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Protein Status Modulates the Rewarding Value of Foods and Meals to Maintain an Adequate Protein Intake

Running title: Protein status and Reward

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24 **Abstract**

25 Proteins are dietary components that contribute to nutritional needs of the body through the
26 provision of nitrogen and amino acids. Protein status is tightly and continuously controlled to prevent
27 or counteract protein deficiency and to maintain or restore an adequate protein status. Animals
28 learn to detect and avoid diets deficient or devoid in protein or in at least one indispensable amino
29 acid and when given a choice reject these diets. Diets restricted marginally in protein or in one or
30 more amino acids more often induce hyperphagia, interpreted as an attempt to increase protein or
31 amino acid intake and to meet the need for protein and amino acids. The increase in energy intake
32 induced by a low protein diet is compensated for by an increased energy expenditure that restrains
33 the gain in adiposity. The status of protein and/or amino acid insufficiency induced by protein or
34 amino acid restricted diets is characterized by a profile of peripheral and central signals that
35 contribute to modulate peripheral metabolic adaptations and central pathways involved in the
36 control of feeding behaviour. These processes impact on the motivation for food and food choice,
37 with an appetite for protein and/or for the limiting amino acid(s) associated with a reward driven
38 sensitivity to protein and amino acid content of food and diets, which leads to restore or maintain an
39 adequate protein status. In contrast to a protein-restricted diet, high-protein diets are usually
40 reported to decrease food intake in both animals and humans, at least for a transient period, in
41 relation to a reported satiating effect of proteins through activation of anorexigenic pathways.

42

43 **Introduction**

44 Proteins are dietary components that contribute to nutritional needs of the body through the
 45 provision of nitrogen and amino acids. The maintenance of normal physiological function and survival
 46 in animals and humans requires a continuous supply of amino acids to tissues to support protein
 47 turnover and other amino acid–dependent metabolic processes. These phenomena depend on a
 48 daily intake of an adequate quantity of protein provided by a diverse array of foods. Protein status,
 49 characterized by amino acid sufficiency in the body to support metabolic functions, is tightly and
 50 continuously controlled and influences both protein and energy metabolism and feeding behavior
 51 (including food motivation, food choice, or food aversion) to prevent or counteract protein deficiency
 52 and to maintain or restore an adequate protein status. In this context, the different diets are
 53 considered, according to their protein content, as severely restricted (2-3% energy), moderately
 54 restricted (5-8% energy), with adequate content (10-20% energy) or with generous or high protein
 55 content (above 25-30% energy). These different diets have various consequences on food intake,
 56 food choice, metabolism, body weight and body composition in relation with the capacity of the
 57 organism to adapt or accommodate or not to the supply of protein provided by these diets.

58 **Severely deficient protein or amino acid diets are rejected**

59 Animals learn to detect and avoid diets severely deficient or devoid in protein or in at least one
 60 indispensable amino acid and when given a choice reject these diets (Rogers, Leung, 1973). These
 61 severely deficient protein diets induce a protein-deficient status characterized by low plasma protein
 62 concentration, and low body weight, fat mass, and lean mass (Du et al, 2000; Chaumontet et al,
 63 2018). Very low-protein diets (2-3% protein) more usually depress food intake (Du et al, 2000).
 64 Animals also avoid diets that are severely imbalanced in at least one amino acid and selectively find
 65 and consume the missing amino acid when offered a choice between different foods (Gietzen et al,
 66 2007). In Humans, consumption of an incomplete protein diet may be detected also and results in a
 67 signal to stop eating, being rather a signal of hunger suppression than of satiation or satiety
 68 (Veldhorst et al., 2009; Nieuwenhuizen et al, 2009).

69 The decrease in food intake induced by an extremely low protein diet is believed to originate from
 70 imbalanced plasma and brain amino acid patterns and the induction of a taste aversion to the amino
 71 acid devoid diet (Feurté et al, 1999, 2002; Even et al, 2000). It has been established that consumption
 72 of a meal inadequate in at least one essential amino acid reduces the concentrations of the deficient
 73 or limiting amino acid in plasma, in cerebrospinal fluid and in the brain where this amino acid
 74 depletion is sensed by general control nonderepressible 2 (GCN2) binding of deacylated tRNA and
 75 subsequent glutamatergic signalling (Hao et al, 2005). As an initial signal amino acid deficiency

induces an increase in uncharged tRNA that binds to GCN2 with subsequent phosphorylation of eIF2 and of the glutamate receptor, GluR1. The associated loss of GABAergic/inhibitory control contributes to activating glutamatergic excitatory circuits which project to different brain regions leading to the modification of feeding behaviour (Anthony, Gietzen, 2013).

