The Role of Protein in Weight Management: A Biochemist's Perspective

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OVERVIEW

Protein, as a macronutrient, functions at a molecular level in the body to impact metabolic pathways that are important role in weight management - from influencing our desire to eat (or satiety), promoting the oxidation of fat or promoting the growth of muscle. Normal growth and development, as well as maintenance of our lean body mass, is dependent upon a daily intake of high quality proteins that provide an adequate amount of essential amino acids. Presented here is a case for how dietary protein is critical to weight management

WEIGHT LOSS VERSUS WEIGHT MANAGEMENT

Research on consumer trends and practices indicate that consumers are thinking less of weight loss, which is a general loss in body mass, both fat and muscle tissue over a short term versus weight management, which is more focused on a longterm reduction of fat mass and maintenance of lean muscle mass (Mellentin 2012). Body Mass Index (BMI) is a measure of whether people are underweight, at normal weight or overweight for their size and has historically been used to predict cardiovascular or diabetic disease risk, however, BMI measures do not accurately reflect differences in people's body shapes, as in the case of athletes versus non-athletes where athletes can get misclassified as obese (Klungland Torstveit and Sundgot-Borgen 2012). Many health practitioners consider waist circumference to be more important than BMI to assess a person's "adiposity" or level of body fat and risk of cardiovascular disease or developing diabetes (Carey, Walters et al. 2004; Empana, Ducimetiere et al. 2004; Flint, Rexrode et al. 2010). Focusing on maintaining lean mass and losing fat mass is particularly important as we age, as a significant loss of muscle mass and function can lead to frailty, a higher risk of falls and bone fractures and loss of independence. Unfortunately as we age we tend to lose muscle mass as a consequence of aging but researchers are showing that a combination of good dietary practices that include adequate levels of protein in addition to exercise can actually attenuate the loss of muscle mass (Symons, Sheffield-Moore et al. 2011).

ROLE OF PROTEIN IN WEIGHT MANAGEMENT

Consuming a diet relatively high in protein (20% of energy from protein or higher) contributes to weight management by several distinct mechanisms (Hu 2005): 1) Protein consumption is associated with increased diet induced thermogenesis and increases overall energy expenditure throughout the day; 2) Protein in the diet attenuates the glycemic response to carbohydrate and reduces the insulin response which, in turn, reduces deposition of fat into adipose; 3) High protein in the diet promotes higher satiety; and 4) Higher protein consumption promotes muscle protein synthesis and lean mass gain (Candow, Burke et al. 2006).

PROTEIN INCREASES ENERGY EXPENDITURE

Total body energy expenditure can be altered by changing the composition of the diet while leaving the total caloric intake the same and specifically, increasing the proportion of protein in the diet favors a higher energy expenditure. Mikkelsen et al. (Mikkelsen, Toubro et al. 2000) conducted a study of 12 men who followed one of three test diets for 4 days with the 5th day being the test day where 24 hr energy expenditure was measured in a whole-body calorimeter. Each subject consumed all three test diets in a blinded, randomized order with several weeks "washout" in between diets. The control diet was a low protein, high carb diet and the two test diets were both high protein, one using pork as 20% of the protein and the other using 20% from soy protein. All diets were matched for calories. The observed 24 hr energy expenditure after consuming the high protein diets, whether pork or soy, was higher than what was observed during the carbohydrate diet phase (Mikkelsen, Toubro et al. 2000). It also appeared that the pork induced a higher

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rate of thermogenesis as more calories were burned by the subjects when consuming the pork diet compared to the soy diet. Overall the higher protein diets resulted in more calories burned compared to the low protein diet. Therefore, over the long term, eating the same amount of calories as protein versus carbohydrate would be expected to lead to less weight gain as more calories are burned in metabolizing the protein vs carbohydrate.

