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## Intestinal gluconeogenesis: metabolic benefits make sense in the light of evolution

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► **To cite this version:**

Amandine Gautier-Stein, Gilles Mithieux. Intestinal gluconeogenesis: metabolic benefits make sense in the light of evolution. *Nature reviews. Gastroenterology & hepatology*, 2023, 20 (3), pp.183-194. 10.1038/s41575-022-00707-6 . hal-04272015

**HAL Id: hal-04272015**

**<https://hal.science/hal-04272015>**

Submitted on 5 Dec 2023

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## 1. INTRODUCTION

The control of plasma glucose level is one of the most demanding homeostatic functions of physiology. Physiological glycaemia ranges between 0.7 and 1.1g/L in post-absorptive state in human and in most mammals, and up to 2-2.5 g/L during food assimilation, this value may vary according to the meal content in carbohydrates. Strikingly, the first signs of neurogluopenia rapidly occur whether glycaemia drops below 0.5g/L. It is generally thought that the energetic supply from glucose to cells is the reason of this demand in plasma glucose level. Instead, we recently argued that glucose could be essential through the supply in 3 carbon-building blocks to the synthesis of amino acids and nucleotides, or other metabolites or proteins that are vital for the cell life, while energy requirements could be more relevantly fulfilled from other sources like lipid-derived metabolites <sup>1</sup>.

Whatever could be the reason for the demand in glucose, ultimately, the source of plasma glucose is food. Therefore, it seems almost paradoxical that humans can undergo fasting for several weeks (up to about 2 months), without any problematic decrease in plasma glucose. The function that makes this possible is called gluconeogenesis, which refers to the capacity of the body to synthesize glucose *de novo* from non-glucidic compounds. Thus, gluconeogenesis is a crucial function for survival. Three organs of the body have the capacity to synthesize glucose and release it in blood: the liver, the kidney and the intestine, since they are the only organs known to express glucose-6 phosphatase (G6Pase), the enzyme dephosphorylating glucose-6 phosphate to produce glucose and inorganic phosphate <sup>1</sup>.

While the gluconeogenic capacity of the liver is known since more than 150 years <sup>2</sup>, that of the kidney has been uncovered in the mid of the 20<sup>th</sup> century <sup>3</sup>, and that of the intestine even more recently, at the end of the nineties <sup>4,5</sup>. It is noteworthy that the discovery of the presence of G6Pase in the rat and human guts <sup>4</sup> and of the evidence of intestinal gluconeogenesis (IGN) in the rat <sup>5</sup> was rapidly followed by the demonstration of the possibility of IGN in humans, during the anhepatic phase of liver transplantation <sup>6</sup>. It must be mentioned that the authors stated that given the analytical precision of the tracer approach utilized, it could not be formally excluded that all glucose produced could derive from the kidney <sup>6</sup>. More recently, IGN was also suggested to take place in obese insulin-resistant patients after they underwent gastric bypass surgery <sup>7,8</sup>.

From a quantitative viewpoint, organ-specific glucose fluxes were estimated *in vivo* using metabolic tracers or organ glucose balance. In the fed post-absorptive state, i.e. at the end

63 of the assimilation of the last meal and between the meals, the liver contributes to about  
64 80% of EGP in rats, through the handling of glycogen stores, a property that the kidney and  
65 intestine have not <sup>1</sup>. In the post-absorptive state, a consensus from previous human and dog  
66 studies has been that the kidney could represent about 20% of EGP in post-absorptive state <sup>9</sup>  
67 . Similar values were later determined in rat <sup>10</sup>. Once fasting takes place, it was early  
68 suggested, from comparisons of splanchnic and kidney glucose balance after 5-6 weeks of  
69 partial but severe (10% of normal calorie intake) fasting in young obese patients, that the  
70 human kidney could increase its contribution to EGP to represent about 50% of glucose  
71 produced in fasting humans <sup>11</sup>. Comparable values were determined later in rodents fasted  
72 for 24-72h, from a glucose tracer approach <sup>10</sup>. It must be mentioned that the net kidney  
73 glucose balance is close to 0, which reveals that the kidney releases the same amount of  
74 glucose as it consumes. This is also true for IGN in several instances, with the exception of  
75 gastric bypass surgery (see below). In the same time, the rat intestine also increases its  
76 participation to EGP during fasting, to represent 20-25% of the total amount of glucose  
77 produced in rats. Consistently with the variations of IGN during fasting, the expression of  
78 intestinal gluconeogenesis genes (*G6pc1*, coding the catalytic subunit of G6Pase and *Pck1*,  
79 coding phosphoenolpyruvate carboxykinase-cytosolic form) is hormonally regulated. It is  
80 inhibited by insulin <sup>4</sup> and activated by glucagon and glucocorticoids <sup>12</sup>.

81 It is not insignificant that IGN releases glucose in blood in the most richly innervated part of  
82 the body, apart from the brain. A major role of the gastrointestinal nervous system relates to  
83 the sensing of nutrients and metabolites from food to regulate energy homeostasis. For  
84 instance, that glucose is sensed by the nervous system surrounding the portal vein (the so-  
85 called « portal glucose signal ») and curbs hunger, is long known <sup>13-15</sup>. Thus, among the  
86 multiple nutrient sensing mechanisms taking place within the gastrointestinal system, IGN  
87 has been recognized as a possible relay of some nutrients to signal to the brain and influence  
88 energy homeostasis <sup>16-18</sup>, e.g., for recent reviews). It is a first purpose of this review to  
89 provide a comprehensive view of the nutritional regulation of IGN and of the central and  
90 peripheral effects by which the function positively influences energy homeostasis *via* gut-  
91 brain-periphery circuits. A second purpose relates to the central mechanisms by which IGN  
92 may account for the modulation of behaviour by food quality. Finally, we will discuss how  
93 the metabolic benefits associated with this function in fed and fasting states may explain  
94 why it has been retained by a mechanism of natural selection.

95

96

## 2. METABOLIC CONTROL

97

### 2.1. Regulation mechanisms by nutrition

98

#### *a. Protein-enriched diets*

99 Protein-enriched diets (PED) have long been associated with satiety in animals and humans  
100 <sup>19,20</sup>. A body of evidence has suggested that PED could exert their satiety action via the  
101 activation of IGN and the portal glucose signal. The proof of this concept was provided from  
102 experiments in the rat <sup>21</sup>. It was shown that the expression of the regulatory genes of  
103 gluconeogenesis (*G6pc1* and *Pck1*) is increased on PED in the kidney <sup>10</sup> and intestine <sup>21</sup>, while  
104 the outflow of glucose from the gut is increased in the post-absorptive period to about 20%  
105 of EGP, such a flow being sufficient to decrease food intake <sup>21</sup>. This was associated with the  
106 activation of the main hypothalamic areas involved in the control of energy homeostasis.  
107 The key role of the gastrointestinal nervous system was ascertained from experiments of  
108 chemical denervation, which prevented the decrease in food intake from taking place <sup>21</sup>.  
109 Satiety has been the readout used to investigate the mechanisms by which PED activates the  
110 expression of IGN regulatory genes. Proteins are not absorbed by the gut mucosa under the  
111 form of amino acids but, instead, under the form of small peptides containing at least 2  
112 amino acids. The bulk of peptides are hydrolysed to amino acids within the mucosa but a  
113 fraction traverses the mucosa to reach the portal blood *via* peptide transporters <sup>22</sup>. Thus, it  
114 was shown that under PED, peptides released in blood bind to and antagonize  $\mu$ -opioid  
115 receptors that are present in the periportal nervous system (**Figure 1**). This activates a neural  
116 reflex arc resulting in the induction of IGN gene expression, which does not take place  
117 whether the gastrointestinal system has been previously inactivated<sup>23</sup>. The causal role of IGN  
118 in the satiety effects of dietary protein was further established from experiments involving a  
119 mouse with targeted deletion of intestinal G6Pase (IG6Pase-KO mice). These mice, indeed,  
120 are insensitive to the hunger-curbing effect of either PED <sup>24</sup> or of infused peptides <sup>23</sup>.

121

122

#### *b. Fibre-enriched diets*

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126

It is long known that dietary fibres exert anti-obesity and anti-diabetic effects in humans  
<sup>25,26</sup>. Dietary fibres are found principally in fruit and vegetables. They are non digestible by  
the enzymes present in the proximal gut of mammals. Thus, they traverse the proximal gut  
to reach the distal portions, where they are fermented by the intestinal microbiota. The

127 most important metabolites produced by the fermentation process are short-chain fatty  
128 acids (SCFAs). Especially propionate and butyrate are considered to represent key relays in  
129 the metabolic benefits of fibres <sup>17</sup>. The mechanism was not known, before IGN has been  
130 uncovered. Propionate had long been described as a possible systemic gluconeogenic  
131 substrate. Since the intestinal mucosa is located between the intestinal lumen and the body,  
132 it was hypothesized that propionate could be converted in glucose by IGN and induce its  
133 benefits by this way, instead of reaching the liver *via* the portal vein. While intestinal glucose  
134 release was around 5% of EGP with a starch-based control diet <sup>27</sup>, supplementing food with  
135 10% (w/w) fibre (as fructo-oligosaccharide) or 5% (w/w) propionate increased intestinal  
136 glucose release at the same level as that determined for PED, i.e. around 17% and 22 % of  
137 EGP, respectively <sup>28</sup>. That propionate is an IGN substrate in this situation was established  
138 from the use of carbon-labelled propionate, which was significantly incorporated into  
139 glucose by passage through the gut mucosa <sup>28</sup>. Moreover, propionate binds to free fatty acid  
140 receptor 3 (also called G protein-coupled receptor 41) present in gastrointestinal nerves to  
141 induce a neural loop recruiting the autonomous nervous system (**Figure 1**). This promotes  
142 the release of the parasympathetic neuromediator vasoactive peptide 1 (VIP) that activates  
143 the cAMP-dependent expression of IGN regulatory genes in the gut mucosa <sup>29</sup>. In parallel,  
144 butyrate contributes to the cAMP-induced activation of expression of IGN regulatory genes,  
145 *via* an ATP-induced activation of adenylate cyclase, independently of any receptor-mediated  
146 process <sup>28</sup> (**Figure 1**).

147 Interestingly, succinate, the precursor of propionate in the bacterial metabolism <sup>30</sup> also  
148 serves as a substrate of IGN, whereas succinate has no action to regulate the expression of  
149 IGN genes <sup>31</sup>. It is noteworthy that the role of succinate to stimulate IGN was recently  
150 extended to allosteric activation of fructose-1,6 bisphosphatase, a well-known regulatory  
151 enzyme of gluconeogenesis in the liver <sup>32</sup>. Since acute regulations of G6Pase activity exist in  
152 the liver <sup>33-35</sup>, the hypothesis that short-term regulation of G6Pase could also exist in the gut  
153 and concur to the control of IGN is attractive and deserves future consideration.

154 It is noteworthy that a general belief in the field is that a change in the microbiota taxonomy  
155 could be a key in the metabolic benefits of dietary fibre. Instead, our data have suggested  
156 that the benefits of fibre may take place from very different microbiota compositions (i.e.  
157 hypercaloric diet versus control starch-based diet), provided that IGN is present to initiate

158 the gut-to-brain glucose signal <sup>28</sup>. Detailed interactions among IGN, SCFAs and gut  
159 microbiota were discussed in a recent review (Mithieux, TEM, 2022).

