Effect of whey protein isolate on strength, body composition and muscle hypertrophy during resistance training

Effect of whey protein isolate on strength, body composition and muscle hypertrophy during resistance training

Alan Hayes and Paul J Cribb

Exercise Metabolism Unit, Center for Aging, Rehabilitation, Exercise and Sport (CARES) and the School of Biomedical Sciences, Victoria University. Victoria, Australia.

Address for correspondence

Dr. Alan Hayes.

Exercise Metabolism Unit.

School of Biomedical Sciences, Victoria University, Victoria, Australia.

PO Box 14428 MCMC Melbourne Vic 8001 Australia

Tel: +61 3 9919 4658 Fax: +61 3 9919 2465

E-mail: Alan.Hayes@vu.edu.au

Abstract

Purpose of Review: Sarcopenia (skeletal muscle wasting with aging) is thought to underlie a number of serious age-related health issues. While it may be seen as inevitable, decreasing this gradual loss of muscle is vital for healthy aging. Thus, it is imperative to investigate exercise and nutrition-based strategies designed to build a reservoir of muscle mass as early as possible.

Recent Findings: Elderly individuals are still able to respond to both resistance training and the anabolic signals provided by protein ingestion, provided specific amino acids, such as leucine, are present. Whey proteins are a rich source of these essential amino acids and rapidly elevate plasma amino acids, thus providing the foundations for preservation of muscle mass. Several studies involving supplementation with whey protein have shown to be effective in augmenting the effects of resistance exercise, in particular when supplementation occurs in the hours surrounding the exercise training.

Summary: While further work is required, particularly in elderly people, simple dietary and exercise strategies that may improve the maintenance of skeletal muscle mass will likely result in a decrease in the overall burden of a number of diseases and improve the quality of life as we age.

Key Words: resistance exercise, protein supplementation, muscle hypertrophy, sarcopenia

Introduction

Athletic performance and vanity aside, there are many important reasons for needing to know more about how to build muscle mass (hypertrophy), not the least being the underestimated role of muscle mass in healthy aging. Aside from a negative impact on health, functional ability and lifestyle quality, an age-related decline in muscle mass is thought to underlie conditions that shorten lifespan such as type-2 diabetes and cardiovascular disease [1-3, 4**]. It has been long known that resistance exercise is still effective in even very elderly populations [5] and current research shows that some of these undesirable diseases can be partially reversed by resistance training [6*,7**]. While some leading researchers in this area believe that sarcopenia may be reversible (at least to a certain extent) [8*], others recommend that tomorrows older adults should be concerned with building a greater “starting reserve capacity” of muscle mass today to ensure they avoid the unknown threshold that precedes physical frailty and compromised health [9].

In this review we highlight the role that protein supplementation may play in the development of muscle strength and hypertrophy. We also feature the results obtained with whey protein isolate (WPI); one particular type of protein supplement that appears to have advantages over others in terms of promoting muscle accretion. Finally, we briefly identify and discuss other compounds namely creatine monohydrate and glucose, that have been shown to provide an additional beneficial effect greater than WPI supplementation alone.

The role of protein supplementation in the development of muscle hypertrophy

Resistance exercise (RE) training is considered an integral component of any attempt to build strength and promote muscle hypertrophy, and offset age-related changes in body composition, strength and functional ability [10,11]. Although RE is fundamentally anabolic, a net gain in muscle mass is only possible via an interaction with protein-containing meals [12]. For this reason, there has been a greater research focus on specific nutritional strategies that may enhance the acute anabolic response (i.e., stimulation of muscle protein synthesis) to RE which in turn may augment chronic physiological adaptations such as muscle hypertrophy and strength [13]. For example, supplementation with protein does affect muscle and whole body protein anabolism and accretion, and this is apparent in both young and older adults [14, 15*]. Data from both acute response studies [15*, 16-19] and longer term (6-12 wk) investigations [20, 21*, 22, 23**, 24*] provide a foundation that suggests the strategic use of protein supplements can play a vital role in the development of muscle hypertrophy during RE training. In fact, supplementation may be vital to restoring the diminished acute response to anabolic stimuli (such as RE or meal consumption) that is characteristic of aging [25-26]. Additionally, the strategic consumption of a supplement containing whole proteins or essential amino acids (EAA) close to RE is consistently shown to dramatically enhance the acute anabolic response to this activity by providing a higher stimulation of protein synthesis and net protein balance compared to placebo treatments [17,18]. Recent data also suggests that supplementation between regular meals may provide an additive effect on net protein accretion due to a more frequent stimulation of muscle protein synthesis [16, 27].

