



HAL
open science

Protein status modulates the rewarding value of foods and meals to maintain an adequate protein intake

Daniel Tomé, Catherine C. Chaumontet, Patrick P. Even, Nicolas Darcel, Dalila D. Azzout-Marniche

► To cite this version:

Daniel Tomé, Catherine C. Chaumontet, Patrick P. Even, Nicolas Darcel, Dalila D. Azzout-Marniche. Protein status modulates the rewarding value of foods and meals to maintain an adequate protein intake. *Physiology & behavior*, 2019, 206, pp.7-12. 10.1016/j.physbeh.2019.03.012 . hal-02346686

HAL Id: hal-02346686

<https://hal.science/hal-02346686>

Submitted on 22 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

3

4 Running title: Protein status and Reward

5

6 Authors: Daniel Tomé, Catherine Chaumontet, Patrick C Even, Nicolas Darcel, Dalila Azzout-
7 Marniche

8 PNCA, INRA, AgroParisTech, Université Paris-Saclay, 75005 Paris, France

9

10

11 Correspondance

12 Daniel Tomé

13 PNCA, INRA, AgroParisTech

14 16 rue Claude Bernard 75005 Paris, France

15 Email : tome@agroparistech.fr

16 Tel : +33144081718

17

18

19 Key words : Protein; feeding behavior; reward; gut hormones, insulin; leptin; FGF21

20

21

22

23

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.

290 **Acknowledgments**

291 The authors are very grateful to Pr. Simon Nigel Thornton for improving the English language of this
292 manuscript.

293 **Bibliography**

- 294 Anthony TG, Gietzen DW. Detection of amino acid deprivation in the central nervous system. *Curr*
295 *Opin Clin Nutr Metab Care*. 2013 Jan;16(1):96-101.
- 296 Beaton JR, Feleki V, Stevenson JA. Insulin hyperphagia in rats fed a low-protein diet. *Can J Physiol*
297 *Pharmacol* 1965;43:225–33.
- 298 Bensaid A, Tome D, L'Heureux-Bourdon D, Even P, Gietzen D, Morens C, Gaudichon C, Larue-
299 Achagiotis C, Fromentin G. A high-protein diet enhances satiety without conditioned taste aversion in
300 the rat. *Physiol Behav* 2003;78:311–20.
- 301 Blais A, Chaumontet C, Azzout-Marniche D, Piedcoq J, Fromentin G, Gaudichon C, Tome D, Even PC.
302 Low protein diet-induced hyperphagia and adiposity are modulated through interactions involving
303 thermoregulation, motor activity, and protein quality in mice. *Am J Physiol Endocrinol Metab*
304 2018;314(2): E139–51.
- 305 Booth DA. Food intake compensation for increase or decrease in the protein content of the diet.
306 *Behav Biol* 1974;12:31–40.
- 307 Born JM, Martens MJ, Lemmens SG, Goebel R, Westerterp-Plantenga MS. Protein v. carbohydrate
308 intake differentially affects liking- and wanting-related brain signalling. *Br J Nutr*. 2013 Jan
309 28;109(2):376-81.
- 310 Carreiro AL, Dhillon J, Gordon S, Higgins KA, Jacobs AG, McArthur BM, Redan BW, Rivera RL, Schmidt
311 LR, Mattes RD. The Macronutrients, Appetite, and Energy Intake. *Annu Rev Nutr*. 2016 Jul 17;36:73-
312 103.
- 313 Chartoumpakis DV, Habeos IG, Ziros PG, Psyrogiannis AI, Kyriazopoulou VE, and Papavassiliou AG.
314 Brown adipose tissue responds to cold and adrenergic stimulation by induction of FGF21. *Molecular*
315 *medicine* 17: 736-740, 2011.
- 316 Chaumontet C, Even PC, Schwarz J, Simonin-Foucault A, Piedcoq J, Fromentin G, Azzout-Marniche D,
317 Tome D. High dietary protein decreases fat deposition induced by high-fat and high-sucrose diet in
318 rats. *Br J Nutr* 2015;114:1132–42.
- 319 Chaumontet C, Azzout-Marniche D, Blais A, Piedcoq J, Tomé D, Gaudichon C, Even PC. Low protein-
320 low methionine/high carbohydrate diets induced hyperphagia, increased energy expenditure and
321 FGF21, decreased IGF1 and modestly affected adiposity in female BalbC mice. *Am J Physiol*, in press