### 161 *c. Gastric bypass surgery*

162 Gastric bypass (GBP) surgery of obesity is another situation of dramatic nutritional change of  
163 nutrient delivery to the gut that is associated with spectacular amelioration in body weight  
164 and glucose control <sup>36</sup>. Deciphering the mechanisms behind has not been an easy task. A  
165 number of studies using genetic mouse models have been unsuccessful <sup>37</sup>. A possible reason  
166 could be that the loss of energy in feces of mice fed with hypercaloric diets is very important,  
167 which is sufficient to hide the effect of genotype <sup>37</sup>. It was by an approach of denervation of  
168 the portal vein that we could highlight that the activation of IGN is tightly associated with  
169 the rapid amelioration of insulin signalling, especially in relation with hepatic glucose  
170 production, in wild-type mice fed with a hypercaloric diet <sup>38</sup>. However, IGN had no role in the  
171 restoration of glucagon-like peptide 1 and insulin secretions, which should therefore depend  
172 on separate processes. It is noteworthy that the GBP-induced activation of IGN was rapidly  
173 confirmed in diabetic rat models of GBP <sup>39-41</sup>.

174 Demonstrating the causal role of IGN in GBP in humans is almost impossible. However, a  
175 very important report was the demonstration of the existence of IGN in the post-absorptive  
176 state after Roux-en-y GBP in obese insulin-resistant humans, which was inferred from the  
177 higher plasma glucose concentration in the portal vein compared to peripheral blood in  
178 post-absorptive state <sup>7,8</sup>. It could be estimated from the intestinal arterio-venous difference  
179 in plasma glucose concentration (0.2 mM) and the blood flow in the human intestine  
180 (around 1L/min) that IGN in this situation could represent at least 25% of EGP <sup>8</sup>. Importantly,  
181 a recent study indicated that the expression of IGN genes is positively associated with the  
182 scale of the benefits in glucose homeostasis after GBP surgery in obese human subjects <sup>42</sup>.

## 184 **2.2. Metabolic benefits**

### 185 *a. Management of body weight*

186 A common feature of the three nutritional situations described above is the favourable  
187 management of body weight <sup>25,26,36,43,44</sup>. A major target is lipid storage in the white adipose  
188 tissue (WAT) (**Figure 2**). The latter is minored upon PED <sup>43</sup>, upon fibre-enriched diets, and  
189 dramatically decreased upon GPB <sup>36,44</sup>. That IGN has a causal role in this feature was

190 ascertained from experiment in rodents involving the denervation of the portal vein or the  
191 suppression of intestinal G6Pase (by deletion of *G6pc1*), which both cancelled the benefits of  
192 related diets or of GBP on body weight<sup>21,23,24,28,31</sup>.

193 Using a novel model of overexpression of G6Pase specifically in the intestine (IG6Pase-  
194 overexpressing mouse), we could document the mechanisms behind the benefits of the IGN-  
195 brain circuit on lipid storage in the context of hypercaloric feedings<sup>45</sup>. A combination of the  
196 activation of thermogenesis in the brown adipose tissue (BAT) and of a browning process in  
197 the WAT (especially epididymal) results in an increase in energy expenditure and conferred  
198 on mice a markedly increased resistance to cold<sup>45</sup> (**Figure 2**). On the contrary, the deficiency  
199 in IGN in IG6Pase-KO mice promotes defects in lipid oxidation and thermogenesis. This is  
200 sufficient to increase lipid storage in the WAT (without obesity), even in the context of a  
201 normocaloric diet<sup>45</sup>. It is noteworthy that increased IGN promotes the activation of gene  
202 expression and secretion of adiponectin, a well-known metabolically favourable adipokine,  
203 more especially of its biologically active high-molecular weight form. Additional benefits of  
204 IGN-overexpression relate to the prevention of inflammation and fibrosis in the WAT<sup>45</sup>.

205

#### 206 *b. Management of glucose control*

207 There are two components in the improvements of glucose and insulin tolerance promoted  
208 by IGN in rodents fed on PED<sup>10</sup> or dietary fibers<sup>28</sup>, undergoing GBP<sup>38</sup>, or overexpressing IGN  
209<sup>46</sup>. The first relates to the enhancement of insulin sensitivity, especially at the hepatic level.  
210 This could be demonstrated, e.g., through increased suppression of EGP by insulin during  
211 hyperinsulinemic euglycemic clamp studies<sup>10,38</sup>, improved insulin tolerance and increased  
212 phosphorylation state of hepatic AKT, the intracellular insulin signalling mediator<sup>46</sup> or  
213 decreased activity of hepatic G6Pase<sup>28</sup>. Conversely, the suppression of IGN in IG6Pase-KO  
214 mice results in defective insulin suppression of EGP during hyperinsulinemic euglycemic  
215 clamp and decreased phosphorylation of hepatic AKT<sup>47</sup>.

216 The second component relates to insulin secretion. Mice deficient in IGN exhibit defective  
217 insulin secretion, which could be dependent on inappropriate alpha-adrenergic regulation<sup>47</sup>.  
218 *In vivo*, the early peak of insulin secretion in response to glucose injection is blunted (**Figure**  
219 **2**). *In vitro* in isolated islets, the glucose-stimulated insulin secretion is blunted as well. Both  
220 defects are corrected in IG6Pase-KO mice infused with glucose in the portal vein at a rate  
221 mimicking IGN<sup>47</sup>. Therefore, it is likely that IGN might exert beneficial action on whole body



222 glucose control both via insulin action on hepatic glucose production and via optimal control  
223 of insulin secretion. As a consequence, hyperinsulinemia and hyperglycemia develop more  
224 rapidly in IG6Pase-KO mice than in WT mice upon feeding a hypercaloric diet <sup>47</sup>. This  
225 strongly suggests that IGN deficiency promotes proneness to insulin resistance and diabetes  
226 in mice.

227

### 228 *c. Prevention of NAFLD*

229 It is noteworthy that the prevention of lipid storage deriving from IGN is not limited to  
230 adipose depots. Indeed, a dramatic reduction in lipid accumulation under hypercaloric diet  
231 also relates to the liver in IG6Pase-overexpressing mice <sup>46</sup> (**Figure 2**). Hepatic steatosis is a  
232 serious problem accompanying obesity. While it can remain without consequences on  
233 hepatic health, it may often initiate the sequence of non-alcoholic fatty liver disease (NAFLD)  
234 beginning with inflammation to evolve towards fibrosis and sometimes cirrhosis or hepatic  
235 carcinoma <sup>48</sup>.

236 Contrasting IG6Pase-overexpressing mice with wild type mice, we reported that increased  
237 IGN markedly prevents hepatic steatosis under hypercaloric diet. The prevention relates to  
238 the reduction of lipogenesis (lowered expression of acetyl CoA carboxylase and fatty acid  
239 synthase) and lipid import (lowered expression of the lipid transporter CD36) in the liver,  
240 associated with decreased local inflammation (lowered expression of gene encoding TNF $\alpha$ ,  
241 interleukin 6 and Mcp1) and fibrosis (lowered expression of vimentin and Acta2) <sup>46</sup>. It is  
242 noteworthy that the benefits promoted by IGN extend to plasma lipid parameters, plasma  
243 cholesterol being decreased in IG6Pase-overexpressing mice and plasma triglycerides  
244 increased in IG6Pase-KO mice <sup>46</sup>.

245 On the contrary, contrasting IG6Pase-KO mice with wild type mice, we observed that IGN  
246 deficiency is sufficient to promote lipogenesis, lipid import and inflammation feature the  
247 liver of IG6Pase-KO mice, even under normal chow feeding. This results in a moderate  
248 hepatic steatosis, associated with a moderate increase in plasma triglycerides<sup>46</sup>.

249 Taken together, these data suggest that the induction of intestinal G6Pase activity is  
250 sufficient in itself to induce IGN and to exert anti-obesity and anti-diabetic effects. Since IGN  
251 is efficient under conditions of established obesity and insulin resistance, it constitutes a  
252 novel target for the treatment of metabolic diseases <sup>1,38,46</sup>. This paves the way towards the

253 search for metabolites that can activate IGN in vivo as a basis for new drugs to combat  
254 metabolic disorders.

255

### 256 **3. NEURAL MECHANISMS**

#### 257 **3.1 Gastrointestinal glucose sensing**

258 First involved in the sensing of nutrients to activate IGN, i.e. via periportal sensitive nerves  
259 expressing  $\mu$ -opioid receptor or GPR41 and perimucosal effector nerves secreting VIP (see  
260 above), the periportal nervous system is also in action to sense glucose produced from IGN.  
261 Indeed, no effect of protein- or fiber-enriched diets and no effect of portal glucose infusion  
262 on food intake or metabolic control take place in rats after local denervation of the portal  
263 vein by capsaicin<sup>21,23,28,49</sup>. On the contrary, portal glucose infusion is effective in rats with  
264 sub-diaphragmatic surgical section of the vagal nerve. Moreover, portal glucose activates in  
265 the brainstem the lateral parabrachial nucleus (PBN), connecting nerves from the spinal  
266 cord, and not the nucleus of the solitary tract, connecting vagal sensitive nerves. This has  
267 suggested that the portal glucose signal could be conveyed not by the vagal nerve, but  
268 instead, by the splanchnic nerves and the spinal cord (**Figure 3**).

269 Three main systems of glucose sensing may operate inside the body, mediated by the  
270 facilitative glucose transporter 2 (GLUT2)<sup>50</sup>, sweet taste receptors<sup>49</sup> or sodium-coupled  
271 glucose transporter 3 (SGLT3), a glucose receptor first identified in the gastrointestinal  
272 nervous system<sup>51,52</sup>. Several arguments, including by not limited to the observation that  
273 mice knockout for *Glut2* or for *TRMP5* (coding for a protein required for the activity of taste  
274 receptors) are still sensitive to the satiety effect of PED have argued for a prominent role of  
275 SGLT3 in the sensing of portal glucose from IGN (<sup>49</sup>, see also <sup>1</sup> for further details). That SGLT3  
276 actually represents a specific sensor of IGN remains to be firmly established from  
277 appropriate targeted approaches of gene deletion.

278

#### 279 **3.2 Hypothalamic mechanisms**

280 It is of note that protein- or fibre-enriched diets or portal glucose activate the main regions  
281 of the hypothalamus involved in energy homeostasis, while no activation takes place in rats  
282 with periportal denervation or IGN-deficient mice. This includes, e.g., the arcuate nucleus,  
283 the ventromedial and dorsomedial nuclei, the lateral nucleus and the paraventricular  
284 nucleus<sup>21,23,28,49</sup> (**Figure 3**). That the PBN seem prominent in the transmission of the portal

285 glucose signal (see above) led us to hypothesize that calcitonin gene related peptide (CGRP)  
286 could have a role in IGN effects. Indeed, CGRP-expressing neurons of the PBN were  
287 previously shown to control food intake<sup>53</sup>. Accordingly, there is no effect on food intake of  
288 portal glucose infusion or fibre-enriched diet in *Cgrp*-knockout mice<sup>54</sup>. Moreover, since both  
289 CGRP and its receptor are expressed in the arcuate nucleus, we hypothesized that a target of  
290 CGRP could be proopiomelanocortin (POMC)-expressing neurons that we previously showed  
291 to be a target of portal glucose. Accordingly, portal glucose does not activate arcuate  
292 (POMC)-expressing neurons in *Cgrp*-KO mice, while in wild type mice it does<sup>54</sup>.

