



## SARCOPENIA AND WHEY PROTEINS

### *Implications, Mechanisms and Potential for Nutritional Intervention*

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*A prevalent illness among older adults, sarcopenia entails the loss of muscle mass, strength and function. Research indicates its onset around 40 with profound repercussions after approximately 75 years of age. The world now witnesses an unprecedented trend in population aging, bearing significant implications for the healthcare industry and the labor force.*

The American Association of Retired Persons predicts that 20-25% of the population in the EU, Japan and the United States will be 65 or older by 2030. Also, life expectancy in these countries has increased by 7-10 years for men and by 8 to nearly 13 years for women since the early 60s. Governments in industrialized nations recognize the importance of a higher participation rate for individuals aged 65 and older in the workforce.

This development redefines the global perspective on aging, demanding a healthy, independent individual with the potential to make valuable

contributions well into their 60s and 70s. Hence, age related illnesses such as sarcopenia warrants attention due to their effect on one out of five older individuals. Increasing awareness of sarcopenia and promoting health enhancing strategies to combat the illness possess numerous benefits; these preventional measures may considerably impact healthcare costs. Studies also demonstrate multiple factors for the etiology of sarcopenia, including loss of motor neurons, degradation in muscle protein synthesis, fluctuations in hormonal levels and a sedentary lifestyle.



This report provides the most recent findings on lifestyle strategies that may prevent/treat sarcopenia; the unexplainable, age-related loss of muscle mass that has a negative impact on strength, power, functional ability and daily living. To fully ascertain the unique potential of whey proteins (WP) in the management of sarcopenia, the reader is provided firstly with a clear understanding of its physiological and metabolic repercussions. This is followed by a brief discussion of the mechanisms thought to underline sarcopenia.

The latest findings on the impact of WP supplementation on protein metabolism are presented in this report and the unique attributes of WP for the prevention and treatment of sarcopenia are also identified. Although sarcopenia is a multifaceted phenomenon, it is clear that only two aspects positively affect protein turnover to promote the maintenance/increase of human muscle mass. Those factors are, resistance exercise and the intake of macronutrients namely, protein. Therefore, this report features the latest developments on dietary and exercise interventions aimed at the treatment and prevention of sarcopenia.

## PHYSIOLOGIC AND METABOLIC IMPLICATIONS OF SARCOPENIA

Athletic performance and vanity aside, there are many important reasons for wishing to know more about how to build muscle. Not the least being the underestimated role of muscle mass in healthy aging. Sarcopenia is the unexplainable, age-related loss of muscle mass which has a negative impact on strength, power, functional ability and daily living (Nair 2005). This phenomenon is wide spread among apparently healthy older adults; recent estimates indicate that approximately 45% of people 65 years or older in the United States exhibit sarcopenia (Janssen et al., 2004) and 20% are functionally disabled (Manton 2001).

An accumulating amount of evidence suggests that sarcopenia underlines many of the undesirable conditions that are associated with aging, such as osteoporosis, diabetes, unwanted weight gain, an increased susceptibility to illness, falls and related injuries (Dutta 1997; Evans 1997; Doherty 2003). The direct healthcare costs attributed to sarcopenia each year in the United States may be as high as US \$18.5 billion (Janssen et al., 2004).

While there is a clear relationship between a quantitative net loss of muscle mass and diminished functional capacity (Frontera et al., 2001), of even greater concern is the longitudinal data (spanning 10-12 years) that shows the decline in leg muscle strength can be 60% greater than estimates from a cross-sectional analysis in the same population (Hughes et al., 2001). The rate of muscle mass decline with age is thought to be fairly consistent; approximately 1-2% per year past the age of 50 years (Sehl et al., 2001). However, a reduction of 30% or more is thought to limit normal function (Bortz 2002). A major problem is that the minimal amount required to maintain health and independent living with advancing age is unknown.

Some researchers suggest that sarcopenia may be reversible (at least to a certain extent) (Roubenoff 2003). Others believe that tomorrow's older adults should be concerned with building a greater "starting reserve capacity" of lean body mass (LBM)<sup>1</sup> today to ensure they avoid the unknown threshold that precedes physical frailty and compromised health (Marcell 2003).

An age-related decrease in LBM is also associated with unfavorable changes in body composition, and these changes have severe metabolic repercussions. While there is a dramatic decline in muscle mass between the age of 50 and 75 years (by approximately 25%), this is accompanied by a substantial increase in body fat. Cross-sectional data suggests that the average adult can expect to gain approximately 1 pound (2.2kg) of fat every year between ages 30 to 60, and lose about a half pound of muscle per year; a change that is equivalent to a 15 pound loss of muscle and a 30 pound gain in fat (Baumgartner et al., 1995; Forbes 1999; Gallagher et al., 1997). These changes in body composition have metabolic repercussions.



<sup>1</sup> Muscle mass constitutes approximately 60% of lean body mass. These terms are often used interchangeably, particularly with regard to changes in body composition.

Muscle tissue has a large working range of ATP turnover rates and tremendous potential to consume energy. Due to its mass, muscle is a highly important thermogenic tissue and a prime determinant of basal metabolic rate (BMR) (which for most of us is the largest single contributor to daily energy expenditure) (Elia et al., 2003). Therefore, muscle tissue is not only important for maintenance of a healthy weight, by virtue of its mass and mitochondrial content, muscle tissue is the largest site of lipid oxidation (Heilbronn et al., 2004). Muscle is also the primary site of glucose disposal in the post-prandial (fed) state (Perez-Martin et al., 2001), and exercised muscle promotes healthy glucose metabolism (Henriksen, 2002). Therefore, maintenance of this metabolically active tissue (with elevated mitochondrial potential) may also reduce the risk for the development of type-II diabetes (Perez-Martin et al., 2001). To confirm these metabolic assumptions, cross-sectional data shows that older men and women generally have a decreased ability to mobilize and oxidize fat, as well as possess a slower BMR in comparison to their younger counterparts (Levadoux et al., 2001; Calles-Escandon et al., 1995; Nargy et al., 1996).



Additionally, this age-related decline in BMR and fat metabolism is suggested to be related more to a reduction in LBM than aging *per se* (Levadoux et al., 2001; Calles-Escandon et al., 1995; Nargy et al., 1996). In fact, the preservation of muscle mass throughout aging may reduce the decline in BMR and possibly reduce the degree of body fat accumulation that is characteristically observed in older adults (Evans 1997; Marcel 2003). Unlike aerobic fitness capacity (Broeder et al., 1992), LBM is an important determinant of BMR (Levadoux et al., 2001; Calles-Escandon et al., 1995; Nargy et al., 1996). For this reason, strategies that preserve LBM are thought to be the cornerstone of any successful attempt at weight loss (Poehlman et al., 1998). Therefore, lifestyle and dietary strategies that focus on maintaining muscle mass throughout the lifespan will enhance the health of a wide sector of the population and may prevent or reduce the severity of many aging-related illnesses, as well as reduce a significant economic burden on the healthcare system (Janssen et al., 2004).

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### FACTORS THAT REGULATE THE SIZE OF HUMAN SKELETAL MUSCLE MASS

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The mechanisms that underline sarcopenia are complex and are yet to be fully elucidated. Essentially, sarcopenia is the end result of a malfunction in the regulatory mechanisms that maintain the size of human muscle mass. The quality and quantity of muscle protein is fundamentally maintained through a continuous remodeling process (protein turnover) that involves continuous synthesis and breakdown (Rasmussen & Phillips 2003). The regulation of protein turnover is multifaceted, but this process is basically controlled by the initiation of protein synthesis and degradation (via proteolytic pathways) (Rennie et al., 2004). Regulators of muscle protein turnover work specifically by stimulation (acceleration) or inhibition of protein synthesis and protein degradation; these are identified in Figure 1.

For example, (shown in red in Figure 1) insulin and insulin-like growth factor (IGF-1) prevent contractile protein degradation (IGF-1 inhibits degradation via the ubiquitin pathway).