- 322 Chalvon-Demersay T, Even PC, Chaumontet C, Piedcoq J, Viollet B, Gaudichon C, Tomé D, Foretz M,
323 Azzout-Marniche D. Modifying the Dietary Carbohydrate-to-Protein Ratio Alters the Postprandial
324 Macronutrient Oxidation Pattern in Liver of AMPK-Deficient Mice. *J Nutr*. 2017 Sep;147(9):1669-
325 1676.
- 326 Chalvon-Demersay T, Even PC, Tomé D, Chaumontet C, Piedcoq J, Gaudichon C, Azzout-Marniche D.
327 Low-protein diet induces, whereas high-protein diet reduces hepatic FGF21 production in mice, but
328 glucose and not amino acids up-regulate FGF21 in cultured hepatocytes. *J Nutr Biochem*. 2016
329 Oct;36:60-67.
- 330 Cheng Y, Zhang Q, Meng Q, et al. Leucine deprivation stimulates fat loss via increasing CRH
331 expression in the hypothalamus and activating the sympathetic nervous system. *Mol Endocrinol*
332 2011; 25:1624–1635.
- 333 Darcel N, Fromentin G, Raybould HE, Gougis S, Gietzen DW, Tome D. Fos-positive neurons are
334 increased in the nucleus of the solitary tract and decreased in the ventromedial hypothalamus and
335 amygdala by a high-protein diet in rats. *J Nutr* 2005;135: 1486–90.
- 336 Davidenko O, Darcel N, Fromentin G, Tome D. Control of protein and energy intake—brain
337 mechanisms. *Eur J Clin Nutr* 2013;67: 455–61.
- 338 de Franca SA, dos Santos MP, Przygodda F, Garofalo MA, Kettelhut IC, Magalhaes DA, Bezerra KS,
339 Colodel EM, Flouris AD, Andrade CM, and Kawashita NH. A Low-Protein, High-Carbohydrate Diet
340 Stimulates Thermogenesis in the Brown Adipose Tissue of Rats via ATF-2. *Lipids* 51: 303-310, 2016.
- 341 de Franca SA, Dos Santos MP, Garofalo MA, Navegantes LC, Kettelhut Ido C, Lopes CF, and Kawashita
342 NH. Low protein diet changes the energetic balance and sympathetic activity in brown adipose tissue
343 of growing rats. *Nutrition* 25: 1186-1192, 2009.
- 344 Dhillon J, Craig BA, Leidy HJ, Amankwaah AF, Osei-Boadi Anguah K, Jacobs A, Jones BL, Jones JB,
345 Keeler CL, Keller CE, McCrory MA, Rivera RL, Slebodnik M, Mattes RD, Tucker RM. The Effects of
346 Increased Protein Intake on Fullness: A Meta-Analysis and Its Limitations. *J Acad Nutr Diet*. 2016
347 Jun;116(6):968-83.
- 348 Dickson SL, Egecioglu E, Landgren S, Skibicka KP, Engel JA, Jerlhag E. The role of the central ghrelin
349 system in reward from food and chemical drugs. *Mol Cell Endocrinol* 2011;340:80–7.
- 350 Donald P, Pitts CC, and Pohl SL. Body weight and composition in laboratory rats: effects of diets with
351 high or low protein concentrations. *Science* 211: 185-186, 1981.

