

Food intake control and body weight regulation by dietary protein

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Conflicts of interest: none

Key points

- Under free choice conditions, animal models, mainly rats, choose protein intake levels far in excess of what is required to maintain the body's protein pool.
- The mechanisms for controlling protein intake are partly independent of those controlling energy intake. This can lead to conflicts and induce excessive calorie intake when the protein content of the diet is too low.
- It has been suggested that rats seek to establish a high protein-to-carbohydrate ratio (0.6-0.8) in their diet because this ratio would induce metabolic benefits.
- The protein leverage hypothesis suggests that, when protein intake is just below requirements, the mechanisms that control protein intake are strong enough to induce an increase in food intake and weight gain.

Summary

The protein requirement is generally defined as the amount necessary to maintain the body's protein pool. However, under free choice conditions, animal models often ingest more protein than required for nitrogen balance (10%-15%). This behavior possibly reflects the search for a high protein-to-carbohydrate ratio (0.6-0.8), inducing metabolic benefits. This indicates that in addition to protein homeostasis, dietary proteins are also involved in energy homeostasis. The mechanisms controlling protein and energy intake are partly independent and in specific conditions, there may be a conflict between the two. Protein density in the human diet has decreased ~2% since the 1970s and, according to the protein leverage hypothesis, this decrease may be responsible for the increase in energy intake and prevalence of obesity observed today.

Keywords:

Amino acid; Food intake; Energy metabolism; Body weight; Low protein diets; Dietary self-selection; Protein leverage hypothesis; Protein quality

Introduction

Nutrient requirements are determined primarily by *impetus* for protein deposition in the growing rat, maintenance of the body protein pool in adults, and partitioning of nutrients between protein accretion, fat reserves and energy production (1). The purpose of this review is, based on data from the literature acquired on animal models, to assess whether even limited decreases in the quantity and quality of protein intake can disrupt energy regulation, and in particular increase food consumption and weight gain. Finally, we will discuss the hypothesis of the protein leverage effect (2), or “protein leverage hypothesis”, which proposes that the satisfaction of the protein intake is important enough to significantly impact energy regulation and that, even a small decrease in the level of protein intake can induce a significant hyperphagia and eventually lead to overweight.

Proteins in food intake control mechanisms

The elegance and success of Mayer’s glucostatic hypothesis (3) focused research attention on the role played by carbohydrates and incidentally lipids (4) in the control of food intake, despite early observations by Mellinkof & al. (5) that the circulating levels of amino acids had a significant effect on food intake. The particular attention paid to the roles played by carbohydrates and fats is linked to the important changes in our diet, especially during the second half of the 20th century. These transitions have particularly affected the quantity and quality of carbohydrates and fats in our diet at the same time as the prevalence of obesity increased. The researchers have thus actively worked to describe precisely the mechanisms involved in the perception of the quantity and quality of carbohydrates and fats. At the same time, the possibility that changes in protein intake, in quantity or quality, may have contributed to the development of obesity has been largely ignored, and this quite logically due to the fact that amino acids provide on average only 10-20% of energy intake, and that this percentage has remained stable over time (FAOSTAT database. 2002 [WWW document]. URL <http://faostat.fao.org/faostat/collections>). In addition, proteins are not considered as a source of energy reserve per se, even if the body proteins of the various tissues are quickly mobilized during fasting and able to ensure the production of glucose for several days without any major alteration of physiological functions.

Nevertheless, since the 1960s, many studies have focused on the mechanisms of recognition by the body of the quantity and quality of protein intake (6, 7, 8), mostly without taking into account interactions with fats and carbohydrates. The aim of these studies was to determine for

humans the minimum protein requirements and their possible variations depending on different physiological, physio-pathological, and environmental situations. In animal models, it was very early demonstrated that amino acids derived from protein digestion send specific signaling to the brain at sites only partially differentiated from the sites of carbohydrate and fat intake recognition, and could possibly interfere with each other (9). Finally, some behavioral studies have suggested the presence of significant interactions between the mechanisms of control of dietary intake by carbohydrates and proteins leading some authors, including us, to suggest that animals would optimize the proteins-carbohydrates (PC) ratio in their diet rather than the proteins-energy (PE) ratio (10, 11, 12, 13, 14, 15).