In another recent acute crossover feeding study by Tan et al. (Tan, Batterham et al. 2010) subjects' energy expenditure was measured over 8 hrs during which time they were fed three types of equal caloric breakfasts and lunches. The only difference between the test diets was the type of protein – all diets had 30% of their energy coming from protein. There was no difference between the meat, dairy or soy protein diets in terms of total energy expenditure or the amount of carbohydrate or fat calories burned (Tan, Batterham et al. 2010). There was a statistically significant difference in protein oxidation between the meat and soy diet – with the meat diet having less protein oxidation than soy and soy not being significantly different than dairy protein. This suggests that eating meat may spare protein oxidation a bit more than eating soy protein, which may, over a long time, translate into a net preservation of more lean mass but this would have to be tested in a longer and larger trial.

In summary, eating a higher protein diet leads to higher energy expenditure through the oxidation of carbohydrates and fats and through the cost of metabolizing the protein itself. While the thermogenic effect is small and only accounts for a few percent of total daily calories, this mechanism is believed to contribute to a meaningful extent when a high protein diet is consumed over a long period of time.

PROTEIN ATTENUATES GLYCEMIC RESPONSE

The glycemic index of a food is a value calculated based on the glycemic response it elicits in normal, healthy people. The rise in glucose (glycemic response) results from the digestion of carbohydrates which can be simple sugars or complex carbohydrates such as starches. The glycemic response to the same amount or type of carbohydrate can be low or high over time depending on various factors as will be described below. Insulin is secreted in response to the rise in plasma glucose in general the higher the glycemic responses the higher the insulin secreted (Riccardi, Rivellese et al. 2008). Insulin is an anabolic hormone and is required in pathways that lead to increased deposition of nutrients into body fat or lean mass. While this role of insulin is critical to normal growth and maintenance of body tissues, excess insulin can lead to excessive weight gain and increased fat deposition as well as insulin resistance.

A recently published study demonstrated that consuming both protein and fat together with carbohydrate reduces the glycemic response to the meal (Hatonen, Virtamo et al. 2011). In this study all the subjects consumed each of six test meals one week apart in a random order and the blood glucose and insulin responses were measured each time. For all meals the total amount of carbohydrate was the same and served as a standard glucose beverage for the control (which was measured twice for each subject) and or as mashed potatoes for the remainder of the meals (Hatonen, Virtamo et al. 2011). Mashed potatoes were served alone and then for the various test meals different foods were added to the mashed potatoes which, on their own, did not contribute any significant carbohydrate to the meal – canola oil, chicken breast, salad or rye bread or a mixture of these. The mashed potatoes alone resulted in a peak of plasma glucose that was as high as the standard glucose beverage (Hatonen, Virtamo et al. 2011). Adding canola oil or a chicken breast to the mashed potatoes reduced the glucose peak and this was only statistically significant for the chicken breast. Adding salad to the mashed potatoes showed no effect on the rise in glucose but adding the oil and chicken breast to the mashed potatoes and salad, even with rye bread added, still significantly reduced the glucose rise. The glycemic index of the foods calculated as an area under the curve for plasma glucose reflected the same results except for the group that also consumed the rye bread; meaning that while the peak glucose was reduced by oil and the chicken breast, rye bread extended the rise in plasma glucose (Hatonen, Virtamo et al. 2011). There was a trend for decreased insulin release in subjects consuming mashed potatoes with chicken breast, oil, and salad versus the chicken breast alone (Hatonen, Virtamo et al. 2011). The trend for lowered insulin upon adding oil and salad with the chicken breast to the mashed potatoes indicates an interaction of the protein insulin response with signals from the oil and salad consumption

Soy protein and soy fiber can also lower the glycemic index of foods. In an unpublished study conducted at Solae LLC, adding 20 g of soy protein to rice crisp bars containing 50 g of carbohydrate significantly lowered the glycemic index from 97 to 57. Adding six grams of soy fiber with 9 grams of soy protein also reduced the glycemic index to 64. Since much of the soy fiber is insoluble it may help reduce the peak in plasma glucose by slowing the stomach digestion and release of digested sugars . Insulin was not measured in this study conducted at Solae but based on these results and those reported by other groups we would expect to have seen lower insulin responses in the subjects.