- 352 Drummen M, Dorenbos E, Vreugdenhil ACE, Stratton G, Raben A, Westerterp-Plantenga MS, Adam
353 TC. Associations of Brain Reactivity to Food Cues with Weight Loss, Protein Intake and Dietary
354 Restraint during the PREVIEW Intervention. *Nutrients*. 2018 Nov 15;10(11).
- 355 Du F, Higginbotham DA, White BD. Food intake, energy balance and serum leptin concentrations in
356 rats fed low-protein diets. *J Nutr* 2000;130:514–21.
- 357 Even PC, Rolland V, Feurté S, Fromentin G, Roseau S, Nicolaïdis S, Tomé D. Postprandial metabolism
358 and aversive response in rats fed a threonine-devoid diet. *Am J Physiol Regul Integr Comp Physiol*.
359 2000 Jul;279(1):R248-54.
- 360 Faipoux R, Tome D, Gougis S, Darcel N, Fromentin G. Proteins activate satiety-related neuronal
361 pathways in the brainstem and hypothalamus of rats. *J Nutr* 2008;138:1172–8.
- 362 Feurté S, Tomé D, Gietzen DW, Even PC, Nicolaïdis S, Fromentin G. Feeding patterns and meal
363 microstructure during development of a taste aversion to a threonine devoid diet. *Nutr Neurosci*.
364 2002 Sep;5(4):269-78.
- 365 Feurte S, Nicolaidis S, Even PC, Tome D, Mahe S, Fromentin G. Rapid fall in plasma threonine
366 followed by increased intermeal interval in response to first ingestion of a threonine-devoid diet in
367 rats. *Appetite*. 1999 Dec;33(3):329-41.
- 368 Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonov A, Flier JS,
369 Maratos-Flier E, and Spiegelman BM. FGF21 regulates PGC-1alpha and browning of white adipose
370 tissues in adaptive thermogenesis. *Genes Dev* 26: 271-281, 2012.
- 371 Forney LA, Wanders D, Stone KP, Pierse A, and Gettys TW. Concentration-dependent linkage of
372 dietary methionine restriction to the components of its metabolic phenotype. *Obesity (Silver Spring,*
373 *Md* 25: 730-738, 2017.
- 374 Fromentin G, Darcel N, Chaumontet C, Marsset-Baglieri A, Nadkarni N, Tome D. Peripheral and
375 central mechanisms involved in the control of food intake by dietary amino acids and proteins. *Nutr*
376 *Res Rev* 2012;25:29–39.
- 377 Gibson EL, Wainwright CJ, Booth DA. Disguised protein in lunch after low-protein breakfast
378 conditions food-flavor preferences dependent on recent lack of protein intake. *Physiol Behav*
379 1995;58:363–71.
- 380 Gietzen DW, Hao S, Anthony TG. Mechanisms of food intake repression in indispensable amino acid
381 deficiency. *Ann Rev Nutr* 2007; 27:63–78.

- 382 Gibson EL, Booth DA. Acquired protein appetite in rats: dependence on a protein-specific need state.
383 *Experientia* 1986;42:1003–4.
- 384 Gosby AK, Conigrave AD, Raubenheimer D, Simpson SJ. Protein leverage and energy intake. *Obes*
385 *Rev.* 2014 Mar;15(3):183-91.
- 386 Griffioen-Roose S, Mars M, Siebelink E, Finlayson G, Tome D, de Graaf C. Protein status elicits
387 compensatory changes in food intake and food preferences. *Am J Clin Nutr* 2012;95:32–8.
- 388 Griffioen-Roose S, Smeets PA, van den Heuvel E, Boesveldt S, Finlayson G, de Graaf C. Human protein
389 status modulates brain reward responses to food cues. *Am J Clin Nutr* 2014;100:113–22.
- 390 Hao S, Sharp JW, Ross-Inta CM, et al. Uncharged tRNA and sensing of amino acid deficiency
391 in mammalian piriform cortex. *Science* 2005; 307:1776–1778.
- 392 Hill CM, Berthoud HR, Munzberg H, and Morrison CD. Homeostatic sensing of dietary protein
393 restriction: A case for FGF21. *Frontiers in neuroendocrinology*, 2018.
- 394 Hill CM, Laeger T, Albarado DC, McDougal DH, Berthoud HR, Munzberg H, and Morrison CD. Low
395 protein-induced increases in FGF21 drive UCP1-dependent metabolic but not thermoregulatory
396 endpoints. *Scientific reports* 7: 8209, 2017.
- 397 Hotta Y, Nakamura H, Konishi M, Murata Y, Takagi H, Matsumura S, Inoue K, Fushiki T, Itoh N.
398 Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for
399 ketogenesis and triglyceride clearance in liver. *Endocrinology* 2009;150:4625–33.
- 400 Jean C, Fromentin G, Tomé D, Larue-Achagiotis C. Wistar rats allowed to self-select macronutrients
401 from weaning to maturity choose a high-protein, high-lipid diet. *Physiology & behavior*. 2002;76:65–
402 73.
- 403 Jean C, Rome S, Mathe V, Huneau JF, Aattouri N, Fromentin G, Achagiotis CL, Tome D. Metabolic
404 evidence for adaptation to a high protein diet in rats. *J Nutr* 2001;131:91–8.
- 405 Journal M, Chaumontet C, Darcel N, Fromentin G, Tome D. Brain responses to high-protein diets. *Adv*
406 *Nutr* 2012;3:322–9.
- 407 Kharitonov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE,
408 Hammond LJ, Moyers JS, Owens RA, et al. FGF-21 as a novel metabolic regulator. *J Clin Invest*
409 2005;115: 1627–35.
- 410 Kinzig KP, Hargrave SL, Hyun J, Moran TH. Energy balance and hypothalamic effects of a high-
411 protein/low-carbohydrate diet. *Physiol Behav.* 2007;92:454–60.

