

The Emerging Role of Dairy Proteins and Bioactive Peptides in Nutrition and Health

Dietary Proteins in the Regulation of Food Intake and Body Weight in Humans¹

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ABSTRACT This review presents 4 lines of evidence supporting a role for proteins in the regulation of food intake and maintenance of healthy body weights. It is concluded that the protein content of food, and perhaps its source, is a strong determinant of short-term satiety and of how much food is eaten. Although the role of protein in the regulation of long-term food intake and body weight is less clear, the evidence reviewed suggests that further research to define its role is merited. Such research has the potential to lead to new functional foods, food formulations, and dietary recommendations for achieving healthy body weights. *J. Nutr.* 134: 974S–979S, 2004.

KEY WORDS: • proteins • food intake • body weight • dairy • high-protein diets

The role of protein in the regulation of long-term energy balance and maintenance of healthy body weights in humans has received little examination. One major criticism advanced in the evaluations of high-protein, low-carbohydrate, high-fat diets is that both their high protein content and high fat content may have adverse health effects (1–3). However, high protein diets remain popular suggesting that there is some perceived benefit in the eyes of the public. One such benefit may be increased satiety arising from protein in the diets, contributing to the ease of adherence to a reduced energy diet and/or contributing to spontaneous reduction in energy intake.

This review presents 4 lines of evidence supporting a role for proteins in the regulation of food intake and healthy body weights. First, proteins suppress food intake more than fats or carbohydrates and do so more than which can be accounted for by their energy content alone. Second, proteins make a stronger contribution to satiety and delay the return of hunger compared with fat and carbohydrates. Third, high-protein diets support the maintenance of lean body mass under circumstances of energy restriction, thereby promoting weight loss primarily as adipose tissue. Fourth, protein digestion leads to the stimulation of many physiological and metabolic responses known to be involved in food intake regulation. As illustrated by using milk as an example, these effects are dependent on the source of protein.

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Proteins and food intake

The first and perhaps strongest line of evidence supporting a role for protein in food intake and body weight regulation arises from the fact that protein consumption suppresses short-term food intake beyond what is expected from its energy content alone in both animals (4,5) and humans (6). For example, in rats food intake suppression was equal in the first hour of feeding after rats received 0.5 g whey, 1 g glucose, or 1.5 g corn oil in water by gavage compared to when given water alone. Compensation for the calories in the gavaged treatments in the first 2 h of feeding was ~300, 150, and 65%, respectively (5). Similarly, if rats are fed a high protein diet with no other choice, they will initially eat less than if fed a diet containing the regular amount of protein. However, they quickly adapt by increasing their capacity to catabolize the excess amino acids. When given a choice of 2 diets, 1 high in protein (e.g., 50%) and 1 low in protein (e.g., 10%) the rat will balance dietary choice to regulate protein intake within a range consistent with normal growth and body weight (7,8). It has been proposed, therefore, that amino acids signal neurochemical systems that contribute not only to the regulation of food intake but also to food choice (8).

In humans, the protein content of a food or meal is also a factor in the short-term reduction of food intake. Although it is clear that a protein content of ~50 g in a food or meal is a stronger determinant of satiety than either fat or carbohydrate, the lack of dose response studies makes it difficult to describe the relationship between the quantity of protein in the pre-meal and the duration of satiety. As with carbohydrates (9), the dose of protein and the time until the next eating occasion are both factors that need to be considered when evaluating the effect of protein on satiety.

There are several designs used in short-term studies that show that the protein content of food contributes to satiety. One approach used is to feed subjects high or low protein foods

or meals of fixed energy content followed by measuring their food intake at a later meal. A second approach is to feed high or low protein meals ad libitum and measure the total amount consumed within the meal. Third, some studies provide high or low protein first courses to a meal and subsequently measure the consumption of the remainder of the meal.

