microbiome could represent a target for interventions aiming to overcome anabolic resistance, to maintain muscle mass and strength in older adults.

6.2. The Metabolome

Studies assessing the specific effect of dietary protein on gut microbiota composition are limited and some of the work done thus far has focused instead on the altered fermentation products. Trials using ^{1}H-nuclear magnetic resonance (NMR) technology have shown a shift in bacterial metabolism with different metabolite profiles according to the source of protein (114) and one study of high-protein, low-carbohydrate diets in 17 obese men reported increased hazardous metabolites (e.g. N-nitrosamines), and decrease in cancer-protective metabolites (e.g. ferulic acid) in their faecal samples (146). Indeed a growing number of studies are using ^{1}H-NMR technology to assess faecal, urinary and plasma metabolomes as measures of metabolic health [e.g. (147)]. More research is needed into the use of the metabolome in the context of dietary protein intake, and the significance of changes in the metabolome for skeletal muscle mass and function.

7. Discussion

As the world's population ages, it has become imperative to gain more understanding of the aging process. Declines in muscle mass and function with age have significant associated morbidity and mortality, and the prevalence of both sarcopenia and frailty is increasing. The care of older people is complex, and a multitude of factors influence lower protein intake and loss of skeletal muscle with age (see Figures 1 and 2).

Anabolic resistance is likely to result from cumulative declines across multiple physiological systems, with effects on both MPS and MPB, a dynamic interaction of multiple factors (see Figure 3). Current thinking must not be limited to one or two mechanisms but focus on anabolic resistance as a complex and multidimensional construct. The aetiologies and mechanisms involved are not understood and may be different for each aging individual, suggesting a possible need for personalised medicine within this population to guide future interventions.

Dietary protein is essential for skeletal muscle and it has been established that older adults require a higher RDA of dietary protein. Research suggests that better quality protein, especially that containing higher quantities of leucine, is likely to benefit muscle health in the older population. The importance of timing of protein administration needs to be more clearly understood, and the potential benefit on skeletal muscle of fast- versus slow-digestive proteins is another question yet to be answered. A significant amount of work has focused on protein source, with animal sources typically containing more leucine, greater digestibility and achieving a higher MPS responses. That said, meat intake declines with age, and chewing and poor oral health can be an issue for older adults. There are also increasing concerns about the environmental impact of animal sourced food (85). Furthermore, research is needed to confirm whether increases in MPS, muscle mass and/or muscle strength lead to meaningful functional outcomes in this older demographic.

Studies show that supplementing protein/EAAs, particularly in combination with resistance exercise, is beneficial for aging muscle. However, many trials have had conflicting results. As with all nutritional studies, it is difficult to ensure the adherence of participants to the intervention, and to quantify the impact of non-compliance on the results. Trials of nutritional supplements in participants already replete have limited usage, and a wide variety of measures and assessments are currently being used. This heterogeneity leads to significant uncertainty amongst current evidence and makes clinical translation of findings extremely difficult. High

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quality studies are needed to establish standardised, feasible measures of muscle mass, strength and physical function for future work in this area. Personalised dietary recommendations may show promise going forward, and this is currently being assessed in a large randomised controlled trial of a multi-component intervention in the management of sarcopenia (148).

Difficulties in carrying out accurate studies in this area are highlighted by the use of such extreme methods as Ferrando et al. (1996), in which volunteers were required to stay for 22 days in the lab, 7 of which were for diet stabilisation, and 15 of which were spent in strict head-down bed rest (149). Others have employed similar methods (42). With limited funding throughout academia, the feasibility of such trials is limited. Some ways in which shortcomings can be addressed include twin studies, reducing heterogeneity at baseline, more consistent use of measures throughout studies, use of standardised diets prior to interventions, and the development of standardised measures of muscle mass, muscle function, physical activity, and diet. In light of the low levels of reliability of our current dietary recording methods (150), the use of the metabolome may represent an objective and reliable way of assessing compliance with dietary interventions going forward (151). Novel techniques in the measurement of MPS, such as the use of oral heavy water as a stable isotope, show significant promise for future research in this field.

Few human studies have evaluated the effects of the gut microbiome on dietary protein metabolism, and the ensuing metabolome or vice versa, and those that have, have had limitations such as highly heterogeneous groups at baseline, short intervention periods, variation in dietary measures used, different sample storage methods, and disparate lab processing. Animal studies have shown promise, and the one available human trial on gut microbiome modulation showed positive improvements in muscle function (140). Research is needed to establish whether a dysbiotic gut microbiome contributes to skeletal muscle loss in the context of acute/or chronic illness, or indeed in the aging process itself. Furthermore, the potential role of the gut microbiome in anabolic resistance warrants further investigation. The plasticity and diversity of the gut microbiome and its metabolome, in comparison to the human genome, represent exciting prospects for personalised medicine, and indeed, in the role of dietary protein in skeletal muscle function of older adults.

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