Proceedings of the Nutrition Society (2011), **70**, 104–113 doi:10.1017/S0029665110003927
 First published online 22 November 2010

The Summer Meeting of the Nutrition Society hosted by the Scottish Section was held at Heriot-Watt University, Edinburgh on 28 June–1 July 2010

Conference on 'Nutrition and health: cell to community'

Symposium 2: Exercise and protein nutrition
Dietary protein and exercise training in ageing

René Koopman

Basic and Clinical Myology Laboratory, Department of Physiology, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Victoria 3010, Australia

Ageing is accompanied by a progressive loss of skeletal muscle mass and strength, leading to the loss of functional capacity and an increased risk for developing chronic metabolic diseases such as diabetes. The age-related loss of skeletal muscle mass results from a chronic disruption in the balance between muscle protein synthesis and degradation. As basal muscle protein synthesis rates are likely not different between healthy young and elderly human subjects, it was proposed that muscles from older adults lack the ability to regulate the protein synthetic response to anabolic stimuli, such as food intake and physical activity. Indeed, the dose–response relationship between myofibrillar protein synthesis and the availability of essential amino acids and/or resistance exercise intensity is shifted down and to the right in elderly human subjects. This so-called 'anabolic resistance' represents a key factor responsible for the age-related decline in skeletal muscle mass. Interestingly, long-term resistance exercise training is effective as a therapeutic intervention to augment skeletal muscle mass, and improves functional performance in the elderly. The consumption of different types of proteins, i.e. protein hydrolysates, can have different stimulatory effects on muscle protein synthesis in the elderly, which may be due to their higher rate of digestion and absorption. Current research aims to elucidate the interactions between nutrition, exercise and the skeletal muscle adaptive response that will define more effective strategies to maximise the therapeutic benefits of lifestyle interventions in the elderly.

Sarcopenia: Nutrition: Exercise training: Muscle hypertrophy

The preservation of muscle function is crucial for maintaining an independent lifestyle and the capacity to perform the activities of daily living in the elderly. One of the important factors in the loss of functional performance is the progressive loss of skeletal muscle mass with ageing, called 'sarcopenia'[1–3]. This apparent muscle wasting in elderly human subjects occurs at a rate of about 0·5–1·0% per year starting at about 40 years of age. Lean muscle mass contributes up to about 50% of the total body mass of young adults but can decline to 25% by 75–80 years of age[4,5]. The loss of muscle mass is most notable in the lower limb muscles, with the cross-sectional area of the *vastus lateralis* reduced by as much as 40% at the age of 80 years[6]. Sarcopenia is associated with a three- to fourfold increased likelihood of disabilities and the loss of muscle mass especially in the lower limbs is associated with an increased risk of falls and impairment in the ability to perform routine activities.

The loss of muscle mass is viewed as a largely inevitable and undesirable consequence of ageing[7], with muscle loss estimated to affect 30% of people older than 60 years and >50% of those older than 80 years[1]. Demographic studies indicate that the world's population aged 60 years and above will triple within the next 50 years, and the subpopulation of older adults aged 80 years and above represents the fastest-growing subpopulation in the

Abbreviations: AA, amino acids; EAA, essential amino acids; mTOR, mammalian target of rapamycin; mTORCI, mTOR complex I; S6K1, S6 protein kinase.

Corresponding author: Dr René Koopman, fax +61 3 8344 5818, email rkoopman@unimelb.edu.au

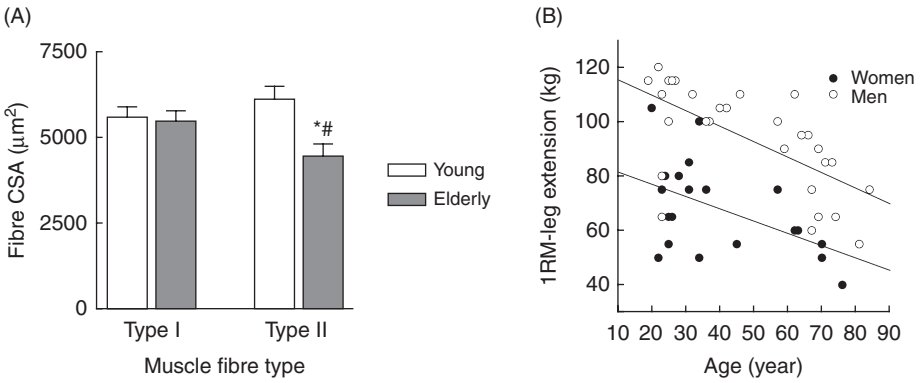

Fig. 1. Muscle fibre cross-sectional area in young (about 20 year) and elderly (about 76 year) men (A). Note the smaller type-II muscle fibres in the elderly men compared with the young controls (adapted from [14]). (B) Correlation between age and one-repetition maximum (1RM) leg press strength (adapted from[146]).

developed world[8]. It is therefore not surprising that the global ageing will have a major impact on our health-care system, as the number of frail elderly requiring hospitalisation and/or institutionalisation increases. Good health is essential for maintaining independence and to continue to actively enjoy family and community life. As such, lifelong health promotion is warranted to prevent or delay the onset of non-communicable and chronic (metabolic) diseases such as heart disease and stroke, cancer and diabetes. Preventing, attenuating and/or reversing the decline in skeletal muscle mass should be the main goal for interventional strategies to promote healthy ageing.

Ageing and protein turnover in skeletal muscle

The loss of skeletal muscle mass in the elderly is characterised by atrophy of type-II (fast) muscle fibres (Fig. 1(A)), fibre necrosis, fibre-type grouping and a reduction in satellite cell content in type-II muscle fibres[6,9–14]. The loss of skeletal muscle mass is accompanied by the loss of muscle strength (Fig. 1(B)), a decline in functional capacity[15–22] and a reduction in whole-body and muscle oxidative capacity[4,23,24]. Together, these alterations at a muscle level have substantial health consequences, since they contribute to the greater risk of developing insulin resistance due to the reduced capacity for blood glucose disposal and a greater likelihood of excess lipid deposition in liver and skeletal muscle tissue leading to hyperlipidaemia, hypertension and cardiovascular co-morbidities.