Marginally restricted protein or amino acid diets induce hyperphagia

In contrast to severely deficient protein diets, marginally restricted protein or amino acid diets (3-8% energy) more often induce hyperphagia, interpreted as an attempt to increase protein or amino acid intake and meet the need for protein and amino acids (Beaton et al, 1965; White et al. 1994, 1998; Du et al, 2000; Sorensen et al, 2008; Carreiro et al, 2016). In addition, there are a large number of studies indicating that diets with marginally reduced dietary protein content increase total food intake in different species, but also increase energy expenditure, reduce growth, alter metabolic pathways within tissues and organs and modify body composition (Du et al, 2000; Sorensen et al, 2008; Blais et al, 2018). In rats fed low-protein diets body fat is increased and body proteins are decreased, and when protein intake is increased the gains in carcass protein and serum protein concentrations are directly related to the protein intake and reach a plateau for an intake considered to indicate protein requirement (Du et al, 2000).

When the protein in the diet is moderately restricted, food intake is primarily determined by the attempt to meet protein needs and animals fed a low-protein diet more usually eat more than the control group fed an adequate protein diet to preserve the protein intake (Blais et al, 2018; Chalvon-Demersay et al, 2016; Sorensen et al, 2008; Du et al, 2000; Leung, Rogers, 1986). Additional evidence also indicates that amino acid composition influences feeding, such that animals increase their consumption of diets that are moderately restricted in one or a few indispensable amino acids, as observed for instance with methionine (Forney et al., 2017). Growing animals restricted in dietary protein enhance their food intake to meet their protein requirement for maintenance and growth of lean tissues but with a risk of depositing excess energy as fat (Blais et al, 2018; Sorensen et al, 2008; White et al. 1994 and 1998). There is however a probable limitation in the increase in energy intake because in most cases, it is far from being enough to compensate for the decrease in the dietary protein content (Du, 2000; Blais et al. 2018). In addition, the increases in energy intake induced by a marginally low-protein diet are compensated for by an increased energy expenditure that decreases food efficiency and dissipates for a part the excess energy, and as a result fat mass accumulates much less than expected thus preventing a gain in adiposity (Donald et al, 1981; White et al. 1994; Du et al, 2000; Pezeshki et al, 2016; Chalvon-Demersay, 2016; Blais et al, 2018). In Humans, dietary protein content of the diet was negatively associated with total energy intake irrespective of whether

carbohydrate or fat were the diluents of protein (Gosby et al, 2004) but overfeeding of a low-protein diet (3%) also increased energy expenditure (Vinales et al, 2018), and this could also prevent, as observed in rodent, the gain in adiposity suspected to be induced by the overfeeding.

The increase in energy expenditure in low-protein fed animals is generally considered as a response to dissipate the surfeit of energy intake, but other studies observed that this increase can occur also in the absence of hyperphagia and therefore could represent a primary and specific response to protein restriction and that the increase in energy intake would be a secondary adjustment to compensate for the increase in energy expenditure (Rothwell et al, 1987; Hill et al, 2017, 2018; Laeger et al, 2016). This increase in energy expenditure and the changes in metabolic efficiencies produced by protein deficient diets have been associated tentatively with the stimulation of adaptive diet-induced thermogenesis with increased activity of brown adipose tissue, possibly white adipose tissues, and in muscles (Chartoumpekis, 2011; de Franca, 2009, 2016; Fisher, 2012; Pezeshki, 2016). However, according to other studies, the increase in total energy expenditure in low-protein fed mice could also result from changes in the pattern of spontaneous activity (in particular less time spent resting and more time spent fidgeting and active) (Blais et al, 2018). Interestingly, the apparently increased thermogenic response to feeding was observed only in mice housed at 22°C, but not at thermal neutrality (30°C, Blais 2018). Detailed analysis of the evolution of the cost of thermoregulation, the cost of activity and the thermogenic response to feeding suggests that the apparently higher thermogenic response to feeding in low-protein fed mice was due to a slower decrease in the energy expended for thermoregulation after ingestion of a test-meal. When this phenomenon was taken into account, the thermogenic response to feeding appeared similar in low- and normo-protein fed mice both at 30°C and 22°C.

Protein and amino acid deficient status induces an appetite for protein

Protein deficiency induces a specific appetite for protein, observed both in animals and in humans. The status of protein and/or amino acid insufficiency induced by protein or amino acid restricted diets is characterized by a profile of peripheral and central signals that contribute to modulate peripheral metabolic adaptations and central pathways involved in the control of feeding behaviour. These processes impact on motivation for food and food choice, with an appetite for protein and/or for the limiting amino acid(s), associated with a reward driven sensitivity to the protein and the amino acid content of foods in the diet, inducing a preference for these foods that can participate in the restoration and/or maintenance an adequate protein status (Figure 1).