- 412 Laeger T, Albarado DC, Burke SJ, Trosclair L, Hedgepeth JW, Berthoud HR, Gettys TW, Collier JJ,
413 Munzberg H, and Morrison CD. Metabolic Responses to Dietary Protein Restriction Require an
414 Increase in FGF21 that Is Delayed by the Absence of GCN2. *Cell reports* 16: 707-716, 2016.
- 415 Laeger T, Henagan TM, Albarado DC, Redman LM, Bray GA, Noland RC, Munzberg H, Hutson SM,
416 Gettys TW, Schwartz MW, et al. FGF21 is an endocrine signal of protein restriction. *J Clin Invest*
417 2014;124:3913–22.
- 418 Lees EK, Krol E, Grant L, Shearer K, Wyse C, Moncur E, Bykowska AS, Mody N, Gettys TW, and
419 Delibegovic M. Methionine restriction restores a younger metabolic phenotype in adult mice with
420 alterations in fibroblast growth factor 21. *Aging Cell* 13: 817-827, 2014.
- 421 Leidy HJ, Lepping RJ, Savage CR, Harris CT. Neural responses to visual food stimuli after a normal vs.
422 higher protein breakfast in breakfast skipping teens: a pilot fMRI study. *Obesity (Silver Spring)*
423 2011;19: 2019–25.
- 424 Leidy HJ, Ortinau LC, Douglas SM, Hoertel HA. Beneficial effects of a higher-protein breakfast on the
425 appetitive, hormonal, and neural signals controlling energy intake regulation in overweight/obese,
426 "breakfast-skipping," late-adolescent girls. *Am J Clin Nutr.* 2013 Apr;97(4):677-88.
- 427 Leidy HJ, Clifton PM, Astrup A, Wycherley TP, Westerterp-Plantenga MS, Luscombe-Marsh ND,
428 Woods SC, Mattes RD. The role of protein in weight loss and maintenance. *Am J Clin Nutr.* 2015
429 Jun;101(6):1320S-1329S.
- 430 L'Heureux-Bouron D, Tome D, Bensaid A, Morens C, Lacroix M, Huneau JF, Fromentin G.
431 Preabsorptive factors are not the main determinants of intake depression induced by a high-protein
432 diet in the rat. *Physiol Behav* 2004;81:499–504.
- 433 Leung PMB, Rogers QR. 1986. Effect of amino acid imbalance and deficiency on dietary choice
434 patterns of rats. *Physiol. Behav.* 37:747–58
- 435 Makarios-Lahham, L.; Roseau, S.M.; Fromentin, G.; Tome, D.; Even, P.C. Rats free to select between
436 pure protein and a fat-carbohydrate mix ingest high-protein mixed meals during the dark period and
437 protein meals during the light period. *J. Nutr.* 2004, 134, 618–624.
- 438 Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, Mohammadi M,
439 Potthoff MJ. Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and
440 overfeeding. *Diabetes* 2014;63:4057–63.