The progressive understanding of the mechanisms controlling food intake led to the recognition of a specific control of protein intake, but which interacts significantly with the control of energy intake. This separation-interaction can lead to conflicts in certain nutritional situations, where the regulations of protein and energy intake are no longer compatible. The question then arose of which mechanism prevails over the other or more pragmatically how the compromise is established between the satisfaction of protein intake and that of energy intake.

Proteins in protein and energy homeostasis

To provide the essential amino acids for the renewal and growth of the body protein pool, a minimum protein intake is required, resulting in a recommended intake of 0.83 g/kg/day for the general population, corresponding in most cases to a PE ratio of 10-15%.

Almost all the amino acids supplied by the diet are oxidized, either during the postprandial period (about 20% of the ingested amino acids are oxidized), or at the end of the processes of proteosynthesis and oxidation of amino acids. After being disaminated in the liver, the carbon skeletons enter the catabolic pathways where they are oxidized either directly or indirectly via gluconeogenesis (16), and participate in the production of energy in the same way as carbohydrates and lipids.

The level of spontaneous protein intake is high, regulated and adapted to environmental and physiological constraints: contribution of free choice experiments

The role of proteins in energy metabolism is all the more important as the level of spontaneous protein intake is high and defended. Indeed, many studies have analyzed the spontaneous dietary intake of the rat in conditions of free choice, i.e. when they can select their level of protein intake independently from the intake of carbohydrates and lipids. Most of these studies reported protein intake levels above those required to maintain nitrogen balance (17, 18, 19,

20). In a study reported in 1974 (18), Musten & al. observed that rats under free choice conditions ingested an average of 30% of their energy in the form of proteins, while 10-15% are sufficient in rats to ensure nitrogen balance. This level of protein is increased if the protein source is diluted by a non-nutritive element (18, 21) or is increased if protein requirements are increased by physiological needs such as lactation to ensure milk synthesis (22, 23). When the relative protein requirements are reduced, because the energy requirements are increased not those of proteins, rats maintain a constant level of protein intake and specifically increase their carbohydrate and fat intakes. This is particularly the case during feeding after a period of food restriction (24), or when the animals are housed in a cold environment that increases the cost of thermoregulation, a process which, in rodents, depends mainly on the oxidation of carbohydrates and lipids in the brown adipose tissue (18, 25). The response to exercise is more complex. Studies in rats show that, depending on the type of exercise and its intensity, the rat can increase the intake of either carbohydrates or fats (26), but in almost all situations, they maintain constant and even slightly increase protein intake whereas amino acids poorly participate as energy substrates during muscular effort. This response is probably related to the fact that the metabolic effects induced in post-exercise period, can change the protein requirements more than the exercise itself (27, 28, 29).

The influence of the variation of other macronutrients compared to proteins was very early demonstrated in free choice experiments. Indeed, it has been shown that the PE ratio selected by rats was generally lower when the lipid intakes were high (30). In 1981 and 1982, studies reported that when the lipid content of the protein-free diet was increased, the selected PE ratio decreased in lean and obese mice (on average from 30 to 35% up to 15 to 20%) (31, 32). Our group also reported that male Wistar rats with a choice between a pure protein diet and either a high-carbohydrate protein-free diet or a high-fat protein-free diet, respectively selected a PE ratio of 40% (20) or only 28% (33). In 1979, Kratz and Levitsky reported that when sucrose was offered to self-selecting rats, they consumed it immediately in large quantities, and the PE ratio was temporarily reduced from 37% to 28%, but quickly restored because the rats decreased selectively intake of the protein-free diet containing sucrose (34). At the same time, rats fed a composite diet could not reduce the consumption of carbohydrates and lipids independently of that of proteins, and maintained a high intake of the composite diet to maintain protein intake. Under free choice condition, the possibility of separating the control of energy and protein intake limited the overconsumption of calories and weight gain when the palatability of the diet was increased by sucrose.

The variations of protein intake as a function of age show that young growing rats do not select diets with higher protein contents than adult rats. The most general trend observed is rather a preference for high-fat diets just after weaning, then an increase in protein preference that reaches a maximum at puberty and then continues, or only slowly decreases (19, 35, 36). In older rats, there is often both a loss of lean body mass and a spontaneous decrease in protein intake (19, 36) which would be linked to alterations in the mechanisms controlling food intake (37, 38).

