

## Protein, amino acids and the control of food intake

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The influence of protein and amino acid on the control of food intake and the specific control of protein and amino acid intakes remains incompletely understood. The most commonly accepted conclusions are: (1) the existence of an aversive response to diets deficient in or devoid of protein or deficient in at least one essential amino acid; (2) the existence of a mechanism that enables attainment of the minimum requirement for N and essential amino acids by increasing intake of a low-protein diet; (3) a decrease in the intake of a high-protein diet is associated with different processes, including the high satiating effect of protein. Ingested proteins are believed to generate pre- and post-absorptive signals that contribute to the control of gastric kinetics, pancreatic secretion and food intake. At the brain level, two major afferent pathways are involved in protein and amino acid monitoring: the indirect neuro-mediated (mainly vagus-mediated) pathway and the direct blood pathway. The neuro-mediated pathway transfers pre-absorptive and visceral information. This information is for the main part transferred through the vagus nerve that innervates part of the oro-sensory zone: the stomach, the duodenum and the liver. Other information is directly monitored in the blood. It is likely that the system responds precisely when protein and essential amino acid intake is inadequate, but in contrast allows a large range of adaptive capacities through amino acid degradation and substrate interconversion.

### Protein: Amino acid: Food intake

The influence of protein and amino acids on the control of food intake and the specific control of protein and amino acid intakes remains incompletely understood. Dietary proteins supply amino acids, which have major metabolic functions in the body. They are the substrates for protein synthesis, and the capacity to provide essential amino acids is of crucial importance for preserving body functions. They are involved in energy metabolism as substrates (directly or through gluconeogenesis) and as precursors of intermediary components of the Krebs cycle. They are also the precursors for numerous nitrogenous components, including nucleic acids, NO, glutathione, polyamines, creatine, taurine, histamine, catecholamines (dopamine, adrenaline, noradrenaline) and serotonin. Taken together, the observations of the relationships between protein, amino acids and food intake strongly suggest that protein- and amino acid-dependent mechanisms are involved in the control of food and protein intake. These mechanisms are associated with peripheral and central signalling processes for these very important nutrients.

### Protein, amino acids and food intake

The influence of protein and amino acids on food intake has been mainly studied in relation to protein and amino acid deficiency, their satiating effect compared with the other macronutrients and in the situation of a high-protein

diet. The most commonly accepted conclusions are: (1) the existence of an aversive response to diets deficient in or devoid of protein or deficient in at least one essential amino acid; (2) the existence of a mechanism enabling attainment of the minimum requirement for N and essential amino acids by increasing low-protein diet intake; (3) a decrease in the intake of a high-protein diet is associated with different processes including the high satiating effect of protein.

The existence of a specific appetite for essential amino acids is strongly suggested, since rats fed a diet devoid in one essential amino acid are able to recognize its presence when offered a choice between different diets with or without this essential amino acid according to different paradigms (Feurté *et al.* 1999, 2002; Gietzen & Magrum 2001). A specific appetite has also been reported for protein after a slight food or protein deprivation (Thibault & Booth, 1999). In addition, when rats were offered a low protein diet (5–8% energy as protein), they tended to increase their food intake in comparison with a standard protein diet (14% energy as protein) in order to increase their protein ingestion to a level allowing attainment of protein requirements; however, when fed a diet containing <5% protein as energy, they reduced their food intake (Du *et al.* 2000).

The control of protein ingestion is also suggested by numerous studies showing that, when given the opportunity,

**Abbreviation:** CCK, cholecystokinin.

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animals usually select a relatively constant level of protein and % protein as energy. Interestingly, it also seems that the spontaneous level of protein ingestion does not correspond to the minimal % protein as energy (10–12 % in the adult rat) required for N balance, but is usually higher and varies according to different factors, including age, the physiological state and the nature of the food. This suggests that different, complex and redundant mechanisms are involved in the control of protein ingestion (Peters & Harper, 1987). For instance, it has been observed that rats given a choice between the three macronutrients select a diet very high in protein (40 % energy as protein) (Jean *et al.* 2002; Wetzler *et al.* 2003). There is also a nyctimeral regulation of macronutrient ingestion in which proteins and lipids are mainly ingested at the end and carbohydrates at the beginning of the night-cycle (Larue-Achagiotis & Thouzeau, 1996).