The ubiquitin pathway is one of the major protein degradation pathways cells rely upon to eliminate cellular proteins. Proteins that are damaged or are no longer needed by a cell are 'tagged' with the protein known as ubiquitin and this targets the tagged protein to a large protein complex known as the proteasome for hydrolysis by proteases. The stress-induced hormone cortisol and the growth factor myostatin are known inhibitors of protein synthesis (Rennie et al., 2004). Cortisol, cytokines (small signaling peptides secreted by cells) and ubiquitin proteins (shown in green) activate protein degradation whereas insulin, amino acids, mechanical loading and the anabolic hormones such as testosterone, growth hormone (GH), IGF-1 and mechano-growth factor (a splice variant of IGF-1 that is

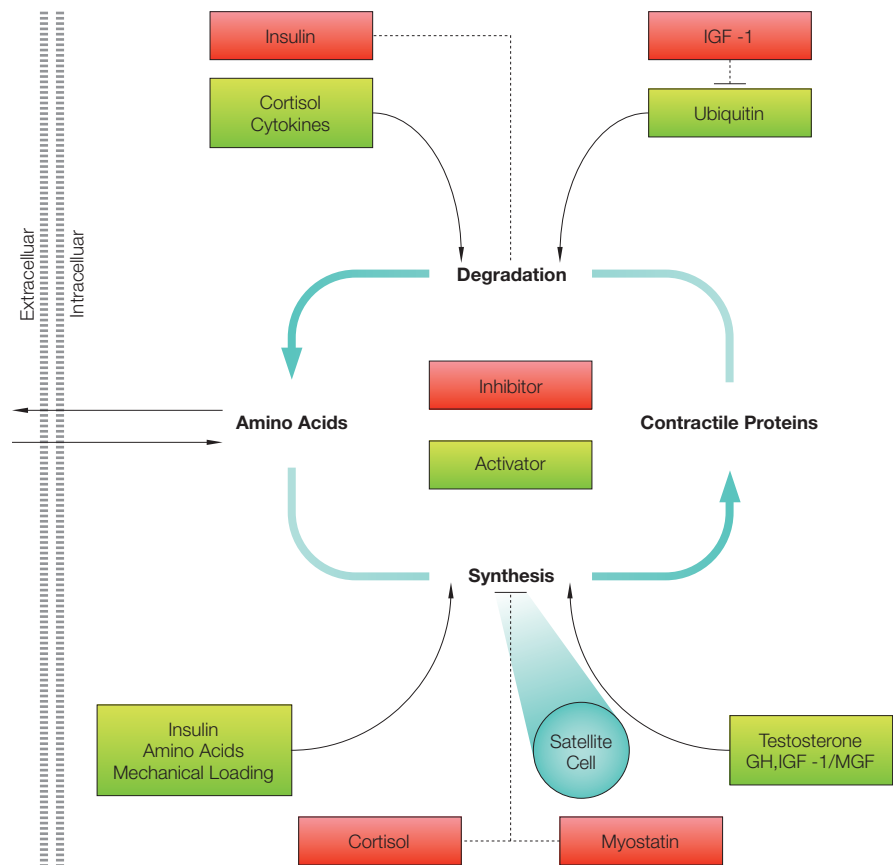
produced in muscle); all stimulate muscle protein synthesis to varying degrees (Florini et al., 1996; Bhasin 2003; Rennie et al., 2004;). It is the balance between these factors, among others, that determine muscle loss or gain as they are regulated temporally throughout a person's life; however, several of these factors also respond to stimuli such as the availability of certain nutrients.

Perhaps the best example of nutrients affecting muscle synthesis and breakdown is that of the anabolic hormone insulin. Binding of insulin to its receptor on cellular membranes initiates a signaling cascade involving phosphorylation/activation of numerous proteins including those required for protein synthesis. One of the rate-limiting steps in the synthesis of cellular protein is that of translation initiation and the assembly of the translation complex. Numerous molecules are involved including ribosomal subunits, tRNAs, mRNA and multiple protein factors known as elongation Initiation Factors (eIF).

Another important molecule in the signaling pathway for translation initiation is mTOR (mammalian target of the drug rapamycin), a protein kinase that activates several eIFs and rpS6 (ribosomal protein S6) by activating the rpS6 kinase p70<sup>S6K</sup>. rpS6 activation is associated with increased translational capacity in a cell. The insulin signaling pathway activates mTOR which then increases levels of active rpS6 and eIFs thereby increasing cellular translation of proteins. In the case of muscle cells, a major result is the increased translation of contractile proteins. Moreover, the BCAA leucine has also been shown to up regulate protein synthesis through activation of mTOR and eIFs. Specific studies that describe the effects of dietary carbohydrate and leucine on cellular protein synthesis are included in the following text.

**Figure 1. Regulators of Muscle Protein**

*A number of regulators (shown in red and green) affect muscle protein turnover within muscle. This ultimately influences the size of lean body mass. Each of these regulators exerts their effects via the stimulation or inhibition of protein synthesis and protein degradation. Sarcopenia may be the result of a malfunction in some or all of these regulators.*



Aside from the regulators of muscle mass described in Figure 1, a clear understanding of the processes that lead to sarcopenia is exacerbated by muscle tissue's multifaceted role in the regulation of whole body protein metabolism. Besides its locomotive and metabolic implications, muscle tissue is the body's largest reservoir of bound and unbound proteins (amino acids) (muscle constitutes 50-75% of all proteins in the human body) (Phillips et al., 2005). While quantitative estimates suggest that about 1-2% of muscle is synthesized and broken down on a daily basis, the mass of this tissue means that it accounts for up to 50% of whole body protein turnover (Rennie & Tipton 2000). This impact (on whole body protein turnover) is a clear reflection of the essential role that muscle tissue plays in the regulation of whole body amino acid metabolism.

Muscle is the reservoir and synthesis site of a number of amino acids (AA) that are constantly exported to meet an array of physiological demands. One example is the amino acid glutamine — the essential fuel that powers many aspects of immune function and cell turnover (Curi et al., 2005).

However, when considering all aspects that may influence the size of human muscle mass, one must keep in mind that the regulatory network that controls this process may not reside exclusively within muscle.

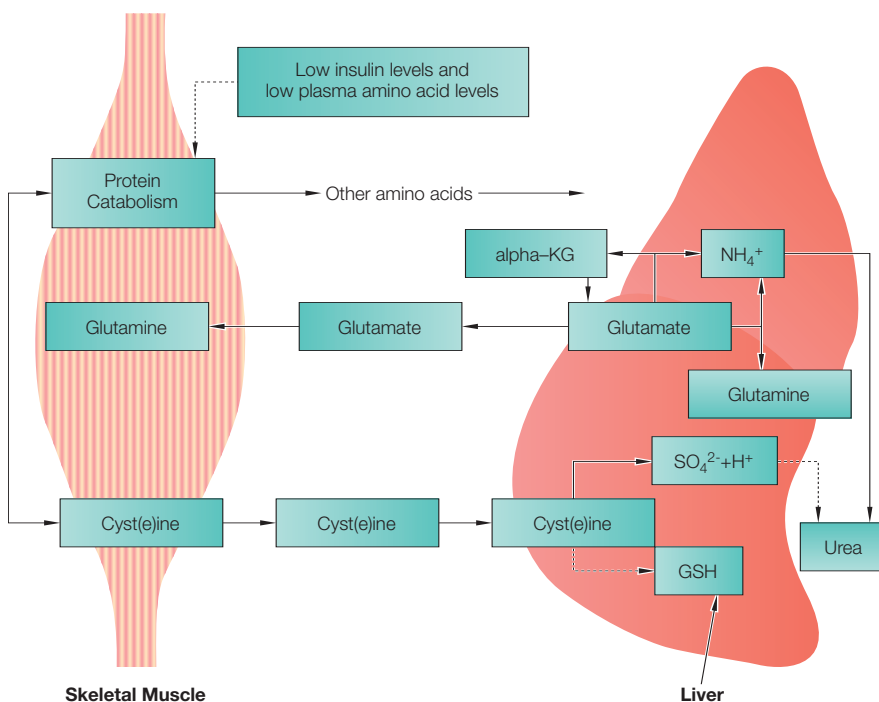
There is a substantial amount of evidence that suggests the regulation of whole body protein metabolism (and the size of muscle mass) involves a regulatory circuit between muscle, blood (plasma) and liver metabolism of AA (see Figure 2). One proposed mechanism of regulation is a link between levels of the amino acid cysteine and muscle catabolism. Both longitudinal (Kinscherf et al., 1996; Hilderbrant et al., 2004) and cross-sectional studies on adults aged 28-70 years suggest that the amount of cysteine in plasma is a regulator of whole body protein metabolism that ultimately influences changes in body composition (Hack et al., 1996, 1997, 1998; Holm et al., 1997). The amount of this "non-essential" AA in plasma regulates whole body protein metabolism via the hepatic catabolism of cyst(e)ine (i.e., both cysteine and its disulphide twin, cystine) into sulphate ( $SO_4^{2-}$ ) and protons ( $H^+$ ), an essential

process that inhibits the rate limiting step in hepatic urea production (the end product of protein degradation) and shifts whole body nitrogen disposal toward glutamine biosynthesis. (The conversion of AA into glucose is linked to the rate at which ammonium ions in the liver are converted into either urea or glutamine, therefore, hepatic cysteine catabolism serves to retain the AA reservoir in muscle).

The term "controlled catabolism" is used to explain the regulatory circuit presented in Figure 2 (Hack et al., 1997). The most important function of this regulatory circuit is to ensure that any time urea production is too high and plasma AA are accordingly too low, controlled muscle catabolism is triggered. This leads to export of cyst(e)ine from muscle, an increase in plasma cyst(e)ine and a down regulation of hepatic urea production (and preservation of LBM).

**Figure 2. Regulatory Circuit Between Muscle, Blood (plasma) and Liver Metabolism of AA**

*The regulation of whole body protein metabolism (and the size of muscle mass) may involve a regulatory circuit between muscle, blood (plasma) and liver cysteine AA metabolism. A process that promotes glutathione (GSH) synthesis and a down regulation of urea production that in turn, shifts whole body nitrogen economy towards muscle preservation.*



However, a few studies have suggested that in cachectic conditions (illnesses that promote muscle wasting such as HIV and various forms of cancer), but also aging, this regulatory process appears to be disturbed. That is, a failure to conserve muscle proteins and convert abnormally large amounts of AA into glucose, and release large amounts of nitrogen as urea. The availability of cyst(e)ine in the blood stream could determine the threshold at which other AA are converted into other forms of chemical energy, which in turn may influence body composition in humans (Hack et al., 1996; 1997; 1998; Kinscherf et al., 1996; Holm et al., 1997).