In the majority of short-term studies, treatments of fixed energy content have been given at lunch and food intake measured at a later meal. The following 3 studies illustrate this approach and show that a high protein lunch suppresses later food intake more than a protein poor lunch of similar energy. First, adults fed a protein rich lunch, containing 40 g of protein, consumed fewer calories at a meal 2 to 3 h later compared to their intake after a low protein (6 g) lunch (10). Second, when young women were given equicaloric 1884 kJ (450 kcal) liquid lunches, they consumed an average of 31% less energy after the lunch high in protein (80 g of a commercial whey protein, 71.5% of energy) and 20% less energy after the mixed lunch (40 g protein and 62 g carbohydrate, 36% and 55% of energy, respectively) at a meal 4.5 to 4.75 h later as compared to when they consumed a lunch high in carbohydrates (113 g of a polydose supplement, 99% of energy) (11). Third, young women fed a high protein (64.5 g protein, 43% of energy) meat casserole for lunch consumed 12% fewer calories at a meal 4 h later compared to when they ate an equicaloric (~2560 kJ or 615 kcal) high carbohydrate (15.5 g, 10% of energy) vegetarian casserole for lunch (12).

If the treatment food is fed ad libitum, the protein content of the food influences how much of it is eaten within a single meal. For example, subjects eating to satiety stopped eating when they had consumed 50 g of protein and only 1721 kJ (411 kcal) from a high protein omelette but when fed a low protein omelette, containing only 25 g of protein, they consumed 2985 kJ (713 kcal) (13). These data might be taken to suggest that while energy and protein content are both factors in determining the amount consumed, protein may have a protective role in preventing excess energy intake.

The importance of both dose and duration of time to the test meal in determining outcome is illustrated in the next 2 studies. Healthy young men given preloads similar in caloric (1172 kJ or 280 kcal) content as an omelette "snack" either high in protein (37 g, 54% of energy), or low in protein (10 g, 15% of energy) and high in fat (25 g, 79% of energy) ate similar amounts at a meal 2 h later. However, with no time interval between the test omelettes and the test meal, an omelette containing only 26 g protein decreased intake in the remainder of the meal compared with the low protein (16 g) omelette even if the later contained twice the calories (13).

Although the previous studies cited refer to possible benefits of protein in reducing short-term caloric intake, there is very little evidence that the regular consumption of high compared with low protein meals might contribute to reduced daily energy intake. In the Iowa breakfast studies of almost forty years ago adolescent girls were given breakfasts that contained different amounts of protein (9, 15, or 24 g), and they consumed each diet for six days duration. The 5 subjects that consumed all breakfasts had 17% lower daily energy intake after eating the breakfast with the highest protein content compared to the breakfast with the lowest protein content (14). Unfortunately, the sample size was small and no additional studies have been reported, leaving uncertain the role of a high protein breakfast on daily caloric intake.

Proteins and satiety

The effect of protein on the return of hunger and satiety provides another line of evidence that protein is unique among macronutrients in its effects on food intake. A stronger short-term satiety effect of protein, compared with that of fat and carbohydrate, has been shown by the delay in time at which food is requested after a protein load. In 1 study, young men were fed an ad libitum lunch followed 4 h later by mandatory snacks containing ~1000 kJ (250 kcal) that were either high in protein (77% of energy), fat (58% of energy), or carbohydrate (84% of energy) (15). The subjects were isolated in a windowless room, devoid of any temporal cues, and were brought to a buffet dinner at their own request. On the control day, when no snack was served, subjects requested dinner ~6 h after the beginning of lunch. Dinner requests were delayed the longest, by 60 min, on the day the high protein snack was consumed, while requests were only delayed by 35 min when a high carbohydrate snack was consumed and by 25 min on the day that a high fat snack was eaten by the subjects.

The effect of high protein foods on food intake is consistent with their effect on subjective measurements of satiety. Visual analogue scales, 100 mm lines anchored at either end with opposing statements, are often used to measure subjective satiety and appetite as they are found to reflect actual food intake (16,17). Accordingly, foods high in protein not only suppress food intake at a later meal more than carbohydrate or fat, as described earlier, but also provide stronger feelings of satiety immediately after their consumption (11,13,18–20).