PROTEIN AND SATIETY

High protein diets can lead to successful weight management over the long term and the major mechanism that appears to be involved is by increasing one's satiety; protein increases the postprandial feeling of fullness (Fromentin, Darcel et al. 2012). Therefore, people are inclined to eat less at that meal and will tend to stay full longer between meals – that way overall reducing their total daily caloric intake.

It appears that all protein sources are comparable in their ability to induce satiety as demonstrated in the study by Tan et al. (Tan, Batterham et al. 2010). In that study, all subjects consumed the equicaloric test meals (breakfast and lunch) containing different protein sources at different visits. The subjects were asked hourly to fill out questionnaires that used a visual analog scale to quantitatively assess their feelings of hunger and satiation just before and after consuming the test meal. There were no differences in feelings of satiety or hunger between the different test diet groups – all protein sources (meat, dairy or soy) gave rise to similar ratings of fullness (Tan, Batterham et al. 2010).

So exactly how does protein in the diet give rise to a feeling of satiety or fullness? That has been the focus of some of our research at Solae – if protein induces satiety is there anything that can do as we process the protein to maintain or even increase the ability of protein to induce this sense of fullness? In order to determine whether processing conditions can affect satiety we have established screening assays to test for the ability of various proteins to enhance the feeling of fullness.

Screening assays have been established at Solae that screen for various proteins and protein hydrolysates' ability to induce the release of two specific satiety hormones (cholescystokinin (CCK) or Glucagon-Like Peptide-1 (GLP-1)) from intestinal enteroendocrine (STC-1) cells (Krul, Li et al. 2010). Both these hormones are secreted into the bloodstream in response to specific nutrients in the intestinal lumen and play a role in inducing a sense of satiety *in vivo* (Bowen, Noakes et al. 2006). The satiety response is a very complex and involves humoral and neural signals and interplay between the timing and absolute levels of the various nutrient signals and satiety hormone responses. For instance, cells that release CCK respond to protein and fat in the gut lumen and are localized in the uppermost part of the gut. GLP-1 secreting enteroendocrine cells are localized more distally in the gut and respond primarily to carbohydrate signals but it is know that these signals are modulated by protein as well (Punjabi, Arnold et al. 2011).

Our screening assay objective is to determine whether proteins or hydrolysates that have an enhanced ability to induce CCK or GLP-1 release by the enteroendocrine cells, do promote increased satiety *in vivo*. In our screening assay, we subject our protein samples to a simulated stomach and upper intestinal digestion process. The resultant digested protein mixture would mimic what would be coming in contact with the sensing cells in the gut *in vivo*.

Proteins from different sources have been tested in the CCK screening assay in their intact form or after being subjected to the simulated digestion procedure. As expected, most undigested proteins do not stimulate CCK release since intact, undigested proteins would not normally be present in the gut and interestingly do not stimulate any significant CCK release (Krul, Li et al. 2010). Simulated gastrointestinal digestion of proteins from various sources increased the ability of the proteins to stimulate CCK release, and the largest increases were seen for beef protein and soy protein (Krul, unpublished data).

Unlike CCK release, GLP-1 release was induced in the presence of the intact proteins (since GLP-1 is stimulated by carbohydrate in the culture media). Simulated digestion of most proteins decreased the amount of GLP-1 secreted, however, GLP-1 release did increase for digested soy protein, beef plasma protein and dairy whey protein (Krul, unpublished data). It is not immediately apparent how proteins modulate GLP-1 release but clearly more work needs to be done in this area.