293 It was previously established that portal glucose activates the phosphorylation of signal  
294 transducer and activator of transcription 3 (STAT3), the classic intracellular messenger of  
295 leptin signaling<sup>47</sup>. In agreement with a key role of a CGRP-pSTAT3 mechanism in the portal  
296 signal action, glucose infusion in the portal vein or CCRP infusion in the third ventricle  
297 activates the phosphorylation of STAT3 in the arcuate nucleus of wild type mice, while in  
298 *Cgrp*-KO mice it does not<sup>54</sup>. These data strongly suggest that CGRP and STAT3 are links in the  
299 mechanistic chain by which portal glucose acts in the arcuate nucleus.

300

### 301 **3.3 Role of the sympathetic nerves**

302 Relating to the final IGN-signaling stage, i.e. the hypothalamic to periphery circuit, it was  
303 previously suggested that the efferent sympathetic nervous system is responsible for the  
304 control of lipogenesis in the liver<sup>55,56</sup> and of browning in the white adipose tissues (WAT)  
305 thermogenesis and energy expenditure<sup>57-60</sup>. In agreement with the latter concept, we  
306 specified that the activation of IGN in the IG6Pase-overexpressing mouse results in the liver  
307 in a large increase in the number of neurons expressing tyrosine hydroxylase (**Figure 2**), the  
308 key enzyme involved in the synthesis of catecholamine<sup>46</sup>. In the brown adipose tissue (BAT)  
309 and WAT of these mice, we observed a marked increase in the expression of neuronal  
310 tyrosine hydroxylase associated with an increased expression of the beta3-adrenergic  
311 receptor in adipose cells<sup>45</sup>. Moreover, local denervation of the BAT with the sympathetic  
312 toxin 6-hydroxydopamine (6-OHDA) suppresses resistance to cold in IG6Pase-overexpressing  
313 mice<sup>45</sup>. These data have strongly suggested that the final stage of IGN-derived benefits  
314 relates to the modulation of the activity of TH-expressing neurons of the SNS in peripheral  
315 IGN targets (**Figure 2**).

316

## 317 **4. MODULATION OF BEHAVIOR**

### 318 **4.1 Brain targets of IGN**

319 It is noteworthy that C-FOS labelling revealed that there are multiple brain targets of IGN  
320 and/or portal glucose signaling throughout the brain, in addition to the hypothalamus and  
321 the aforementioned nuclei connecting sensitive nerves in the brain stem, such as the PBN  
322 <sup>21,23,28</sup>. These relates to several brain areas involved in the so-called limbic system, which is  
323 considered a neuroanatomic and functional interface between the vegetative and the  
324 cognitive life. Especially, portal glucose activates the central amygdala, an important area,  
325 e.g., in fear conditioning and food aversion, and the nucleus accumbens, the orbitofrontal  
326 cortex and the olfactory bulb <sup>61</sup>, which are involved in reward processes and motivation <sup>62</sup>  
327 **(Figure 3)**. In agreement with the latter, portal glucose infusion was previously associated  
328 with a promotion of food preference, while the mechanism was not studied up to now <sup>14</sup>.  
329 Additional target areas of IGN in the brain relate to the dentate gyrus in the hippocampus <sup>63</sup>,  
330 which controls the hypothalamic-pituitary-adrenal (HPA) axis and anxiety-related behavior  
331 <sup>64,65</sup> **(Figure 3)**.

### 332

### 333 **4.2 IGN and anxiety-related behavior**

334 The impact of IGN on anxiety/stress-related behavior was investigated using standardized  
335 validated tests like the open field test or the forced swimming test <sup>63</sup>. We compared the  
336 effects of IGN gain of function under PED- or fibre-feeding and of the loss of function in  
337 IG6Pase-KO mice. The results were perfectly consistent. The gain of function of IGN  
338 decreases anxiety, promotes exploratory behavior and decreases neophobia (open field),  
339 and improves perseverance and decreases resignation (forced swimming). The loss of  
340 function exerts opposite actions on the same parameters, revealing that IGN-deficiency  
341 induces proneness to neophobia and anxiety/stress-related behavior, as it induces insulin  
342 resistance and diabetes (see above). In addition, IG6Pase-KO mice exhibit impaired retro-  
343 control of the HPA axis <sup>63</sup>. It is noteworthy that the link between insulin resistance and  
344 diabetes induced by hypercaloric feeding and anxiety-related behavior was previously  
345 established <sup>66</sup>.

346 On a molecular viewpoint, IGN deficiency promotes impairment in the differentiation stage  
347 of hippocampal neurogenesis, revealed by the decreased expression of doublecortine, a  
348 protein required for the normal migration of neurons, and of brain-derived neurotrophic

349 factor (BDNF). This initiates a decrease in the expression of the hippocampal glucocorticoid  
350 receptor, a major driver of the retro-control of the HPA axis, and consequently an increased  
351 expression of the precursor of corticotropin-releasing factor (CRH) in the hypothalamus.  
352 Consistently, these defects are corrected by portal glucose infusion <sup>63</sup>.  
353 Most importantly, both protein- and fibre-enriched diets increase the expression of the  
354 glucocorticoid receptor in the hippocampus of wild type mice, which does not place in  
355 IG6Pase-KO mice <sup>63</sup>. There is a vast literature reporting the benefits of dietary fibre or dietary  
356 protein on emotional behavior in rodents and in humans, while supportive mechanisms  
357 remained elusive <sup>67,68</sup>. It is noteworthy that the nutrient-induced expression of the  
358 hippocampal glucocorticoid receptor, with IGN as a central mechanistic link in this chain, has  
359 provided an underlying mechanism accounting for these behavioral benefits linked to food  
360 quality. This could be related to a potentiation of glucocorticoid signaling and of the retro-  
361 control of the HPA axis in the hippocampus. Finally, since brain areas like the orbitofrontal  
362 cortex and the hippocampus are important regions involved in memory and are also targets  
363 of IGN <sup>61,63</sup>, the hypothesis that IGN and nutrients might influence this key cognitive function  
364 deserves further investigation.

365

## 366 5. ATTEMPT AT AN EVOLUTIONARY PERSPECTIVE

367 Thus, IGN is a function providing benefits in relation with glucose metabolism and energy  
368 homeostasis and with emotional behaviour. It is noteworthy that these fields have already  
369 been popularly associated in the concept of “hanger” (a mix of hunger and anger) illustrating  
370 the more than frequent feeling of disquiet or irritability accompanying the feeling of hunger.  
371 The articulation between both feelings could be of special importance, as discussed below.

372

### 373 5.1. EGP and IGN in evolution

374 There exist 2 isoforms of G6PC1, the catalytic subunit of G6Pase, carrying out EGP and  
375 expressed in the liver, kidneys and intestine specifically <sup>1</sup>. G6PC2 is mainly expressed in the  
376 beta-cell of the islets of Langherans (insulin-secreting cells) and G6PC3 is ubiquitous. It is not  
377 the matter to discuss their function here; the important observation is that both of these  
378 isoforms have no gluconeogenic activity, i.e. no capacity to lead to glucose release in the  
379 bloodstream <sup>69</sup>. It is noteworthy that, seeking for orthologs of human G6PC1, one can find

380 them in about 2/3 of eukaryotes, 3/4 of metazoans, 95% of chords and 100% of vertebrates  
381 **(Figure 4A).**

382 Interestingly, ancestral G6PC that gave rise to the 3 G6PC isoforms is more closely related to  
383 the G6PC3 paralogs, while G6PC1 paralogs are only found in vertebrates **(Figure 4B)**. This in  
384 line with the dogma that the exchange of glucose between gluconeogenic and non-  
385 gluconeogenic organs, and thus the need of EGP, has featured organisms once a circulatory  
386 system has been a basis for life. Most importantly, the existence of IGN or of G6PC1 in the  
387 gut has been demonstrated in all vertebrates studied up to now, from the most evolved to  
388 the least evolved. This relates to humans <sup>4,7,8,42</sup> but also to various mammals as carnivores  
389 (cat, dog <sup>70,71</sup>) herbivores (cow, sheep, pig, rabbit <sup>72-75</sup>) and rodents (mouse, rat (see above),  
390 guinea pig <sup>76</sup>), to birds (hen <sup>77</sup> and also to various fishes (herbivorous as carp <sup>78</sup>, omnivorous  
391 like tilapia <sup>79</sup> or carnivorous like catfish of trout <sup>78,80</sup>). It is of interest that the regulation of  
392 intestinal expression of G6PC1 by dietary protein content of the diet was demonstrated in  
393 trout <sup>80</sup>. These data suggest that IGN and its nutritional regulation by nutrients have  
394 appeared in evolution with vertebrates and the process of EGP, i.e. about 500 million years  
395 ago. This excludes that its anti-obesity-diabetes properties may have an evolutionary role,  
396 since these metabolic diseases are human-related and recent. Therefore, the question arises  
397 as to what makes IGN so essential that it is has been maintained in all vertebrates by a  
398 process of natural selection.

399

## 400 **5.2. Importance of IGN in starvation**

401 One must have in mind that the metabolism of glucose-6 phosphate through glycolysis and  
402 the pentose phosphate shunt is a universal basis of living cells. Indeed, both pathways  
403 synthesize from the carbon-building blocks of glucose all molecules, such as amino-acids and  
404 nucleotides, necessary for the synthesis of proteins, enzymes, co-enzymes, mRNAs, DNA,  
405 without which cell life and division would not be possible. This makes of the maintenance of  
406 glucose in blood and thus gluconeogenesis a function absolutely crucial in resistance to  
407 starvation.

408 In this context, that IGN and renal gluconeogenesis significantly increase their contribution  
409 to EGP in relation to the liver during fasting presents several advantages <sup>5,11,27,81</sup>. Indeed,  
410 glutamine is a major substrate for both renal and intestinal gluconeogenesis <sup>5,9</sup>. On the  
411 contrary, the liver is unable to metabolize glutamine because due to its kinetic properties

412 liver glutaminase cannot use glutamine at physiological concentration<sup>82</sup>. Therefore, IGN and  
413 renal gluconeogenesis allow the body to engage glutamine carbons in gluconeogenesis, a  
414 process that the liver is unable to do itself. One must keep in mind that glutamine is the  
415 most abundant amino acid in blood and in muscular protein and is considered a major  
416 gluconeogenic reservoir of the body<sup>1</sup>.

417 Most importantly, relating to IGN, glutamate (produced from glutamine by glutaminase) is  
418 metabolized by glutamate-pyruvate carboxylase, a two-substrate enzyme utilizing pyruvate  
419 as a second substrate, and releasing alanine from pyruvate<sup>83,84</sup>. Pyruvate is also utilized by  
420 lactate dehydrogenase to produce lactate. Alanine and lactate, released in the portal vein,  
421 are then captured by the liver, in which they constitute the main substrates of  
422 gluconeogenesis. Therefore, a major consequence of the use of glutamine by IGN is the  
423 feeding of hepatic gluconeogenesis<sup>85</sup>.