High protein diets, food intake, and body weight regulation

A third line of evidence that dietary protein contributes to food intake and body weight regulation arises from studies of high protein diets. The suggestion that a high protein diet of reduced energy content is more satiating and encourages adherence more than 1 low in protein appeared in the literature over 50 years ago. More specifically, an isocaloric weight-reducing diet (7536 kJ or 1800 kcal/d) high in protein (26%) was reported to be more satiating than a diet containing only 8% protein (21). Since that time many high protein diets have been promoted for weight loss and they have become very popular with the public (1,22,23). The 2 with the highest protein content are the Atkins and the Protein Power diets averaging as a percentage of energy, 35% protein, over 50% fat and a very low carbohydrate content of 8% or less. The Sugar Busters and The Zone diets average 28% of energy as protein, but allow 40% of energy from carbohydrate and 32% from fat (1).

These high protein diets have been criticized on the prediction that they will have adverse effects on calcium balance, the progression of cardiovascular disease and on renal and liver function, in addition to not being nutritionally balanced or being of benefit to weight loss (1–3). In contrast to these hypothetical concerns, the Daily Reference Intake Macronutrient Report defines an acceptable range of protein intake for adults to be 10–35% of energy intake (24), which suggests that the popular diets are not unsafe because of their protein content. Indeed, the report concludes that there is insufficient data to support the hypothesis that higher protein diets are associated with chronic diseases including osteoporosis, renal insufficiency, and coronary artery disease (24).

Both the Atkins diet and the Protein Power diet have been found to contribute to weight loss and to reduce risk factors for cardiovascular disease in obese subjects. Unfortunately, the role of protein in contributing to the beneficial effects attrib-

uted to the diets remains unclear because none of the following studies achieved protein intakes recommended by either the Atkins or the Protein Power diets. Subjects classified as obese (22,25) and severely obese (23) on these low-carbohydrate diets had greater weight loss and reduced risk factors for cardiovascular disease than those following a low-fat diet. In the first study of obese subjects (22), in which the test diet was the Atkins diet, the diet was associated with a more favorable effect on weight at 3 and 6 mo, but not at 1 y, and with more favorable changes in HDL cholesterol and triglycerides at 1 y. Despite neither the composition nor the quantity of food consumed being recorded, the greater initial weight loss (7% of total body weight) on the Atkins diet was suggested to be due to lower energy intake (22). Protein is not mentioned as a possible factor. Similarly, in a study of obese women placed on either a low carbohydrate diet (<20 g carbohydrate/d for 2 wk, subsequently raised to 40–60 g/d) or an energy restricted high carbohydrate diet (55% carbohydrate, 15% protein, 30% fat energy), those in the low carbohydrate group had greater weight loss at 3 and 6 mo (25). Again, neither the dietary protein intake nor the effect of the diet on satiety was reported. In the study of the severely obese subjects (23), the low-carbohydrate diet was the Protein Power diet and at six months the average composition of the energy intake of the subjects was 22% protein, 37% carbohydrate and 41% fat. For those on the low fat diet the distribution was 16, 51 and 33%, respectively. Reported energy intake was decreased more on average for those on the Protein Power diet, but the decrease was not statistically different from those on the low fat diet despite their significantly greater weight loss (5.8 kg versus 1.9 kg).

Although not a randomized control trial, when overweight and obese subjects were counseled to follow a very low carbohydrate intake, but with energy intake not restricted, individuals achieved a reported protein intake of 32% when group diet reinforcement meetings were held biweekly for the first 3 mo and monthly for the last 3 mo (26). Their weight decreased by an average of 10% over the 6-mo study period and a more favorable blood lipid profile was observed. These benefits of high protein diets on decreasing cardiovascular disease risk factors are consistent with a prospective women's cohort study which concluded that protein intake was inversely associated with ischemic heart disease among those women with the highest total protein intake (27).