The progressive muscle wasting with ageing must be due to a disruption in the regulation of skeletal muscle protein turnover, leading to a chronic imbalance between muscle protein synthesis and degradation. Although it was originally reported that healthy older adults had decreased rates of basal muscle protein synthesis[5,25–31], more recent studies have failed to reproduce these findings and generally show little or no differences in basal muscle protein synthesis rates between young and old adults[32–39]. These discrepancies may be due to the standardisation of prior physical activity[24], selection of subjects[30] or the selection of different precursor pools to calculate muscle protein synthesis[40]. It seems unlikely that basal muscle protein fractional synthesis rates are diminished by 20–30% as reported previously[25,26,28,29] and/or that muscle protein breakdown is elevated by as much as 50% in the elderly compared to younger adults[41]. Such opposing alterations in the rates of protein synthesis and breakdown would be accompanied by more rapid muscle wasting than what is typically observed (3–8% per decade[20,42]), and it therefore seems unlikely that basal muscle protein fractional synthesis rates could be diminished by 20–30% during ageing as reported previously[25,26,28,29]. The relatively slow rate of muscle loss during ageing must mean that the mismatch between the average diurnal rate of muscle protein synthesis and breakdown is small. It is currently accepted that basal fasting protein synthesis and/or breakdown rates are not (substantially) different between young and elderly human subjects[26,32–39,43]. To better understand the skeletal muscle wasting in the elderly, researchers have started to focus on the muscle anabolic response to anabolic stimuli such as physical activity, food intake and anabolic hormones such as insulin. It was well established that the protein turnover in skeletal muscle is highly responsive to exercise and nutrient intake in healthy young individuals[44]. Interestingly, data from recent studies suggest that the muscle protein synthetic response to resistance exercise[39] and following the ingestion of a small amount of amino acids (AA) with[36,45] or without carbohydrate[32,33] is reduced in the elderly when compared with young controls. The latter is believed to represent a key factor responsible for the age-related decline in skeletal muscle mass[46].

Anabolic response to exercise

Exercise is a powerful stimulus to promote net muscle protein anabolism, resulting in specific metabolic and morphological adaptations in skeletal muscle. Endurance training can increase whole-body and muscle oxidative capacity and endurance[47], whereas resistance exercise training can increase muscle mass and strength, and thus improve physical performance and functional capacity[48]. It generally takes weeks to months before training-induced changes in skeletal muscle mass become apparent[49]. The prolonged time course for hypertrophy is a reflection of the slow turnover rate of muscle proteins, i.e. about 1% per

https://doi.org/10.1017/S0029665110003927 Published online by Cambridge University Press

day for contractile proteins[25,50,51]. Although muscle hypertrophy occurs at a slow rate, a single bout of resistance exercise can rapidly (within 2–4 h[52]) stimulate muscle protein synthesis, and increase protein synthesis rates, particularly the myofibrillar protein synthesis[28,31,47], which persist for up to 16 h in trained[53] and 24–48 h in untrained individuals[52–54]. Muscle protein breakdown is also stimulated following exercise, albeit to a lesser extent than protein synthesis[52,55], and results in an improved net muscle protein balance that persists for up to 48 h in untrained individuals[52].

It has been generally accepted that the increase in protein synthesis following exercise is due to increased mRNA translation[56]. Many laboratories have shown that the signalling pathway involving a mammalian target of rapamycin (mTOR) complex I (mTORCI) plays a crucial role in the control of mRNA translation initiation and elongation[57–59]. The activity of mTORCI determines the activity of downstream effectors such as the 70-kDa S6 protein kinase (S6K1) and the eukaryotic initiation factor 4E-binding protein[60]. Both play key regulatory roles in modulating translation initiation, and control the binding of mRNA to the 40S ribosomal subunit[60]. Studies have shown that the mTORCI signalling pathway is activated after acute resistance exercise in healthy human subjects[47,59,61,62]. Moreover, Drummond *et al.*[63] showed elegantly that early acute contraction-induced increase in human protein synthesis in human subjects can be blocked with rapamycin treatment indicating that mTORCI signalling is crucial during the early post-exercise recovery. In addition, it was shown that the phosphorylation status of S6K1 following resistance exercise is a good marker for the long-term increase in skeletal muscle mass in rats[64] and human subjects[65]. Moreover, significant correlations were reported between S6K1 phosphorylation/activation and muscle protein synthesis following exercise in young healthy human subjects[39], highlighting the importance of this signalling pathway in the adaptive response to resistance exercise.

Ageing and the anabolic response to exercise

Muscle protein synthesis is responsive to resistance and endurance exercise in both young and elderly human subjects[29,30,39,61,66,67]. Some studies have reported subtle differences in changes in gene expression and anabolic signalling[68], with early studies indicating that the protein synthetic response to resistance-type exercise did not differ considerably between the young and elderly[26,31]. In contrast, an elegant study by Kumar *et al.*[39] showed anabolic resistance of anabolic signalling (i.e. 4E-binding protein and S6K1) and muscle protein synthesis after resistance exercise (performed in the fasted state) in elderly men compared with young controls, which became apparent especially at higher exercise intensities. This study demonstrated that the sigmoidal response of muscle protein synthesis to resistance exercise of different (increasing) intensities was shifted downward in older men compared to younger men[39]. Interestingly, this study shows that the linear relationship between S6K1 phosphorylation and muscle protein synthesis after resistance exercise, which is observed in young healthy adults, was not present in the elderly, indicating that anabolic signalling regulating mRNA translation is impaired in the older human subjects[39].

Compared to protein synthesis, not many studies have actually measured muscle protein breakdown using stable isotope tracers. Most studies rely on measurements of mRNA or protein expression of proteins involved in protein degradation such as Atrogin-1, MuRF-1, calpains and their regulators. It has been suggested that mRNA expression of proteolytic regulators, such as Atrogin-1, are elevated in muscles from old compared with young adults at rest and these levels increased even further in the elderly in response to resistance exercise. These findings from Raue *et al.*[69] suggest that the regulation of ubiquitin proteasome-related genes involved in muscle atrophy might be altered in the elderly and protein breakdown may be increased in elderly human subjects. However, whether these changes in mRNA expression translate to actual changes in protein expression and altered proteasome activity has yet to be established. Thus, there is a paucity of data regarding the measurement of muscle protein breakdown in response to exercise in the elderly and it is clear that further research is needed to assess the impact of exercise and specific exercise modalities on post-exercise muscle protein synthesis and breakdown rates and associated myocellular signalling in young and elderly human subjects.

Anabolic response to food intake

Protein turnover in skeletal muscle is highly responsive to nutrient intake[70]. Ingestion of AA and/or protein strongly stimulates muscle protein synthesis[35,37,51,70,71]. Besides serving as a substrate for polypeptide biosynthesis, AA were shown to directly activate regulatory proteins in mRNA translation, while non-essential AA do not induce a substantial increase in muscle protein synthesis. In contrast, essential amino acids (EAA) increase muscle protein synthesis in the absence of increased non-essential AA availability. The branched-chain amino acid, leucine, is of particular interest since it has the unique ability to directly increase signalling through mTOR and its downstream targets 4E-binding protein and S6K1 and ribosomal S6. The EAA[72,73], and leucine in particular[74,75], seem to represent the main anabolic signals responsible for the post-prandial increase in muscle protein synthesis. The observations that EAA show a dose-dependent stimulation of muscle protein synthesis without increasing plasma insulin[76], and that carbohydrate ingestion does not affect protein synthesis[77], suggest that insulin is rather permissive instead of modulatory[46,76,78]. Greenhaff *et al.*[78] showed that insulin in the range of 30–150 μU/ml does not further stimulate muscle protein synthesis. In contrast to protein synthesis, muscle protein degradation seems to be very responsive to relatively small changes in insulin concentrations. Insulin levels of 15 μU/ml can almost maximally reduce muscle protein breakdown[79] and there seems to be no further inhibition above 30 μU/ml[78]. These data suggest that protein breakdown can be already maximally reduced by slightly increased insulin concentrations which can be achieved by the intake of a small breakfast in healthy young men[46].

https://doi.org/10.1017/S0029665110003927 Published online by Cambridge University Press

Ageing and the anabolic response to food intake

Data from recent studies suggest that the muscle protein synthetic response to the ingestion of a small amount of EAA[32,33] is attenuated in the elderly, and is now believed to represent one of the key factors responsible for the age-related decline in skeletal muscle mass. The so-called 'anabolic resistance' in elderly human subjects was demonstrated by a rightward and downward shift of the dose–response relationship between myofibrillar protein synthesis and the availability of leucine in the plasma[32]. Cuthbertson *et al.*[46] showed that even a very large (40 g) dose of EAA is not able to bring the curve back to values for young subjects, suggesting that supplementation with extra protein, EAA or leucine will not be sufficient to restore the rate of muscle protein synthesis in older adults, relative to those found in the young.

