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

Amandine Gautier-Stein, Gilles Mithieux

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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 ¹.

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 ¹.

While the gluconeogenic capacity of the liver is known since more than 150 years ², that of the kidney has been uncovered in the mid of the 20th century ³, and that of the intestine even more recently, at the end of the nineties ^{4,5}. It is noteworthy that the discovery of the presence of G6Pase in the rat and human guts ⁴ and of the evidence of intestinal gluconeogenesis (IGN) in the rat ⁵ was rapidly followed by the demonstration of the possibility of IGN in humans, during the anhepatic phase of liver transplantation ⁶. 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 ⁶. More recently, IGN was also suggested to take place in obese insulin-resistant patients after they underwent gastric bypass surgery ^{7,8}.

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 ¹. 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 ⁹
67 . Similar values were later determined in rat ¹⁰. 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 ¹¹. Comparable values were determined later in rodents fasted
72 for 24-72h, from a glucose tracer approach ¹⁰. 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 ⁴ and activated by glucagon and glucocorticoids ¹².

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 ¹³⁻¹⁵. 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 ¹⁶⁻¹⁸, 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.

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2. METABOLIC CONTROL

2.1. Regulation mechanisms by nutrition

a. Protein-enriched diets

Protein-enriched diets (PED) have long been associated with satiety in animals and humans^{19,20}. A body of evidence has suggested that PED could exert their satiety action via the activation of IGN and the portal glucose signal. The proof of this concept was provided from experiments in the rat²¹. It was shown that the expression of the regulatory genes of gluconeogenesis (*G6pc1* and *Pck1*) is increased on PED in the kidney¹⁰ and intestine²¹, while the outflow of glucose from the gut is increased in the post-absorptive period to about 20% of EGP, such a flow being sufficient to decrease food intake²¹. This was associated with the activation of the main hypothalamic areas involved in the control of energy homeostasis. The key role of the gastrointestinal nervous system was ascertained from experiments of chemical denervation, which prevented the decrease in food intake from taking place²¹. Satiety has been the readout used to investigate the mechanisms by which PED activates the expression of IGN regulatory genes. Proteins are not absorbed by the gut mucosa under the form of amino acids but, instead, under the form of small peptides containing at least 2 amino acids. The bulk of peptides are hydrolysed to amino acids within the mucosa but a fraction traverses the mucosa to reach the portal blood *via* peptide transporters²². Thus, it was shown that under PED, peptides released in blood bind to and antagonize μ -opioid receptors that are present in the periportal nervous system (**Figure 1**). This activates a neural reflex arc resulting in the induction of IGN gene expression, which does not take place whether the gastrointestinal system has been previously inactivated²³. The causal role of IGN in the satiety effects of dietary protein was further established from experiments involving a mouse with targeted deletion of intestinal G6Pase (IG6Pase-KO mice). These mice, indeed, are insensitive to the hunger-curbing effect of either PED²⁴ or of infused peptides²³.

b. Fibre-enriched diets

It is long known that dietary fibres exert anti-obesity and anti-diabetic effects in humans^{25,26}. 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 ¹⁷. 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 ²⁷, 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 ²⁸. 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 ²⁸. 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 ²⁹. 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 ²⁸ (**Figure 1**).

147 Interestingly, succinate, the precursor of propionate in the bacterial metabolism ³⁰ also
148 serves as a substrate of IGN, whereas succinate has no action to regulate the expression of
149 IGN genes ³¹. 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 ³². Since acute regulations of G6Pase activity exist in
152 the liver ³³⁻³⁵, 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 ²⁸. 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 ³⁶. Deciphering the mechanisms behind has not been an easy task. A
165 number of studies using genetic mouse models have been unsuccessful ³⁷. 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 ³⁷. 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 ³⁸. 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 ^{39–41}.

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 ^{7,8}. 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 ⁸. 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 ⁴².

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 ^{25,26,36,43,44}. A major target is lipid storage in the white adipose
188 tissue (WAT) (**Figure 2**). The latter is minored upon PED ⁴³, upon fibre-enriched diets, and
189 dramatically decreased upon GPB ^{36,44}. 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^{21,23,24,28,31}.

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 feedings⁴⁵. 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 cold⁴⁵ (**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 diet⁴⁵. 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 WAT⁴⁵.

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 PED¹⁰ or dietary fibers²⁸, undergoing GBP³⁸, or overexpressing IGN
209⁴⁶. 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^{10,38}, improved insulin tolerance and increased
212 phosphorylation state of hepatic AKT, the intracellular insulin signalling mediator⁴⁶ or
213 decreased activity of hepatic G6Pase²⁸. 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 AKT⁴⁷.