424 In addition, it is a widely accepted dogma that gluconeogenesis is endergonic and consumes  
425 energy. This is indeed true for hepatic gluconeogenesis from lactate and alanine. On the  
426 contrary, glucose production from glutamine by renal and intestinal gluconeogenesis is  
427 exergonic and produces 4 ATP per mole of glucose synthesized<sup>85</sup>. Therefore, a major  
428 advantage of the replacement of hepatic gluconeogenesis by IGN and renal gluconeogenesis  
429 during starvation is that this permits to fulfill the need of glucose homeostasis and in the  
430 same time this preserves the energetic balance of the body.

431 At last, there is a rebound of hepatic glycogen stores as fasting prolongs<sup>33</sup>, a process in  
432 which the activation of IGN must unavoidably have a role. This could be crucial in the  
433 situations requiring an urging availability in blood glucose while starving. This relates for  
434 example to situations requiring to run, e.g. to escape a predator, to catch a prey or to fight.  
435 Therefore, the repartition of gluconeogenesis among the liver, kidney and intestine in  
436 fasting might be a key for survival in the wild.

437

### 438 **5.3. Importance of IGN in fed state**

439 Intestinal gluconeogenesis may have been essential in evolution also for the specificities of  
440 its nutritional regulation in the fed state. Indeed, IGN is activated during the post-absorptive  
441 period by two specific nutrient types, proteins and fibres (not complex carbohydrates like  
442 starch or lipids). Proteins are digested slowly compared to carbohydrates or lipids, and along  
443 the whole (proximal and distal) intestine. Fibres are digested lately since they must traverse

444 the proximal intestine before to be fermented in SCFAs in the distal gut. This explains that  
445 IGN activated by these nutrients takes place during the early and late post-absorptive period  
446 (see <sup>1</sup> for further discussion on the time course of the induction of IGN by nutrients). The  
447 other key feature of both proteins and fibres is that they provide *via* amino acids and  
448 microbiota-derived metabolites the carbon blocks to build glucose in the gut mucosa, by not  
449 only. Propionate is metabolised by the intestine essentially <sup>86</sup>. This could also be the case of  
450 glutamine and aspartate from luminal origin <sup>83</sup>. This is not the case for other amino acids  
451 that are only partly utilized by the intestine and can be released in blood to fuel  
452 gluconeogenesis in the liver and kidney. For example, alanine is released by the gut and is a  
453 major glucose precursor in the liver <sup>83</sup>. Amino acids and especially glutamine are thus the  
454 gluconeogenic reservoir of the body <sup>1</sup>. This is of special importance in the maintenance of  
455 plasma glucose in post-absorptive state and during the night, at a time when the liver  
456 glycogen is depleted. Thus, we have previously suggested that IGN could be a signal to  
457 inform the brain that food that has been ingested previously is suitable to satisfy glucose  
458 homeostasis for a while <sup>1</sup>.

459

#### 460 **5.4. What is the advantage provided in fed state?**

461 It is established that the fall in plasma glucose is a major driver of hunger <sup>87</sup>. Due to the  
462 intense glycolytic activity of the gut mucosa, hypoglycaemia primarily occurs in the portal  
463 vein once glucose from food is no longer released at the end of the postprandial period. This  
464 corresponds to the reset of hunger and of the associated energy sparing processes that are  
465 promoted by energy deficiency <sup>88</sup>. However, portal hypoglycaemia is hindered to take place  
466 whether IGN replaces glucose from food by glucose from gluconeogenic origin. This blunts  
467 the reset of hunger and of energy sparing processes that are promoted by the lack of food.  
468 Interestingly, these processes are mainly controlled by the melanocortin receptors of type 4  
469 and 3, respectively, in neurons of the paraventricular nucleus <sup>89</sup>, the latter being a key target  
470 of IGN <sup>21,23,28,49</sup>.

471 In itself, to feel less hungry and store less energy from food does not seem an advantage,  
472 unless it is associated with decreased anxiety and increased serenity and exploratory  
473 behaviour, as is the case for IGN <sup>63</sup>. One must bear in mind that the life in the wild is stressful  
474 almost all the time. The living beings must ceaselessly arbitrate between coping with hunger  
475 and the risk of starvation and escaping from eventual predators, both being stressful



476 themselves. Thus, foraging in a mental state of anxiety or fear occupies most of the waking  
477 time in animals (and in humans before discovering agriculture and livestock breeding). In this  
478 case, that nutrients previously ingested promote satiety and decrease anxiety offers a period  
479 of time of “serenity”. This could be very useful for example for exploring environment,  
480 finding a new food source, finding a new hiding place to protect themselves, or storing in a  
481 safe place food that has been collected but not yet eaten. This could also be useful to remain  
482 safely hidden instead of being forced to forage in a potentially hazardous environment  
483 because of the possible presence of predators. These could be very favourable for the  
484 survival of the individual. This could also provide some quiet time for breeding. This could be  
485 favourable for the survival of the species.

486

### 487 **5.5. IGN through human evolution**

488 In the Paleolithic period, before discovering agriculture and livestock breeding, dietary habits  
489 of hunters-gatherers were mainly based on the consumption of low-energy food composed  
490 of roots or tubers, berries, insects or grubs, and sometimes of fishes or small animals. This  
491 corresponds to a macronutrient composition very rich in fibre and protein, which has been  
492 estimated to represent for each about 3 times the content of current human diet <sup>90</sup>. This  
493 could be alternated with infrequent periods of food abundance, when available animal meat  
494 was abundant, which could from time to time increase the protein content of the diet by up  
495 to 60% of calories ingested <sup>90</sup>. It has even been estimated that humans (both sapiens and  
496 non-sapiens) had adopted a carnivorous type of feeding, requiring strong physiological  
497 adaptation, during the ice ages of the Paleolithic period <sup>91</sup>. These nutritional conditions were  
498 conducive to the frequent establishment of IGN, once the food had been assimilated (**Figure**  
499 **5**). The human diet has begun to change in the Neolithic period, with the gradual increase in  
500 dairy products (richer in fat) and crops (richer in starch), which was less and less conducive  
501 to the establishment of IGN.

502 Today, human eating habits, featured by regular meals comprising around 50%  
503 carbohydrates under the form of starch and sugars and 30-35 % lipids, are not conducive to  
504 the establishment of IGN between the meals and during the night (**Figure 5**). Indeed, IGN in  
505 the post-absorptive period was measured to amount to 0-5 % of EGP in rats under such  
506 nutritional conditions <sup>5,27,92</sup>. The exceptions, of course, are the specific nutritional conditions  
507 described in paragraph 2.1 above, under which IGN is induced.

508 In addition to the fact that the *G6pc1* gene was only identified in the 1990s<sup>1</sup>, this may be a  
509 further explanation of why IGN was not detected in human studies until its existence and  
510 nutritional regulation was discovered in rodents.

511

## 512 **5.6. Similitude with leptin features**

513 The resemblance between the physiology of IGN and that of leptin, an essential regulator of  
514 energy homeostasis released by the adipose tissue, are striking. Regarding energy  
515 metabolism, IGN like leptin opposes the hunger reset and energy storing processes  
516 promoted, e.g., by energy deficiency<sup>1,89</sup>. Regarding glucose control, IGN like leptin improves  
517 systemic glucose tolerance and hepatic insulin sensitivity and suppresses hepatic glucose  
518 production<sup>47,93</sup>. To do so, IGN like leptin activates the aforementioned hypothalamic areas  
519 and the parabrachial nucleus in the hindbrain<sup>49,94</sup>. Leptin and IGN also share a STAT3-  
520 phosphorylation process as an intracellular signalling pathway in the arcuate nucleus,  
521 especially in POMC-expressing neurons<sup>47,89</sup>. Leptin increases energy expenditure via a  
522 hypothalamic-driven activation of the sympathetic system in the BAT<sup>95</sup>, what IGN does as  
523 well<sup>45</sup>.

524 The similarities also relate to the anti-anxiety activity of IGN and leptin. The central  
525 amygdala, a target of IGN<sup>49</sup>, is also a target of leptin in relation with its anxiolytic-like  
526 activity<sup>96</sup>. The hippocampal neurogenesis is a well-known target of anti-depressants<sup>97</sup>.  
527 Hippocampal neurogenesis is defective in IGN-deficient mice and stimulated by IGN-  
528 activated by nutrients<sup>63</sup>. Moreover, hippocampal neurogenesis has been identified as a  
529 target of leptin in relation with the hormone anti-depressive-like features<sup>98</sup>.

530 Because of these similarities of effects, it is tempting to speculate that IGN and leptin should  
531 have related roles in evolution. It is noteworthy that the leptin signalling has appeared in  
532 vertebrates like IGN<sup>99</sup>. Leptin is widely regarded as a signal to the brain of the evolution of  
533 lipid energy reserves<sup>89,94,95,99</sup>. Accordingly, the decrease in circulating leptin promotes  
534 hunger and energy sparing processes when adipose storage decreases, which compromises  
535 survival. This is a long-term signage of special importance during fasting.

536 At the opposite end of the time scale, gastrointestinal hormones, like cholecystokinin and  
537 glucagon-like peptide 1, are secreted in response to the meal and signal to the brain the  
538 presence of food in the gut<sup>88</sup>. The presence of food results in decreased hunger and the  
539 absence of food in hunger reset<sup>88</sup>. This is a short-term signage, of importance in the fed

540 state. It is noteworthy that these two hormones regulate glucose homeostasis in the same  
541 sense as leptin and IGN<sup>100</sup> and also exhibit anti-anxiety properties<sup>101,102</sup>.

542 We propose that IGN fits ideally into the temporal sequence of signalling between  
543 gastrointestinal hormones and leptin (**Figure 6**). While gastrointestinal hormones signal to  
544 the brain the state of the food supply and leptin the state of the energy stores, IGN should  
545 signal the filling level of the gluconeogenic reservoir available from the food previously  
546 ingested. This could be a mid-term signage, delaying the reset of hunger and associated  
547 anxiety, with the potential benefits described above and sum up below.

548

## 549 **6. CONCLUSION**

550 Intestinal gluconeogenesis has been documented up to now in all vertebrates in which the  
551 question of its presence was raised. It contributes to EGP and maintenance of plasma  
552 glucose during fasting. In the fed state, it is a key signal to the brain. It is activated by  
553 nutrients previously ingested that are capable of fuelling gluconeogenesis and maintain  
554 plasma glucose during the post-absorptive period, such as dietary protein and fermentable  
555 fibres. Thus, it opposes the hunger reset and the energy sparing processes triggered by  
556 portal hypoglycaemia in absence of food. It also opposes the mental state of anxiety linked  
557 to foraging. The genetic activation of IGN via overexpression of intestinal G6Pase is sufficient  
558 in itself to oppose the pro-obesity and pro-diabetes effects of high-calorie foods. These  
559 metabolic benefits take place in insulin-resistant and obese-diabetic states in animal and  
560 humans. Therefore, IGN, a function from ancient times, represents a novel target for the  
561 future treatment of metabolic disorders.

