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

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4 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

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

80 **Marginally restricted protein or amino acid diets induce hyperphagia**

81 In contrast to severely deficient protein diets, marginally restricted protein or amino acid diets (3-8%
82 energy) more often induce hyperphagia, interpreted as an attempt to increase protein or amino acid
83 intake and meet the need for protein and amino acids (Beaton et al, 1965; White et al. 1994, 1998;
84 Du et al, 2000; Sorensen et al, 2008; Carreiro et al, 2016). In addition, there are a large number of
85 studies indicating that diets with marginally reduced dietary protein content increase total food
86 intake in different species, but also increase energy expenditure, reduce growth, alter metabolic
87 pathways within tissues and organs and modify body composition (Du et al, 2000; Sorensen et al,
88 2008; Blais et al, 2018). In rats fed low-protein diets body fat is increased and body proteins are
89 decreased, and when protein intake is increased the gains in carcass protein and serum protein
90 concentrations are directly related to the protein intake and reach a plateau for an intake considered
91 to indicate protein requirement (Du et al, 2000).

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

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

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

131 **Protein and amino acid deficient status induces an appetite for protein**

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

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

142 21 (FGF21); and in the hypothalamus high levels of Neuropeptide Y (NPY) and Corticotropin Releasing
143 Hormone (CRH) levels and low levels of Proopiomelanocortin (POMC). In low protein fed rats, the
144 fasting concentration of the orexigenic hormone ghrelin was higher, and the fasting concentrations
145 of the anorexigenic hormones insulin and leptin were lower, in comparison to rats fed a normal
146 protein diet (Chaumontet et al, 2018). These profiles are associated also with higher expression of
147 the orexigenic neuropeptide NPY and of the stress-related neuropeptide CRH in the hypothalamus in
148 low protein fed rats, which is in line with an overall increased motivation for food and higher stress in
149 protein-deficient animals (White et al, 1994; Chaumontet et al, 2018). Indeed, orexigenic ghrelin
150 notably increases food intake reward with central NPY and opioid signalling as the necessary
151 mediators (Skibicka et al, 2011; Dickinson et al, 2011). Conversely, leptin and insulin contribute to
152 decreasing food reward by modulation of dopamine-signalling pathways and this process is
153 downregulated in low protein fed animals (Pfaffly et al, 2010; Chaumontet et al, 2018). Amino acid
154 and protein deficiency also induce activation of the sympathetic nervous system via increased CRH
155 with increased energy expenditure and activation of lipolysis genes (Cheng et al, 2011).

156 Hepatic FGF21 production and plasma concentrations, which are stimulated by low protein-high
157 carbohydrate diets and blunted under a high protein-low carbohydrate diet in rodents, have been
158 proposed as a signal of protein deficiency (Chaumontet et al, 2019), but could be also controlled by a
159 combination with glucose (Chalvon-Demersay et al, 2016) and high triglyceride concentrations in the
160 liver (Chalvon-Demersay et al, 2017). It was shown recently also in human that plasma FGF21
161 concentration increased only after overfeeding of very low-protein (3%) diets irrespective of whether
162 the diet was high in carbohydrate (75%) or high in fat (46%), with larger increases in FGF21 being
163 associated with greater increases in 24 hour energy expenditure (Vinales et al, 2018). The expression
164 of FGF21 seems to be controlled in part by the serine/threonine-protein kinase General control
165 nonderepressible 2 (GCN2) that senses amino acid deficiency, and signalling through the Activating
166 transcription factor 4 (ATF4) pathway (Hill et al, 2018). FGF21 is involved in numerous metabolic
167 processes, such as hepatic lipid oxidation, ketogenesis (Kharitononkov et al, 2005; Hotta et al, 2009),
168 and glucose uptake in different organs, such as brown adipose tissue and skeletal muscle (Mashili et
169 al, 2011; Markan et al, 2014). High FGF21 is believed to be involved in the stimulation of adaptive
170 diet-induced thermogenesis in response to low-protein diet feeding {Chalvon-Demersay, 2016; Hill,
171 2018; Laeger, 2014} and would also mediate the thermogenic responses to essential amino acid
172 leucine and methionine restriction (Lees, 2014; Wanders, 2015, 2017). FGF21 is involved also in
173 feeding behaviour, as intracerebroventricular infusion of FGF21 increases both energy expenditure
174 and food intake without affecting body composition (Sarruf et al, 2010), and the increase in food
175 intake induced by low-protein diets is not observed in FGF21-knockout mice (Laeger et al, 2014).