Characteristically, the anabolic response to protein containing meals is diminished in older adults when compared to younger adults [25]. However, some data suggests that strategic supplementation may attenuate this and help restore the acute anabolic response to meals. Using old (22 month) and young (8 month) rats, Combaret et al., 2005 [28] demonstrated that when the

older rodent's diet was supplemented with leucine (a key amino acid in anabolic activation) the ability of feeding to block protein breakdown was rejuvenated. This has subsequently been confirmed in both aged rats [29**] and elderly humans [30,31]. Thus, in older adults, strategic supplementation that is rich in certain amino acids, such as leucine, may help restore the acute anabolic response to meals [25,28-31]. Therefore, protein supplementation appears to have at least three prominent roles in augmenting muscle accretion and promoting hypertrophy. Firstly, by supplementing close to RE to ensure a greater stimulation of muscle anabolism in response to this activity. Secondly, some data suggests that supplementation between meals may promote more frequent stimulation of muscle protein synthesis, thereby promoting a higher net gain in muscle protein on a daily basis. Finally, strategic supplementation with protein that is rich in EAA and in particular, leucine may help restore the acute anabolic response to meals which characteristically diminishes with aging.

Whey Protein Supplementation

It has been established that certain types of protein affect whole body protein anabolism and accretion [14] and therefore, have the potential to affect muscle and strength development during RE training [32*]. The type of protein consumed may influence results from RE training due to variable speeds of absorption, differences in amino acid profiles, unique hormonal response, or positive effects on antioxidant defense [33]. Whey protein (WP) is the collective term for the soluble protein fractions extracted from dairy milk. WP supplements, that is, 80%+ protein concentrates (WPC 80) or 90%+ protein isolates (WPI 90) have become popular among athletes and others interested in gaining muscle mass [33]. Rather than merely increasing the quantity of protein in the diet, these particular proteins may also provide some unique nutritional advantages. Characteristically, these WP supplements contain a very high concentration of EAA (45-55g/100g of protein) with minimal fat, carbohydrate and lactose. They are the richest known source of branch chain amino acids (BCAA), in particular, leucine (up to 14g/100g protein) [34]. Leucine is an established modulator of muscle protein metabolism and has been identified as a key regulator in the translation initiation pathway of muscle protein synthesis [35]. The importance of a leucine rich diet to promote muscle anabolism, healthy blood glucose metabolism and the management of a healthy weight are now recognized [36]. In a similar manner to BCAA administration, supplementation with WPI (25grams) is shown to augment the phosphorylation of p70-S6k; a major regulatory kinase in the activation of muscle protein synthesis in response to RE [37]. That is, supplementation with WPI after RE resulted in greater activation of key proteins

in the translation initiation complex that stimulate muscle protein synthesis. Additionally, in this study, supplementation resulted in significantly greater (eccentric) strength after 12 weeks of training (25% greater than placebo) in young but not in older adults [37]. However, the WPI-treated older participants demonstrated greater phosphorylation (activation) of the translational protein kinase p70-S6K1 after 12 weeks of RE when compared to the placebo group. Also, the older adults given WPI 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 [37].