The mechanisms responsible for the proposed anabolic resistance to protein and/or AA administration in the elderly are yet to be elucidated fully. Cuthbertson *et al.*[32] reported decrements in amounts of signalling protein in the protein kinase B/mTORCI pathway in old muscle and showed an attenuated rise in the activation of key signalling proteins in this pathway after ingesting 10 g EAA in the elderly *v.* the young. These findings seem to be consistent with previous observations by Guillet *et al.*[45] who showed reduced S6K1 phosphorylation following combined AA and glucose infusions in the elderly. Combined, these data suggest that anabolic signalling is impaired in skeletal muscles of older compared to younger adults[32,76], which may be in part due to insulin resistance in the elderly. Recent data suggest that muscle protein breakdown is not strongly inhibited by insulin in the elderly[80], whereas other reports suggested that muscle protein synthesis is resistant to the anabolic action of insulin in the elderly[36,43]. It has been proposed that the anabolic resistance can be attributed to a less responsive impact of physiological hyperinsulinemia on the increase in skeletal muscle blood flow and subsequent AA availability in aged muscle[43,81], which would agree with the reduced activation of the phosphatidylinositol-3 kinase–protein kinase B–mTOR signalling pathway and with the lesser increase in the muscle protein synthetic rate after AA/protein ingestion in the elderly[32].

Another mechanism that has been suggested to contribute to the anabolic resistance to food intake in elderly men is an impairment in dietary protein digestion and/or absorption[82]. Recent data show that the digestion rate of protein is an independent regulating factor of post-prandial protein anabolism[83]. As such, it seems plausible to assume that any impairment in protein digestion and/or absorption will reduce the appearance rate of dietary AA in the bloodstream, thereby reducing AA delivery to the muscle and subsequently attenuating the muscle protein synthetic response. To accurately assess the appearance rate of AA derived from dietary protein, the labelled AA need to be incorporated in the dietary protein source[84–86]. As free AA and protein-derived AA exhibit a different timing and efficiency of intestinal absorption[85], simply adding labelled free AA to a drink containing protein does not provide an accurate measure of the digestion and absorption

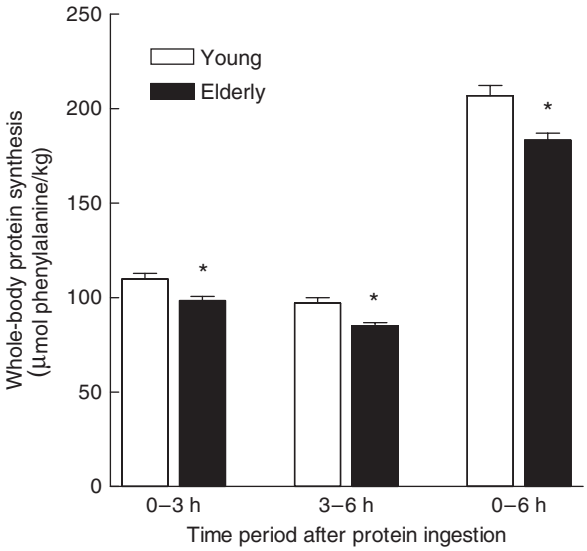

Fig. 2. Whole-body protein synthesis rates, calculated over 3 h or 6 h periods, following the ingestion of 35 g of casein protein in young (about 23 year, *n* 10) and elderly (about 64 year, *n* 10) men. Whole-body protein synthesis rates, calculated per kg body weight, are significantly lower in the elderly compared to the young controls (*$P<0{\cdot}05$). Adapted from Koopman *et al.*[88].

kinetics of the ingested dietary protein[87]. These methodological restrictions represent the main reasons why only a few researchers have investigated the differences in digestion and absorption kinetics of specific dietary protein sources and the disparity in anabolic response between young and elderly human subjects. These studies have suggested that AA utilisation in the splanchnic area is elevated in the elderly[82], which would imply that less of the ingested AA are available for muscle protein synthesis[82]. We have recently repeated similar experiments, comparing the appearance rate of dietary L-[1-^{13}C]-phenylalanine in the circulation following the intake of 35 g intact intrinsically labelled casein protein[88]. Our data clearly show that splanchnic extraction is not altered significantly in elderly men, and that over a 3 and 6 h period the same amount of dietary phenylalanine appears in the circulation[88]. Although we did not observe any impairment in digestion and absorption in the elderly, we observed substantially (about 12%) lower rates of whole-body protein synthesis and phenylalanine hydroxylation following protein ingestion in the elderly men compared to the young men (Fig. 2), calculated over the first 3 h, subsequent 3 h or total 6 h time period after protein ingestion. Consistent with these observations, we observed a 14% difference in muscle protein synthesis rates between young and elderly men over the 6 h period, although this difference did not reach statistical significance[88]. Not all researchers have found impaired muscle protein synthetic response to protein intake in the elderly as similar protein synthetic rates were observed in young and elderly human subjects after ingestion of large amounts of carbohydrate and proteins[89], and following ingestion of large[90] and small amount of beef[90,91]. Discrepancies may arise from differences in timing of biopsy collection, the precursor

https://doi.org/10.1017/S0029665110003927 Published online by Cambridge University Press

pool used to calculate muscle protein synthesis or the age of the elderly volunteers studied. Clearly, more research is warranted to determine the extent of an anabolic resistance to food (i.e. intact protein) intake that exists in elderly human subjects.