Cross-sectional investigations on AA exchange rates in adults aged from 28-70 years have reported the following:

- Firstly, plasma cysteine levels show by far, the strongest age-dependant change of any AA. (Hack et al., 1997; 1998; Holm et al., 1997; Kinscherf et al., 1996).
- Secondly, older adults (60 years and over) exhibit significantly lower glutamine exchange rates and glutamine/ cysteine ratios than their younger counterparts (Hack et al., 1997; 1998).
- Thirdly, a highly significant ( $P < 0.001$ ) correlation between a low glutamine/ cysteine ratio and increased body fat has been observed across all age groups (Hack et al., 1997).

It is also important to note that the data presented in these studies suggests an age-related decrease in the efficiency of hepatic cyst(e)ine to regulate urea production (Hack et al., 1997). That is, the liver of an older person with a given plasma cysteine level converts less AA to glutamine than a young, healthy individual. Therefore, muscle stores are relied upon increasingly with advancing age to meet the metabolic demands for glutamine. These researchers suggest the end result is a steady but aggressive catabolism of muscle tissue throughout the lifespan (Hack et al., 1997; 1998).

The regulatory circuit presented in Figure 2 may be disturbed by circulating cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ). High levels of these cytokines are characteristic of condition that promote muscle wasting (Strassmann et al., 1992; Kotler 2000). However, high levels of these cytokines are also a characteristic of older adults (Visser et al., 2002; Krabbe et al., 2004).

An increase in circulatory cytokines increases the requirements not only for glutamine but also glutathione (L-gamma-glutamyl-L-cysteinylglycine).

Glutathione (GSH) is the centerpiece of all cellular antioxidant defenses that regulate many aspects of metabolism such as re-dox status, synthesis of DNA and protein, cell proliferation and apoptosis as well as cytokine production (Townsend et al., 2003; Wu 2004). Cysteine is the rate limiting AA in GSH synthesis (Wu 2004). Conditions related to chronic inflammation (excessive cytokine production) such as cancer, HIV and also aging, create an unfavorable competition for GSH via a limited hepatic cyst(e)ine reservoir. A situation that leads to oxidative stress and an environment that promotes the loss of muscle tissue (Bounous & Molson 2003; Hack et al., 1997; Townsend et al., 2003).

Collectively, this research underlines the importance of maintaining an adequate supply of bioavailable cysteine in the blood during aging. Dietary intervention with cysteine-rich compounds is shown to increase plasma cysteine concentrations, boost GSH production and improve body composition in adults (Kinscherf et al.,

1996; Lands et al., 1999; Hilderbrandt et al., 2004). Therefore, dietary intervention with cysteine-rich compounds may also be an effective strategy that influences whole body protein metabolism in older adults to offset the age-related loss of muscle mass. This is an important topic that is yet to receive investigation by the scientific community.

However, no matter how tempting it is to speculate, the mechanism(s) that underline sarcopenia is likely to be multifaceted and involve some or all of the regulators identified in Figures 1 and 2. Despite the complexity of sarcopenia, a review of the literature clearly shows that there are only two factors that positively affect protein turnover to promote the maintenance/ increase of human muscle mass. Those factors are resistance exercise (RE) and the intake of macronutrients, namely protein and carbohydrates. Therefore, the focus of this report now shifts to the most recent findings on the effects of macronutrient consumption and exercise in older adults on protein metabolism and body composition. In particular, how these two important aspects interact to positively affect the size of human muscle mass and therefore, offer an intervention to minimize the effects of sarcopenia.



## EXERCISE IN AGING MUSCLE: THE LATEST RESEARCH

The results of recent studies now confirm that a lifelong exercise program offers real protection against the increasing levels of oxidative stress that damage cellular structures and cause aging of tissues and organs (Rosa et al., 2005). However, RE particularly is regarded as fundamental to the development and maintenance of muscle mass in adults (Rasmussen & Phillips 2003). This is based on two recent confirmations. Firstly, RE is a most effective form of mechanical loading and therefore, a potent stimulus to increase the rate of protein synthesis within muscles (Phillips et al., 2005). Secondly, the stimulation of muscle protein synthesis (MPS) is now recognized as the facilitating process that underlines changes in the size of muscle mass (Rennie et al., 2004; Cuthbertson et al., 2005). That is, a high stimulation of MPS is essential not only to increasing muscle mass (Baar & Esser 1999) but also the preservation of muscle mass in aging humans (Rennie et al., 2004).

Conventional RE typically involves the controlled movement of weighted devices such as barbells, dumbbells and machines (with fulcrums and loaded weight stacks). During conventional RE, muscles undergo concentric (shortening), isometric (static) and eccentric (lengthening) actions against a constant external load; the magnitude of which is limited by the individual's concentric strength. Conventional RE training typically involves the use of heavy loads lifted in sets of 1 to 12 maximum effort repetitions. This activity is shown to be a safe and effective intervention that stimulates MPS and promotes the maintenance of muscle mass in older populations (Barry & Carson 2004; Hunter et al., 2004) including the frail elderly (90+ years old) (Fiatarone et al., 1994).



Advancing age appears to reduce resting MPS rates, the amount of anabolic hormones in circulation, maximum voluntary muscle strength and power as well as the expression of muscle-specific genes (Short & Nair 2001; Yarasheski 2003). However, it is truly fascinating that RE training is capable of reversing (or at least improving) each of these aspects (Yarasheski et al., 2001; Hunter et al., 2004; Hasten et al., 2000; Jubrias et al., 2001; Newton et al., 2002). In line with these findings, other studies have shown that aging *per se* does not diminish the capacity to increase muscle size (Frontera et al., 1988; Hikida et al., 2000) or the ability to gain muscle mass during RE (Frontera et al 1988; Binder et al., 2005). While a major contributor to the age-related decline in muscle mass is thought to be the loss of alpha-motor neuron input (Candow & Chilibeck 2005), the research available suggests that older adults respond with strength and power improvements in a similar manner to young adults (Hakkinen et al., 2001; Newton et al., 2002).

As discussed in the previous section, some evidence suggests that the age-related decline in muscle mass may be associated with chronic inflammation and the increased levels of cytokines (such as TNF- $\alpha$  and IL-6)

(Visser et al., 2002; Krabbe et al., 2004; Toth et al., 2005). However, RE training has been shown to reduce cytokine levels in the muscles of older adults (Greiwe et al., 2001). Additionally, this reduction was associated with an increased rate of MPS. Others have shown that RE training can induce favorable changes in heat-shock proteins (Hsp70) in monocytes and lymphocytes in older adults, and these changes were associated with improvements in strength and a reduction in circulating cytokines (Bautmans et al., 2005). The Hsp's protect cellular integrity during stressful situations such as the production of excessive amounts of free radicals (that damage cells and cause illness and infection). Hsp70 is thought to play a role in the muscle regeneration process during RE-training (Kilgore et al., 1998). However, production decreases with age as cytokine levels increase (Njemini et al., 2002). Nevertheless, RE-training appears to induce positive changes in cytokine and Hsp production that reduce age-related chronic inflammation and promotes the maintenance of muscle mass (Greiwe et al., 2001; Bautmans et al., 2005). Despite these clear benefits of RE to an aging population, when responses in young (20-30 years) and older adults (60 years and over) are compared directly, some clear differences have been observed.

- In response to a single bout of RE (of the same relative intensity), the stimulation of protein synthesis is shorter-lived in older adults (Sheffield-Moore et al., 2005). This response is accompanied by a greater release of muscle AA and a more vigorous acute-phase (immune) response of plasma proteins (particularly albumin). These results suggest a differential hepatic and muscle protein response to RE in older adults as compared to younger adults (Sheffield-Moore et al., 2005).
- Some, (Balagopal et al., 1997; Toth et al., 2005) but not all (Volpi et al., 2001) studies report that older adults possess basal (resting) MPS rates that are 19-40% lower than younger adults. While aging may or may not affect resting protein turnover rates, it is clear that older mammals (including humans) possess a diminished capacity to synthesize new muscle in response to anabolic stimuli (such as RE) when compared directly to their younger counterparts (Alway et al., 2005).
- This is supported by longitudinal RE training studies that have compared chronic adaptations (such as hypertrophy, strength and LBM gains) in older and younger adults directly (Lemmer et al., 2001; Hakkinen et al., 1998). For example, Dionne et al., (2004) assessed the impact of a 6-month controlled RE program on LBM, resting energy expenditure (REE) and glucose disposal (insulin sensitivity) in 19 younger (18-35 years) and 12 older (55-70 years) non-obese caucasian women. In younger women, the RE program increased body weight due to an increase in LBM. The exercise program also increased REE and glucose disposal in the younger women. On the other hand, the older women lost fat mass and showed a lesser capacity to gain LBM. No improvement in insulin sensitivity or REE was detected in the older women. Thus, younger women showed greater metabolic changes in body composition, REE and insulin sensitivity in response to RE than older women (Dionne et al., 2004).
- Aside from a diminished response to anabolic stimuli, other age-related aspects that may influence the capacity to build and/or preserve muscle include a higher concentration of circulating cytokines that retard protein synthesis rates (Visser et al., 2002; Toth et al., 2005), lower concentrations of anabolic hormones in circulation (Bhasin 2003; Kraemer & Ratamass 2005; Toth et al., 2005), and differences in muscle gene expression in response to exercise (Welle et al., 2003).
- Finally, although aging may reduce the capacity to build and replace muscle protein, no studies have attempted to assess at what stage in life this starts to take place. Also, it is not known what the main instigators are that cause these chemical and physical alterations. For example, it is unclear whether it is a person's level of physical activity, or quality of nutrition. It is also uncertain if these aspects have a combined effect on gene regulation. Additionally, is not known whether a life-long commitment to RE training may provide a preventative effect against this age-related decline in muscle mass. These are important considerations that will need to be addressed in the near future before wide scale intervention recommendations can be made to an aging population.