Aside from strategic supplementation after exercise, regularly incorporating WP supplements into the daily diet may also promote the maintenance of lean body mass (LBM). WP supplements are a rich rare source of cyst(e)ine residues; up to 4-fold higher than other high quality proteins such as casein and soy [34]. A series of cross-sectional studies and intervention trials involving humans living with and without cachectic conditions strongly suggest that the hepatic catabolism of cyst(e)ine (cysteine and its disulphide twin, cystine) is a key regulator of whole body protein metabolism and changes in muscle mass [38-40]. Briefly, an abundant supply of cyst(e)ine in the blood is necessary for hepatic catabolism of this AA into sulfate and protons; a process that down-regulates urea production, promotes glutathione synthesis and shifts whole body nitrogen disposal in favor of preserving the muscle AA pool (which is synonymous with muscle anabolism) [38-40]. WP supplementation (up to 1g/kg/day) is the only protein source shown to augment this pathway of protein metabolism [40, 41]. And, it may do so possibly in a dose-dependant manner [42]. Aside from a favorable amino acid profile for the preservation of muscle mass, WP supplements possess rather unique digestion/absorption kinetics. The acute response to a single dose of WP is a very high (but transient) blood AA peak and stimulation of (whole body) protein synthesis when compared to other high quality protein sources such as casein (the other major bovine milk protein). What is more relevant is that when WP supplements are consumed as part of a mixed-macronutrient meal, this protein's rapid absorption kinetics and ability to stimulate a very high rate of muscle protein synthesis is unaltered but the result is a more prolonged state of anabolism and inhibition of protein degradation as well as a higher net gain in whole body protein [14]. This virtue has been demonstrated in both young and older adults, in direct comparison to isonitrogenous meals containing casein [14]. Due to its amino acid profile and ability to promote higher net protein accretion, the incorporation of WP into the diet may enhance the development of muscle hypertrophy and improve body composition during RE training.

Whey protein isolate and resistance training

Several human [20, 21*, 24*, 40] and rodent trials [43-45] demonstrate WP's ability to improve body composition (increase in muscle mass and/or a decrease in fat mass) as well as promote a physiological response that may explain these changes. For example, in a group of healthy adults, in direct comparison to supplementation with casein, WP supplementation (30 grams/day) improved body composition (maintain lean mass, reduce fat mass) via enhanced antioxidant (GSH) status. This improvement was obtained without exercise training [40]. In rodents, WP administration is shown improve body composition via more efficient fat oxidation in the hours after exercise [43], enhanced muscle insulin sensitivity [44] and the suppression of hepatic fatty acid synthesis along side an increased in fat utilization by muscle [45]. With regard to RE training specifically, one study reported that a dose of WPI (1.2g/kg/day) in RE-trained individuals resulted in an almost 2-fold higher gain (2.1 vs. 1.2kg) in LBM and a better gain in bench press strength compared to a CHO-supplemented group [20]. In another double-blinded study that used two groups of matched, RE-trained young men, our laboratory has also demonstrated a significantly greater gain in LBM and strength in a group provided WPI (1.5g/kg/day) compared to a matched group given an equivalent dose of casein [21*]. To our knowledge, this study is the first to directly compare the effects of two different protein sources on body composition and strength changes during a structured, supervised RE training program using experienced participants. In our investigation [21*] dietary analyses concurred that the participant's eating patterns were characteristic of bodybuilders, i.e., they consumed a high energy/protein intake divided into small, frequent, mixed macronutrient meals over the 24 hour period. The supplement dose (120 grams per day for an 80kg individual) was divided into 4 small doses and consumed with meals throughout the day. In comparison to an equivalent dose of casein, treatment with the WPI resulted in a 5-fold better gain in LBM and better gains in strength after the 10 week RE training program. To date, this is the only study that has examined the effects of replacing a large portion of dietary protein with one particular source, such as WPI, in athletic individuals undertaking an intense exercise training.

Although a number of RE training studies involving supplementation have reported changes in strength and body composition, very few have compared the changes alongside hypertrophy responses at the cellular level, such as fiber-specific (i.e., type-I, IIa, IIx) hypertrophy as well as the sub-cellular level, such as contractile protein content. Therefore, based on our previous work [21*] our laboratory completed a series of studies [22-24*] that aimed to