Early work from the laboratory of Yves Boirie[82–84,86,92] showed that ingestion of a slowly digested protein (casein) led to a more positive whole-body protein balance (averaged over a 7 h period) when compared with the ingestion of a fast digestible protein (whey) or a mixture of free AA in healthy, young subjects[83]. In contrast, ingestion of a fast protein resulted in greater (whole-body) net protein retention compared to a slow protein when provided to healthy, older men[82,84,86,92]. The latter response might be attributed to the reported anabolic resistance of the muscle protein synthetic machinery to become activated in the elderly. In accordance with the fast *v*. slow protein concept, we tested the hypothesis that the ingestion of a casein protein hydrolysate, i.e. enzymatically pre-digested casein, would enhance protein digestion and the absorption rate in elderly men[93]. We expected that this enhanced AA uptake in the gut would result in a greater increase in plasma AA availability and might improve the post-prandial muscle protein synthetic response. Elderly men ingested 35 g intrinsically L-[1-^{13}C]phenylalanine labelled casein or casein hydrolysate and we assessed the appearance rate of dietary phenylalanine in the circulation and the subsequent muscle protein synthetic response. The ingestion of casein hydrolysate accelerated the appearance rate of dietary phenylalanine in the circulation, lowered splanchnic phenylalanine extraction, increased post-prandial plasma AA availability and tended to augment the subsequent muscle protein synthetic response *in vivo* in human subjects, compared to the ingestion of intact casein[93]. The difference in the appearance rate of dietary protein between intact and hydrolysed casein was particularly evident in the first 3 h after the protein ingestion, with about 50 % more dietary phenylalanine appearing in the circulation after ingestion of the casein hydrolysate[93]. Consistent with these findings, it was reported that protein pulse feeding (providing up to 80 % of daily protein intake in one meal) leads to greater protein retention than ingesting the same amount of protein provided over four meals throughout the day (spread-feeding) in elderly women[94,95]. These findings may indicate that part of the proposed anabolic resistance in the elderly might be compensated for, in part, by enhancing AA availability during the post-prandial period.

Ageing and the anabolic response to combined exercise and nutrition

We have shown previously that muscle protein synthesis rates are lower in the elderly (about 75 year) compared to young controls under conditions in which resistance-type exercise is followed by food intake[96]. However, combined ingestion of carbohydrate and protein during recovery from physical activity resulted in similar increases in mixed muscle protein synthesis rates, measured over a 6-h period, in young and elderly men[96]. Consistent with our findings, Drummond *et al.*[61] reported similar post-exercise muscle protein synthesis rates over a 5-h recovery period in young *v*. elderly subjects following the ingestion of carbohydrate with an EAA mixture. However, their data indicated that the anabolic response to exercise and food intake was delayed in the elderly. During the first 3 h of post-exercise recovery, the young subjects showed a substantial increase in the muscle protein synthesis rate, which was not observed in the elderly. The delayed activation of muscle protein synthesis in the elderly may be attributed to a more pronounced activation of AMP-activated protein kinase and/or reduced extracellular-signal-regulated kinases1/2 activation during exercise, which seems to be consistent with an attenuated rise in 4E-binding protein phosphorylation following resistance-type exercise in older adults[39]. These data highlight the importance of measuring muscle protein synthesis over different time periods (0–3 h and 3–6 h) following exercise and/or food intake to gain more information about impairments in activation of protein synthesis in the elderly. The mechanisms responsible for the delayed intracellular activation of the mTOR pathway in skeletal muscle remain unclear, but might include differences in muscle recruitment, muscle fibre-type composition, the capacity and/or sensitivity of the muscle protein synthetic machinery, the presence of an inflammatory state and/or the impact of stress on the cellular energy status of the cell between young and older adults.

Long-term interventions

The clinical relevance of nutritional and/or exercise intervention in the elderly stems from the long-term impact on skeletal muscle mass and strength, and the implications for functional capacity. In accordance with the previously discussed findings, the muscle protein synthetic machinery is able to respond to anabolic stimuli, albeit maybe to a lesser extent[46], until very old age[97,98]. Although it was suggested previously that elderly human subjects need more protein[99], more recent studies by Campbell *et al.*[100], who performed very comprehensive nitrogen balance experiments, clearly showed that dietary protein requirements did not increase with age, and that a dietary protein allowance of 0·85 g/kg per day is adequate. Some researchers believe that the attenuated muscle protein synthetic response to food intake in the elderly can, at least partly, be compensated for by increasing the leucine content of a meal[34,101]. However, we have shown previously that additional leucine intake does not further increase muscle protein synthesis after resistance exercise when ample protein is ingested by elderly men[102]. In addition, we investigated the effect of 3 months of leucine supplementation with each main meal (7·5 g/d) on skeletal muscle mass and strength and on glycemic control in healthy elderly men[103]. Consistent with our observations from our acute post-exercise study, we did not observe any effect of leucine supplementation on skeletal muscle mass and strength. In addition, no improvements in indices of whole-body insulin sensitivity blood-glycated Hb content, or the plasma lipid profile were observed. We concluded that long-term leucine supplementation (7·5 g/d) does not augment skeletal muscle mass or strength and does not improve glycaemic control or the blood lipid profile in healthy elderly men.

https://doi.org/10.1017/S0029665110003927 Published online by Cambridge University Press

Resistance exercise training interventions were shown effective in augmenting skeletal muscle mass, increasing muscle strength and/or improving functional capacity in the elderly[97,98,104–119]. In addition, endurance[97,98,104–110] exercise was shown to enhance the skeletal muscle oxidative capacity, resulting in greater endurance capacity[5,120]. Although the muscle regenerative capacity seems to decline at a more advanced age, the reduced satellite cell pool size[119] does not compromise the capacity for muscle hypertrophy to occur even at an advanced age[121–123] and resistance exercise training was shown to increase muscle fibre size[124–127]. Recently, Verdijk *et al.*[119] assessed the effects of 12 weeks of leg resistance exercise training on fibre-type specific hypertrophy and satellite cell content in healthy, elderly men. Prolonged training resulted in a 28% increase in the size of type-II muscle fibres and a concomitant 76% increase in type-II muscle fibre satellite cell content in elderly males[119]. The apparent differences in fibre size and/or satellite cell content between type-I and type-II muscle fibres prior to intervention were no longer evident after 12 weeks of training. Overall, these findings suggest that satellite cells are instrumental in the generation of new myonuclei to facilitate muscle fibre hypertrophy[128].