Finally, in rat models of obesity, early studies suggested that a specific appetite for protein would motivate hyperphagia because protein synthesis was less effective (39). Subsequent studies have shown that in free choice conditions, obese rats selected a lower PE ratio than healthy animals. However, because they had a much higher total dietary intake, in absolute terms, the amount of protein ingested was equivalent to that measured in healthy rats (40). Measurements of protein synthesis finally converged to indicate that there is in fact no protein synthesis defect in most animal models of obesity (8, 41).

The energy supply by proteins

In an attempt to understand why animals ingest seemingly much more protein than they need, several researchers have hypothesized that a high protein content in the diet may be motivated by the search for an optimal PC ratio. In favor of this hypothesis, it is observed that animals ingesting a high-fat diet generally consume less protein than rats ingesting a high-carbohydrate diet (32), and several studies have shown that by doing so, rats do tend to maintain a relatively constant PC ratio (31, 33). The majority of studies reported PE ratios in a range of 25-35% and PC ratios in a range of 0.6-0.8, including in obesity models (42).

Thus, we may wonder why the PC ratio is defended at such a high value knowing that, in the human diet as well as in the established diets for laboratory rats, the PC ratio is rather in the order of 0.2-0.3. One hypothesis is that a high PC ratio would be a way to reduce the dependence of the metabolism on insulin, and ultimately reduce the incidence of co-morbidity factors such as insulin resistance and obesity (43). Indeed, under conditions where the carbohydrate content of the diet is high (low PC ratio), the body must adapt to consume and/or store large amounts of glucose, a process that strongly depends on insulin. When carbohydrate intake is reduced, in particular in favor of proteins (high PC ratio), more glucose is produced from the catabolism of dietary amino acids and their conversion to glucose via gluconeogenesis in liver. This mode of glucose production may have metabolic benefits such as a better regulation of blood glucose levels (44, 45). In 2003, Layman & al. (12) showed in humans that a dietary PC ratio of 1 during

a calorie restriction diet, led to more weight and fat loss, and a greater decrease in plasma triglycerides and in the triglyceride/HDL cholesterol ratio. The same type of diet fed to rats in 2011 (14) demonstrated comparable results and showed improvement of the insulin response to eating a meal. In the same study, the expression levels of the Akt and P70-S6 Kinase 1 genes in muscle and adipose tissue revealed an increase in insulin sensitivity in the muscle and a decrease in glucose uptake by adipose tissue. Finally, in rats it was also shown that a very high protein diet (PE=50%; PC=1.2) for 6 months reduced food intake and weight gain (46).

Several studies have looked for a central control mechanism of the PC ratio, in particular through the evolution of blood tryptophan concentrations and their consequences on serotonin synthesis at the central level. However, to date, no evidence of such a relationship has been found (47, 48, 49).

The influence of the level of protein intake on the regulation of weight and body composition

Increasing the protein content of the diet only marginally alters the energy balance

In response to ingestion of a high-protein diet, the protein component of dietary intake control appears to be reduced, and dietary intake is primarily controlled by the energy component of the diet (8, 50). In the rat model, an increase in protein content of the diet appears to have only moderate effects on food intake, but these effects can last for several months (46, 51). It was also observed that the rat adjusts rapidly and accurately its diet intake in response to the dilution of the dietary protein to maintain its level of protein intake, but it reacts only slowly and incompletely to an increase in the protein concentration in its diet (52). Our laboratory has conducted several studies on the responses to high-protein diets in animal models. The response to these diets indicates that there is a rapid adaptation of metabolic pathways in the liver allowing deamination of amino acids and production of carbonaceous skeletons, as well as mechanisms for renal elimination of urea resulting from hepatic catabolism of amino acids (16, 51, 53). These adaptive processes make it possible to manage a very high protein content in the diet, up to 50% and even beyond (54), and to regulate the energy balance normally.