In rodents a low-protein diet induces both low plasma concentrations of leptin, insulin and insulin-like growth factor-1 (IGF1), and high plasma concentrations of ghrelin and Fibroblast growth factor

21 (FGF21); and in the hypothalamus high levels of Neuropeptide Y (NPY) and Corticotropin Releasing Hormone (CRH) levels and low levels of Proopiomelanocortin (POMC). In low protein fed rats, the fasting concentration of the orexigenic hormone ghrelin was higher, and the fasting concentrations of the anorexigenic hormones insulin and leptin were lower, in comparison to rats fed a normal protein diet (Chaumontet et al, 2018). These profiles are associated also with higher expression of the orexigenic neuropeptide NPY and of the stress-related neuropeptide CRH in the hypothalamus in low protein fed rats, which is in line with an overall increased motivation for food and higher stress in protein-deficient animals (White et al, 1994; Chaumontet et al, 2018). Indeed, orexigenic ghrelin notably increases food intake reward with central NPY and opioid signalling as the necessary mediators (Skibicka et al, 2011; Dickinson et al, 2011). Conversely, leptin and insulin contribute to decreasing food reward by modulation of dopamine-signalling pathways and this process is downregulated in low protein fed animals (Pfaffly et al, 2010; Chaumontet et al, 2018). Amino acid and protein deficiency also induce activation of the sympathetic nervous system via increased CRH with increased energy expenditure and activation of lipolysis genes (Cheng et al, 2011).

Hepatic FGF21 production and plasma concentrations, which are stimulated by low protein-high carbohydrate diets and blunted under a high protein-low carbohydrate diet in rodents, have been proposed as a signal of protein deficiency (Chaumontet et al, 2019), but could be also controlled by a combination with glucose (Chalvon-Demersay et al, 2016) and high triglyceride concentrations in the liver (Chalvon-Demersay et al, 2017). It was shown recently also in human that plasma FGF21 concentration increased only after overfeeding of very low-protein (3%) diets irrespective of whether the diet was high in carbohydrate (75%) or high in fat (46%), with larger increases in FGF21 being associated with greater increases in 24 hour energy expenditure (Vinales et al, 2018). The expression of FGF21 seems to be controlled in part by the serine/threonine-protein kinase General control nonderepressible 2 (GCN2) that senses amino acid deficiency, and signalling through the Activating transcription factor 4 (ATF4) pathway (Hill et al, 2018). FGF21 is involved in numerous metabolic processes, such as hepatic lipid oxidation, ketogenesis (Kharitonov et al, 2005; Hotta et al, 2009), and glucose uptake in different organs, such as brown adipose tissue and skeletal muscle (Mashili et al, 2011; Markan et al, 2014). High FGF21 is believed to be involved in the stimulation of adaptive diet-induced thermogenesis in response to low-protein diet feeding {Chalvon-Demersay, 2016; Hill, 2018; Laeger, 2014} and would also mediate the thermogenic responses to essential amino acid leucine and methionine restriction (Lees, 2014; Wanders, 2015, 2017). FGF21 is involved also in feeding behaviour, as intracerebroventricular infusion of FGF21 increases both energy expenditure and food intake without affecting body composition (Sarruf et al, 2010), and the increase in food intake induced by low-protein diets is not observed in FGF21-knockout mice (Laeger et al, 2014).

After food or protein deprivation, a specific appetite for protein and a preference for protein-rich foods has been observed in rodents (Booth et al, 1974; Gibson, Booth, 1986). Animals offered a marginally protein-deficient diet tend to meet their target protein needs with different strategies, including an increase in food intake or when given a choice a preference for protein-rich foods (Blais et al, 2018; Chaumontet et al, 2018). In rats fed a protein-deficient diet, the neuronal activation of the Nucleus Accumbens is significantly and positively related to the protein content of the meal when rats are offered calibrated meals with different protein contents. Whereas when rats are fed a diet with an adequate protein content the Nucleus Accumbens is not sensitive to the protein content of the meal (Chaumontet et al, 2018). Under protein-deficient conditions, when there is a need to increase protein intake to compensate for protein deficiency and reach an adequate protein status, the Nucleus Accumbens activity is positively correlated to the protein content in meals whereas animals show an increased motivation for protein. In Humans, studies have also shown a higher appetite for protein-rich foods after protein restricted diets (Griffioen-Roose et al, 2012, 2014), as well as a preference for foods associated with a protein-rich flavor after a low-protein preload (Gibson et al, 1995). Moderately deficient protein status has been shown to increase the response of central reward regions to savory food cues in Humans (Griffioen-Roose et al 2014).