Some of our preliminary work with soy protein had suggested that treating the soy protein during processing with food enzymes may increase their ability to induce CCK and GLP-1 and it was noted that different types of hydrolysates varied in their relative potencies with regard to this effect (Krul, Li et al. 2010). We have screened 170 different soy protein hydrolysates and have identified a subset of hydrolysates that have an enhanced ability to induce both CCK and GLP-1 release (Krul, unpublished data). We have determined that these hydrolysates, once exposed to the simulated gastrointestinal digestion, still retain their satiety hormone releasing activity. Future work in this area will include a human clinical study to determine whether the soy protein hydrolysates with enhanced CCK and GLP-1 inducing activity *in vitro* can promote increased satiety *in vivo*. If enhanced satiety is observed with the hydrolysates versus intact protein, this will provide an opportunity to process proteins in a way that will be beneficial as ingredients in products designed for weight management.

PROTEIN AND LEAN MASS MAINTENANCE OR GROWTH

The final way in which high protein meals contribute to weight management is by promoting the maintenance or growth of lean muscle mass. Studies have indicated that you need approximately 20 g protein at any one meal event to get a boost in muscle protein synthesis (Moore, Robinson et al. 2009). Significantly less than 20 g may results in the amino acids not getting incorporated into muscle, but may end up getting incorporated into other tissues, getting oxidized or possibly getting converted to sugars for energy. Exercise also stimulates muscle protein synthesis and muscle growth but you need to be consuming protein in order to see this net increase in muscle protein synthesis (Atherton and Smith 2012). Any high quality protein can effectively help build muscle mass (Rodriguez, DiMarco et al. 2009). For example, in the study of Tang et al. (Tang, Moore et al. 2009), healthy men exercised one leg while one leg stayed at rest. Immediately following the exercise, a beverage containing 22 g of protein was consumed – the only difference between the groups was the source of protein – dairy whey, casein or soy protein. Even in the resting leg muscle protein synthesis was stimulated, with whey and soy protein showing greater muscle protein synthesis after 180 mins compared to casein. Muscle protein synthesis in the exercised leg was higher as expected and all protein sources supported the enhanced protein synthesis with whey > soy > casein at 180 mins.

Muscle protein synthesis is coordinated through an intracellular pathway that has at it's core a protein called mTOR (mammalian Target of Rapamycin), a regulatory enzyme that integrates input from many hormones, nutrients and mechanical stimuli (Dodd and Tee 2012). mTOR activates genes that initiate muscle protein synthesis. Insulin or Insulin-like growth factor work through mTOR to promote muscle growth. The amino acid leucine has been recognized to be a critical signal that is recognized by mTOR by a mechanism which still has to be worked out. Once mTOR has been activated by leucine, this is sufficient to initiate muscle protein synthesis through the activated transcription factors that initiate protein synthesis – 4EBP1, p70s6k and eIF4G and muscle protein synthesis proceeds as long as there is sufficient delivery of all the amino acids to the muscle.

There have been a few studies that have looked at meat proteins and muscle protein synthesis. In one example, Symons et al. (Symons, Sheffield-Moore et al. 2011) demonstrated that both younger and older men in the fasted (post-absorptive) state exhibited a basal level of muscle protein synthesis that was similar for both age groups. This group had previously shown that consuming the beef alone without exercise resulted in a significant increase in muscle protein synthesis (Symons, Sheffield-Moore et al. 2009). In the more recent study (Symons, Sheffield-Moore et al. 2011), men had a muscle biopsy taken then consumed a meal containing 90 g of beef protein (which we assume is beyond what is needed to stimulate muscle protein synthesis). After an hour, the men completed a bout of resistance exercise and another biopsy was taken after 5 hrs. Consuming the beef and then exercising resulted in an approximate 30% increase in the rate of muscle protein synthesis compared to just eating the protein meal alone (Symons, Sheffield-Moore et al. 2011).