In overweight subjects, it has been proposed that a low-calorie high-protein diet is more effective than an unmodified diet, in particular by reducing hunger, by limiting the loss of lean mass, and by limiting weight recovery during refeeding (55). However, studies in animals and humans do not confirm this hypothesis. In rats that were made obese and then submitted to a 40% caloric restriction, weight loss was the same when the diet had a regular or a high protein

content (56). In obese subjects following a moderate energy restriction (about 1600 kcal/day), a daily supplementation of 30g of protein did not reduce the loss of lean mass, which accounted for about 20% of total weight loss (57). After bariatric surgery, subjects receiving a protein supplementation of 10 to 15 g/day for 6 months lost as much lean mass as control subjects who received isocaloric supplement but the loss of lean body mass was similar in both groups (58). In a Sacks study carried out for two years in obese subjects following a weight loss diet, with groups differing in macronutrient composition, the subjects lost and regained as much weight and fat with high protein diets (59). These results suggest that it is the intensity of the restriction that prevails over the composition and the protein content of the diet.

In ad libitum situation, high-protein diets can be effective at preventing weight gain by reducing the feeling of hunger. One of the most frequently proposed hypotheses is that a high-protein diet provides large amounts of circulating and temporarily stored amino acids that can be catabolized through hepatic gluconeogenesis (53, 60, 61). According to this hypothesis, blood glucose levels in rats after a night of fasting are usually slightly higher in those fed a high protein diet than in those fed a normal protein diet (14, 62). However, this hypothesis was advanced (63) and contradicted by the same authors (64). We also showed that after a meal, the participation of dietary proteins in gluconeogenesis is very low (65). In a context of energy restriction, a high-protein diet could partially preserve protein homeostasis and avoid adding a negative protein balance to the negative energy balance. However, supplementation studies in humans show that an additional protein intake does not completely counteract this effect. As well as described by Dulloo & al. (66), during total or partial refeeding, a high-protein diet can also prevent that a too large fraction of energy from being directed towards the reconstitution of lipid reserves.

Decreased protein content has immediate and strong effects on body composition

When dietary protein intake drops below the levels necessary to ensure the renewal of body proteins, we observe major effects on the control of food intake and on body composition. In the young rat, growth remains very constant for protein intakes greater than 20%, but decreases rapidly when the protein content of the diet is decreased (67, 68). In adult rats, Du & al. (69) showed a significant decrease in protein gain relative to the protein intake level as soon as the PE ratio fell below 12.5%. This occurred in conjunction with an increase in body fat accumulation relative to that of proteins, leading to a gradual increase in body fat content. This result has been observed in many other studies, and in our laboratory, we recently reported similar results on the mouse model (70). We also observed that low-protein diets increased the

level of activity in mice, a phenomenon also reported in rats (71), and increased the energy cost of activity. Combined, these two phenomena induce significant increases in total energy expenditure that almost compensate hyperphagia and limit fat gain. Other studies have also reported increases in energy expenditure related to an increase in dietary thermogenesis supported by an activation of the activity of the brown adipose tissue (72, 73, 74, 75).

In rats, the increase in energy intake is observed only within a fairly narrow range of protein restriction, immediately below the levels required to maintain the nitrogen balance, i.e. around 8-12% protein in the diet (9, 69, 76). Some authors suggested that animals would sacrifice energy regulation and increase energy intake to ensure protein homeostasis, and that this response would be maximized when the level of protein intake is just below requirements (18, 77), i.e. in conditions where an increase in protein intake can successfully restore nitrogen balance.

The influence of protein quality

The quality of a protein is defined as its ability to provide enough of the essential amino acids that are not synthesized by the body, in order to ensure the renewal of the body protein pool. In general, animal-based proteins (egg, milk, meat and fish) have a good balance of essential amino acids. Plant-based proteins have, for the most part, insufficient levels of a few essential amino acids, primarily lysine in cereals and oilseeds, and to some extent methionine in legumes, which makes them less metabolically effective. Protein levels in plants are also generally lower than in animal sources. Thus, even by combining the ingestion of cereals and legumes, a relative deficiency in some essential amino acids may commonly persists. In response, when it has the opportunity, and if the deficiency is not too acute, the animal can be expected to ingest more plant-based proteins to ensure a sufficient intake of limiting amino acid. We were able to verify this hypothesis on a mouse model (70) where we observed that the metabolic and behavioral responses to protein restriction were stronger with soy protein (methionine deficient) than with casein (78). It was also observed that self-selecting rats fed a plant-based protein had to select a higher PE ratio to maintain a weight gain similar as those fed with a protein that was not deficient in essential amino acids (79, 80, 81). For example, Musten & al. showed that self-selecting rats ingested on average 40% of their energy intake in the form of wheat protein (gluten), deficient in lysine, against 30% with casein (18). A few years later, Kishi & al. (82) reported similar results and calculated that in their study as well as in the Musten & al. study,

the increase in protein intake induced by the low nutritional values of the plant proteins matched that required to preserve the intake of essential amino acids.