- 441 Mashili FL, Austin RL, Deshmukh AS, Fritz T, Caidahl K, Bergdahl K, Zierath JR, Chibalin AV, Moller DE,
442 Kharitononkov A, et al. Direct effects of FGF21 on glucose uptake in human skeletal muscle:
443 implications for type 2 diabetes and obesity. *Diabetes Metab Res Rev* 2011;27:286–97.
- 444 Min DK, Tuor UI, Koopmans HS, Chelikani PK. Changes in differential functional magnetic resonance
445 signals in the rodent brain elicited by mixed-nutrient or protein-enriched meals. *Gastroenterology*.
446 2011; 141:1832–41.
- 447 Morrison CD, Xi X, White CL, Ye J, Martin RJ. Amino acids inhibit Agrp gene expression via an mTOR-
448 dependent mechanism. *Am J Physiol Endocrinol Metab*. 2007;293:E165–71.
- 449 Nieuwenhuizen AG, Hochstenbach-Waelen A, Veldhorst MA, Westerterp KR, Engelen MP, Brummer
450 RJ, Deutz NE, Westerterp-Plantenga MS. Acute effects of breakfasts containing alpha-lactalbumin, or
451 gelatin with or without added tryptophan, on hunger, 'satiety' hormones and amino acid profiles. *Br J*
452 *Nutr*. 2009 Jun;101(12):1859-66.
- 453 Paddon-Jones D, Leidy H. Dietary protein and muscle in older persons. *Curr Opin Clin Nutr Metab*
454 *Care*. 2014 Jan;17(1):5-11.
- 455 Pezeshki A, Zapata RC, Singh A, Yee NJ, and Chelikani PK. Low protein diets produce divergent effects
456 on energy balance. *Scientific reports* 6: 25145, 2016.
- 457 Pfaffly J, Michaelides M, Wang GJ, Pessin JE, Volkow ND, Thanos PK. Leptin increases striatal
458 dopamine D2 receptor binding in leptin deficient obese (ob/ob) mice. *Synapse* 2010;64:503–10.
- 459 Rogers QR, Leung PM. The influence of amino acids on the neuroregulation of food intake. *Fed Proc*
460 1973;32:1709–19.
- 461 Rothwell NJ, and Stock MJ. Influence of carbohydrate and fat intake on diet-induced thermogenesis
462 and brown fat activity in rats fed low protein diets. *J Nutr* 117: 1721-1726, 1987.
- 463 Sarruf DA, Thaler JP, Morton GJ, German J, Fischer JD, Ogimoto K, Schwartz MW. Fibroblast growth
464 factor 21 action in the brain increases energy expenditure and insulin sensitivity in obese rats.
465 *Diabetes* 2010;59:1817–24.
- 466 Skibicka KP, Dickson SL. Ghrelin and food reward: the story of potential underlying substrates.
467 *Peptides* 2011;32:2265–73.
- 468 Sørensen A, Mayntz D, Raubenheimer D, Simpson SJ. Protein-leverage in mice: the geometry of
469 macronutrient balancing and consequences for fat deposition. *Obesity (Silver Spring)*. 2008
470 Mar;16(3):566-71.

