# Dietary protein to support active aging

# SUMMARY

*Age-related muscle loss, physical activity and dietary protein*

Older individuals display a blunted muscle protein synthetic response to dietary protein ingestion. This *anabolic resistance* at least partly explains the age-related decline in skeletal muscle mass. Physical activity, and resistance-type exercise in particular, is the most potent strategy to increase muscle protein synthesis rates. Dietary protein ingestion is required to further stimulate muscle protein synthesis rates and achieve a positive net muscle protein balance during post-exercise recovery.

*Amount of protein*

In **Chapter 2**, we defined the amount of protein required to stimulate near-maximal muscle protein synthesis rates in healthy older men. Ingestion of at least 30 g protein results in a measurable rise in post-exercise muscle protein synthesis rates when compared to placebo ingestion. By applying intrinsically L-[1-$^{13}$C]-phenylalanine-labeled protein, we were able to demonstrate a protein dose-dependent increase in the incorporation of L-[1-$^{13}$C]-phenylalanine into myofibrillar protein, with ingestion of 45 g protein providing a greater amount of dietary protein-derived phenylalanine for *de novo* muscle protein synthesis. This finding demonstrates that whereas muscle protein synthesis rates were stimulated after ingestion of 30-45 g protein, ingestion of greater protein doses provide greater amounts of amino acid precursors that are utilized to support the increase in *de novo* muscle protein synthesis rates.

*Leucine co-ingestion*

Ingesting 30-45 g protein in a single mixed meal may be practically challenging for certain older populations. The amino acid leucine has been established as one of the most anabolic amino acids due to its ability to phosphorylate key anabolic signaling proteins (*i.e.*, mTORC1 and S6K) in skeletal muscle tissue. Therefore, in **Chapter 3**, we assessed post-prandial protein handling and the muscle protein synthetic response to the ingestion of a smaller, 15 g protein dose with or without additional free leucine (1.5 g) during recovery from resistance-type exercise in older individuals. Interestingly, free leucine co-ingestion seemed to compromise protein digestion and/or amino acid absorption as dietary protein-derived phenylalanine availability was lower over the entire 6 h post-prandial period compared with ingestion of only 15 g protein. Nevertheless, we demonstrate that free leucine co-ingestion with 15 g protein stimulates greater muscle protein synthesis rates compared with ingestion of only 15 g protein.

*Pre-sleep dietary protein ingestion*

Besides augmenting the muscle protein synthetic response to a post-exercise meal, optimal *timing* of meal ingestion may also aid in compensating for anabolic resistance in older individuals. In **Chapter 4,** we assessed whether a bout of resistance-type exercise could augment the overnight muscle protein synthetic response dietary protein ingested prior to sleep in older men. We observed that approximately 55 % of the ingested dietary protein became available in the circulation during sleep. Furthermore, the prior bout of exercise substantially increased overnight muscle protein rates, with 28 % more dietary protein-derived amino acids being directed toward *de novo* overnight muscle protein synthesis.
The ingestion of more rapidly digestible protein sources results in a greater post-prandial rise in circulating amino acid concentrations, which thereby further increases muscle protein synthesis rates. We speculated that protein digestion and absorption might also be modulated by an external factor as simple as body position (*i.e.,* sitting upright or lying down). In **Chapter 5**, we tested the impact of protein ingestion in an upright position compared with a head-down tilted (-20 °) supine body position. As expected, peak leucine concentration and plasma amino acid availability were lower after protein ingestion in the inverted position when compared with the upright seated position. These proof-of-principle findings may not necessarily carry over to real-life situations. Therefore, in **Chapter 6**, we tested the impact of protein ingestion in a horizontal, supine body position compared with an upright seated position. We found that gastric emptying was accelerated and the rise in circulating leucine concentration was greater in the upright seated position compared with the horizontal supine position. These findings indicate that body position modulates rate of dietary protein digestion and absorption and impacts amino acid availability in the circulation, which may impact post-prandial muscle protein synthesis rates.

*Long-term assessment of muscle protein synthesis rates*

Recently, the application of deuterated water ($^2H_2O$) has re-emerged in the field as an approach to assess muscle protein synthesis rates over multiple days or weeks *in vivo* in humans. We applied oral deuterated water dosing in **Chapter 7** to assess the impact of resistance-type exercise training on local muscle protein synthesis rates over a three-day period in healthy, younger males. We found that that daily resistance-type exercise stimulated muscle protein synthesis rates over multiple days. Whereas acute labeled amino acid infusion studies have shown that resistance-type exercise increases muscle protein synthesis rates by 50–100 %, we observed a lower impact of resistance-type exercise on the muscle protein synthetic response (~25 %) under free-living conditions. This discrepancy between tracer methodologies likely reflects the impact of other anabolic factors, such as dietary protein intake, habitual physical activity, sleep and hormonal fluctuations, which are incorporated into the more prolonged assessment of muscle protein synthesis rates.

*Prolonged resistance-type exercise training*

In **Chapter 8**, we hypothesized that supplementation with leucine-enriched whey protein provided immediately after training and every night prior to sleep would further augment the muscle protein synthetic response during post-exercise recovery and therefore lead to greater gains in muscle mass and strength following more prolonged training in active older men. While twelve weeks of resistance-type exercise training increased whole body lean mass and maximal leg extension strength, protein supplementation did not further increase muscle mass or strength gains over the 12-week training intervention. These findings demonstrate that protein supplementation may not provide added benefit to muscle mass and strength gains following resistance-type exercise training in healthy, active older individuals who already consume ample amounts of protein.
In order to assess the muscle anabolic response to protein supplementation *during* training, we provided subjects with deuterated water ($^2H_2O$) throughout week 12 of the training intervention and collected additional muscle biopsy samples. In line with our findings on differences in muscle mass and strength, we observed no difference in myofibrillar protein synthesis rates between the placebo- and protein-supplemented groups.

*Future application of deuterated water methods to study muscle protein metabolism*

In the final chapter of this dissertation, we discuss novel methodology based around deuterated water administration to study human muscle protein metabolism. In particular, application of deuterated water methods within long-term intervention studies may provide us with a better understanding of the dynamic regulation of muscle protein synthesis underlying skeletal muscle hypertrophy and atrophy. Deuterated water ingestion combined with recent developments in high-throughput analytical techniques allows for simultaneous *in vivo* assessment of the fractional synthesis rates of hundreds of individual proteins. This proteome dynamics approach represents an important methodological development in the field of human muscle protein metabolism and future application will provide more complete insight into the dynamic regulation of skeletal muscle protein reconditioning.