562

## 563 **ACKNOWLEDGEMENTS:**

564 The authors would like to thank their team and the countless number of past members and  
565 collaborators, without whom the work analyzed herein would not have been possible, and  
566 more particularly Fabienne Rajas and Justine Vily-Petit for critical reading and help in the  
567 preparation of the manuscript. The authors are also grateful to the University Claude  
568 Bernard Lyon1 and INSERM for hosting and funding their laboratory and the INRAe and the  
569 CNRS for funding their salary.

570

571 **COMPETING INTERESTS:**

572 The authors declare no conflict of interest in relation with this article.

573 **KEY POINTS:**

574 - Like the liver and kidney, the intestine is able to carry out gluconeogenesis and release  
575 glucose in blood in the fasting state to contribute to the maintenance of plasma glucose.

576 - In the fed post-absorptive state, intestinal glucose is sensed by gastrointestinal nerves and  
577 initiates a signal to the brain regions controlling energy homeostasis and anxiety/stress-  
578 related behavior.

579 - The activation of intestinal gluconeogenesis promotes anti-obesity and anti-diabetic effects  
580 associated with minimization of anxiety/stress-related behavior.

581 - Intestinal gluconeogenesis is activated after gastric bypass surgery in rodents and in obese  
582 insulin-resistant humans and was shown in obese mice to account for the metabolic benefits  
583 observed.

584 - Intestinal gluconeogenesis is activated by macronutrients capable of fuelling systemic  
585 gluconeogenesis and to maintain plasma glucose for a while, thereby delaying the need to  
586 return to foraging and store energy.

587 - Intestinal gluconeogenesis might be a mid-term signage related to plasma glucose,  
588 between short-term signage related to food, by gastrointestinal hormones, and long-term  
589 signage related to energy stores, by leptin.

590

591 **REFERENCES**

592

- 593 1. Soty, M., Gautier-Stein, A., Rajas, F. & Mithieux, G. Gut-Brain Glucose Signaling in  
594 Energy Homeostasis. *Cell Metab.* **25**, 1231–1242 (2017).
- 595 2. Bernard, C. *Nouvelle fonction du foie considéré comme organe producteur de matière*  
596 *sucrée chez l'homme et les animaux.* (1853).
- 597 3. Krebs, H. A. RENAL GLUCONEOGENESIS. *Adv. Enzyme Regul.* **1**, 385–400 (1963).
- 598 4. Rajas, F., Bruni, N., Montano, S., Zitoun, C. & Mithieux, G. The glucose-6 phosphatase  
599 gene is expressed in human and rat small intestine: Regulation of expression in fasted  
600 and diabetic rats. *Gastroenterology* **117**, 132–139 (1999).
- 601 5. Croset, M. *et al.* Rat small intestine is an insulin-sensitive gluconeogenic organ.  
602 *Diabetes* **50**, 740–746 (2001).

- 603 6. Battezzati, A. *et al.* Nonhepatic glucose production in humans. *Am. J. Physiol.*  
604 *Endocrinol. Metab.* **286**, E129-135 (2004).
- 605 7. Hayes, M. T., Foo, J., Besic, V., Tychinskaya, Y. & Stubbs, R. S. Is intestinal  
606 gluconeogenesis a key factor in the early changes in glucose homeostasis following  
607 gastric bypass? *Obes Surg* **21**, 759–762 (2011).
- 608 8. Mithieux, G. Comment about intestinal gluconeogenesis after gastric bypass in human  
609 in relation with the paper by Hayes et al., *Obes. Surg.* 2011. *Obesity Surgery* **22**, 1920–  
610 1922 (2012).
- 611 9. Gerich, J. E., Meyer, C., Woerle, H. J. & Stumvoll, M. Renal gluconeogenesis: its  
612 importance in human glucose homeostasis. *Diabetes Care* **24**, 382–391 (2001).
- 613 10. Pillot, B., Soty, M., Gautier-Stein, A., Zitoun, C. & Mithieux, G. Protein feeding promotes  
614 redistribution of endogenous glucose production to the kidney and potentiates its  
615 suppression by insulin. *Endocrinology* **150**, 616–624 (2009).
- 616 11. Owen, O. E., Felig, P., Morgan, A. P., Wahren, J. & Cahill, G. F. Liver and kidney  
617 metabolism during prolonged starvation. *J. Clin. Invest.* **48**, 574–583 (1969).
- 618 12. Mutel, E. *et al.* Control of blood glucose in the absence of hepatic glucose production  
619 during prolonged fasting in mice: induction of renal and intestinal gluconeogenesis by  
620 glucagon. *Diabetes* **60**, 3121–3131 (2011).
- 621 13. Russek, M. Participation of hepatic glucoreceptors in the control of intake of food.  
622 *Nature* **197**, 79–80 (1963).
- 623 14. Tordoff, M. G. & Friedman, M. I. Hepatic portal glucose infusions decrease food intake  
624 and increase food preference. *Am J Physiol* **251**, R192-196 (1986).
- 625 15. Nijijima, A. Glucose-sensitive afferent nerve fibres in the hepatic branch of the vagus  
626 nerve in the guinea-pig. *J Physiol* **332**, 315–323 (1982).
- 627 16. Fournel, A. *et al.* Glucosensing in the gastrointestinal tract: Impact on glucose  
628 metabolism. *Am J Physiol Gastrointest Liver Physiol* **310**, G645-658 (2016).
- 629 17. Zmora, N., Suez, J. & Elinav, E. You are what you eat: diet, health and the gut  
630 microbiota. *Nat Rev Gastroenterol Hepatol* **16**, 35–56 (2019).
- 631 18. Duca, F. A., Waise, T. M. Z., Pepller, W. T. & Lam, T. K. T. The metabolic impact of small  
632 intestinal nutrient sensing. *Nat Commun* **12**, 903 (2021).
- 633 19. Booth, D. A., Chase, A. & Campbell, A. T. Relative effectiveness of protein in the late  
634 stages of appetite suppression in man. *Physiol Behav* **5**, 1299–1302 (1970).
- 635 20. Rolls, B. J., Hetherington, M. & Burley, V. J. The specificity of satiety: the influence of  
636 foods of different macronutrient content on the development of satiety. *Physiol Behav*  
637 **43**, 145–153 (1988).
- 638 21. Mithieux, G. *et al.* Portal sensing of intestinal gluconeogenesis is a mechanistic link in  
639 the diminution of food intake induced by diet protein. *Cell Metab* **2**, 321–9 (2005).
- 640 22. Lee, V. H. Membrane transporters. *Eur J Pharm Sci* **11 Suppl 2**, S41-50 (2000).
- 641 23. Duraffourd, C. *et al.* Mu-opioid receptors and dietary protein stimulate a gut-brain  
642 neural circuitry limiting food intake. *Cell* **150**, 377–88 (2012).

- 643 24. Penhoat, A. *et al.* Protein-induced satiety is abolished in the absence of intestinal  
644 gluconeogenesis. *Physiol. Behav.* **105**, 89–93 (2011).
- 645 25. Ray, T. K. *et al.* Long-term effects of dietary fiber on glucose tolerance and gastric  
646 emptying in noninsulin-dependent diabetic patients. *Am. J. Clin. Nutr.* **37**, 376–381  
647 (1983).
- 648 26. Mendeloff, A. I. Dietary fiber and human health. *N Engl J Med* **297**, 811–814 (1977).
- 649 27. Mithieux, G., Gautier-Stein, A., Rajas, F. & Zitoun, C. Contribution of intestine and  
650 kidney to glucose fluxes in different nutritional states in rat. *Comp. Biochem. Physiol. B,*  
651 *Biochem. Mol. Biol.* **143**, 195–200 (2006).
- 652 28. De Vadder, F. *et al.* Microbiota-generated metabolites promote metabolic benefits via  
653 gut-brain neural circuits. *Cell* **156**, 84–96 (2014).
- 654 29. De Vadder, F., Plessier, F., Gautier-Stein, A. & Mithieux, G. Vasoactive intestinal peptide  
655 is a local mediator in a gut-brain neural axis activating intestinal gluconeogenesis.  
656 *Neurogastroenterol. Motil.* **27**, 443–448 (2015).
- 657 30. Flint, H. J., Scott, K. P., Louis, P. & Duncan, S. H. The role of the gut microbiota in  
658 nutrition and health. *Nat Rev Gastroenterol Hepatol* **9**, 577–589 (2012).
- 659 31. De Vadder, F. *et al.* Microbiota-Produced Succinate Improves Glucose Homeostasis via  
660 Intestinal Gluconeogenesis. *Cell Metabolism* **24**, 151–157 (2016).
- 661 32. Wang, K. *et al.* Parabacteroides distasonis Alleviates Obesity and Metabolic  
662 Dysfunctions via Production of Succinate and Secondary Bile Acids. *Cell Rep* **26**, 222-  
663 235.e5 (2019).
- 664 33. Minassian, C., Ajzannay, A., Riou, J. P. & Mithieux, G. Investigation of the mechanism of  
665 glycogen rebound in the liver of 72-hour fasted rats. *J Biol Chem* **269**, 16585–16588  
666 (1994).
- 667 34. Mithieux, G., Daniele, N., Payrastra, B. & Zitoun, C. Liver microsomal glucose-6-  
668 phosphatase is competitively inhibited by the lipid products of phosphatidylinositol 3-  
669 kinase. *J Biol Chem* **273**, 17–19 (1998).
- 670 35. Daniele, N. *et al.* Phosphatidylinositol 3-kinase translocates onto liver endoplasmic  
671 reticulum and may account for the inhibition of glucose-6-phosphatase during  
672 refeeding. *J. Biol. Chem.* **274**, 3597–3601 (1999).
- 673 36. Pories, W. J. Diabetes: the evolution of a new paradigm. *Ann Surg* **239**, 12–13 (2004).
- 674 37. Barataud, A. *et al.* Metabolic benefits of gastric bypass surgery in the mouse: The role  
675 of fecal losses. *Mol Metab* **31**, 14–23 (2020).
- 676 38. Troy, S. *et al.* Intestinal gluconeogenesis is a key factor for early metabolic changes  
677 after gastric bypass but not after gastric lap-band in mice. *Cell Metab* **8**, 201–11 (2008).
- 678 39. Kim, M., Son, Y. G., Kang, Y. N., Ha, T. K. & Ha, E. Changes in Glucose Transporters,  
679 Gluconeogenesis, and Circadian Clock after Duodenal–Jejunal Bypass Surgery. *OBES*  
680 *SURG* **25**, 635–641 (2015).
- 681 40. Sun, D. *et al.* Duodenal-jejunal bypass surgery up-regulates the expression of the  
682 hepatic insulin signaling proteins and the key regulatory enzymes of intestinal  
683 gluconeogenesis in diabetic Goto-Kakizaki rats. *Obes Surg* **23**, 1734–1742 (2013).