A high-protein diet is usually associated with a decrease in food intake. When rats previously adapted to a normal-protein diet are offered a high-protein diet, they immediately decrease their food intake and progressively but incompletely re-increase food intake on the following days (Harper & Peters, 1989; Jean *et al.* 2001; Morens *et al.* 2000, 2001). A decrease in food intake was also observed in rhesus monkeys fed a high-protein diet (Hannah *et al.* 1990). This decrease in high-protein diet intake has been thought to arise from the low palatability of the diet, the time required for metabolic adaptation and a satiating effect (Harper & Peters, 1989; Reid & Hetherington, 1997). In contrast, despite previous hypotheses, it is unlikely that a high-protein diet induces a conditioned taste aversion, except for diets with very high levels of protein, i.e. 70–80 % energy (Harper & Peters, 1989; Bensaid *et al.* 2003).

The satiating effect of protein has been the subject of numerous studies. Satiety is defined as the absence of hunger during the interprandial period, whereas satiation describes the overall mechanisms that lead to cessation of eating. It seems that protein has the highest satiating effect when compared with other macronutrients in human subjects and rats (Porrini *et al.* 1997; Reid & Hetherington, 1997; Trigazis *et al.* 1997; Bensaid *et al.* 2002). However, some discrepancies originate from the physiological state, the duration of eating and the way by which nutrients are administered, i.e. oral, intra-gastric or intravenous (Burton-Freeman *et al.* 1997; Reid & Hetherington, 1997; Trigazis *et al.* 1997; Eisenstein *et al.* 2002; Raben *et al.* 2003). Moreover, the nature of the protein can influence the satiating effect, but the mechanism involved remains unclear, although it is unlikely that it originates from the presence of some specific amino acid as a precursor of neuromediators, i.e. tryptophan and serotonin or tyrosine and dopamine (Burton-Freeman *et al.* 1997). Moreover, some interactions between the satiating effects of the three macronutrients are suspected.

#### Peripheral events associated with the influence of protein and amino acid on food intake

The events associated with the control of food intake involve: (1) short-term mechanisms arising during the meal, mainly related to oro-pharyngeal and intestinal sensory processes; (2) postprandial meal-induced visceral

and metabolic signals; (3) long-term signals related to energy stores, mainly in adipose tissues. Ingested proteins are believed to generate pre- and post-absorptive signals that contribute to the control of gastric kinetic, pancreatic secretion and food intake.

Regarding short-term oro-pharyngeal signals, proteins are thought to have a low palatability that varies according to the protein source and is only slightly modified by the addition of sucrose. In addition, proteins or amino acids are very likely to generate pre-absorptive signals while still in the digestive tract. Chemoreceptors able to detect luminal nutrients (carbohydrates, amino acids, peptides, fatty acids or triacylglycerol) located in the small intestine could trigger the release of hormones, such as cholecystokinin (CCK), by mucosal enteroendocrine cells in response to luminal protein (Philips & Powley, 1996; Eastwood *et al.* 1998; Mathis *et al.* 1998; Phifer & Berthoud, 1998; Schwartz, 2000). At the periphery CCK directly modulates exocrine pancreatic secretion and possibly gastric kinetics. In addition, the proximity of vagal afferent axons and CCK immunoreactive cells supports the idea that CCK released from enteroendocrine cells acts on vagal sensory fibres in a paracrine fashion (Berthoud & Patterson, 1996). CCK could thus act on gastric kinetic through a vago-vagal loop and also stimulate satiety through low affinity vagal CCK<sub>A</sub>-receptors. Contradictory results were, however, obtained from surgical and chemical vagal blockage to investigate the role of the vagus nerve in the anorectic effect of oral, intragastric or intraduodenal amino acid and protein administration (Schwartz *et al.* 1999; Lheureux-Bouron *et al.* 2003).

Among the post-absorptive metabolic factors, an increased metabolic rate produces indirect vagal-mediated and direct signals recorded by the central nervous system. Proteins are thought to produce a greater thermogenic effect than other macronutrients participating in protein-induced satiety (Crovetto *et al.* 1998), but this effect is not always observed (Eisenstein *et al.* 2002; Raben *et al.* 2003). Amino acid-induced gluconeogenesis also prevents a decrease in glycaemia that contributes to satiety (Holt *et al.* 1996; Morens *et al.* 2003). Last, the variations in plasma hormones and free amino acid concentrations can be directly recorded by the central nervous system mainly through the concomitant variations in their intracerebral levels (Peters & Harper, 1987; Feurté *et al.* 1999; Farnsworth *et al.* 2003; Morens *et al.* 2003; Raben *et al.* 2003). This could involve a central nutrient chemosensor system for essential amino acids (Gietzen & Magrum, 2001). It could also involve other specific mechanisms associated with the central availability of specific amino acid precursors (histidine, tryptophan and tyrosine) of the neurotransmitters histamine, serotonin and the catecholamines (dopamine, adrenaline, norepinephrine; Fernstrom, 2000). In addition, in the case of a very-high-protein diet, the excess of a specific amino acid, including methionine, tryptophan and histidine, can produce a toxic effect associated with the amino acid or its metabolites, leading to an aversive anorexic response. Some results also suggest that the capacity to prevent the increase in blood NH<sub>3</sub> could play a role as a signal to limit protein intake (Herrero *et al.* 1994).