Evidence for long-term benefits of high protein diets, independent of a low carbohydrate intake, come from trials that alter protein relative to the carbohydrate content of the diet and hold fat constant. One of the trials was a 6-mo study where subjects consumed a low fat (30% of energy) diet containing either 25 or 12% of energy as protein. Those subjects on the 25% protein diet had a greater reduction in body weight (8.9 kg), primarily from fat (7.6 kg), than did the subjects consuming the 12% protein diet, who lost only 5.1 kg of body weight, of which 4.3 kg was body fat (28). The subjects voluntarily ate less on the high protein diet and the authors suggest that this was due to the enhanced satiety caused by the high-protein diet. The second study was a crossover design where the subjects were fed a diet ad libitum for 6 d that as a percentage of energy was 31% protein, 32% fat and 37% carbohydrate (29). Subjects spontaneously consumed 25% less energy on the 31% protein diet compared with when they consumed the American Heart Association phase 1 diet that contained only 15% of energy as protein. A role for protein in contributing to satiety, and thus dietary adherence, is supported by a recent study in which women were fed isoenergetic diets containing different carbohydrate to protein ratios for 10

wk. Women following a diet with a carbohydrate to protein ratio of 1.4 (125 g protein/d) reported similar weight loss but greater satiety than those fed a diet containing a carbohydrate to protein ratio of 3.5 (68 g protein/d) (30).

In addition to promoting fat loss, high protein diets that are energy reduced for purposes of achieving weight loss have the added benefit of maintaining lean body mass (30) and resting energy expenditure (31), which is a major component of total energy expenditure. Protein given alone, as described in the protein-sparing modified fasting treatment of severe obesity, prevents a negative nitrogen balance and maintains lean body mass (32). Even under circumstances such as trauma and surgery that result in rapid protein catabolism, an intravenous amino acid infusion equivalent to ~2 g/kg of protein spares body protein (33).

Protein source and food and body weight regulation

Protein source, as a factor affecting the short-term feeding response of humans, has received little investigation and the results are mixed. However, studies in rats suggest that protein source is a factor. For example, food intake suppression in the next hour of feeding is greater following gavage with whey compared with egg-albumin and soy protein (34). This variation among proteins in their effect on food intake may be due to their physiological actions in the gut and independent of their nutritional qualities as judged traditionally by their essential amino acid composition (35). In the past, explanation for the effects of proteins on food intake was sought in their effects on plasma and brain amino acids concentration and was based on the aminostatic hypothesis of food intake regulation (7). However, because plasma and especially brain amino acid concentrations rise relatively late after protein consumption (36,37) it seems more likely that satiety signals arising from protein digestion begin in the gastrointestinal tract.

The mechanisms by which the peptide products of protein digestion exert their effect on food intake via the gut include slowing stomach emptying (38), perhaps via opioid receptors (39,40), and direct or indirect stimulation of gut hormone receptors, including cholecystokinin (CCK) (39,41) and glucagon-like peptide-1 (5,42).

Experimental evidence that protein source is a factor affecting short-term satiety and food intake in humans is mixed. In perhaps the earliest study reported on the effect of protein source on satiety, young men fed a meal containing 50 g of protein from lean fish were less hungry, as measured by visual analogue scales, over a 3-h period than when they were fed an equivalent amount of protein as either beef or chicken (43). The level of satiety produced by the meal of fish also declined more slowly over time than those of the other meals. Unfortunately, food intake was not measured at a later meal, leaving uncertain the effect of protein source on food intake.