High protein diets decrease food intake

In contrast to protein-restricted diets, high-protein diets are usually reported to decrease food intake in both animals and humans, at least for a transient period, in relation to a reported satiating effect of proteins through activation of anorexigenic pathways (Journel et al, 2012; Westerterp-Plantenga et al, 2009; Fromentin et al, 2012; Davidenko et al, 2013) (figure 2).

Rodents fed a high protein diet (above 25% energy) compared with rats fed a normal protein diet (10-20% energy) have a lower daily energy intake in relation to higher satiety and lower body weight gain associated with lower fat mass without a difference in lean mass (Jean et al, 2001; Bensaid et al, 2003). Food intake depression induced by a high-protein diet is not due to palatability factor but to higher satiety in the rat (L'Heureux-Bouron et al. 2004). Under conditions of protein sufficiency, with a normal protein diet there is no specific motivation for protein intake, only the energy component is involved, and the homeostatic control relates mainly, or even only, to energy control. This effect is further reinforced with a generous intake of protein, which could explain the slight decrease in energy intake observed with a high protein diet compared with a normal protein diet. This could be through conditions of a lack of motivation for a protein-rich food sustained by inhibition of the reactivity of the reward system to protein-rich foods. Interestingly, in diet selection experiments, animals with the possibility to self-select macronutrients match their protein intake and even choose

an appropriate and specific quantity of energy from protein at a level most often about two times above their protein requirement defined from nitrogen balance (Jean et al, 2002; Makarios-Lahham et al, 2004; Azzout-Marniche et al, 2016). It can be hypothesized that at this relatively high level of intake of protein there is no appetite for protein.

In rats, high protein feeding activates satiety-related neuronal pathways in the brainstem and hypothalamus, with neuronal activity increased in the nucleus of the solitary tract (NTS) and decreased in the ventromedial hypothalamus and amygdala (Faipoux et al, 2008; Darcel et al, 2005). Protein and amino acid ingestion induce the secretion of gut neuropeptides in different parts of the small intestine, such as Cholecystokinin (CCK) in the duodenum or peptide YY (PYY) and Glucagon like peptide-1 (GLP-1) in the ileum. Some of these gut hormones (mainly CCK and GLP-1) then activate the vagus nerve. The involvement of this pathway in protein sensing and signalling to the brain goes along with the finding that infusion of proteins into the duodenum activates vagal afferent fibers in rats in a CCK-dependent manner (Tomé et al, 2009). In rats, activation of noradrenergic neurons and the increased expression of c-Fos in the Nucleus of the Tractus Solitary has been observed after high-protein feeding compared with normal protein feeding (Faipoux et al, 2008; Schwarz et al, 2010). In another study, high-protein feeding in mice potentiated the vagally mediated NTS response to CCK, as shown by increased c-Fos activation (Fromentin et al, 2012). In addition, a high-protein load compared with a normal protein load leads to decreased messenger RNA expression of the vagal receptor of orexin-1 in nodose ganglia (Journel et al, 2012).

High protein feeding is associated also with low ghrelin and high leptin plasma concentrations, and with low NPY and high POMC levels in the hypothalamus (Chaumontet et al, 2018). High-protein diets have been shown to regulate both catabolic and anabolic neuronal pathways in the arcuate nucleus. Proteins inhibit anabolic neuronal signalling (decreased NPY and agouti-related protein mRNA levels) and activate the catabolic signaling (POMC neurons producing α -melanocyte-stimulating hormone) in the hypothalamus (Morrison et al, 2007; Kinzig et al, 2007; Faipoux et al, 2008). A manganese-enhanced magnetic resonance imaging study showed that mice adapted to a high protein diet compared with a high carbohydrate diet and have lower basal activation in the hypothalamus, particularly in the paraventricular nucleus and the lateral hypothalamus (Zeeni et al, 2010). This is associated with lower orexin neuron activity in the lateral hypothalamus (Journel et al, 2012). Proteins are thought to stimulate satiety centers (the Nucleus of the Tractus Solitary and the arcuate nucleus), but also to reduce reward mechanisms in the brain. In rats, the satiety effect of proteins is associated with a decrease in blood-oxygen-level-dependent signal (the blood-oxygen-level-dependent contrast imaging the change in blood flow related to energy use by brain cells), specifically in the amygdala, which is a part of the limbic system and involved in the memory of

emotional reactions, including sensory stimuli and appetitive conditioning (Min et al, 2011). Basal neuronal activation in the Accumbens Nucleus after a meal is lower in high protein fed animals compared to normal protein fed animals with a lower sensitivity of dopamine-dependent reward pathways to protein (Chaumontet et al, 2018).