- 684 41. Yan, Y. *et al.* Roux-en-Y Gastric Bypass Surgery Suppresses Hepatic Gluconeogenesis  
685 and Increases Intestinal Gluconeogenesis in a T2DM Rat Model. *Obes Surg* **26**, 2683–  
686 2690 (2016).
- 687 42. Gutierrez-Repiso, C. *et al.* Jejunal gluconeogenesis associated with insulin resistance  
688 level and its evolution after Roux-en-Y gastric bypass. *Surg Obes Relat Dis* **13**, 623–630  
689 (2017).
- 690 43. Jean, C. *et al.* Metabolic evidence for adaptation to a high protein diet in rats. *J Nutr*  
691 **131**, 91–98 (2001).
- 692 44. Sjöström, L. *et al.* Lifestyle, diabetes, and cardiovascular risk factors 10 years after  
693 bariatric surgery. *N. Engl. J. Med.* **351**, 2683–2693 (2004).
- 694 45. Vily-Petit, J. *et al.* Intestinal Gluconeogenesis Regulates Brown and White Adipose  
695 Tissues Functions in mice. *bioRxiv* 2021.10.25.465675 (2021)  
696 doi:10.1101/2021.10.25.465675.
- 697 46. Vily-Petit, J. *et al.* Intestinal gluconeogenesis prevents obesity-linked liver steatosis and  
698 non-alcoholic fatty liver disease. *Gut* **69**, 2193–2202 (2020).
- 699 47. Soty, M. *et al.* A gut-brain neural circuit controlled by intestinal gluconeogenesis is  
700 crucial in metabolic health. *Mol Metab* **4**, 106–117 (2015).
- 701 48. Parlati, L., Régnier, M., Guillou, H. & Postic, C. New targets for NAFLD. *JHEP Rep* **3**,  
702 100346 (2021).
- 703 49. Delaere, F. *et al.* The role of sodium-coupled glucose co-transporter 3 in the satiety  
704 effect of portal glucose sensing. *Mol Metab* **2**, 47–53 (2012).
- 705 50. Thorens, B. GLUT2, glucose sensing and glucose homeostasis. *Diabetologia* **58**, 221–232  
706 (2015).
- 707 51. Diez-Sampedro, A. *et al.* A glucose sensor hiding in a family of transporters. *Proceedings*  
708 *of the National Academy of Sciences* **100**, 11753–11758 (2003).
- 709 52. Soták, M., Marks, J. & Unwin, R. J. Putative tissue location and function of the SLC5  
710 family member SGLT3: Developments in SGLT3 biology. *Exp Physiol* **102**, 5–13 (2017).
- 711 53. Carter, M. E., Soden, M. E., Zweifel, L. S. & Palmiter, R. D. Genetic identification of a  
712 neural circuit that suppresses appetite. *Nature* **503**, 111–114 (2013).
- 713 54. Soty, M. *et al.* Calcitonin Gene-Related Peptide-Induced Phosphorylation of STAT3 in  
714 Arcuate Neurons Is a Link in the Metabolic Benefits of Portal Glucose.  
715 *Neuroendocrinology* **111**, 555–567 (2021).
- 716 55. Nogueiras, R., López, M. & Diéguez, C. Regulation of lipid metabolism by energy  
717 availability: a role for the central nervous system. *Obes Rev* **11**, 185–201 (2010).
- 718 56. Lee, S. B. *et al.* Intermittent restraint-induced sympathetic activation attenuates  
719 hepatic steatosis and inflammation in a high-fat diet-fed mouse model. *Am. J. Physiol.*  
720 *Gastrointest. Liver Physiol.* **317**, G811–G823 (2019).
- 721 57. Morrison, S. F., Madden, C. J. & Tupone, D. Central Neural Regulation of Brown Adipose  
722 Tissue Thermogenesis and Energy Expenditure. *Cell Metabolism* **19**, 741–756 (2014).
- 723 58. Bachman, E. S. *et al.* betaAR signaling required for diet-induced thermogenesis and  
724 obesity resistance. *Science* **297**, 843–845 (2002).

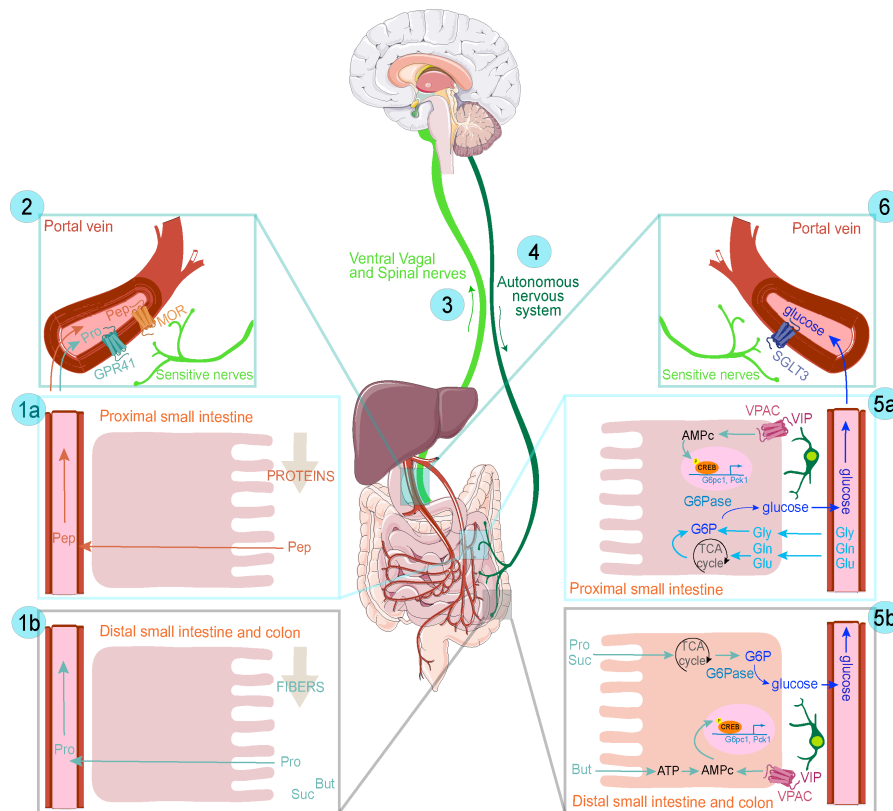
- 725 59. Cannon, B. & Nedergaard, J. Brown adipose tissue: function and physiological  
726 significance. *Physiol. Rev.* **84**, 277–359 (2004).
- 727 60. Harms, M. & Seale, P. Brown and beige fat: development, function and therapeutic  
728 potential. *Nat. Med.* **19**, 1252–1263 (2013).
- 729 61. Delaere, F., Akaoka, H., De Vadder, F., Duchampt, A. & Mithieux, G. Portal glucose  
730 influences the sensory, cortical and reward systems in rats. *Eur. J. Neurosci.* **38**, 3476–  
731 3486 (2013).
- 732 62. Berland, C., Small, D. M., Luquet, S. & Gangarossa, G. Dietary lipids as regulators of  
733 reward processes: multimodal integration matters. *Trends Endocrinol Metab* **32**, 693–  
734 705 (2021).
- 735 63. Sinet, F. *et al.* Dietary Fibers and Proteins Modulate Behavior via the Activation of  
736 Intestinal Gluconeogenesis. *Neuroendocrinology* **111**, 1249–1265 (2021).
- 737 64. Pariante, C. M. & Miller, A. H. Glucocorticoid receptors in major depression: relevance  
738 to pathophysiology and treatment. *Biol Psychiatry* **49**, 391–404 (2001).
- 739 65. Zhu, L.-J. *et al.* The different roles of glucocorticoids in the hippocampus and  
740 hypothalamus in chronic stress-induced HPA axis hyperactivity. *PLoS One* **9**, e97689  
741 (2014).
- 742 66. Zemdegs, J. *et al.* High-fat diet-induced metabolic disorders impairs 5-HT function and  
743 anxiety-like behavior in mice. *Br J Pharmacol* **173**, 2095–2110 (2016).
- 744 67. Spencer, S. J., Korosi, A., Layé, S., Shukitt-Hale, B. & Barrientos, R. M. Food for thought:  
745 how nutrition impacts cognition and emotion. *NPJ Sci Food* **1**, 7 (2017).
- 746 68. Wolfe, A. R. *et al.* Dietary protein and protein-rich food in relation to severely  
747 depressed mood: A 10 year follow-up of a national cohort. *Prog Neuropsychopharmacol*  
748 *Biol Psychiatry* **35**, 232–238 (2011).
- 749 69. Hutton, J. C. & O'Brien, R. M. Glucose-6-phosphatase catalytic subunit gene family. *J*  
750 *Biol Chem* **284**, 29241–29245 (2009).
- 751 70. Hübscher, G., West, G. R. & Brindley, D. N. Studies on the fractionation of mucosal  
752 homogenates from the small intestine. *Biochem J* **97**, 629–642 (1965).
- 753 71. Shittu, S. T., Alada, A. R. A. & Oyebola, D. D. O. Metabolic Fate of the Glucose Taken up  
754 by the Intestine During Induced Hyperglycaemia in Dogs. *Niger J Physiol Sci* **33**, 37–49  
755 (2018).
- 756 72. Lohrenz, A.-K. *et al.* Glucose transporters and enzymes related to glucose synthesis in  
757 small intestinal mucosa of mid-lactation dairy cows fed 2 levels of starch. *J Dairy Sci* **94**,  
758 4546–4555 (2011).
- 759 73. Tournaire, D., Bastide, P. & Dastugue, G. [Enzymatic activities in the small intestine of  
760 the lamb. (Acid and alkaline phosphatases, glucose-6-phosphatase, alpha-D-  
761 methylglucosidase, invertase and lactase)]. *C R Seances Soc Biol Fil* **160**, 1597–1600  
762 (1966).
- 763 74. Radecki, S. V., Ku, P. K., Bennink, M. R., Yokoyama, M. T. & Miller, E. R. Effect of dietary  
764 copper on intestinal mucosa enzyme activity, morphology, and turnover rates in  
765 weanling pigs. *J Anim Sci* **70**, 1424–1431 (1992).