In contrast, 2 studies report that protein source in a mixed meal is not a factor in food intake suppression at a later meal (44,45). However, the size of the mixed lunch and the duration of time until supper, when food intake was measured, may have contributed to the negative conclusion of these studies. In the initial study by Lang et al. (44), six dietary protein sources including egg-albumin, casein, gelatin, soy protein, pea protein, and wheat gluten were fed in mixed 5200 kJ (1242 kcal) macronutrient meals. Total protein content of the six meals varied from 61 to 74 g, and the treatment protein contributed 40 to 47 g. No difference in caloric intake was measured at a dinner 8 h later. In a follow-up study, 3 protein sources (casein, gelatin and soy) were each given in 2 amounts (25 g or 50 g protein), as part of a mixed meal of 1800 kJ (425

kcal) or 3600 kJ (860 kcal) respectively, for a total of 6 treatments (45). The latency for appetite to recover was significantly longer after the higher energy meals, but was affected by source only among the higher protein lunches, where latency for satiety with the gelatin lunch was longer than for the casein lunch. Similar to the first study, protein source did not affect food intake at a self-selected buffet meal 8 h later. However, the return of hunger 3 to 7 h after the lunches were served may be an indicator that food intake measurements 8 h after treatments is too long a delay to detect differential effects of protein source on food intake, once again illustrating the importance of timing between the test meal and the measurement of food intake.

In support of the argument that the previous studies were not well designed to test for the effect of protein source on food intake in humans, a recent study found that drinks containing 1700 kJ (~400 kcal) and 48 g of whey resulted in higher subjective satiety and lower food intake at a buffet meal 90 min later compared to when the subjects received drinks containing an equivalent amount of casein (46).

Based on the products of digestion and the physiological and metabolic actions of milk proteins, it should not be surprising that whey and casein differ in their effect on food intake. The faster absorption rate and hormonal response to whey, compared with casein, may be 1 explanation (47). Whey consumption leads to higher plasma concentrations of factors known to contribute to satiety (48) including amino acids, glucose-dependent insulinotropic polypeptide, glucagon-like peptide-1, and CCK (46).

Another explanation for the differences between the effects of casein and whey on food intake may reside in the peptide products derived from them and their physiological effects preabsorptively. Whey protein rapidly enters the jejunum mostly in the form of the intact protein, whereas casein is slow to appear and mainly in the form of degraded peptides (47). This difference between the 2 proteins is largely attributed to the clotting and/or precipitation of the casein (unlike the soluble whey) in the acidic media of the stomach, giving it longer exposure to gastric peptic hydrolysis (49).

Milk protein proteolysis products of relevance to food intake regulation include casomorphins, caseinomacropeptide (CMP) and the BCAA leucine. Casomorphins are peptides released upon the digestion of casein and are known to interact with gastric opioid receptors thus slowing gastrointestinal motility (50). Casomorphins may play a role in regulating food intake because the opioid receptor antagonist, naloxone methiodide, prevents the reduction in food intake after a casein preload is given to rats (39,40).

CMP is the first digestion product of casein to be released from the stomach (51). In rats, CMP stimulates pancreatic secretion (a marker of CCK release) in a dose dependent manner with an ~20-fold greater potency than whey protein (52) and in humans it has been reported to be a very potent stimulant of CCK release (53). Thus CMP may have potential as an appetite suppressant but only 1 published clinical trial of the effect of CMP on food intake was found in the scientific literature (54). In this study, no effect of CMP on food intake was found at a lunch served 1 h after men and women consumed 0.4 g or 2.0 g of CMP in a preload drink of 34 J (8 kcal). Before concluding that CMP has little effect in contributing to satiety in humans, studies at higher doses are required.

Commercial whey products prepared by ultrafiltration contain 15–20% CMP (55) as a by-product of cheese making. During the formation of the cheese curd, the enzyme chymosin cleaves CMP from casein and it becomes water soluble, thus moving out of the curd with the whey fraction. One would

therefore expect that if CMP is the active component of milk that leads to reduced food intake, this would be a documented benefit of many of the commercial whey products available. Although this has not occurred it raises the possibility that the difference between the effects of whey and casein on food intake reported by Hall et al. (46) could be because the whey product was prepared by ultrafiltration and thus contained high levels of CMP.