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

192 **High protein diets decrease food intake**

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

197 Rodents fed a high protein diet (above 25% energy) compared with rats fed a normal protein diet
198 (10-20% energy) have a lower daily energy intake in relation to higher satiety and lower body weight
199 gain associated with lower fat mass without a difference in lean mass (Jean et al, 2001; Bensaid et al,
200 2003). Food intake depression induced by a high-protein diet is not due to palatability factor but to
201 higher satiety in the rat (L'Heureux-Bouron et al. 2004). Under conditions of protein sufficiency, with
202 a normal protein diet there is no specific motivation for protein intake, only the energy component is
203 involved, and the homeostatic control relates mainly, or even only, to energy control. This effect is
204 further reinforced with a generous intake of protein, which could explain the slight decrease in
205 energy intake observed with a high protein diet compared with a normal protein diet. This could be
206 through conditions of a lack of motivation for a protein-rich food sustained by inhibition of the
207 reactivity of the reward system to protein-rich foods. Interestingly, in diet selection experiments,
208 animals with the possibility to self-select macronutrients match their protein intake and even choose

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

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

228 High protein feeding is associated also with low ghrelin and high leptin plasma concentrations, and
229 with low NPY and high POMC levels in the hypothalamus (Chaumontet et al, 2018). High-protein
230 diets have been shown to regulate both catabolic and anabolic neuronal pathways in the arcuate
231 nucleus. Proteins inhibit anabolic neuronal signalling (decreased NPY and agouti-related protein
232 mRNA levels) and activate the catabolic signaling (POMC neurons producing α -melanocyte-
233 stimulating hormone) in the hypothalamus (Morrison et al, 2007; Kinzig et al, 2007; Faipoux et al,
234 2008). A manganese-enhanced magnetic resonance imaging study showed that mice adapted to a
235 high protein diet compared with a high carbohydrate diet and have lower basal activation in the
236 hypothalamus, particularly in the paraventricular nucleus and the lateral hypothalamus (Zeeni et al,
237 2010). This is associated with lower orexin neuron activity in the lateral hypothalamus (Journel et al,
238 2012). Proteins are thought to stimulate satiety centers (the Nucleus of the Tractus Solitary and the
239 arcuate nucleus), but also to reduce reward mechanisms in the brain. In rats, the satiety effect of
240 proteins is associated with a decrease in blood-oxygen-level-dependent signal (the blood-oxygen-
241 level-dependent contrast imaging the change in blood flow related to energy use by brain cells),
242 specifically in the amygdala, which is a part of the limbic system and involved in the memory of

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

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

265 **Conclusion**

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

276 and high energy expenditure progressively decrease and are no longer observed when protein status
277 is restored and protein intake is at a level meeting or even above protein and amino acid
278 requirement as determined from nitrogen balance. This is particularly observed with a high-protein
279 diet that reduces the reward-driven motivation for food and for protein, an effect associated with
280 the satiating effect of protein. These processes involved in the control of the protein content of diets
281 that efficiently allow maintaining an adequate protein status, are associated with complex
282 interactions between peripheral and central signals that remains incompletely understood. It
283 remains to identify the primary and secondary signals of protein and amino acid deficiency, how
284 these primary and secondary mediators and signals to trigger a cascade of activation at the central
285 level leading to the modulation of the reward system, what is the learned association between
286 protein deficiency and central sensation and perception, and how these processes induce a
287 conditioned preference for protein to adapt protein intake when needed. In addition, further
288 research on how a low dietary protein diet could be used in the control of food intake, including the
289 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