Protein/AA ingestion before, during and/or after exercise acutely stimulates muscle protein synthesis and reduces muscle protein breakdown to facilitate muscle fibre hypertrophy. Remarkably, little evidence exists that dietary interventions can further augment the adaptive response to prolonged exercise training in the elderly. The proposed importance of ample dietary protein intake in the long-term adaptive response to resistance training in the elderly has been a topic of intense debate[129–131]. Some researchers suggest that the current RDA for habitual protein intake of 0·8 g/kg per day[132,133] is marginal to allow lean mass accretion following resistance exercise training[99] or even insufficient for long-term maintenance of skeletal muscle mass in sedentary elderly human subjects[134]. However, others have shown that when habitual dietary protein intake is standardised at 0·9 g/kg per day, exercise-induced increases in muscle mass become apparent and further increases in protein intake does not provide any additional effect[114]. In addition, data from Walrand *et al.*[135] indicated that although increased protein intake in the elderly further improved nitrogen balance (by increasing AA oxidation), no beneficial effects on muscle protein synthesis and muscle function were observed. These observations might explain why most studies fail to observe any additional benefit of nutritional co-intervention on the skeletal muscle adaptive response to prolonged resistance exercise training in the elderly[97,98,106,113,114,117,118,136–139]. However, it has been suggested that it is not the total protein amount *per se*, but the timing of protein intake that is crucial for its stimulatory effect on muscle protein synthesis and muscle fibre hypertrophy. Esmarck *et al.*[140] concluded that the intake of a protein supplement immediately after each bout of resistance-type exercise was required for skeletal muscle hypertrophy to occur with a 12-week intervention in the elderly. Although the absence of any hypertrophy in the control group seems to conflict with previous studies that show muscle hypertrophy following resistance training without any dietary intervention, the proposed importance of nutrient timing is supported by more recent studies investigating the impact of AA or protein co-ingestion prior to, during and/or after exercise on the acute muscle protein synthetic response[141,142]. Verdijk *et al.*[117] compared increases in skeletal muscle mass and strength following 3 months of resistance exercise training with or without protein ingestion prior to and immediately after each exercise session in elderly males. Timed protein supplementation prior to and after each exercise bout did not further increase skeletal muscle hypertrophy in healthy, elderly men who habitually consumed about 1·0 g protein/kg per day. Taken together, the available data suggest that sufficient habitual protein intake (about 0·9 g/kg per day) combined with a normal meal pattern (i.e. providing ample protein three times daily) will allow for substantial gains in muscle mass and strength with resistance exercise training in the elderly. Additional protein supplementation does not seem to provide large surplus benefits to the exercise intervention in healthy, elderly males. Additional protein intake may reduce subsequent voluntary food consumption in the elderly[143] and consequently some have suggested that supplementation with EAA would be more efficient[144]. Clearly, acute studies have shown benefits of timed supplementation with small (7–15 g) amounts of EAA on muscle protein synthesis[33,35,71]. However, well-designed, double-blind, placebo-controlled long-term studies to investigate beneficial and adverse effects of long-term EAA supplementation in the elderly are yet to be performed[145].

Conclusions

The loss of skeletal muscle mass with ageing is associated with reduced muscle strength, the loss of functional capacity and an increased risk for developing chronic metabolic disease. The progressive loss of skeletal muscle mass does not appear to be attributed to age-related changes in basal muscle protein synthesis and/or rates of protein breakdown. Recent studies suggest that the muscle protein synthetic response to the main anabolic stimuli, i.e. food intake and/or physical activity, is blunted in the elderly. Despite this potential anabolic resistance to food intake and/or physical activity, resistance exercise training can stimulate net muscle protein accretion significantly. Prolonged resistance exercise training has proved to be an effective intervention for attenuating and/or treating the loss of muscle mass and strength in the elderly. Further research is warranted to provide insight into the interactions between nutrition, exercise and skeletal muscle adaptations in order to define more effective nutritional, exercise and/or pharmaceutical interventional strategies to prevent and/or treat sarcopenia.

Acknowledgements

R. K. is a C.R. Roper Senior Research Fellow in the Faculty of Medicine, Dentistry and Health Sciences at The University of Melbourne and his research is currently funded by grants/fellowships from the Ajinomoto Amino Acid Research Program (3ARP, Ajinomoto, Japan) and the European Society for Clinical Nutrition and Metabolism (ESPEN). The author declares no conflict of interest.

References

1. Baumgartner RN, Koehler KM, Gallagher D *et al.* (1998) Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol* **147**(8), 755–763.
2. Forbes GB & Reina JC (1970) Adult lean body mass declines with age: some longitudinal observations. *Metabolism* **19**, 653–663.
3. Melton LJ, 3rd, Khosla S, Crowson CS *et al.* (2000) Epidemiology of sarcopenia. *J Am Geriatr Soc* **48**, 625–630.
4. Short KR & Nair KS (2000) The effect of age on protein metabolism. *Curr Opin Clin Nutr Metab Care* **3**(1), 39–44.
5. Short KR, Vittone JL, Bigelow ML *et al.* (2004) Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab* **286**, E92–E101.
6. Lexell J (1995). Human aging, muscle mass, and fiber type composition. *J Gerontol A Biol Sci Med Sci* **50**, 11–16.
7. Paddon-Jones D & Rasmussen BB (2009) Dietary protein recommendations and the prevention of sarcopenia. *Curr Opin Clin Nutr Metab Care* **12**, 86–90.
8. WHO (2008) Available from: http://www.who.int/topics/ageing/.
9. Kadi F, Charifi N, Denis C *et al.* (2004) Satellite cells and myonuclei in young and elderly women and men. *Muscle Nerve* **29**, 120–127.
10. Larsson L (1978) Morphological and functional characteristics of the ageing skeletal muscle in man. A cross-sectional study. *Acta Physiol Scand Suppl* **457**, 1–36.
11. Larsson L, Sjodin B & Karlsson J (1978) Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22–65 years. *Acta Physiol Scand* **103**, 31–39.
12. Lexell J, Henriksson-Larsen K & Sjostrom M (1983) Distribution of different fibre types in human skeletal muscles. 2. A study of cross-sections of whole m. vastus lateralis. *Acta Physiol Scand* **117**(1), 115–122.
13. Lexell J, Henriksson-Larsen K, Winblad B *et al.* (1983) Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. *Muscle Nerve* **6**, 588–595.
14. Verdijk LB, Koopman R, Schaart G *et al.* (2007) Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly. *Am J Physiol Endocrinol Metab* **292**, E151–E157.
15. Bassey EJ, Fiatarone MA, O'Neill EF *et al.* (1992) Leg extensor power and functional performance in very old men and women. *Clin Sci (Lond)* **82**, 321–327.
16. Brown M, Sinacore DR & Host HH (1995) The relationship of strength to function in the older adult. *J Gerontol A Biol Sci Med Sci* **50**, 55–59.
17. Frontera WR, Hughes VA, Lutz KJ *et al.* (1991) A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. *J Appl Physiol* **71**, 644–650.
18. Landers KA, Hunter GR, Wetzstein CJ *et al.* (2001) The interrelationship among muscle mass, strength, and the ability to perform physical tasks of daily living in younger and older women. *J Gerontol A Biol Sci Med Sci* **56**, B443–B448.
19. Larsson L & Karlsson J (1978) Isometric and dynamic endurance as a function of age and skeletal muscle characteristics. *Acta Physiol Scand* **104**, 129–136.
20. Lindle RS, Metter EJ, Lynch NA *et al.* (1997) Age and gender comparisons of muscle strength in 654 women and men aged 20–93 yr. *J Appl Physiol* **83**, 1581–1587.
21. Petrella JK, Kim JS, Tuggle SC *et al.* (2005) Age differences in knee extension power, contractile velocity, and fatigability. *J Appl Physiol* **98**, 211–220.
22. Wolfson L, Judge J, Whipple R *et al.* (1995) Strength is a major factor in balance, gait, and the occurrence of falls. *J Gerontol A Biol Sci Med Sci* **50**, 64–67.
23. Nair KS (1995) Muscle protein turnover: methodological issues and the effect of aging. *J Gerontol A Biol Sci Med Sci* **50** Spec No, 107–112.
24. Nair KS (2005) Aging muscle. *Am J Clin Nutr* **81**, 953–963.
25. Balagopal P, Rooyackers OE, Adey DB *et al.* (1997) Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol* **273**(4 Pt 1), E790–E800.
26. Hasten DL, Pak-Loduca J, Obert KA *et al.* (2000) Resistance exercise acutely increases MHC and mixed muscle protein synthesis rates in 78–84 and 23–32 yr olds. *Am J Physiol Endocrinol Metab* **278**, E620–E626.
27. Rooyackers OE, Adey DB, Ades PA *et al.* (1996). Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. *Proc Natl Acad Sci USA* **93**, 15364–15369.
28. Welle S, Thornton C, Jozefowicz R *et al.* (1993) Myofibrillar protein synthesis in young and old men. *Am J Physiol* **264**(5 Pt 1), E693–E698.
29. Welle S, Thornton C & Statt M (1995) Myofibrillar protein synthesis in young and old human subjects after three months of resistance training. *Am J Physiol* **268**(3 Pt 1), E422–E427.
30. Yarasheski KE, Welle S & Nair KS (2002) Muscle protein synthesis in younger and older men. *Jama* **287**(3), 317–318.
31. Yarasheski KE, Zachwieja JJ & Bier DM (1993). Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am J Physiol* **265**(2 Pt 1), E210–E214.
32. Cuthbertson D, Smith K, Babraj J *et al.* (2005) Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *Faseb J* **19**, 422–424.
33. Katsanos CS, Kobayashi H, Sheffield-Moore M *et al.* (2005) Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. *Am J Clin Nutr* **82**, 1065–1073.
34. Katsanos CS, Kobayashi H, Sheffield-Moore M *et al.* (2006) A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* **291**, E381–E387.
35. Paddon-Jones D, Sheffield-Moore M, Zhang XJ *et al.* (2004) Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* **286**, E321–E328.
36. Volpi E, Mittendorfer B, Rasmussen BB *et al.* (2000) The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab* **85**, 4481–4490.
37. Volpi E, Mittendorfer B, Wolf SE *et al.* (1999) Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol* **277**(3 Pt 1), E513–E520.
38. Volpi E, Sheffield-Moore M, Rasmussen BB *et al.* (2001) Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *JAMA* **286**, 1206–1212.
39. Kumar V, Selby A, Rankin D *et al.* (2009) Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol* **587**(Pt 1), 211–217.
40. Tipton KD (2001) Muscle protein metabolism in the elderly: influence of exercise and nutrition. *Can J Appl Physiol* **26**, 588–606.