The leverage effect of proteins, myth or reality?

The protein leverage hypothesis suggests that a decrease in the protein content in the diet has the potential to induce an increase in food intake, and eventually a significant increase in weight gain and fat mass. It was theorized by Simpson and Raubenheimer in 2005 (2), on the ground of numerous experimental data on insect and rodents models (83), and recently updated (84). In addition, the concept is not completely new since in 1981, Emmans (85) proposed that to understand the control mechanisms of food intake, it was better to consider that, rather than eating to obtain energy, individuals eat to optimize the intake of their most limiting food resource. In 1993, Webster (1) already defended the idea that the mechanisms controlling protein intake could become predominant.

In the reasoning mode of Simpson and Raubenheimer, it is because proteins represent only a small part of the energy intake (~15%) that small variations in the protein content of the diet are likely to have significant effects on the control of food intake and regulation of energy balance. The authors describe how, between 1970 and 2010, the increase in the availability of palatable and inexpensive foods rich in fats and sugars have reduced the protein content of the food from 14% to 12.5% in the USA. According to their model, if the level of protein intake is precisely regulated, a decrease from 14% to 12.5% in the protein content of the diet requires to increase food intake by 14% to maintain a constant level of protein intake (2, 84) (See Figure 1). This necessarily implies an increase in weight if compensatory mechanisms, such as physical activity, are not put in place. According to the authors, such a risk is amplified because the ultra-processed Western diet is energetically dense and humans have an ancestral tendency to look for very palatable fatty and sweet foods (86). The experiments carried out in humans by the authors of this hypothesis (87, 88, 89), and by other teams (90, 91), tend to confirm that the leverage effect of proteins affects the control of food intake (evolution from the black point to the gray point in Figure 1). However other studies report more mixed results (92, 93) in which it appears that the control of food intake by proteins may be quite strongly constrained by the mechanisms controlling energy intake (evolution from the black point to the white point in Figure 1). Other studies have shown that the level of protein intake is very constant in different social groups and that in these groups the protein content in the diet is inversely correlated with the energy ingested (94), thus suggesting that energy intake is

increased to maintain protein intake constant. The fact that the diet has evolved towards ultra-processed foods therefore seems to favor a decrease in the level of protein intake, and an increase in food intake and obesity (95). Finally, the authors of the hypothesis recently analyzed a large number of studies in humans and showed that a reinterpretation of these studies reveals a very pronounced protein leverage effect (84, 96).

Despite the interest for this hypothesis and the accumulation of results that suggest its validity, including very recent studies (97, 98), there is at this time, no formal demonstration of a causal relationship between the increase in obesity prevalence and the decrease in the dietary protein content. Many confounding effects, such as the sugar and fat content, palatability, energy density, vitamins and minerals contents, n-6/n-3 ratio in fats, and physical activity levels are also likely to play a role on the actual prevalence of obesity. In an article published in 2019, Hall (91) showed that even a very dominant protein leverage effect could explain only 2/3 of the weight gain observed in the USA since 1970, which confirms that other parameters are certainly involved. Indeed, as discussed above, experiments to restrict the level of protein intake in animal models never induced significant increases adiposity levels because a decrease in dietary protein content appears to increase energy expenditure, which offset the increase in food intake (78). Measurements of energy expenditure must also be done in human to check whether this type of adaptation is also occurring. Despite these limitations, the protein leverage hypothesis must be considered seriously in a context where, for utterly defensible reasons of sustainable development and reasoned agriculture, the nutritional policies propose a reduction in the level of protein intake and partial replacement of animal-based proteins by plant-based proteins.