- 471 Schwarz J, Burguet J, Rampin O, Fromentin G, Andrey P, Tome D, Maurin Y, Darcel N. Three-
472 dimensional macronutrient-associated Fos expression patterns in the mouse brainstem. PLoS ONE.
473 2010;5:e8974.
- 474 Tomé D, Schwarz J, Darcel N, Fromentin G. Protein, amino acids, vagus nerve signaling, and the brain.
475 Am J Clin Nutr. 2009;90:838S–43S.
- 476 Veldhorst MA, Nieuwenhuizen AG, Hochstenbach-Waelen A, Westerterp KR, Engelen MP, Brummer
477 RJ, Deutz NE, Westerterp-Plantenga MS. A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP
478 lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. Clin
479 Nutr. 2009 Apr;28(2):147-55.
- 480 Vinales KL, Begaye B, Bogardus C, Walter M, Krakoff J, Piaggi P. FGF21 is a Hormonal Mediator of the
481 Human "Thrifty" Metabolic Phenotype. Diabetes. 2018 Sep 26. pii: db180696. doi: 10.2337/db18-
482 0696.
- 483 Wanders D, Forney LA, Stone KP, Burk DH, Pierse A, and Gettys TW. FGF21 Mediates the
484 Thermogenic and Insulin-Sensitizing Effects of Dietary Methionine Restriction but Not Its Effects on
485 Hepatic Lipid Metabolism. *Diabetes* 66: 858-867, 2017.
- 486 Wanders D, Stone KP, Dille K, Simon J, Pierse A, and Gettys TW. Metabolic responses to dietary
487 leucine restriction involve remodeling of adipose tissue and enhanced hepatic insulin signaling.
488 *BioFactors* 41: 391-402, 2015.
- 489 Westerterp-Plantenga MS, Nieuwenhuizen A, Tome D, Soenen S, Westerterp KR. Dietary protein,
490 weight loss, and weight maintenance. Annu Rev Nutr 2009;29:21–41.
- 491 White BD, He B, Dean RG, Martin RJ. Low protein diets increase neuropeptide Y gene expression in
492 the basomedial hypothalamus of rats. J Nutr 1994;124:1152–60.
- 493 White, B. D., Dean, R. G. & Martin, R. J. (1998) An association between low levels of dietary protein,
494 elevated NPY gene expression in the basomedial hypothalamus and increased food intake. Nutr.
495 Neurosci. 1: 173–182.
- 496 Zeeni N, Nadkarni N, Bell JD, Even PC, Fromentin G, Tome D, Darcel N. Peripherally injected
497 cholecystokinin-induced neuronal activation is modified by dietary composition in mice. Neuroimage.
498 2010;50:1560–5.
- 499
- 500

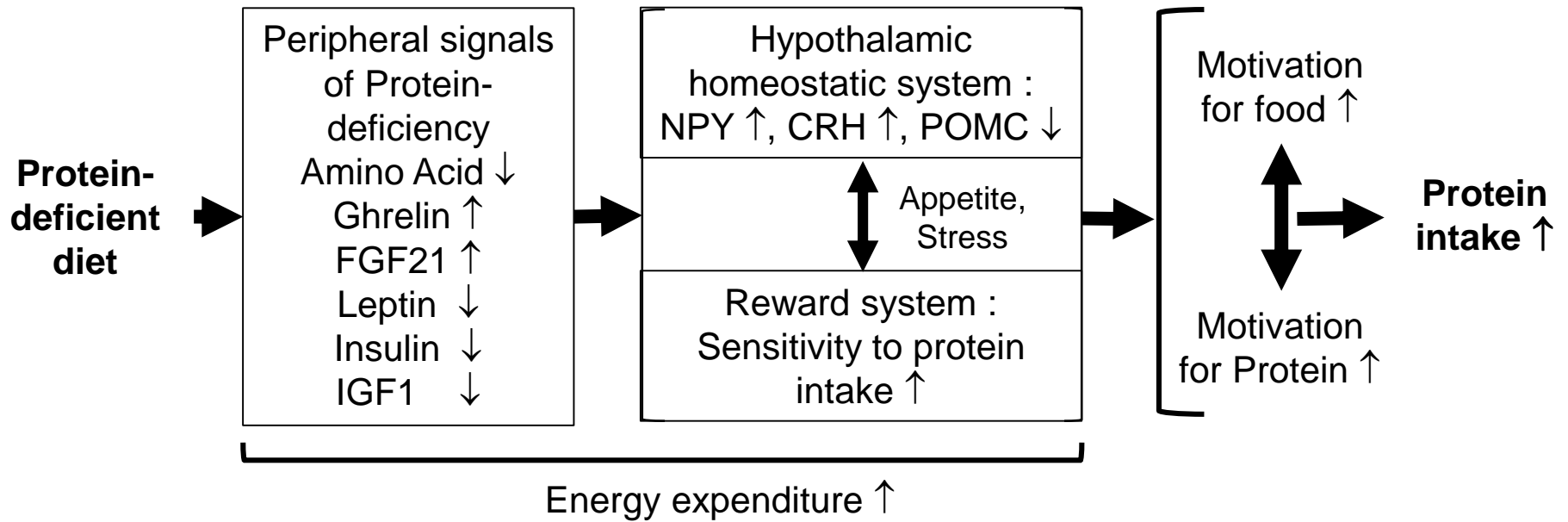
501 **Legend to figures**

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



**Adequate
to High
Protein
diet**