The main reason RE is thought to be so important for the prevention and treatment of sarcopenia is that it has a remarkable impact on protein turnover. A single bout of RE results in the acute stimulation of MPS (up to 100% above basal values) that peaks within 3-24 hours but remains elevated (at a diminishing rate) for up to 48 hours post-exercise (Phillips et al., 2002; Kim et al., 2005). However, studies that have assessed both muscle protein breakdown (MPB) and MPS rates report that both processes are accelerated after RE so that net protein balance (NPB) remains negative until an exogenous source of protein is supplied (Biolo et al., 1995; 1997). The combination of protein-containing meals and RE act synergistically; they provide a net gain in muscle protein that is much greater than the sum of either aspect alone. This is why nutritional intervention with protein-rich dietary sources during RE is considered essential in any attempt to maintain or increase muscle mass (Phillips et al., 2005). The combination of RE and strategic intake of nutrients after this activity appears to be the only way to increase the amount of protein within the muscles of both younger (Phillips et al., 2005) and older adults (Esmark et al., 2001).



## THE EFFECTS OF MACRONUTRIENT INTAKE ON MUSCLE PROTEIN METABOLISM

In healthy adults, earlier studies (that utilized isotope labeled AA and tracer hind limb exchange methods) established that the large increase in whole-body synthesis observed after the consumption of a meal is due mainly to the changes in protein synthesis rates in muscle. In fact, in response to feeding, muscle mass contributes more than half of the total increase in whole body protein synthesis (Rennie et al., 1982). More recent studies have used similar methods to establish the following:

1. The consumption of a meal (a combination of protein, carbohydrate and fats) stimulates an increase in MPS. This anabolic response is due primarily to the protein (or AA) content of the meal (Svanberg et al., 1996; 1997; 1999; 2000).
2. Most of this stimulatory effect is due to the essential amino acids (EAA) (Volpi et al., 2003). Of these, the branch chain amino acids (BCAA) are most potent (Anthony et al., 2002).
3. The consumption of a meal stimulates muscle anabolism via an increase in MPS and inhibition of MPB. However, a net gain of muscle protein observed after a meal is due mostly to the large increase in MPS; inhibition of MPB contributes but to a lesser extent (Rennie et al., 2002).
4. This anabolic response to a meal is transient. During the post-prandial phase (1-4 hours after the meal) MPS is elevated while MPB is reduced. MPB increases in the post-absorptive state (4 hours after the meal) and MPS rates decline. Therefore, frequent consumption of protein-rich meals (throughout the day) is necessary for muscle protein accretion.

5. The recommended dietary allowance for protein appears not to be adequate for older people to maintain skeletal muscle mass (Campbell et al., 2001). While some renowned scientists in this area recommend that high protein intakes are not necessary to build muscle (Rennie et al., 2004), they suggest that protein quality may be a key aspect (Rennie 2005). Other experts caution that the underlying biology of maintenance AA needs may be much more complicated than simply the support of protein metabolism itself; there are so many gaps in our knowledge of this subject that until the various functions of AA are better understood at both the mechanistic and quantitative level, the current dietary recommendations for both healthy and sick humans will remain at an intellectually unsatisfactory empirical level (Reeds & Biolo 2002).
6. An individual's habitual protein intake may prove to be one of the more important variables that influence the size of human muscle mass since recent work has confirmed that the concentration of EAA in the blood (plasma) regulate protein synthesis rates within muscle (Bohe et al., 2003). That is, when plasma EAA levels are low, MPS rates decline (Kobayashi et al., 2003). Conversely, MPS rates increase in a linear fashion with increased EAA availability (Bohe et al., 2001). Therefore, dietary intervention with protein sources that are rich in EAA may create and maintain a high concentration of EAA in the blood. This would activate a key mechanism (i.e., the stimulation of MPS) that preserves muscle mass.

The important role of dietary protein supplementation in promoting muscle gains has recently been highlighted (Paddon-Jones et al., 2005a). Supplementation (15g of EAA and 30g of glucose) was shown to produce a greater anabolic effect in muscle (increase in net phenylalanine balance) than ingestion of a regular meal, despite the fact that both contained a similar dose of EAA. Furthermore, the consumption of the supplement did not interfere with the normal anabolic response to the meal consumed 3 hours later. The findings from this study suggest that protein supplements could be used in between regular meals to stimulate a higher rate of MPS and muscle anabolism. Other studies have already shown that the anabolic effects of daily protein supplementation are accumulative; they provide a higher net increase in muscle mass over time (Paddon-Jones et al., 2004b).



## NUTRITIONAL INTERVENTION IN OLDER ADULTS: RECENT DEVELOPMENTS

In addition to other age-related causes of muscle loss, there also appears to be a diminished acute response to anabolic stimuli such as RE or meal consumption.

- For example, in both old rats and humans aged between 60-70 years, a diminished anabolic response to protein supplementation was shown to be due to a decreased activation of the molecular signaling proteins within muscle that initiate protein synthesis (Cuthbertson et al., 2005; Guillet et al., 2004). However, this finding is controversial. Other research groups have shown that oral consumption of EAA (15 g) stimulates MPS similarly in both elderly and young adults (Volpi et al., 1999; Paddon-Jones et al., 2004a). Nonetheless, other age-related differences in the response to meal/supplement consumption have been identified.
- First-pass splanchnic extraction of AA in response to oral protein supplementation is higher in older adults, but the delivery of AA to muscles appears to be the same in both young and older adults (Volpi et al., 1999). This difference in intestinal absorption may be due to the increased importance of the splanchnic tissues in the regulation of protein turnover in aging (Fukagawa & Young 1987).
- In young adults the combination of carbohydrate (CHO) (in the form of glucose) and EAA elicits an anabolic response greater than achieved by AA supplementation alone (Miller et al., 2003). However, when this macronutrient combination is given to elderly subjects, the anabolic response is blunted by the addition of CHO (Volpi et al., 2000). Therefore, dietary supplementation with protein only may provide a more calorie-efficient means of stimulating protein synthesis in older populations (Paddon Jones et al., 2004a).
- It has been reported that smaller amounts of protein (approx. 7 g) do not stimulate an anabolic response in the muscles of older adults (Katsanos et al., 2005). This not only confirms a diminished anabolic sensitivity to protein-containing meals in older adults, it also suggests that larger doses of protein (15 g or more) may need to be consumed by older adults to ensure that they obtain an anabolic response to feeding.
- However, a recent study indicated that enrichment of a 7 g EAA mixture with a higher percentage of leucine increased the level of protein synthesis in elderly subjects. In contrast, enrichment with leucine did not result in an increase in muscle protein synthesis in young adults. These findings suggest leucine has a unique and crucial role for protein synthesis in the elderly even when the amount of EAA consumed is relatively small (Katsanos et al., 2006).
- Another important finding is that the inhibitory effect of protein-containing meals on protein breakdown appears to be reduced in older adults (Boirie et al., 2001). An age-related difference in the inhibitory effect of protein meals on (whole body) protein breakdown was confirmed in a recent study. Using old (22 month) and young (8 month) rats, this research demonstrated that the normal feeding-related reduction in MPB was much less prominent in the older rats. However, when the old rats were fed a diet supplemented with leucine, the ability of meals to block protein breakdown was rejuvenated. (Combaret et al., 2005). While some discrepancies are evident, recent studies generally confirm that aging “blunts” the normal anabolic response to meals.

Insulin is a key hormone in the regulation of whole body protein metabolism. However, the role of insulin in the regulation of muscle protein during aging is complex and yet to be fully elucidated (Wolfe & Volpi 2001). The effects of age on insulin sensitivity and  $\beta$ -cell function are also controversial; some investigators suggest that insulin sensitivity declines with age (DeFronzo 1979), others report that insulin sensitivity is maintained while  $\beta$ -cell function is impaired with age in glucose-tolerant individuals (Chiu et al., 2000). Insulin only appears to stimulate MPS when AA are present, even then, only small amounts are needed (Kimball et al., 2002). Insulin's main effect on protein metabolism is its ability to reduce protein breakdown in many tissues, including muscle (Bennet et al., 1990; O'Brien & Granner 1996; Wolfe 2000). With regards to the impact of aging, a recent study (Paddon Jones et al., 2004a), reported that although a 15 g dose of EAA stimulates muscle anabolism, it failed to stimulate insulin secretion in older adults (unlike younger adults). This may be one reason why older adults demonstrate a slower uptake of AA. In general, insulin plays only a permissive role in the regulation of MPS, its main role in protein metabolism is related to nutrient transport but also the ability to reduce the breakdown of protein in tissue.