Conclusion

Amino acids provided by dietary proteins participate effectively in the control of dietary intake, first via specific signals that allows the body to estimate the quantity and balance of amino acids provided by the diet. The main response to this type of signal is to direct the individual's behavior towards the search for foods richer in protein or containing the limiting amino acid(s) in order to rebalance his intake. Amino acids then act as energetic substrates, in the same way as carbohydrates and lipids, and ensure that intake levels match energy expenditure levels.

Experimental evidence suggests that conflicts between the mechanisms that control protein and energy intake levels are resolved in favor of the energy regulation mechanisms. Excess protein intake does not really pose a problem of regulation, because amino acids are quickly integrated

into the catabolic pathways and used as energy substrates instead of carbohydrates and lipids whose levels in the food are necessarily reduced. To some extent, it even seems that in rats, a high intake level (30-50%) may have rather favorable effects on weight and body fat, probably because a high PC ratio allows for less insulin-dependent energy regulation.

A drop in protein intake levels below the requirements of the nitrogen balance quickly creates problems in maintaining lean body mass. No experiments have reported increases in dietary intake sufficient to compensate substantially for the deficit in amino acid intake. However, there appears to be a narrow window, at the lower limit of protein requirements (close to current feeding conditions in westernized societies), in which limited but continuous increases in dietary intake can develop to correct, at least partially, the amino acid intake deficit.

Knowing that the development of obesity is caused by the daily addition of very small errors between energy inputs and outputs, the leverage effect of proteins, even if it is limited by the mechanisms of energy regulation, could induce a small increase in food intake, which in long term would induce significant weight variations. Should this hypothesis be confirmed, it would have to be taken into account in the evaluation of the effects of the dietary transition towards a decrease in protein consumption. Although the majority of the experimental results reported in this review have been obtained in laboratory rodents which, due to their small size, high metabolism and continuous growth, probably have protein requirements and regulatory mechanisms that may differ at the margin from those prevailing in humans, the most recent experimental and epidemiological data in humans are largely consistent with the experimental results obtained in these models.

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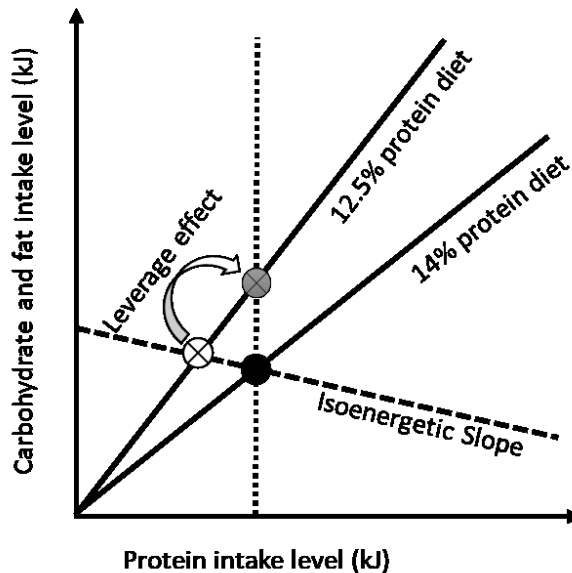
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Figure 1: Representation of the "Protein leverage" concept

- Optimal level of protein intake (by energy)
- ⊗ Preservation of the optimal level of protein intake (Full Leverage Effect)
- ⊗ Preservation of the optimal level of energy intake (Zero Leverage Effect)



The figure derived from the model proposed by Simpson and Raubenheimer represents the level of protein intake vs. the level of carbohydrate and lipid intake according to the protein content of the diet, i.e. in this example 14% vs. 12.5% (n.b. the scales are not respected for more visibility of the mechanism). If we consider a 14% protein level in the diet as optimum in humans, the protein, carbohydrate and lipid intake levels that are established on this diet are optimum (black point). If the level of protein intake is defended at the expense of the level of carbohydrate and lipid intake, the decrease in the protein content of the diet from 14% to 12.5% will force to consume more carbohydrates and lipids ($\sim +14\%$) to maintain the optimal level of protein intake (evolution from black point to grey point). If, on the contrary, energy regulation predominates, the intake of carbohydrates, lipids and proteins will slide along the iso-energetic slope, the total food intake will remain constant and the level of protein intake will decrease in proportion to the decrease in protein content in the diet (evolution of the food intake from the black point to the white point).