examine the effects of WP alone and in combination with other compounds on three different levels of physiology; body composition, muscle fiber cross-sectional area, and contractile protein accrual. Using groups of adult males (under 35 yrs) two of these trials examined the effects of WPI and creatine monohydrate (CrM) supplementation both separately and in combination during structured RE programs [22, 24*]. The groups consumed 1.5gram/kg/day of protein supplement, CrM treatment involved the addition 0.3-0.1g/kg/day of CrM to the supplement (a protocol used in many other CrM supplementation studies). Assessments after the 10-11 week RE program revealed that supplementation provided significantly greater 1RM strength improvements compared to a CHO-treated group. However, the hypertrophy responses among the groups varied at the three levels assessed. For example, although strength improvements among the CrM and WPI treated groups were similar, the presence of CrM resulted in much greater gains in body weight, lean body mass and muscle hypertrophy [22, 24*] The resounding conclusion from these trials was that if maximum gains in body weight and muscle mass are desired during an RE program, the addition of CrM to a WP supplement is highly beneficial. However, if strength improvements are desired with only a moderate increase in muscle mass, then supplementation with WPI (without CrM) may be more appropriate. The beneficial effects of protein supplementation have been further confirmed at the cellular level by a recent study that showed greater increases in fat-free mass, muscle strength and myosin heavy chain gene expression following 10 weeks of resistance training and ingestion of a whey/casein/amino acid mixture compared to a dextrose placebo [46].

Supplement-timing (i.e., the strategic consumption of a protein supplement before and/or after each workout) is thought to be the ideal strategy for promoting muscle hypertrophy from RE [13]. However, studies are yet to examine whether this strategy may provide greater muscle hypertrophy or strength development compared to supplementation at other times during the day. Therefore, using a WPI-based protein supplement, our laboratory recently completed a trial that examined the effects of supplement-timing on muscle hypertrophy in comparison to supplementation in the hours not close RE [23**]. In this study, the resistance-trained males were randomly matched for strength, completed a fully supervised 10 week RE program and consumed their supplement (1g/kg/body wt containing WPI and CrM) immediately before and after RE (PRE-POST). The second group (MOR-EVE) consumed the same dose of the same supplement in the morning and late evening (times that were at least 4-5 hours outside of training). No other supplementation was permitted and the athletes followed their regular eating patters. Results showed that the group who performed the supplement-timing strategy (PRE-POST) demonstrated

a gain in lean body mass that was almost double the MOR-EVE group. This group also demonstrated a greater improvement in 1RM strength, which were supported by a greater increase in hypertrophy of the type-II fibers and muscle contractile protein content in this group [23**]. Therefore, supplement-timing with WPI-based supplement represents a simple but effective strategy that enhances the adaptations that are desired from RE-training. Clearly, this strategy would be of benefit to most healthy adults that perform RE. However, this protocol may also have important implications for populations that require improvements in strength and body composition but have a reduced capacity for exercise such as, the frail elderly, cardiac rehabilitation patients or others living with conditions that compromise health such as HIV, cancer and the various muscular dystrophies. Importantly, aged individuals showed the same significant elevation in amino acid levels within 2 hours of ingesting a protein-rich meal as their young counterparts [47**] and also displayed the same elevation in acute protein synthesis. Thus, it is likely that supplementing the diet with essential amino acids surrounding a resistance training session would similarly augment the effects of training in elderly individuals, although further work is required in this area.

Conclusion

Some important roles that protein supplementation may play in the development of muscle strength and hypertrophy have been identified in this review. However, different types of protein may have the potential to affect muscle and strength development during resistance training. In this review we have highlighted the results obtained with WPI supplementation during RE. WP supplements in general appear to have several advantages over other protein sources in terms of promoting muscle accretion and hypertrophy. These include a profile rich in the amino acids known to promote muscle anabolism as well as rapid digestion/absorption kinetics (even when consumed in mixed macronutrient meals) shown to promote higher net gains in whole body protein. In trials involving healthy participants, supplementation with WPI is shown to promote better gains in strength, lean body mass and muscle fiber hypertrophy compared to equivalent does of protein or carbohydrate. The addition of CrM to WP is shown to provide an even greater benefit particularly in terms of gains in body mass and muscle fiber hypertrophy. Additionally, the strategic consumption of a WPI-based supplement just before and immediately after RE represents a simple but effective strategy that enhances muscle hypertrophy and strength gains during RE-training.