Is there a protein that is optimal for building and/or maintaining muscle? Given that people do not normally consume a single source of protein we, at Solae, hypothesized that blends of proteins may possibly offer more benefit for developing products for sports nutrition than any single source of protein (Paul 2009). Whey protein is currently the market leader in protein for sports nutrition products, but casein and soy are high quality proteins that are also formulated into sports nutrition products. Protein sources can be complementary to each other; even high quality proteins like whey, casein and soy while containing all the essential amino acids have different limiting amino acids. Thus a blend would "level out" the essential amino acid profiles. Perhaps the most important feature of a protein blend for supporting muscle growth or maintenance is the difference in digestion rates of the individual proteins. In the case of whey, casein and soy, whey protein is the most quickly digested, soy protein is intermediate and caseinate is the slowest. We hypothesized that a blend of whey, caseinate and soy proteins would offer a more metered and sustained delivery of amino acids for a longer period of muscle protein synthesis. Despite the fact that many companies are "blending proteins" in various sports nutrition products, there had been no clinical study conducted that tested this hypothesis directly.

We conducted an animal study using two different whey: caseinate: soy blends versus whey or soy protein alone to test the hypothesis that a blend would be better at promoting muscle protein synthesis than a single source of protein. Muscle protein synthesis (MPS) was stimulated to a greater extent (greater fractional synthetic rate (FSR)) and over a longer period with a blend of 25% whey protein: 50% caseinate: 25% soy protein (Butteiger, Cope et al. 2012).

A clinical study was recently completed to determine whether the protein blend that showed a benefit to increase muscle protein synthesis in the animal study would translate to benefits in humans (Reidy, Walker et al. 2012). Healthy men and women had catheters inserted to administer a stable isotopically labelled amino acid (phenylalanine) and to obtain blood samples. Muscle biopsies were taken to measure the incorporation of the amino acid into muscle protein (FSR). Subjects performed a period of resistance exercise and then consumed a single beverage containing approximately 20 g of the blend protein or whey protein. Muscle protein synthesis was measured in the muscle biopsy specimens taken at 1, 3 and 5 hr post exercise. The increase in muscle protein synthesis (MPS) seen after exercise and protein consumption was similar with the soy/dairy protein blend or whey protein supplementation following resistance exercise however, the soy/dairy protein blend had a prolonged effect on muscle protein synthesis which was significant throughout the 3-5 hours after exercise and consumption of the protein blend (Reidy, Walker et al. 2012). The increase seen with whey protein in the 1-3 hour period did not continue in the late 3-5 hr period as seen with the blend (Reidy, Walker et al. 2012). Therefore, these data support our hypothesis that soy and dairy protein blends may offer more benefit to increase muscle mass than a single source protein alone. We believe that the differences in the relative digestion rates of the different proteins and sustained release of amino acids over time contributed to the extended period of upregulated muscle protein synthesis seen in this study.

In conclusion, it is worth noting that other protein blends such as meat and soy proteins may prove to be beneficial for muscle protein synthesis. Not only is there a possibility of showing benefits for muscle protein synthesis but by adding soy protein to meats such as beef, one can reduce the fat content and increase the % protein per serving weight.

SUMMARY

Consuming high protein diets promotes increased energy expenditure and fat oxidation compared to low protein diets thereby contributing to reduced overall energy intake and less weight gain.

High protein meals reduce the glycemic index for a given amount of carbohydrate; this reduces the "hyper" secretion of insulin and prevents insulin mediated fat deposition

Different proteins induce satiety hormone release in cell-based assays to varying degrees; partial enzyme hydrolysis of soy protein appears to enhance CCK & GLP-1 release

High quality proteins promote muscle protein synthesis & protein blends may have additional benefits by stimulating muscle protein synthesis for longer periods & offers ways to increase the relative protein to lipid content of product offerings

REFERENCES

Atherton, P. J. and K. Smith (2012). "Muscle protein synthesis (MPS) in response to nutrition and exercise." *J Physiol 590*(Pt 5): 1049-1057.

Bowen, J., M. Noakes, et al. (2006). "Appetite regulatory hormone responses to various dietary proteins differ by body mass index status despite similar reductions in ad libitum energy intake." *J Clin Endocrinol Metab 91*(8): 2913-2919.

Butteiger, D. N., M. Cope, et al. (2012). "A soy, whey and caseinate blend extends postprandial skeletal muscle protein synthesis in rats." *Clin Nutr*, in press.