41. Trappe T, Williams R, Carrithers J *et al.* (2004) Influence of age and resistance exercise on human skeletal muscle proteolysis: a microdialysis approach. *J Physiol* **554**(Pt 3), 803–813.
42. Lynch NA, Metter EJ, Lindle RS *et al.* (1999) Muscle quality. I. Age-associated differences between arm and leg muscle groups. *J Appl Physiol* **86**, 188–194.
43. Rasmussen BB, Fujita S, Wolfe RR *et al.* (2006) Insulin resistance of muscle protein metabolism in aging. *Faseb J* **20**, 768–769.
44. Koopman R, Saris WH, Wagenmakers AJ *et al.* (2007) Nutritional interventions to promote post-exercise muscle protein synthesis. *Sports Med* **37**, 895–906.
45. Guillet C, Prod'homme M, Balage M *et al.* (2004) Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *Faseb J* **18**, 1586–1587.
46. Rennie MJ (2009) Anabolic resistance: the effects of aging, sexual dimorphism, and immobilization on human muscle protein turnover. *Appl Physiol Nutr Metab* **34**, 377–381.
47. Wilkinson SB, Phillips SM, Atherton PJ *et al.* (2008) Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J Physiol* **586**(Pt 15), 3701–3717.
48. Evans WJ (1995) Effects of exercise on body composition and functional capacity of the elderly. *J Gerontol A Biol Sci Med Sci* **50**, 147–150.
49. Rennie MJ & Tipton KD (2000) Protein and amino acid metabolism during and after exercise and the effects of nutrition. *Annu Rev Nutr* **20**, 457–483.
50. Nair KS, Halliday D & Griggs RC (1988) Leucine incorporation into mixed skeletal muscle protein in humans. *Am J Physiol* **254**(2 Pt 1), E208–E213.
51. Volpi E, Ferrando AA, Yeckel CW *et al.* (1998) Exogenous amino acids stimulate net muscle protein synthesis in the elderly. *J Clin Invest* **101**, 2000–2007.
52. Phillips SM, Tipton KD, Aarsland A *et al.* (1997) Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol* **273**(1 Pt 1), E99–E107.
53. Tang JE, Perco JG, Moore DR *et al.* (2008) Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am J Physiol Regul Integr Comp Physiol* **294**, R172–R178.
54. MacDougall JD, Tarnopolsky MA, Chesley A *et al.* (1992) Changes in muscle protein synthesis following heavy resistance exercise in humans: a pilot study. *Acta Physiol Scand* **146**, 403–404.
55. Biolo G, Maggi SP, Williams BD *et al.* (1995) Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol* **268**(3 Pt 1), E514–E520.
56. Laurent GJ, Sparrow MP & Millward DJ (1978) Turnover of muscle protein in the fowl. Changes in rates of protein synthesis and breakdown during hypertrophy of the anterior and posterior latissimus dorsi muscles. *Biochem J* **176**, 407–417.
57. Bodine SC, Stitt TN, Gonzalez M *et al.* (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* **3**, 1014–1019.
58. Bolster DR, Kubica N, Crozier SJ *et al.* (2003) Immediate response of mammalian target of rapamycin (mTOR)-mediated signalling following acute resistance exercise in rat skeletal muscle. *J Physiol* **553**(Pt 1), 213–220.
59. Dreyer HC, Fujita S, Cadenas JG *et al.* (2006) Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. *J Physiol* **576**(Pt 2), 613–624.
60. Kimball SR, Farrell PA & Jefferson LS (2002) Invited Review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. *J Appl Physiol* **93**, 1168–1180.
61. Drummond MJ, Dreyer HC, Pennings B *et al.* (2008) Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. *J Appl Physiol* **104**, 1452–1461.
62. Koopman R, Zorenc AH, Gransier RJ *et al.* (2006) The increase in S6K1 phosphorylation in human skeletal muscle following resistance exercise occurs mainly in type II muscle fibers. *Am J Physiol Endocrinol Metab* **290**, E1245–E1252.
63. Drummond MJ, Fry CS, Glynn EL *et al.* (2009) Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *J Physiol* **587**(Pt 7), 1535–1546.
64. Baar K & Esser K (1999) Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol* **276**(1 Pt 1), C120–C127.
65. Terzis G, Georgiadis G, Stratakos G *et al.* (2008) Resistance exercise-induced increase in muscle mass correlates with p70S6 kinase phosphorylation in human subjects. *Eur J Appl Physiol* **102**, 145–152.
66. Fujita S, Rasmussen BB, Cadenas JG *et al.* (2007) Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and Akt/mammalian target of rapamycin signaling. *Diabetes* **56**, 1615–1622.
67. Sheffield-Moore M, Yeckel CW, Volpi E *et al.* (2004) Postexercise protein metabolism in older and younger men following moderate-intensity aerobic exercise. *Am J Physiol Endocrinol Metab* **287**, E513–E522.
68. Hameed M, Orrell RW, Cobbold M *et al.* (2003) Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *J Physiol* **547**(Pt 1), 247–254.
69. Raue U, Slivka D, Jemiolo B *et al.* (2007) Proteolytic gene expression differs at rest and after resistance exercise between young and old women. *J Gerontol A: Biol Sci Med Sci* **62**, 1407–1412.
70. Rennie MJ, Edwards RH, Halliday D *et al.* (1982) Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clin Sci (Lond)* **63**, 519–523.
71. Paddon-Jones D, Sheffield-Moore M, Katsanos CS *et al.* (2006) Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. *Exp Gerontol* **41**, 215–219.
72. Tipton KD, Gurkin BE, Matin S *et al.* (1999) Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J Nutr Biochem* **10**, 89–95.
73. Volpi E, Kobayashi H, Sheffield-Moore M *et al.* (2003) Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* **78**, 250–258.
74. Smith K, Barua JM, Watt PW *et al.* (1992) Flooding with L-[1–13C]leucine stimulates human muscle protein incorporation of continuously infused L-[1–13C]valine. *Am J Physiol* **262**(3 Pt 1), E372–E376.
75. Norton LE & Layman DK (2006) Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. *J Nutr* **136**, 533S–537S.