References

1 Doherty TJ. Aging and sarcopenia J Appl Physiol 2003; 95: 1717-1727

2. Janssen, I, Ross, R. Linking age-related changes in skeletal muscle mass and composition with metabolism and disease. J Nutr Health Aging 2005; 9: 408-419

3. Zacker, RJ. Health-related implications and management of sarcopenia. JAAPA 2006; 19: 24-29

** 4. Thomas, DR. Loss of skeletal muscle mass in aging: Examining the relationship of starvation, sarcopenia and cachexia. Clin Nutr 2007; 26: 389-399
Excellent review on separation of sarcopenia from other muscle-wasting conditions and discusses potential treatment strategies for each

5. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Eng J Med 1994; 330: 1769-1775

* 6. Levinger I, Goodman C, Hare DL, et al. The effect of resistance training on functional capacity and quality of life in individuals with high and low number of metabolic risk factors. Diabetes Care 2007; 30: 2205-2210
Evidence of effectiveness of resistance exercise in improving functional outcomes

** 7. Williams MA, Haskell WL, Ades PA, et al. Resistance exercise in individuals with and without cardiovascular disease: 2007 Update. A Scientific Statement From the American Heart Association Council on Clinical Cardiology and Council on Nutrition, Physical Activity, and Metabolism. Circulation 2007; 116: 572-584
Updated guidelines for implementation of resistance exercise programs in a range of people

* 8. Melov S, Tarnapolsky MA, Beckman K, et al. Resistance exercise reverses aging in human skeletal muscle. PLoS ONE 2007; 2(5): e465, 1-9
Good summary of potential sarcopenia treatments in new open-access format.

9. Evans W, Functional and Metabolic Consequences of Sarcopenia. J Nutr 1997; 127: 998S–1003S

10. Henwood TR, Taaffe DR. Short-term resistance training and the older adult: the effect of varied programmes for the enhancement of muscle strength and functional performance. Clin Physiol Funct Imaging 2006; 26: 305-313

11. Hazell T, Kenno K, Jakobi J. Functional benefit of power training for older adults. J Aging Phys Act 2007; 15: 349-359

12. Phillips SM, Hartman JW, Wilkinson SB.Dietary protein to support anabolism with resistance exercise in young men. J Am Coll Nutr 2005; 24: 134S-139S

13. Volek JS. Influence of nutrition on responses to resistance training. Med Sci Sports Exerc 2004 ; 36: 689-696

14. Dangin M, Guillet C, Garcia-Rodenas C, et al. The rate of protein digestion affects protein gain differently during aging in humans. J Physiol 2003; 549: 635-644

*15. Paddon-Jones D, Sheffield-Moore M, Katsanos CS, et al. Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. Exp Gerontology 2006; 41: 215-219
Interesting comparison of essential amino acid supplementation compared to whey protein

16. Paddon-Jones D, Sheffield-Moore M, Urban RJ, et al. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days bedrest. J Clin Endocrinol Metab 2004; 89: 4351-4358

17. Tipton KD, Borsheim E, Wolf S, et al. Acute response of net muscle protein balance reflects 24-h balance after exercise and amino acid ingestion. Am J Physiol Endocrinol Metab 2003; 284: E76-E89

18. Tipton KD, Elliott TA, Cree MG, et al. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. Med. Sci. Sports Exerc. 2004 ; 36: 2073-2081

19. Tipton KD, Elliott TA, Cree MG, et al. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. Am J Physiol Endocrinol Metab 2007; 292: E71-76

20. Burke DG, Chilibeck PD, Davidson KS, Candow DG, Farthing J, Smith-Palmer T. The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. Int J Sport Nutr Exerc Metab 2001; 11: 349-64

* 22. Cribb, PJ, Williams AD, Hayes A, Carey MF. The effect of whey isolate on strength, body composition and plasma glutamine. Int. J Sports Nutr Exerc Metab 2006; 16: 494-509
Important consideration was use of a supplement dose and protocol that is characteristic of strength athletes

22. Cribb PJ, Williams AD, Hayes A. A creatine-protein-carbohydrate supplement enhances responses to resistance training. Med Sci Sports Exerc. 2007 Nov *in press*

** 23. Cribb, PJ, Hayes A. Effect of supplement-timing and resistance training on skeletal muscle hypertrophy. Med Sci Sports Exerc 2006; 38: 1918-1925
Provided the functional evidence alluded to by many studies of the benefit of protein ingestion in the hours surrounding resistance exercise

* 24. Cribb PJ, Williams AD, Stathis CG et al. Effects of whey isolate, creatine, and resistance training on muscle hypertrophy. Med Sci Sports Exerc 2007; 39: 298-307.
Strength of this study was the use of previously trained athletes who also undertook 2-3 months supervised pre-training and did not alter their normal daily diet

25. Fujita S, Volpi E. Amino acids and muscle loss with aging. J Nutr 2006; 136: 277S-2780S

26. Katsanos C.S., Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe R.R. 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.