## BCAA AND PROTEIN METABOLISM

- The BCAA leucine has been established as a regulator of whole body and skeletal muscle protein metabolism in humans (Nair et al., 1992). Furthermore, leucine supplementation has been reported to stimulate MPS through both insulin dependent and insulin independent mechanisms, via activation (phosphorylation) of key proteins involved in the regulation of protein synthesis in muscle. This has been consistently observed in both rat (Anthony 2000; 2002) and human skeletal muscle (Karlsson et al., 2004; Lui et al., 2002). However, as mentioned previously, the presence of leucine also appears to play a role in minimizing protein breakdown (Combaret et al., 2005).
- Diets that are rich in the BCAA but particularly leucine, are now considered to be effective for treatment of many age-related conditions such as obesity, type 2 diabetes and Metabolic Syndrome X (Layman et al., 2006) as well as sarcopenia (Rennie 2005).

The BCAA play a pivotal role in muscle metabolism in several ways:

- Firstly, they serve as direct precursors for glutamine production (Holecek 2002) which is the essential fuel that powers many aspects of immune function and cell turnover (Curi et al., 2005).
- Secondly, the BCAA, but leucine in particular, is thought to be primarily responsible for the activation of MPS via activation (phosphorylation) of several translation initiation factors (eIFs) (Anthony et al., 2002).
- However, leucine also appears to regulate oxidative use of glucose by muscle through stimulation of glucose recycling via the glucose-alanine cycle (Layman et al., 2006). This promotes protein sparing and provides a stable glucose environment with low insulin responses during energy-restricted periods (Layman et al., 2006).

For these reasons, several leading research groups suggest that a diet rich in the BCAA would play an important role in the prevention and treatment of many age-related conditions such as obesity, type 2 diabetes, and sarcopenia (Layman 2006; Rennie 2005).

What does all of this research tell us about nutrient intervention against sarcopenia? It tells us that the quality of protein intake is important. Other studies have already clarified that various dietary protein sources have different effects on whole body protein anabolism and accretion (Boirie et al., 1997; Dangin et al., 2003; Bos et al., 2003), and therefore, have the potential to influence changes in muscle mass. In light of these recent findings on leucine, a protein's leucine content is now considered a determinant of quality. Even conservative scientists now suggest that the consumption of leucine-rich protein sources may be a key strategy that helps prevent/reduce sarcopenia (Rennie 2005).



## WP's EFFECTS ON MUSCLE PROTEIN METABOLISM: THE LATEST RESEARCH

- The consumption of macronutrients close to RE is consistently shown to provide a positive NPB (Levenhagen et al., 2002; Miller et al., 2003; Rasmussen et al., 2000) and promote muscle gains in older adults (Esmarck et al., 2001). A recent study (Koopman et al., 2005) attempted to extend these findings by investigating whether additional leucine to a (whey) protein-CHO supplement could further promote muscle protein anabolism.

The study by Koopman et al., (2005) used healthy young males given repeated doses of a whey/CHO supplement (0.2 g of whey/kg of body weight each hour) with or without additional leucine (0.1 g/kg) for 5 hours after an RE workout. This study simultaneously assessed whole body protein turnover as well as the fractional synthesis rates in muscle (by the incorporation of labeled phenylalanine). The results obtained suggest that the addition of the leucine significantly increased whole body net protein balance and provided a higher anabolic response within muscle. However, it is important to note that the total amount of leucine administered in the trials was very different. The whey/CHO supplement provided only 0.02 g/kg/hr (a 1.6 g dose per hour for an 80 kg individual); much lower than leucine-enriched supplement which provided a total of 0.12 g/kg/hr (or 9.6 g per hour for an 80 kg person).

- Another recently published study (Paddon-Jones et al., 2005b) quantified net MPS in healthy elderly individuals (65-79 years) following oral consumption of WP (15g) or EAA (15g). Results showed that both supplements significantly stimulated MPS in older adults. This confirms previous work that a dose of whole dairy proteins (20g) (casein or whey) are capable of stimulating MPS in young adults in a manner comparable to that achieved with free form AA (Tipton et al., 2004). The study by Paddon-Jones et al. (2005b) concluded that compared to the isocaloric quantity of WP, the dose of EAA provided a more energetically efficient stimulation of MPS. However, this was due to the fact that the EAA supplement contained more than twice the dose of EAA than the WP.



However, the percentage of phenylalanine taken up by the leg was consistent with the relative proportion of EAA in each supplement. Therefore, relative to the dose of EAA contained within each supplement, the WP was just as efficient at promoting muscle anabolism.

- The ability of WP to stimulate MPS and promote muscle anabolism has been confirmed at the molecular level (Farnfield et al., 2005). In this research, supplementation with WP (isolate) (25 g) after RE activated key proteins in the translation initiation complex that leads to MPS (Farnfield et al., 2005). Supplementation resulted in significantly greater (eccentric) strength after training (25% greater than placebo) in young but not in older adults. However, the whey-supplemented older participants demonstrated greater phosphorylation (activation) of the translational protein kinase p70<sup>S6</sup>K1 after 12 weeks of RE when compared to the placebo group. This effect was not observed in the younger groups. Also, the older adults given WP demonstrated a 17.3 fold increase in the Pax7 gene (marker of muscle-growth activation) compared to a 2.6 fold increase in the placebo group. These findings provide molecular evidence of WP's ability to augment MPS after RE.

## THE UNIQUE ROLE OF WP IN THE MANAGEMENT OF SARCOPENIA

When considering nutritional interventions in the prevention and treatment of sarcopenia, it should be remembered that WPs provide a number of unique advantages.

- WP is a rich, rare source of bioavailable cyst(e)ine. Most WPs (80%+ protein concentrates and isolates) contain at least a 3 to 4 fold higher concentration of cysteine compared to other protein sources, including casein and soy (Bucci & Unlu 2000). As identified previously in this report, cysteine plays a key role in the synthesis of GSH and regulation of whole body protein metabolism that may result in improvements in body composition.

*Important: unlike other protein sources (such as casein or soy), supplementation with WP is shown in research to augment this pathway of protein metabolism, boost GSH production and provide a positive influence on body composition (more muscle and less fat mass). This has been confirmed in both rodent (Bouthegeourd et al., 2002; Belobrajdic et al., 2004; Marriotti et al., 2004) and human trials (Lands et al., 1999; Bounous & Molson 2003; Middleton et al., 2004; Moreno et al., 2005; Cribb et al., 2006).*

- WPs provide all the correct AA (the building blocks of protein) in approximate proportion to their ratios in skeletal muscle (Ha & Zemel 2003).

*Important: the synthesis of protein within muscle is a continuous activity that requires not only leucine but also a balanced supply of twenty different AA (Rennie et al., 2004).*

- Also, WPs generally contain a higher concentration of the EAA than other protein sources (Bucci & Unlu 2000) and have rapid absorption kinetics (Dangin et al., 2001; 2003). Supplementation results in a higher blood AA peak and stimulation of protein synthesis compared to other protein sources such as casein and soy (Bos et al., 2003; Dangin et al., 2003). When consumed as part of a mixed-macronutrient meal, WP's unique absorption kinetics (and ability to stimulate muscle protein synthesis) remain unaffected (Dangin et al., 2003). In fact, when WPs are consumed in mixed-macronutrient meals, they provide a stronger anabolic effect via stimulation of protein synthesis and inhibition of protein breakdown (Dangin et al., 2003).

*Important: the concentration of EAA in the blood (plasma) is now considered by many to be a key regulator of protein synthesis rates within muscle, (Bohe et al., 2003) which is the facilitating process that underlines changes in the size of muscle mass (Cuthbertson et al., 2005). Recent work has confirmed that a single dose of WP (15-20g) stimulates MPS comparative to free form EAA (Tipton et al., 2004; Paddon-Jones et al., 2005b). Therefore, supplementing the diet with WP can create and maintain a high concentration of EAA in the blood and stimulate an important mechanism that preserves muscle mass.*



- WP is the richest known source of BCAA; the BCAA comprise up to 30% of WPs AA profile (Bucci & Unlu 2000). In particular, 80%+ WP concentrates and isolates provide up to 14g of leucine, per 100g of protein.

*Important: this is a particularly important attribute when the multifaceted role of the BCAA is considered. Supplementing the diet with WP will not only provide valuable precursors for glutamine synthesis, but also the AA that activate protein synthesis and prevent excessive protein breakdown as well as promote stable blood glucose/insulin metabolism that is conducive to fat loss.*

- Last but definitely not least, it is important to remember that unlike other protein sources, whey (both native and hydrolyzed) is shown in research to modulate an array of immune functions that improve immune responses and maintain competence (as detailed in the Whey Proteins and Immunity monograph).