https://doi.org/10.1017/S0029665110003927 Published online by Cambridge University Press

76. Bohe J, Low A, Wolfe RR *et al.* (2003) Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. *J Physiol* **552**(Pt 1), 315–324.
77. Borsheim E, Cree MG, Tipton KD *et al.* (2004) Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. *J Appl Physiol* **96**, 674–678.
78. Greenhaff PL, Karagounis LG, Peirce N *et al.* (2008) Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* **295**, E595–E604.
79. Wilkes EA, Selby AL, Atherton PJ *et al.* (2009) Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *Am J Clin Nutr* **90**, 1343–1350.
80. Wilkes E, Selby A, Patel R *et al.* (2008) Blunting of insulin-mediated proteolysis in leg muscle of elderly subjects may contribute to age-related sarcopenia (Abstract). *Proc Nutr Society* **67**(OCE5), E153.
81. Fujita S, Rasmussen BB, Cadenas JG *et al.* (2006) Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. *Am J Physiol Endocrinol Metab* **291**, E745–E754.
82. Boirie Y, Dangin M, Gachon P *et al.* (1997) Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci USA* **94**, 14930–14935.
83. Dangin M, Boirie Y, Garcia-Rodenas C *et al.* (2001) The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab* **280**, E340–E348.
84. Beaufrere B, Dangin M & Boirie Y (2000) The 'fast' and 'slow' protein concept. *Nestle Nutr Workshop Ser Clin Perform Program* **3**, 121–131; discussion 31–3.
85. Boirie Y, Gachon P, Corny S *et al.* (1996) Acute postprandial changes in leucine metabolism as assessed with an intrinsically labeled milk protein. *Am J Physiol* **271**(6 Pt 1), E1083–E1091.
86. Dangin M, Boirie Y, Guillet C *et al.* (2002) Influence of the protein digestion rate on protein turnover in young and elderly subjects. *J Nutr* **132**, 3228S–3233S.
87. Boirie Y, Fauquant J, Rulquin H *et al.* (1995). Production of large amounts of [13C]leucine-enriched milk proteins by lactating cows. *J Nutr* **125**, 92–98.
88. Koopman R, Walrand S, Beelen M *et al.* (2009) Dietary protein digestion and absorption rate and the subsequent muscle protein synthetic response are not different between young and elderly men. *J Nutr* **139**, 1707–1713.
89. Koopman R, Verdijk LB, Manders RJF *et al.* (2006) Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. *Am J Clin Nutr* **84**, 623–632.
90. Symons TB, Sheffield-Moore M, Wolfe RR *et al.* (2009) A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. *J Am Diet Assoc* **109**, 1582–1586.
91. Symons TB, Schutzler SE, Cocke TL *et al.* (2007) Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr* **86**, 451–456.
92. Dangin M, Guillet C, Garcia-Rodenas C *et al.* (2003) The rate of protein digestion affects protein gain differently during aging in humans. *J Physiol* **549**(Pt 2), 635–644.
93. Koopman R, Crombach N, Gijsen AP *et al.* (2009) Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. *Am J Clin Nutr* **90**, 106–115.
94. Arnal MA, Mosoni L, Boirie Y *et al.* (2000) Protein feeding pattern does not affect protein retention in young women. *J Nutr* **130**, 1700–1704.
95. Arnal MA, Mosoni L, Boirie Y *et al.* (1999) Protein pulse feeding improves protein retention in elderly women. *Am J Clin Nutr* **69**, 1202–1208.
96. Koopman R, Verdijk L, Manders RJ *et al.* (2006) Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. *Am J Clin Nutr* **84**(3), 623–632.
97. Fiatarone MA, Marks EC, Ryan ND *et al.* (1990) High-intensity strength training in nonagenarians. Effects on skeletal muscle. *Jama* **263**, 3029–3034.
98. Frontera WR, Meredith CN, O'Reilly KP *et al.* (1988) Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J Appl Physiol* **64**, 1038–1044.
99. Campbell WW, Trappe TA, Jozsi AC *et al.* (2002) Dietary protein adequacy and lower body versus whole body resistive training in older humans. *J Physiol* **542**(Pt 2), 631–642.
100. Campbell WW, Johnson CA, McCabe GP *et al.* (2008) Dietary protein requirements of younger and older adults. *Am J Clin Nutr* **88**(5), 1322–1329.
101. Rieu I, Balage M, Sornet C *et al.* (2006) Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *J Physiol* **575**(Pt 1), 305–315.
102. Koopman R, Verdijk LB, Beelen M *et al.* (2008) Co-ingestion of leucine with protein does not further augment post-exercise muscle protein synthesis rates in elderly men. *Br J Nutr* **99**, 571–580.
103. Verhoeven S, Vanschoonbeek K, Verdijk LB *et al.* (2009) Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. *Am J Clin Nutr* **89**, 1468–1475.
104. Ades PA, Ballor DL, Ashikaga T *et al.* (1996) Weight training improves walking endurance in healthy elderly persons. *Ann Intern Med* **124**, 568–572.
105. Bamman MM, Hill VJ, Adams GR *et al.* (2003) Gender differences in resistance-training-induced myofiber hypertrophy among older adults. *J Gerontol A Biol Sci Med Sci* **58**, 108–116.
106. Fiatarone MA, O'Neill EF, Ryan ND *et al.* (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* **330**, 1769–1775.
107. Frontera WR, Hughes VA, Krivickas LS *et al.* (2003) Strength training in older women: early and late changes in whole muscle and single cells. *Muscle Nerve* **28**, 601–608.
108. Frontera WR, Meredith CN, O'Reilly KP *et al.* (1990) Strength training and determinants of VO2max in older men. *J Appl Physiol* **68**, 329–333.
109. Lexell J, Downham DY, Larsson Y *et al.* (1995) Heavy-resistance training in older Scandinavian men and women: short- and long-term effects on arm and leg muscles. *Scand J Med Sci Sports* **5**, 329–341.
110. Vincent KR, Braith RW, Feldman RA *et al.* (2002) Resistance exercise and physical performance in adults aged 60 to 83. *J Am Geriatr Soc* **50**, 1100–1107.
111. Brose A, Parise G & Tarnopolsky MA (2003) Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. *J Gerontol A Biol Sci Med Sci* **58**, 11–19.
112. Ferri A, Scaglioni G, Pousson M *et al.* (2003) Strength and power changes of the human plantar flexors and knee extensors in response to resistance training in old age. *Acta Physiol Scand* **177**, 69–78.