### Integration of the information in the central nervous system

At the brain level, two major afferent pathways are involved in protein and amino acid monitoring: the indirect neuro-mediated (mainly vagus-mediated) and the direct blood pathways. The neuro-mediated pathway transfers pre-absorptive and visceral information. This information is transferred through the vagus nerve that innervates a part of the oro-sensory zone: the stomach, the duodenum and the liver. Other information is received directly in the blood. This and other information participate in the overall central processes, leading to the control of food intake in which the hypothalamus plays a central role (Berthoud, 2002).

The first areas identified as being involved in the transfer of ingested-protein-related information to the brain are the nucleus of the solitary tract, the area postrema, the parabrachial nucleus and the anterior piriform cortex. Localized in the brain stem, the nucleus of the solitary tract is the main projection of the vagus nerve and integrates sensory information of oro-pharyngeal, intestinal and visceral origin. Intraduodenal amino acids increase neuron activity in the nucleus of the solitary tract. Signals circulating in the blood related to the level of protein ingestion (amino acids, peptides, hormones, etc.) have different targets in the brain, including the area postrema and the anterior piriform cortex for amino acids and the arcus nucleus for hormones (insulin, leptin) (Phifer & Berthoud, 1998; Schwartz, 2000; Gietzen & Magrum, 2001). The area postrema, which is near the nucleus of the solitary tract, collects information directly from the blood. The existence of an amino acid chemosensor system, which is in the anterior piriform cortex of the brain, has been convincingly demonstrated for essential amino acids in experiments using essential amino acid-devoid diets, which produce an anorectic response (Gietzen *et al.* 1998; Gietzen & Magrum, 2001; Blais *et al.* 2003). The lateral parabrachial nucleus is the main relay for area postrema and nucleus of the solitary tract, and plays a role in the treatment of the information from visceral origin (Cubero *et al.* 2001).

Peripheral information is further centralized in the hypothalamus, which participates in the control of energy homeostasis and food intake. The paraventricular nucleus is thought to be involved in satiety (Rowland *et al.* 1997). The arcus nucleus, which projects on different nuclei including the paraventricular nucleus, also produces peptide Y, which stimulates food intake (Williams *et al.* 2000). The lateral hypothalamus plays a role in the control of meal size through a dopaminergic control (Yang *et al.* 1997). The ventro-median hypothalamus, associated with the sympathetic system, is also involved in the control of food intake; a lesion of this zone produces hyperphagia and an increase in body weight (Tokunaga *et al.* 1989). This area is thought to be involved in the recognition of the nature of ingested nutrients. The accumbens nucleus and the amygdalia, which strongly interact with the hypothalamus, are involved in the association, reinforcement and recording of signals (Ono *et al.* 1995). The way in which the information arising from ingestion of protein and other nutrients leads to control of the intake of energy and specific nutrients is highly complex and remains incompletely understood.

### Conclusion

The input signals associated with the ingestion of amino acids and other energy-providing nutrients originate from visceral and metabolic mechanisms and involve both indirect (mainly vagus-mediated) and direct information (plasma levels of nutrients and hormones) recorded by the central nervous system. The protein and energy homeostasis control could involve: (1) an indirect visceral sensitivity for both nutrient tasting and signalling mechanisms that mainly records information regarding availability of energy substrates (gut sensitivity, ATP turnover, temperature, thermogenesis); (2) a central nutrient and metabolic chemosensor system for plasma essential amino acid, glucose and hormones (insulin and leptin); (3) specific mechanisms associated with the central availability of specific amino acid precursors (e.g. histidine and tryptophan) of neurotransmitters such as histamine and serotonin (as well as the catecholamines); (4) other emergency signals related to toxicity of some components, including toxic level of amino acid or of their metabolites or NH<sub>3</sub>. The level of blood amino acid is, like glucose, under regulation, and the homeostatic mechanism tolerates fluctuations in the blood concentrations of both glucose and amino acids of about 20%. It is likely that the system responds precisely when protein and essential amino acid intake is inadequate, but allows a large range of adaptive capacities through amino acid degradation and substrate interconversion. By providing alternative substrates to carbohydrates through gluconeogenesis, amino acids may improve the regulation of energy metabolism. These effects contribute to the influence of protein on food intake, endocrine regulation and body composition, and to the greater satiating effect of protein.

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