In conclusion, sarcopenia exacerbates with age, resulting in muscle loss and muscle strength. Since many of its causes appear to be uncontrollable, treatments to decelerate its effects include resistance exercise and consumption of whey proteins (aside from proteins derived from meals). Whey proteins not only appear to be biochemically tailored to preserve valuable muscle mass and maintain immune competence, now evidence suggests that whey proteins have a very favorable affect on protein metabolism and have the capacity to promote the mechanisms that preserve muscle mass and improve body composition. Therefore, supplementing the diet with whey proteins, particularly in combination with activities such as resistance training, represent a research-based, non-pharmaceutical strategy that can be easily incorporated into the lifestyles of adults to help maintain valuable muscle mass that preserves health throughout the aging process.

REFERENCES

Always SE, Siu PM, Murlasits Z and Butler DC. Muscle hypertrophy models: applications for research on aging. *Can J Appl Physiol* 30(5):591-624, 2005.

Anthony JC, Anthony TG, Kimball SR, Vary TC and Jefferson LS. Orally administered leucine stimulates protein synthesis in skeletal muscle of post absorptive rats in association with increased eIF4F formation. *J Nutr* 130:139-145, 2000.

Anthony JC, Reiter AK, Anthony TG, Crozier SJ, Lang CH, MacLean DA, Kimball SR and Jefferson LS. Orally administered leucine enhances protein synthesis in skeletal muscle of diabetic rats in the absence of increases in 4E-BP1 or S6K1 phosphorylation. *Diabetes* 51:928-936, 2002.

Baar K and Esser K. Phosphorylation of the p70<sup>S6K</sup> correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol Cell Physiol* 276:C120-C127, 1999.

Balagopal P, Rooyackers OE, Adey DB, Ades PA and Nair KS. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol* 273: E790-800, 1997.

Barry BK and Carson RG. The consequences of resistance training for movement control in older adults. *J Gerontol A Biol Sci Med Sci* 59(7): 730-754, 2004.

Baumgartner RN, Stauber PM, McHugh D, Koehler KM and Garry PJ. Cross-sectional age differences in body composition in persons 60+ years of age. *J Gerontol Med Sci* 50A:M307-M316, 1995.

Bautmans I, Njemini R, Vasseur S, Chabert H, Moens L, Demanet C and Mets T. Biochemical changes in response to intensive resistance exercise training in the elderly. *Gerontology* 51(4):253-265, 2005.

Bennet WM, Connacher AA, Scrimgeour CM, Jung RT and Rennie MJ. Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissue during hyperaminoacidemia. *Am J Physiol Endocrinol Metab* 259:E185-E194, 1990.

Belobrajdic DP, McIntosh GH and Owens JA. A high-whey-protein diet reduces body weight gain and alters insulin sensitivity relative to red meat in wistar rats. *J Nutr* 134(6):1454-1458, 2004.

Bhasin S. Testosterone supplementation for aging-associated sarcopenia. *J Gerontol A Biol* 58(11):1002-1008, 2003.

Binder EF, Yarasheski KE, Steger-May K, Sinacore DR, Brown M, Schechtman KB and Holloszy JO. Effects of progressive resistance training on body composition in frail older adults: results of a randomized, controlled trial. *J Gerontol A Biol Sci Med Sci* 60(11): 1425-1431, 2005.

Biolo G, Maggi SP, Williams BD, Tipton KD and Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol* 268:E514-E520, 1995.

Biolo G, Tipton KD, Klein S and Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 273:E122-E129, 1997.

Bohè J, Low A, Wolfe RR and Rennie MJ. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. *J Physiol* 552: 315-324, 2003.

Bohe J, Low JF, Wolfe RR and Rennie MJ. Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol* 532:575-579, 2001.

Boirie Y, Gachon P, Cordat N, Ritz P and Beaufrere B. Differential insulin sensitivities of glucose, amino acid, and albumin metabolism in elderly men and women. *J Clin Endocrinol Metab* 86:638-644, 2001.

Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL and Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci USA* 23:94:14930-14935, 1997.

Bortz WM, II. A conceptual framework of frailty: a review. *J Gerontol Med Sci* 57A:M283-M288, 2002.

Bos C, Metges CC, Gaudichon C, et al. Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. *J Nutr* 133(5): 1308-1315, 2003.

Bounous G and Molson JH. The antioxidant system. *Anticancer Res* 1411-1415, 2003.

Bouthegourd JC, Roseau SM, Makarios-Lahham L, Leruyet PM, Tome DG and Even PC. A preexercise alpha-lactalbumin-enriched whey protein meal preserves lipid oxidation and decreases adiposity in rats. *Am J Physiol Endocrinol Metab* 283(3): E565-E572, 2002.

Broeder CE, Burrhus KA, Svanevik LS and Wilmore JH. The effects of aerobic fitness on resting metabolic rate. *Am J Clin Nutr* 55(4):795-801, 1992.

Bucci L and Unlu L. Proteins and amino acid supplements in exercise and sport. In: *Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition*. Driskell J, Wolinsky I eds. CRC Press Boca Raton, FL, 191-212, 2000.

Calles-Escandon J, Arciero PJ and Gardner AW. Basal fat oxidation decreases with aging in women. *J Appl Physiol* 78(1):266-271, 1995.

Campbell WW, Trappe TA, Wolfe RR and Evans WJ. 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(6): M373-M380, 2001.

Candow DG and Chilibeck PD. Differences in size, strength, and power of upper and lower body muscle groups in young and older men. *J Gerontol A Biol Sci Med Sci* 60(2):148-156, 2005.

Chiu KC, Lee NP, Cohan P and Chuang LM. Beta cell function declines with age in glucose tolerant Caucasians. *Clin Endocrinol (Oxf)* 53:569-575, 2000.

Combaret L, Dardevet D, Rieu I, Pouch MN, Bechet D, Taillandier D, Grizard J and Attaix D. A leucine-supplemented diet restores the defective postprandial inhibition of proteasome-dependent proteolysis in aged rat skeletal muscle. *J Physiol* 569:489-499, 2005.

Cribb PJ, Williams AD, Hayes A and Carey MF. The effect of whey isolate on strength, body composition and plasma glutamine. In *J Sports Nutr Exerc Metab*, in press 2006.

Curi R, Lagranha CJ, Doi SO, Sellitti DF, Procopio J, Pithon-Curi TC, Corless M, Newsholme P. Molecular mechanisms of glutamine action. *J Cell Physiol* 204(2): 392-401, 2005.

Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM and Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 19: 422-424, 2005.

Dangin M, Guillet C, Garcia-Rodenas C, Gachon P, Bouteloup-Demange C, Reiffers-Magnani, Fauquant J, Ballèvre O and Beaufrière B. The rate of protein digestion affects protein gain differently during aging in humans. *J Physiol* 549.2:635-644, 2003.

Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballèvre O and Beaufrière B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab* 280:E340-E348, 2001.

DeFronzo RA. Glucose intolerance and aging: evidence for tissue insensitivity to insulin. *Diabetes* 28:1095-1101, 1979.

Dionne IJ, Melancon MO, Brochu M, Ades PA and Poelhman ET. Age-related differences in metabolic adaptations following resistance training in women. *Exp Gerontol* 39(1):133-138, 2004.

Doherty TJ. Aging and sarcopenia. *J Appl Physiol* 95: 1717-1727, 2003.

Dutta C. Significance of Sarcopenia in the Elderly. *J Nutr* 127(5):992-992, 2001.

Elia M, Stratton R and Stubbs J. Techniques for the study of energy balance in man. *Proc Nutr Soc* 62: 529-537, 2003.

Esmarck B, Anderson JL, Olsen S, Richter EA, Mizuno M and Kjaer M. Timing of post-exercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J Physiol* 535:301-311, 2001.

Evans W. Functional and Metabolic Consequences of Sarcopenia. *J Nutr* 127:998S-1003S, 1997.

Farnfield MM, Carey KA and Cameron-Smith D. Whey protein supplementation and resistance training to enhance muscle growth in young and older adults. *Asia Pac J Clin Nutr* 14 Suppl:S69, 2005.

Florin JR, Ewton DZ and Coolican SA. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocrine Rev* 17:481-517, 1996.

Forslund AH, Hambraeus L, Olsson RM, El-Khoury AE, Yu YM and Young VR. The 24-h whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults. *Am J Physiol* 275:E310-E320, 1998.

Frontera WR, Meredith CN, O'Reilly KP, Knuttgen HG and Evans WJ. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J Appl Physiol* 64(3):1038-1044, 1988.

Frontera WR, Hughes VA, Krivickas LS and Roubenoff R. Contractile properties of aging skeletal muscle. *Int J Sport Nutr Exerc Metab* 11: S16-S20, 2001.

Forbes GB. Longitudinal changes in adult fat-free mass: influence of body weight. *Am J Clin Nutr* 70: 1025-1031, 1999.

Fukagawa NK and Young VR. Protein and amino acid metabolism and requirements in older persons. *Clin Geriatr Med* 3(2):329-341, 1987.