Dairy proteins are much higher in the BCAA, especially leucine, compared with meat or plant proteins and this has been proposed to be of benefit to food intake regulation and the maintenance of lean body mass on energy reduced diets. Monkeys fed a liquid diet using a system that allowed manipulation of the intragastric diet, but kept oral factors constant, ate 25% fewer calories when the intragastric diet was 50% casein compared to when it was only 14% (56). Unlike rats (7), the monkeys did not adapt to the high protein diet and had sustained elevated BCAA intake. The authors propose this to be a factor in the reduced energy intake. Layman (57) has also hypothesized that the beneficial effects of high protein diets are due to higher BCAA levels, above that required for protein synthesis. However, he suggests that the benefits are derived from leucine and its metabolic roles in maintaining glucose homeostasis and stimulating protein synthesis, particularly when subjects are on a weight reducing diet with exercise (57). In support of this hypothesis, a high protein diet, providing 1.5 g/kg and 10 g leucine/kg for 16 wk with a 5 d/wk exercise program, produced a significantly greater weight loss, primarily as body fat, than did the low protein diet with the same exercise regime. In addition, at wk 4, 10, and 16 individuals on the high protein diet were found to have higher lean body mass. These results are consistent with other studies of the protein sparing effect of energy restricted, weight reducing diets high in protein (28,30), but the role of leucine in the mediation of this response remains to be established.

Although animal protein consumption has been hypothesized to be a factor contributing to obesity (58), support for the notion that dairy products and their components have some benefits in the maintenance of healthy body weights has been recently provided (59–61). An inverse relationship has been found between ready-to-eat breakfast cereal consumption and the BMI of 4–12 y old children (62) and between the number of servings of dairy products and body fat in preschool children (59). In addition, dairy consumption had a strong inverse association with the ten-year cumulative incidence of obesity and with the insulin resistance syndrome in adults in a large multicenter, population based, prospective observational study (60). In accordance with this association, 1 study reported a 70% greater weight loss over 16 wk in obese adults fed only milk and yoghurt compared to the equicaloric control group (61).

Calcium, the major mineral component in milk, has also been hypothesized to be a factor accounting for the inverse association found between dairy consumption and body weight. Based on a retrospective analysis of several studies, Heaney et al. proposed that a daily increase of 300 mg of calcium, or approximately 1 dairy serving, was associated with a yearly reduction of ~1 kg of body fat in children and 2.5 to 3.0 kg of body weight in adults (63). The relationship between calcium and body weight has been hypothesized to be due to lower intracellular calcium resulting from high calcium intakes, which reduces lipogenesis while increasing lipolysis, thus diminishing adiposity (64). However, dairy products exert a significantly greater anti-obesity effect than supplemental calcium alone suggesting that there are other milk components important in the associations found between dairy consump-

tion and body weight (65). Further studies are necessary to define these relationships (66).

SUMMARY AND CONCLUSIONS

The protein content of food, and perhaps its source, is a strong determinant of short-term satiety and of how much food is eaten. However, the role of protein in the regulation of long-term food intake and body weight is less clear, but several lines of evidence suggest that further research to define its role is merited. In this context, questions raised in this review include: a) What is the optimal dose, composition, and time of day for consuming protein? b) Is the low protein content of popular foods a contributor to excess energy intake? c) Are there differences between and among animal and plant proteins? d) Are there functional peptides contained in food proteins (e.g., milk proteins) that are selectively beneficial? e) What are the mechanisms of action of proteins and peptides? f) Do high-protein diets reduce spontaneous caloric intake because of their satiating properties? g) Are there long-term benefits to high-protein diets? h) Do the protein components of dairy products explain associations between dairy product consumption and body weight?

Answers to these questions have the potential to lead to new functional foods, food formulations, and dietary recommendations for achieving healthy body weights.

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