- 766 75. Bismut, H., Hers, H. G. & Van Schaftingen, E. Conversion of fructose to glucose in the  
767 rabbit small intestine. A reappraisal of the direct pathway. *Eur J Biochem* **213**, 721–726  
768 (1993).
- 769 76. Anderson, J. W. & Rosendall, A. F. Gluconeogenesis in jejunal mucosa of guinea pig.  
770 *Biochim Biophys Acta* **304**, 384–388 (1973).
- 771 77. Palmer, M. F. & Rolls, B. A. The activities of some metabolic enzymes in the intestines of  
772 germ-free and conventional chicks. *Br J Nutr* **50**, 783–790 (1983).
- 773 78. Su, J. *et al.* The characteristics of glucose homeostasis in grass carp and Chinese  
774 longsnout catfish after oral starch administration: a comparative study between  
775 herbivorous and carnivorous species of fish. *Br J Nutr* **123**, 627–641 (2020).
- 776 79. Chen, Y.-J. *et al.* Simultaneous stimulation of glycolysis and gluconeogenesis by feeding  
777 in the anterior intestine of the omnivorous GIFT tilapia, *Oreochromis niloticus*. *Biol*  
778 *Open* **6**, 818–824 (2017).
- 779 80. Kirchner, S., Seixas, P., Kaushik, S. & Panserat, S. Effects of low protein intake on extra-  
780 hepatic gluconeogenic enzyme expression and peripheral glucose phosphorylation in  
781 rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol B Biochem Mol Biol* **140**,  
782 333–340 (2005).
- 783 81. Penhoat, A., Fayard, L., Stefanutti, A., Mithieux, G. & Rajas, F. Intestinal  
784 gluconeogenesis is crucial to maintain a physiological fasting glycemia in the absence of  
785 hepatic glucose production in mice. *Metabolism* **63**, 104–111 (2014).
- 786 82. Hartmann, F. & Plauth, M. Intestinal glutamine metabolism. *Metabolism* **38**, 18–24  
787 (1989).
- 788 83. Windmueller, H. G. & Spaeth, A. E. Identification of ketone bodies and glutamine as the  
789 major respiratory fuels in vivo for postabsorptive rat small intestine. *J. Biol. Chem.* **253**,  
790 69–76 (1978).
- 791 84. Watford, M. Glutamine metabolism in rat small intestine: synthesis of three-carbon  
792 products in isolated enterocytes. *Biochim. Biophys. Acta* **1200**, 73–78 (1994).
- 793 85. Mithieux, G., Rajas, F. & Gautier-Stein, A. A novel role for glucose 6-phosphatase in the  
794 small intestine in the control of glucose homeostasis. *J Biol Chem* **279**, 44231–4 (2004).
- 795 86. Blaak, E. E. *et al.* Short chain fatty acids in human gut and metabolic health. *Benef*  
796 *Microbes* **11**, 411–455 (2020).
- 797 87. Geiselman, P. J. & Novin, D. The role of carbohydrates in appetite, hunger and obesity.  
798 *Appetite* **3**, 203–223 (1982).
- 799 88. Little, T. J. & Feinle-Bisset, C. Effects of dietary fat on appetite and energy intake in  
800 health and obesity--oral and gastrointestinal sensory contributions. *Physiol Behav* **104**,  
801 613–620 (2011).
- 802 89. Schwartz, M. W. & Morton, G. J. Obesity: keeping hunger at bay. *Nature* **418**, 595–597  
803 (2002).
- 804 90. Eaton, S. B. & Konner, M. Paleolithic nutrition. A consideration of its nature and current  
805 implications. *N Engl J Med* **312**, 283–289 (1985).

- 806 91. Ben-Dor, M., Sirtoli, R. & Barkai, R. The evolution of the human trophic level during the  
807 Pleistocene. *Am J Phys Anthropol* **175 Suppl 72**, 27–56 (2021).
- 808 92. Mithieux, G., Rajas, F. & Zitoun, C. Glucose utilization is suppressed in the gut of insulin-  
809 resistant high fat-fed rats and is restored by metformin. *Biochem. Pharmacol.* **72**, 1757–  
810 1762 (2006).
- 811 93. Andreelli, F. *et al.* Liver adenosine monophosphate-activated kinase- $\alpha$ 2 catalytic  
812 subunit is a key target for the control of hepatic glucose production by adiponectin and  
813 leptin but not insulin. *Endocrinology* **147**, 2432–2441 (2006).
- 814 94. Flak, J. N. & Myers, M. G. Minireview: CNS Mechanisms of Leptin Action. *Mol Endocrinol*  
815 **30**, 3–12 (2016).
- 816 95. Enriori, P. J., Sinnayah, P., Simonds, S. E., Garcia Rudaz, C. & Cowley, M. A. Leptin action  
817 in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue  
818 in spite of systemic leptin resistance. *J Neurosci* **31**, 12189–12197 (2011).
- 819 96. Liu, J., Perez, S. M., Zhang, W., Lodge, D. J. & Lu, X.-Y. Selective deletion of the leptin  
820 receptor in dopamine neurons produces anxiogenic-like behavior and increases  
821 dopaminergic activity in amygdala. *Mol Psychiatry* **16**, 1024–1038 (2011).
- 822 97. Jacobs, B. L. Adult brain neurogenesis and depression. *Brain Behav Immun* **16**, 602–609  
823 (2002).
- 824 98. Garza, J. C., Guo, M., Zhang, W. & Lu, X.-Y. Leptin restores adult hippocampal  
825 neurogenesis in a chronic unpredictable stress model of depression and reverses  
826 glucocorticoid-induced inhibition of GSK-3 $\beta$ / $\beta$ -catenin signaling. *Mol Psychiatry* **17**,  
827 790–808 (2012).
- 828 99. Londraville, R. L., Prokop, J. W., Duff, R. J., Liu, Q. & Tuttle, M. On the Molecular  
829 Evolution of Leptin, Leptin Receptor, and Endospinin. *Front Endocrinol (Lausanne)* **8**, 58  
830 (2017).
- 831 100. Ricardo-Silgado, M. L., McRae, A. & Acosta, A. Role of Enteroendocrine Hormones in  
832 Appetite and Glycemia. *Obes Med* **23**, 100332 (2021).
- 833 101. Kim, Y.-K., Kim, O. Y. & Song, J. Alleviation of Depression by Glucagon-Like Peptide 1  
834 Through the Regulation of Neuroinflammation, Neurotransmitters, Neurogenesis, and  
835 Synaptic Function. *Front Pharmacol* **11**, 1270 (2020).
- 836 102. Crespi, F. On the Role of Cholecystokinin (CCK) in Fear and Anxiety: A Review and  
837 Research Proposal. *Journal of Human Psychology* (2019).
- 838 103. Ruan, J. *et al.* TreeFam: 2008 Update. *Nucleic Acids Res* **36**, D735–740 (2008).
- 839 104. Guindon, S. *et al.* New algorithms and methods to estimate maximum-likelihood  
840 phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* **59**, 307–321 (2010).
- 841 105. Letunic, I. & Bork, P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic  
842 tree display and annotation. *Nucleic Acids Res* **49**, W293–W296 (2021).
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**Figure 1: Sequence from nutrient induction of IGN to glucose detection in the portal vein.**



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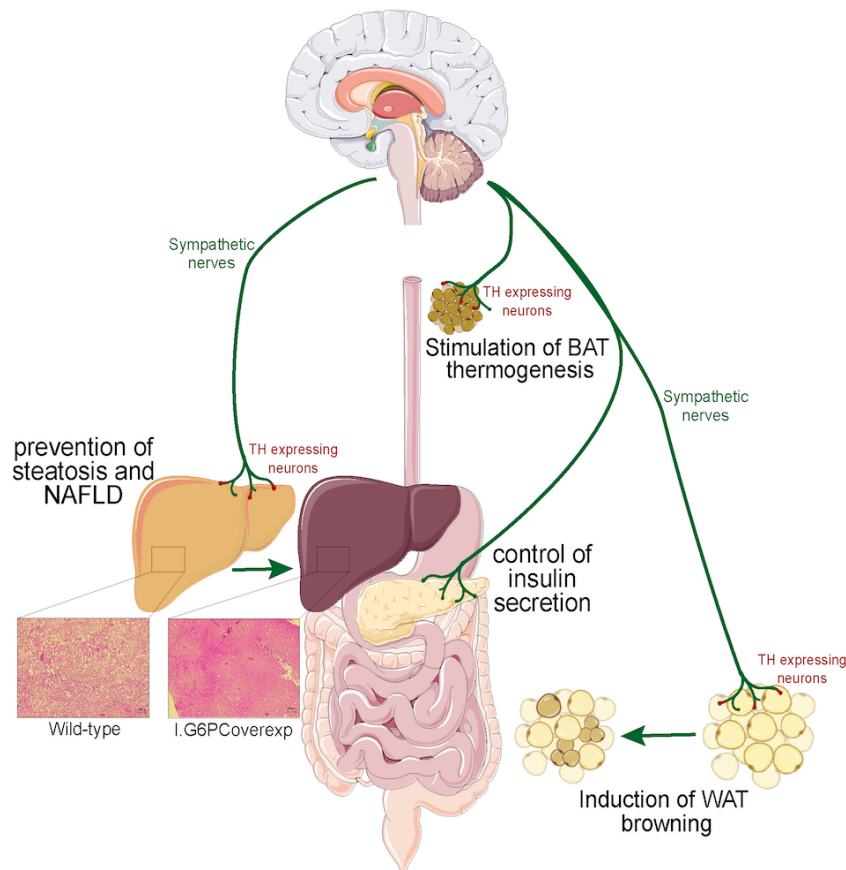
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Proteins are digested in peptides (Pep) in the proximal small intestine and a fraction traverses the mucosa to reach the portal blood via peptide transporters (1a). In the portal vein, Pep act as antagonists on the  $\mu$ -opioid receptor (MOR) to signal to the brain (2) via vagal and spinal nerves (3). Soluble fibers that are not metabolized by mammalian digestive enzymes traverse the proximal intestine to reach the distal gut where they are fermented by the microbiota in short-chain fatty acids as propionate (Pro) and butyrate (But) and other metabolites as succinate (Suc). Pro crosses the mucosa to reach the portal blood (1b), where it acts as an agonist on FFAR3/GRP41 (2), to signal to the brain via ventral vagal and spinal nerves (3). This in turn activates the effector autonomic system (4), resulting in release of the vasoactive peptide (VIP) (5a). In the proximal gut enterocytes (4), the activation of the VIP receptor (VPAC) activates adenylate cyclase and the production of cAMP that next activates the expression of IGN genes (*G6pc1*: catalytic unit of the glucose-6-phosphatase, *Pck1*: phosphoenolpyruvate carboxykinase-cytosolic form). Increased expression of the gluconeogenic genes stimulates the conversion of glutamate (Glu), glutamine (Gln) or Glycerol (Gly) (from digested nutrients or from the bloodstream) into glucose (5a). In enterocytes of the distal small intestine and in colonocytes, the activation of the VIP

864 receptor (VIPR) induces IGN gene expression via cAMP. Additionally, But increases cAMP *via*  
865 ATP production (5b). Finally, Pro and Suc serve as substrates of IGN (5b). The glucose  
866 produced is sensed by SGLT3 in the portal area and signals to the brain *via* spinal nerves (6).