Candow, D. G., N. C. Burke, et al. (2006). "Effect of whey and soy protein supplementation combined with resistance training in young adults." *Intl J Sport Nutr Exerc Metab 16*: 233-244.

Carey, V. J., E. E. Walters, et al. (2004). "Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women - The nurses health study." *Am J Epidemiol 145*(7): 614-619.

Dodd, K. M. and A. R. Tee (2012). "Leucine and mTORC1: a complex relationship." *Am J Physiol Endocrinol Metab 302*(11): E1329-1342.

Empana, J. P., P. Ducimetiere, et al. (2004). "Sagittal abdominal diameter and risk of sudden death in asymptomatic middle-aged men: the Paris Prospective Study I." *Circulation 110*(18): 2781-2785.

Flint, A. J., K. M. Rexrode, et al. (2010). "Body mass index, waist circumference, and risk of coronary heart disease: a prospective study among men and women." *Obes Res Clin Pract 4*(3): e171-e181.

Fromentin, G., N. Darcel, et al. (2012). "Peripheral and central mechanisms involved in the control of food intake by dietary amino acids and proteins." *Nutr Res Rev*: 1-11.

Hatonen, K. A., J. Virtamo, et al. (2011). "Protein and fat modify the glycaemic and insulinaemic responses to a mashed potato-based meal." *Br J Nutr*: 1-6.

Hu, F. B. (2005). "Protein, body weight and cardiovascular health." Am J *Clin Nutr 82*(suppl): 242S-247S.

Klungland Torstveit, M. and J. Sundgot-Borgen (2012). "Are under- and overweight female elite athletes thin and fat? A controlled study." *Med Sci Sports Exerc 44*(5): 949-957.

Krul, E. S., J. Li, et al. (2010). "Cholescystokinin release by STC-1 cells in response to differentially hydrolyzed soy protein." *FASEB J. 24*: 330.338.

Mellentin, J. (2012). Ten Key Trends in Food, Nutrition & Health 2012. New Nutrition Business, The Centre for Food & Health Studies**:** 1-88.

Mikkelsen, P. B., S. Toubro, et al. (2000). "Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate." *Am J Clin Nutr 72*: 1135-1141.

Moore, D. R., M. J. Robinson, et al. (2009). "Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men." *Am J Clin Nutr 89*(1): 161-168.

Paul, G. L. (2009). "The rationale for consuming protein blends in sports nutrition." *J Am Coll Nutr 28*(4): 464S-472S.

Punjabi, M., M. Arnold, et al. (2011). "Peripheral glucagon-like peptide-1 (GLP-1) and satiation." *Physiol Behav 105*(1): 71-76.

Reidy, P. T., D. K. Walker, et al. (2012). "Protein blend ingestion following resistance exercise promotes human muscle protein synthesis." Manuscript submitted.

Riccardi, G., A. A. Rivellese, et al. (2008). "Role of glycemic index and glycemic load in the healthy state in prediabetes, and in diabetes." *Am J Clin Nutr 87*(suppl): 269S-274S.

Rodriguez, N. R., N. M. DiMarco, et al. (2009). "Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance." *Journal of the American Dietetic Association 109*(3): 509-527.

Symons, T. B., M. Sheffield-Moore, et al. (2011). "The anabolic response to resistance exercise and a protein-rich meal is not diminished by age." *J Nutr Health Aging 15*(5): 376-381.

Symons, T. B., M. Sheffield-Moore, et al. (2009). "A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects." *J Am Diet Assoc 109*(9): 1582-1586.

Tan, S. Y., M. Batterham, et al. (2010). "Energy expenditure does not differ, but protein oxidation rates appear lower in meals containing predominantly meat versus soy sources of protein." *Obes Facts* **3**(2): 101-104.

Tang, J. E., D. R. Moore, et al. (2009). "Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men." *J Appl Physiol 107*(3): 987-992.