High-protein diets have been proposed to prevent or treat obesity by improvements in body weight management through modulations in energy metabolism, appetite, and energy intake (Westerterp-Plantenga et al, 2009; Leidy et al, 2015). In humans, higher protein preloads increase fullness ratings more than lower protein preloads (Dhillon, et al, 2016) and there is a protein threshold of at least 30 g protein to elicit satiety responses (Paddon-Jones D, Leidy, 2014). In overweight “breakfast skipping” adolescent girls, the addition of breakfast, particularly one rich in protein, led to reductions in brain activation responses to food stimuli in limbic regions associated with food motivation and reward (notably in the hippocampus, amygdala, anterior cingulate, and parahippocampus), and appeared as a useful strategy to improve satiety, reduce food motivation and reward, and improve diet quality (Leidy et al, 2011, 2013). These data indicate that activation of specific brain regions in the corticolimbic system are involved in the response to protein intake and that a high-protein diet leads to reduce reward-driven eating behavior. Accordingly, ingesting high-protein v. high-carbohydrate food differentially affects liking and wanting task-related brain signalling and subsequent macronutrient intake (Born et al 2013). In an intervention study in overweight and obese subjects that assessed the effects of a weight loss and subsequent weight maintenance period, changes in food reward-related brain activation were inversely associated with protein intake and dietary restraint during weight maintenance after weight loss and positively associated with changes in body weight and body-fat percentage (Drummen et al, 2018).

Conclusion

Protein status is tightly controlled and influences protein and energy metabolism but also protein and energy intake through reward driven sensitivity and an appetite for protein that participates in maintaining or restoring an adequate protein intake. With a status of protein deficiency induced by a protein deficient diet there are both a specific appetite for protein leading to a preference for protein-rich foods and also a higher motivation for foods that can lead to an increase, according to dietary conditions, in energy intake that is however compensated for in part by a concomitant increase in energy expenditure. The relation between protein intake, energy intake and energy expenditure is complex and it is likely that a low protein diet induces a specific increase in energy expenditure even in the absence of hyperphagia. When the protein content of the diet is increased in previously food restricted subjects, hyperphagia, reward driven sensitivity, an appetite for protein,

and high energy expenditure progressively decrease and are no longer observed when protein status is restored and protein intake is at a level meeting or even above protein and amino acid requirement as determined from nitrogen balance. This is particularly observed with a high-protein diet that reduces the reward-driven motivation for food and for protein, an effect associated with the satiating effect of protein. These processes involved in the control of the protein content of diets that efficiently allow maintaining an adequate protein status, are associated with complex interactions between peripheral and central signals that remains incompletely understood. It remains to identify the primary and secondary signals of protein and amino acid deficiency, how these primary and secondary mediators and signals to trigger a cascade of activation at the central level leading to the modulation of the reward system, what is the learned association between protein deficiency and central sensation and perception, and how these processes induce a conditioned preference for protein to adapt protein intake when needed. In addition, further research on how a low dietary protein diet could be used in the control of food intake, including the relation with thermoregulation, represents also an interesting question.

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501 **Legend to figures**

502 **Figure 1:** Protein deficiency is associated with a cascade of events from the periphery to the brain
503 that involves lower amino acid concentrations, enhanced fasting ghrelin, enhanced FGF21, lower
504 leptin, lower insulin, lower IGF1, enhanced hypothalamic NPY, and enhanced activity of the reward
505 system in response to protein intake. FGF21, Fibroblast growth factor 21; CRH, corticotropin
506 releasing hormone; NPY, neuropeptide Y; POMC, Proopiomelanocortin.

507 **Figure 2:** Adequate to High Protein diet is associated with peripheral and central signals that involve
508 enhanced amino acid concentration, enhanced gut neuropeptide CCK, GLP1 and PYY, enhanced
509 plasma insulin and IGF1, low plasma FGF21, enhanced NTS activity, enhanced hypothalamic POMC
510 level, low hypothalamic NPY level, low reward system activity in the Accumbens Nucleus. CCK,
511 Cholecystokinin; GLP1, Glucagon like peptide 1; PYY, Peptide YY; FGF21, Fibroblast growth factor 21;
512 CRH, corticotropin releasing hormone; NPY, neuropeptide Y; POMC, Proopiomelanocortin; NTS,
513 Nucleus of the Tractus Solitary.

514

Figure 1