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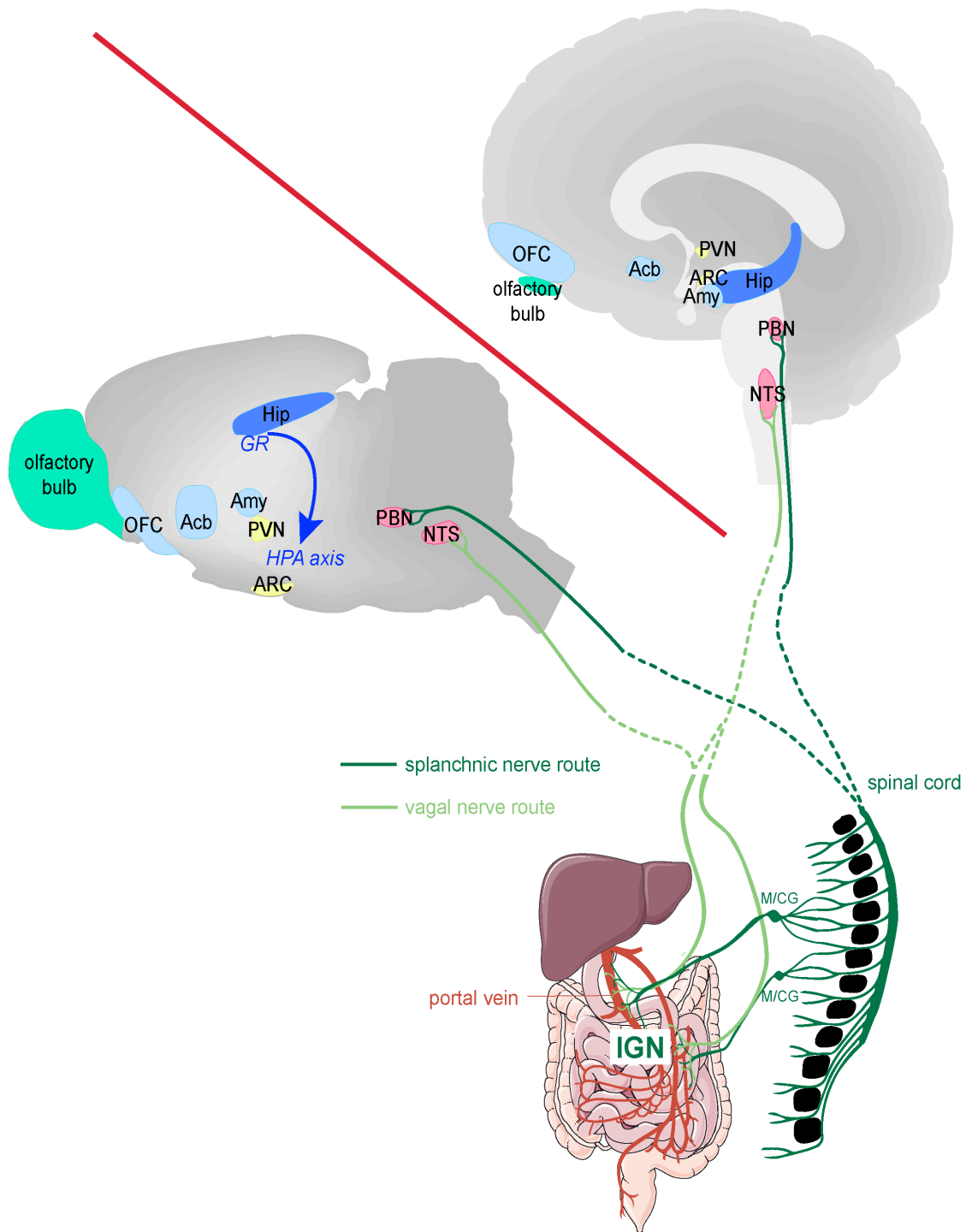
868 **Figure 2: Metabolic processes target of IGN.**



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870 The induction of IGN leads to a decrease in lipogenesis and lipid import in the liver, leading  
871 to prevention of steatosis and further evolution of NAFLD on hypercaloric diet. The induction  
872 of IGN leads to an increase in energy expenditure by adipose tissues, thanks to an activation  
873 of thermogenesis in the brown adipose tissue (BAT) and a browning process in the white  
874 adipose tissue (WAT). The absence of IGN leads to an impairment of glucose-induced insulin  
875 secretion that is corrected by a portal glucose infusion mimicking IGN. The mechanisms by  
876 which IGN exerts these metabolic benefits are associated with the activation of the  
877 sympathetic nerves in these different tissues.

878 **Figure 3: Brain targets of IGN.**

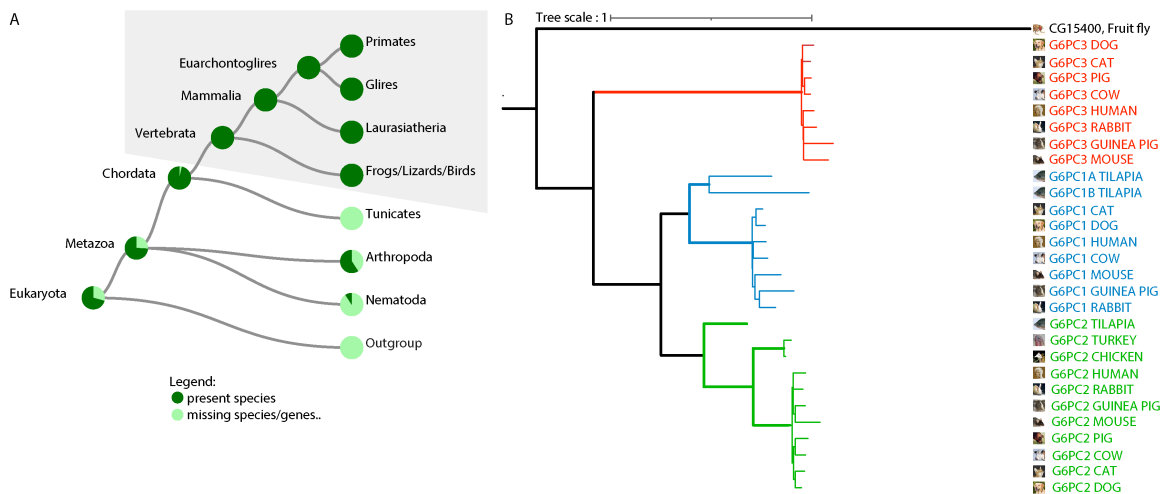


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880 Intestinal gluconeogenesis and portal glucose signal activate several brain nuclei involved in  
881 the regulation of energy homeostasis (*in yellow*, the hypothalamic Arcuate (ARC),

882 paraventricular (PVN), lateral and ventromedial nuclei), and in the regulation of reward  
 883 processes and motivation (*in blue*, the amygdala (Amy), Nucleus accumbens (Acb), the  
 884 orbitofrontal cortex (OFC), *in green*, the olfactory bulb). Intestinal gluconeogenesis and  
 885 portal glucose signal also target the hippocampus (*in dark blue*), improving the retro-control  
 886 of the hypothalamic-pituitary adrenal (HPA) axis by the glucocorticoid receptor (GR). The  
 887 diagram on the left shows the target areas identified in a mouse brain, the diagram on the  
 888 right is a hypothetical transposition of the IGN targets into a human brain.

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890 **Figure 4: Evolution of G6PC genes.**



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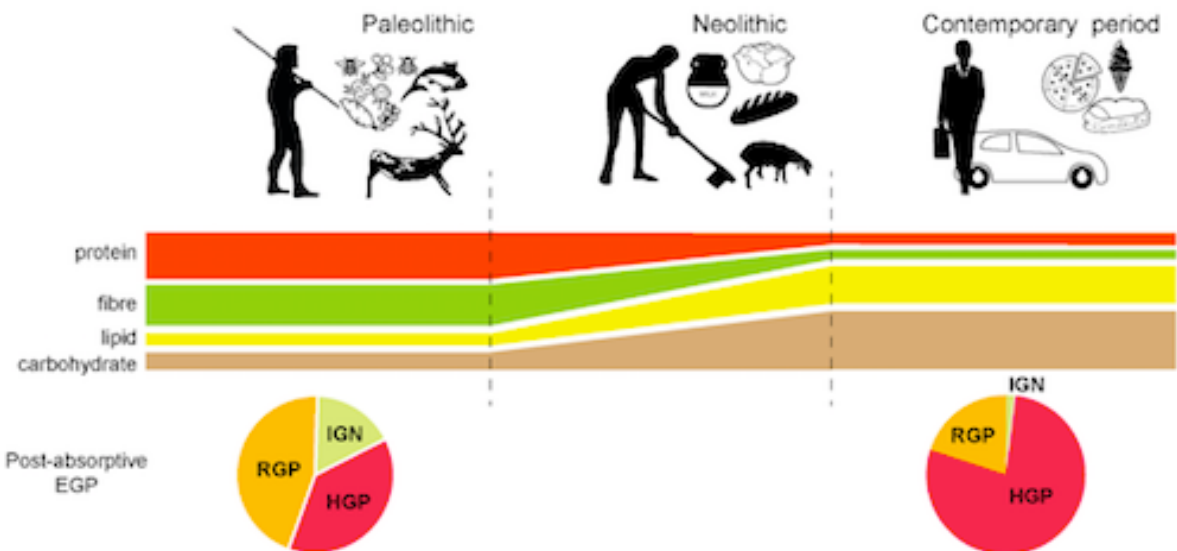
892 **A.** Summary of the evolutionary history of G6PC. Among the predicted orthologous  
 893 sequences, species having an annotated G6PC gene are represented in dark green and  
 894 predicted orthologous sequence, which are still not annotated are represented in light  
 895 green. **B.** Phylogenetic tree indicating the evolution of G6PC genes. The species in which the  
 896 intestinal G6PC1 gene has been identified are represented. G6PC3 paralogs appear in red,  
 897 G6PC2 paralogs in green, G6PC1 paralogs in blue.

898 Data were obtained from the TreeFam family NaN (NaN, TF324388). The sequences (174  
 899 sequences from 77 species) identified by TreeFam in databases (Ensembl, Ensembl

900 Genomes, JGI and Wormbase) were aligned using MCoffee. The final alignment was 1447 AA  
901 long and on average 44% conserved. TreeBest<sup>103</sup> was used to build a gene tree and  
902 reconciles it with the species tree. TreeBest build 5 source trees using nj and Phylml<sup>104</sup>. It  
903 then merges them into one tree trying to minimize the number of duplications using a  
904 species tree. The tree was then edited with iTOL<sup>105</sup>.

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**Figure 5: Changes of EGP in human evolution in relation with food habits.**

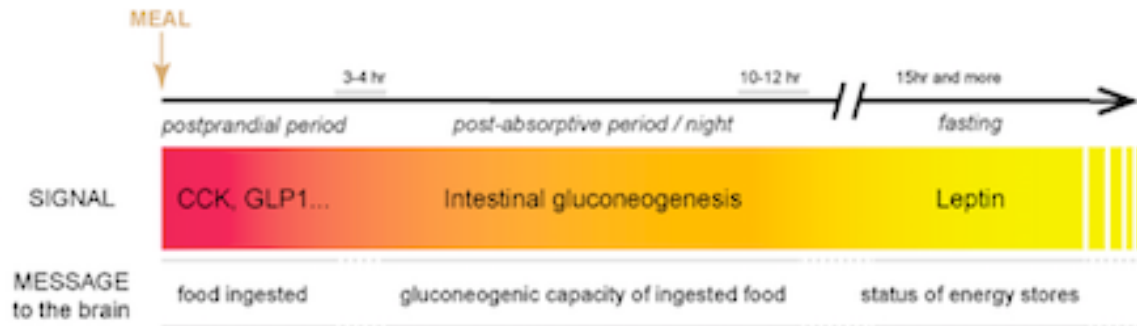


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The proportions of the various macronutrients and their evolution over time are only indicative. The distribution of glucose production between gluconeogenic organs to illustrate the Paleolithic period is derived from our measurements in the post-absorptive period in rats fed a high protein diet<sup>10</sup>. The distribution of glucose production between gluconeogenic organs to illustrate the contemporary period is derived from our measurements in the post-absorptive period in rats fed a starch-based chow diet (see related text). HGP: hepatic glucose production, RGP: renal glucose production.

922 **Figure 6: Proposed sequence of signage to the brain in relation with the presence/absence**  
 923 **of food and its composition.**

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927 The duration of the postprandial period (3-4h) is only indicative and may vary according to  
 928 the quantity and nature of the food ingested. The same applies to the duration of the  
 929 establishment of IGN during the post-absorptive period and/or during the night. CCK:  
 930 cholecystokinin, GLP1: glucagon-like peptide 1.