Gallagher D, Visser M, De Meersman RE, et al. Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. *J Appl Physiol* 83: 229-239, 1997.

Greenhaff PL, Peirce N, Simpson E, Hazell M, Babraj J, Waddell T, Smith K and Rennie M (2004). *J Physiol* 558P, C10.

Greife JS, Cheng B, Rubin DC, Yarasheski KE and Semenkovich CF. Resistance exercise decreases skeletal muscle tumor necrosis factor (alpha) in frail elderly humans. *FASEB J* 15:475-482 2001.

Guillet C, Prod'homme M, Balage M, Gachon P, Giraudet C, Morin L, Grizard J and Boirie Y. Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *FASEB J* 18:1586-1587, 2004.

Hack V et al. Elevated venous glutamate levels in (pre)catabolic conditions result at least partly from a decrease glutamate transport activity. *J Mol Med* 74:337-343 1996.

Hack V, Schmid D, Breikreutz R, Stahl-Henning C, Drings P, Kinscherf R, Taut F, Holm E and Droge W. Cystine levels, cystine flux, and protein catabolism in cancer cachexia, HIV/SIV infection and senescence. *FASEB J* 11:84-92, 1997.

Hack V, Breikreutz R, Kinscherf R, Rohrer H, Bartsch P, Taut F, Benner A and Droge W. The redox state as a correlate of senescence and wasting and as a target for therapeutic intervention. *Blood* 92(1):59-67, 1998.

Hakkinen K, Newton RU, Gordon SE, et al. Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. *J Gerontol A Biol Sci Med Sci* 53:B415-B423, 1998.

Hakkinen K, Kraemer WJ, Newton RU and Alen M. Changes in electromyographic activity, muscle fibre and force production characteristics during heavy resistance/power strength training in middle-aged and older men and women. *Acta Physiol Scand* 171(1): 51-62, 2001.

Hasten DL, Pak-Loduca J, Obert KA and Yarasheski KE. 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, 2000.

Heilbronn L, Smith SR and Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. *Int J Obes Relat Metab Disord* 28 Suppl 4:S12-S21, 2004.

Henriksen EJ. Invited review: Effects of acute exercise and exercise training on insulin resistance. *J Appl Physiol* 93:788-796, 2002.

Hikida RS, Staron RS, Hagerman FC, Walsh S, Kaiser E, Shell S and Hervey S. Effects of high-intensity resistance training on untrained older men. II. Muscle fiber characteristics and nucleocytoplasmic relationships. *J Gerontol A Biol Sci Med Sci* 55(7):B347-B354, 2000.

Hildebrandt W, Hamann A, Krakowski-Roosen H, Kinscherf R, Dugi K, Sauer R, Lacher S, Nobel N, Bodens A, Bellou V, Edler L, Nawroth P and Droge W. Effect of thiol antioxidant on body fat and insulin reactivity. *J Mol Med* 82:336-344, 2004.

Holm E, Hack V, Tokus M, Breikreutz R, Babylon A and Droge W. Linkage between post absorptive amino acid release and glutamate uptake in skeletal muscle tissue of healthy young subjects, cancer patients, and the elderly. *J Mol Med* 75(6): 454-461, 1997.

Holecek M. Relation between glutamine, branched-chain amino acids, and protein metabolism. *Nutrition* 18(2):130-133, 2002.

Hughes VA, Frontera WR, Wood M, et al. Longitudinal muscle strength changes in the elderly: influence of health, physical activity and body composition. *J Gerontol* 56A:B209-217, 2001.

Hunter GR, McCarthy JP and Bamman MM. Effects of resistance training on older adults. *Sports Med* 34(5):329-348, 2004.

Janssen I, Shepard DS, Katzmarzyk PT and Roubenoff R. The cost of sarcopenia in the United States. *J American Geriatrics Society* 52(1):80-85, 2004.

Jubrias SA, Esselman PC, Price LB, Cress ME and Conley KE. Large energetic adaptations of elderly muscle to resistance and endurance training. *J Appl Physiol* 90(5):1663-1670, 2001.

Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A and Wolfe RR. Aging is associated with diminished accretion of muscle proteins following ingestion of a small bolus of amino acids. *Am J Clin Nutr* 82:1065-73, 2005.

Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A and Wolfe RR. 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* 2006 Feb 28 [Epub ahead of print]

- Kraemer WJ and Ratamess NA. Hormonal responses and adaptations to resistance exercise and training. *Sports Med* 35:339-361, 2005.
- Karlssoon HK, Nilsson PA, Nilsson J, Chibalin AV, Zierath JR and Blomstrand E. Branched-chain amino acids increase p70<sup>S6K</sup> phosphorylation in human skeletal muscle after resistance exercise. *Am J Physiol Endocrinol Metab* 287:E1-E7, 2004.
- Kinscherf R, Hack V, Fischbach T, Friedmann B, Weiss C, Edler L, Bartsch P and Droge W. Low plasma glutamine in combination with high glutamate levels indicate risk for loss of body cell mass in healthy individuals: the effect of N-acetyl-cysteine. *J Mol Med* 74(7):393-400, 1996.
- Kim PL, Staron RS and Phillips SM. Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *J Physiol* 568: 283-290, 2005.
- Kimball SR, Farrell PA and Jefferson LS. Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. *J Appl Physiol* 93(3):1168-1180, 2002.
- Kobayashi H, Borsheim E, Anthony TG, Traber DL, Badalamenti J, et al. Reduced amino acid availability inhibits muscle protein synthesis and decreases activity of initiation factor eIF2B. *Am J Physiol Endocrinol Metab* 284:E48898, 2003.
- Koopman R, Wagenmakers AJ, Manders RJ, Zorenc AH, Senden JM, Gorselink M, Keizer HA and van Loon LJ. Combined ingestion of protein and free leucine with carbohydrate increases post exercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab* 288(4): E645-E653, 2005.
- Krabbe KS, Pedersen M and Bruunsgaard H. Inflammatory mediators in the elderly. *Exp Gerontol* 39(5):687-699, 2004.
- Kilgore JL, Musch TI and Ross CR. Physical activity, muscle, and the HSP70 response. *Can J Appl Physiol* 23(3):245-260, 1998.
- Kotler DP. Cachexia. *Ann Intern Med* 133:622-634, 2000.
- Lands LC, Grey VL and Smountas AA. Effect of supplementation with a cysteine donor on muscular performance. *J Appl Physiol* 87:1381-1385, 1999.
- Layman DK. Protein quantity and quality at levels above the RDA improves adult weight loss. *J Am Coll Nutr* 23:631S-636S, 2004.
- Layman DK and Walker DA. Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr* 136(1):319S-323S, 2006.
- Lemmer JT, Ivey FM, Ryan AS, Martel GF, Hurlbut DE, Metter JE, Fozard JL, Fleg JL and Hurley BF. Effect of strength training on resting metabolic rate and physical activity: age and gender comparisons. *Med Sci Sports Exerc* 33(4):532-541, 2001.
- Levadoux E, Morio B, Montaurier C, et al. Reduced whole-body fat oxidation in women and in the elderly. *Int J Obes Relat Metab Disord* 25(1):39-44, 2001.
- Levenhagen DK, Carr C, Carlson MG, Maron DJ, Borel MJ and Flakoll PJ. Postexercise protein intake enhances whole body and leg protein accretion in humans. *Med Sci Sports Exerc* 34:828-837, 2002.
- Liu Z, Jahn LA, Long W, Fryburg DA, Wei L and Barrett EJ. Branched chain amino acids activate messenger ribonucleic acid translation regulatory proteins in human skeletal muscle, and glucocorticoids blunt this action. *J Clin Endocrinol Metab* 86:2136-2143, 2001.
- Nair KS, Schwartz RG and Welle S. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. *Am J Physiol Endocrinol Metab* 263:E928-E934, 1992.
- Nair KS. Aging muscle. *Am J Clin Nutr* 81(5): 953-963, 2005.
- Nagy TR, Goran MI, Weinsier RL, Toth MJ, et al. Determinants of basal fat oxidation in healthy Caucasians. *J Appl Physiol* 80(5):1743-1748, 1996.
- Newton RU, Hakkinen K, Hakkinen A, McCormick M, Volek J and Kraemer WJ. Mixed-methods resistance training increases power and strength of young and older men. *Med Sci Sports Exerc* 34(8):1367-1375, 2002.
- Njemiri R, Abeele MV, Demanet C, Lambert M, Vandebosch S and Mets T. Age-related decrease in the inducibility of heat-shock protein 70 in human peripheral blood mononuclear cells. *J Clin Immunol* 22(4):195-205, 2002.
- Manton KG and Gu X. Changes in the prevalence of chronic disability in the United States black and non-black population above age 65 from 1982 to 1999. *Proc Natl Acad Sci USA* 2001;98:6354-6359.
- Marcell TJ. Sarcopenia: causes, consequences, and preventions. *J Gerontol A Biol Sci Med Sci* 58: M911-M916, 2003.
- Mariotti F, Simbelie KL, Makarios-Lahham L, Huneau J, Laplaize B, Tomé D and Even P. Acute ingestion of dietary proteins improves post-exercise liver glutathione in rats in a dose-dependent relationship with their cysteine content. *J Nutr* 134: 128-131, 2004.
- Miller SL, Tipton KD, Chinkes DL, Wolf SE and Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. *Med Sci Sports Exerc* 35:449-455, 2003.
- Middleton N, Jelen P and Bell G. Whole blood and mononuclear cell glutathione response to dietary whey protein supplementation in sedentary and trained male human subjects. *Inter J Food Sci Nutr* 55:2:131-141, 2004.
- Moreno YF, Sgarbieri VC, da Silva MN, Toro A and Vilela MM. Features of Whey Protein Concentrate in Children with Rapidly Progressive HIV Infection. *J Trop Pediatr* 13, 2005.
- O'Brien RM and Granner DK. Regulation of gene expression by insulin. *Physiol Rev* 76:1109-1161, 1996.
- Paddon-Jones D, Sheffield-Moore M, Creson DL, Sanford AP, Wolf SE, Wolfe RR and Ferrando AA. Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids. *Am J Physiol Endocrinol Metab* 284:E946-E953, 2003.
- Paddon-Jones D, Sheffield-Moore M, Zhang XJ, Volpi E, Wolf SE, Aarsland A, Ferrando AA and Wolfe RR. Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* 286:E321-E328, 2004a.
- Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR and Ferrando AA. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab* 89:4351-4358, 2004b.
- Paddon-Jones D, Sheffield-Moore M, Aarsland A, Wolfe RR and Ferrando AA. Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion. *Am J Physiol Endocrinol Metab* 288: E761-E767, 2005a.
- Paddon-Jones D, Sheffield-Moore M, Katsanos CS, Zhang XJ and Wolfe RR. Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. *Exp Gerontol* Nov 22, 2005b.
- Perez-Martin A, Raynaud E and Mercier J. Insulin resistance and associated metabolic abnormalities in muscle: effects of exercise. *Obes Rev* 7:47-59, 2001.
- Phillips SM, Tipton KD, Aarsland A, Wolf SE and Wolfe RR. Mixed muscle protein synthesis breakdown after resistance exercise in humans. *Am J Physiol Endocrinol Metab* 273:E99-E107, 1997.
- Phillips SM, Parise G, Roy BD, et al. Resistance-training-induced adaptations in skeletal muscle protein turnover in the fed state. *Can J Physiol Pharmacol* 80:1045-1053, 2002.
- Phillips SM, Hartman JW and Wilkinson SB. Dietary protein to support anabolism with resistance exercise in young men. *J Am Coll Nutr* 24:134S-139S, 2005.
- Poehlman ET and Melby C. Resistance training and energy balance. *Int J Sport Nutr* 8(2):143-159, 1998.
- Rasmussen BB and Phillips SM. Contractile and nutritional regulation of human muscle growth. *Exerc Sport Sci Rev* 31(3):127-131, 2003.
- Rasmussen BB, Tipton KD, Miller SL, Wolf SE and Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J Appl Physiol* 88:386-392, 2000.
- Reeds PJ and Biolo G. Non-protein roles of amino acids: an emerging aspect of nutrient requirements. *Curr Opin Clin Nutr Metab Care* 5:43-45, 2002.
- Rennie MJ, Edwards RH, Halliday D, Matthews DE, Wolman SL and Millward DJ. Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clin Sci* 63: 519-523, 1982.