27. Paddon-Jones D, Sheffield-Moore M, Aarsland A, et al. Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion, Am J Physiol Endocrinol Metab 2005; 288: E761–E767

28. Combaret L, Dardevet D, Rieu I, Pouch MN, Bechet D, Taillandier D, Grizard J, Attaix D. A leucine-supplemented diet restores the defective postprandial inhibition of proteasome-dependent proteolysis in aged rat skeletal muscle. J Physiol 2005; 569:489-99

**29. Rieu I, Balage M, Sornet C, et al. Increased availability of leucine with leucine-rich whey proteins improves postprandial muscle protein synthesis in aging rats. Nutrition 2007; 23: 323-31
Important article confirming the potential use of leucine-rich diets for the reversal/prevention of sarcopenia

30. Katsanos CS, Kobayashi H, Sheffield-Moore M, et al. 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; 291: E381-7

31. Rieu I, Balage M, Sornet C, et al. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. J Physiol 2006; 575: 305-15

* 32. Phillips SM. Dietary protein for athletes: from requirements to metabolic advantage. Appl Physiol Nutr Metab 2006; 31: 647-54
Athletes and elderly may both be in need of protein ingestion higher than the RDA

33. Cribb PJ. Whey proteins in Sports Nutrition. United States Dairy Export Council, 2005.

34. Bucci L, Unlu L. Proteins and amino acid supplements in exercise and sport. In: Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition. Edited by Driskell J, Wolinsky I (editors). Boca Raton FL, CRC Press 2000; pp 191-212

35. Norton LE, Layman DK. Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. J Nutr 2006; 136 :533S-537S

36. Layman DK, Walker DA. Potential importance of leucine in treatment of obesity and the metabolic syndrome. J Nutr 2006; 136: 319S-323S.

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

38. Hack V, Schmid D, Breitkreutz R, et al. Cystine levels, cystine flux, and protein catabolism in cancer cachexia, HIV/SIV infection and senescence. FASEB J 1997; 11: 84-92

39. Holm E, Hack V, Tokus M, et al. Linkage between postabsorptive amino acid release and glutamate uptake in skeletal muscle tissue of healthy young subjects, cancer patients, and the elderly. J Mol Med 1997; 75: 454-461

40. Lands LC, Grey VL, Smountas AA. Effect of supplementation with a cysteine donor on muscular performance. J Appl Physiol 1999; 87: 1381-1385

41. Middleton N, Jelen P, 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 2004; 55: 131-141

42. Mariotti F, Simbelie KL, Makarios-Lahham L, et al. Acute ingestion of dietary proteins improves post-exercise liver glutathione in rats in a dose-dependent relationship with their cysteine content. J Nutr 2004 ; 134: 128-131

43. Bouthegourd JJ, Roseau SM, Makarios-Lahham L, et al. A preexercise lactalbumin-enriched whey protein meal preserves lipid oxidation and decreases adiposity in rats. Am J Physiol 2002; 283: E565-E572

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

45. Morifuji M, Sakai K, Sanbongi C, Sugiura K. Dietary whey protein downregulates fatty acid synthesis in the liver, but upregulates it in skeletal muscle of exercise-trained rats. Nutrition 2005; 21: 1052-1058

46. Willoughby DS, Stout JR, Wilborn CD. Effects of resistance training and protein plus amino acid supplementation on muscle anabolism, mass and strength. Amino Acids 207; 32: 467-77

**47. Symons TB, Schutzler SE, Cocke TL et al. Aging does not impair the anabolic response to a protein-rich meal. Am J Clin Nutr 2007; 86: 451-456
Importantly shows elderly subjects can still obtain the same anabolic signal from ingestion of a protein-rich lean beef meal similar in amino acid content as whey protein