NS Proceedings of the Nutrition Society

113. Godard MP, Williamson DL & Trappe SW (2002) Oral amino-acid provision does not affect muscle strength or size gains in older men. *Med Sci Sports Exerc* **34**, 1126–1131.
114. Iglay HB, Thyfault JP, Apolzan JW *et al.* (2007) Resistance training and dietary protein: effects on glucose tolerance and contents of skeletal muscle insulin signaling proteins in older persons. *Am J Clin Nutr* **85**, 1005–1013.
115. Kosek DJ, Kim JS, Petrella JK *et al.* (2006) Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. *older adults. J Appl Physiol* **101**, 531–544.
116. Martel GF, Roth SM, Ivey FM *et al.* (2006) Age and sex affect human muscle fibre adaptations to heavy-resistance strength training. *Exp Physiol* **91**, 457–464.
117. Verdijk LB, Jonkers RAM, Gleeson BG *et al.* (2009) Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy following resistance training in elderly men. *Am J Clin Nutr* **89**, 608–616.
118. Haub MD, Wells AM, Tarnopolsky MA *et al.* (2002) Effect of protein source on resistive-training-induced changes in body composition and muscle size in older men. *Am J Clin Nutr* **76**, 511–517.
119. Verdijk LB, Gleeson BG, Jonkers RAM *et al.* (2009) Skeletal muscle hypertrophy following resistance training is accompanied by a fiber type-specific increase in satellite cell content in elderly men. *J Gerontol A Biol Sci Med Sci* **64**, 332–339.
120. Short KR, Vittone JL, Bigelow ML *et al.* (2003) Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. *Diabetes* **52**, 1888–1896.
121. Dedkov EI, Borisov AB, Wernig A *et al.* (2003) Aging of skeletal muscle does not affect the response of satellite cells to denervation. *J Histochem Cytochem* **51**, 853–863.
122. Shefer G, Van de Mark DP, Richardson JB *et al.* (2006) Satellite-cell pool size does matter: defining the myogenic potency of aging skeletal muscle. *Dev Biol* **294**, 50–66.
123. Thornell LE, Lindstrom M, Renault V *et al.* (2003) Satellite cells and training in the elderly. *Scand J Med Sci Sports* **13**, 48–55.
124. Kadi F, Schjerling P, Andersen LL *et al.* (2004) The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. *J Physiol* **558**(Pt 3), 1005–1012.
125. Kadi F & Thornell LE (2000) Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. *Histochem Cell Biol* **113**, 99–103.
126. Petrella JK, Kim JS, Cross JM *et al.* (2006) Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *Am J Physiol Endocrinol Metab* **291**(5), E937–E946.
127. Olsen S, Aagaard P, Kadi F *et al.* (2006) Creatine supplementation augments the increase in satellite cell and myonuclei number in human skeletal muscle induced by strength training. *J Physiol* **573**(Pt 2), 525–534.
128. Snijders T, Verdijk LB & van Loon LJ (2009) The impact of sarcopenia and exercise training on skeletal muscle satellite cells. *Ageing Res Rev* **8**, 328–338.
129. Campbell WW & Evans WJ (1996) Protein requirements of elderly people. *Eur J Clin Nutr* **50** Suppl 1, S180–S183; discussion S3–5.
130. Morais JA, Chevalier S & Gougeon R (2006) Protein turnover and requirements in the healthy and frail elderly. *J Nutr Health Aging* **10**, 272–283.
131. Campbell WW & Leidy HJ (2007) Dietary protein and resistance training effects on muscle and body composition in older persons. *J Am Coll Nutr* **26**, 696S–703S.
132. Rand WM, Pellett PL & Young VR (2003) Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am J Clin Nutr* **77**(1), 109–127.
133. Trumbo P, Schlicker S, Yates AA *et al.* (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc* **102**, 1621–1630.
134. Campbell WW, Trappe TA, Wolfe RR *et al.* (2001) The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci* **56**, M373–M380.
135. Walrand S, Short KR, Bigelow ML *et al.* (2008) Functional impact of high protein intake on healthy elderly people. *Am J Physiol Endocrinol Metab* **295**, E921–E928.
136. Campbell WW, Crim MC, Young VR *et al.* (1995) Effects of resistance training and dietary protein intake on protein metabolism in older adults. *Am J Physiol* **268**(6 Pt 1), E1143–E1153.
137. Freyssenet D, Berthon P, Denis C *et al.* (1996) Effect of a 6-week endurance training programme and branched-chain amino acid supplementation on histomorphometric characteristics of aged human muscle. *Arch Physiol Biochem* **104**, 157–162.
138. Meredith CN, Frontera WR, O'Reilly KP *et al.* (1992) Body composition in elderly men: effect of dietary modification during strength training. *J Am Geriatr Soc* **40**, 155–162.
139. Welle S & Thornton CA (1998) High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women. *Am J Physiol* **274**(4 Pt 1), E677–E683.
140. Esmarck B, Andersen JL, Olsen S *et al.* (2001) Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol* **535**(Pt 1), 301–311.
141. Beelen M, Koopman R, Gijsen AP *et al.* (2008) Protein coingestion stimulates muscle protein synthesis during resistance-type exercise. *Am J Physiol Endocrinol Metab* **295**(1), E70–E77.
142. Tipton KD, Rasmussen BB, Miller SL *et al.* (2001) Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab* **281**, E197–E206.
143. Fiatarone Singh MA, Bernstein MA, Ryan AD *et al.* (2000) The effect of oral nutritional supplements on habitual dietary quality and quantity in frail elders. *J Nutr Health Aging* **4**, 5–12.
144. Timmerman KL & Volpi E (2008) Amino acid metabolism and regulatory effects in aging. *Curr Opin Clin Nutr Metab Care* **11**, 45–49.
145. Henderson GC, Irving BA & Nair KS (2009) Potential application of essential amino Acid supplementation to treat sarcopenia in elderly people. *J Clin Endocrinol Metab* **94**, 1524–1526.
146. Verdijk LB, van Loon L, Meijer K *et al.* (2009) One-repetition maximum strength test represents a valid means to assess leg strength in vivo in humans. *J Sports Sci* **27**, 59–68.

https://doi.org/10.1017/S0029665110003927 Published online by Cambridge University Press