Rennie MJ, Wackerhage H, Spangenburg EE and Booth FW. Control of the size of the human muscle mass. *Annu Rev Physiol* 66:799-828, 2004.

Rennie MJ and Tipton KD. Protein and amino acid metabolism during and after exercise and the effects of nutrition. *Annu Rev Nutr* 20:457-483, 2000.

Rennie MJ, Bohe J and Wolfe RR. Latency, duration and dose response relationships of amino acid effects on human muscle protein synthesis. *J Nutr* 132(10):3225S-3227S, 2002.

Rennie MJ. A role for leucine in rejuvenating the anabolic effects of food in old rats. *J Physiol* 1; 569:357, 2005.

Rosa EF, Silva AC, Ihara SS, Mora OA, Aboulafia J and Nouailhetas VL. Habitual exercise program protects murine intestinal, skeletal, and cardiac muscles against aging. *J Appl Physiol* 99(4):1569-1575, 2005.

Roubenoff R. Sarcopenia: effects on body composition and function. *J Gerontol A Biol Sci Med Sci* 58(11):1012-1017, 2003.

Roy BD, Tarnopolsky MA, Macdougall JD, Fowles J and Yarasheski KE. Effect of glucose supplement timing on protein metabolism after resistance training. *J Appl Physiol* 82:1882-1888, 1997.

Sehl ME and Yates FE. Kinetics of human aging: I. Rates of senescence between ages 30 and 70 years in healthy people. *J Gerontol Biol Sci* 56: B198-B208, 2001.

Shek PN and Shephard RJ. Physical exercise as a human model of limited inflammatory response. *Can J Physiol Pharmacol* 76(5):589-597, 1998.

Sheffield-Moore M, Paddon-Jones D, Sanford AP, Rosenblatt JJ, Matlock AG, Cree MG and Wolfe RR. Mixed muscle and hepatic derived plasma protein metabolism is differentially regulated in older and younger men following resistance exercise. *Am J Physiol Endocrinol Metab* 288(5):E922-E929, 2005.

Short KR and Nair KS. Muscle protein metabolism and the sarcopenia of aging. *Int J Sport Nutr Exerc Metab* 11 Suppl:S119-S127, 2001.

Singh MA, Ding W, Manfredi TJ, Solares GS, O'Neill EF, Clements KM, Ryan ND, Kehayias JJ, Fielding RA and Evans WJ. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. *Am J Physiol* 277:E135-E143, 1999.

Strassmann G, Fong M, Kenney JS and Jacob CO. Evidence for the involvement of interleukin 6 in experimental cancer cachexia. *J Clin Invest* 89(5): 1681-1684, 1992.

Svanberg E, Ennion S, Isgaard J and Goldspink G. Postprandial resynthesis of myofibrillar proteins is translationally rather than transcriptionally regulated in human skeletal muscle. *Nutr* 16(1):42-46, 2000.

Svanberg E, Moller-Loswick AC, Matthews DE, Korner U, Andersson M and Lundholm K. Effects of amino acids on synthesis and degradation of skeletal muscle proteins in humans. *Am J Physiol*: E718-E724, 1996.

Svanberg E, Moller-Loswick AC, Matthews DE, Korner U, Andersson M and Lundholm K. The role of glucose, long-chain triglycerides and amino acids for promotion of amino acid balance across peripheral tissues in man. *Clin Physiol* 19(4):311-320, 1999.

Svanberg E, Jefferson LS, Lundholm K and Kimball SR. Postprandial stimulation of muscle protein synthesis is independent of changes in insulin. *Am J Physiol* 272:E841-E847, 1997.

Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP and Wolfe RR. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. *Med Sci Sports Exerc* 36:2073-2081, 2004.

Toth MJ, Matthews DE, Tracy RP and Previs MJ. Age-related differences in skeletal muscle protein synthesis: relation to markers of immune activation. *Am J Physiol Endocrinol Metab* 288(5):E883-E891, 2005.

Townsend DM, Tew KD and Tapiero H. The importance of glutathione in human disease. *Biomedicine & Pharmacology* 57 3-4:145-155, 2003.

Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women. The Health ABC study. *J Gerontol Med Sci* 57A: M326-M332, 2002.

Volpi E, Mittendorfer B, Wolf SE and Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol* 277:E513-E520, 1999.

Volpi E, Mittendorfer B, Rasmussen BB and Wolfe RR. 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, 2000.

Volpi E, Sheffield-Moore M, Rasmussen BB and Wolfe RR. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *JAMA* 286:1206-1212, 2001.

Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B and Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* 78(2):250-8, 2003.

Welle S, Brooks AI, Delehanty JM, Needler N and Thornton CA. Gene expression profile of aging in human muscle. *Physiol Genomics* 7;14(2):149-159, 2003.

Wolfe RR and Volpi E. Insulin and protein metabolism. In: *Handbook of Physiology*, edited by Jefferson L and Cherrington A. New York: Oxford Univ Press, 2001, sect. 7, vol. 2, p. 735-757.

Wolfe RR. Effects of insulin on muscle tissue. *Curr Opin Clin Nutr Metab Care* 3:67-71, 2000.

Wu G, Fang Y, Yang S, Lupton JR and Turner ND. Glutathione Metabolism and Its Implications for Health. *J Nutr* 134:489-492, 2004.

Yarasheski KE, Pak-Loduca J, Hasten DL, Obert KA, Brown MB and Sinacore DR. Resistance exercise training increases mixed muscle protein synthesis rate in frail women and men >=76 yr old. *Am J Physiol* 277:E118-E125, 2001.

Yarasheski KE. Exercise, aging, and muscle protein metabolism. *J Gerontol A Biol Sci Med Sci* 58: M918-M922, 2003.

Zhang XJ, Chinkes DL and Wolfe RR. Leucine supplementation stimulates net protein synthesis in skin wound and muscle. *Experimental Biology Meeting* A572.2, 2003.



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