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 regulation⁴⁷.
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 IGN⁴⁷. 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 ⁴⁷. 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 ⁴⁶ (**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 ⁴⁸.

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 α ,
241 interleukin 6 and Mcp1) and fibrosis (lowered expression of vimentin and Acta2) ⁴⁶. 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 ⁴⁶.

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 triglycerides⁴⁶.

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 ^{1,38,46}. 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 μ -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^{21,23,28,49}. 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)⁵⁰, sweet taste receptors⁴⁹ or sodium-coupled
271 glucose transporter 3 (SGLT3), a glucose receptor first identified in the gastrointestinal
272 nervous system^{51,52}. 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 (⁴⁹, see also ¹ 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^{21,23,28,49} (**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 intake⁵³. Accordingly, there is no effect on food intake of
288 portal glucose infusion or fibre-enriched diet in *Cgrp*-knockout mice⁵⁴. 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 does⁵⁴.

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 signaling⁴⁷. 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 not⁵⁴. 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^{55,56} and of browning in the white adipose tissues (WAT)
305 thermogenesis and energy expenditure⁵⁷⁻⁶⁰. 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 catecholamine⁴⁶. 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 cells⁴⁵. Moreover, local denervation of the BAT with the sympathetic
312 toxin 6-hydroxydopamine (6-OHDA) suppresses resistance to cold in IG6Pase-overexpressing
313 mice⁴⁵. 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 ^{21,23,28}. 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 ⁶¹, which are involved in reward processes and motivation ⁶²
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 ¹⁴.
329 Additional target areas of IGN in the brain relate to the dentate gyrus in the hippocampus ⁶³,
330 which controls the hypothalamic-pituitary-adrenal (HPA) axis and anxiety-related behavior
331 ^{64,65} **(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 ⁶³. 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 ⁶³. It is noteworthy that the link between insulin resistance and
344 diabetes induced by hypercaloric feeding and anxiety-related behavior was previously
345 established ⁶⁶.

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 ⁶³.
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 ⁶³. 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 ^{67,68}. 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 ^{61,63}, 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 ¹. 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 ⁶⁹. 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 ^{4,7,8,42} but also to various mammals as carnivores
389 (cat, dog ^{70,71}) herbivores (cow, sheep, pig, rabbit ⁷²⁻⁷⁵) and rodents (mouse, rat (see above),
390 guinea pig ⁷⁶), to birds (hen ⁷⁷ and also to various fishes (herbivorous as carp ⁷⁸, omnivorous
391 like tilapia ⁷⁹ or carnivorous like catfish of trout ^{78,80}). 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 ⁸⁰. 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 ^{5,11,27,81}. Indeed,
410 glutamine is a major substrate for both renal and intestinal gluconeogenesis ^{5,9}. 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 concentration⁸². 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 body¹.

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^{83,84}. 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 gluconeogenesis⁸⁵.

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 synthesized⁸⁵. 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 prolongs³³, 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 ¹ 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 ⁸⁶. This could also be the case of
450 glutamine and aspartate from luminal origin ⁸³. 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 ⁸³. Amino acids and especially glutamine are thus the
454 gluconeogenic reservoir of the body ¹. 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 ¹.

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 ⁸⁷. 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 ⁸⁸. 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 ⁸⁹, the latter being a key target
470 of IGN ^{21,23,28,49}.

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 ⁶³. 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 ⁹⁰. 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 ⁹⁰. 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 ⁹¹. 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 ^{5,27,92}. 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 1990s¹, 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^{1,89}. Regarding glucose control, IGN like leptin improves
517 systemic glucose tolerance and hepatic insulin sensitivity and suppresses hepatic glucose
518 production^{47,93}. To do so, IGN like leptin activates the aforementioned hypothalamic areas
519 and the parabrachial nucleus in the hindbrain^{49,94}. 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^{47,89}. Leptin increases energy expenditure via a
522 hypothalamic-driven activation of the sympathetic system in the BAT⁹⁵, what IGN does as
523 well⁴⁵.

524 The similarities also relate to the anti-anxiety activity of IGN and leptin. The central
525 amygdala, a target of IGN⁴⁹, is also a target of leptin in relation with its anxiolytic-like
526 activity⁹⁶. The hippocampal neurogenesis is a well-known target of anti-depressants⁹⁷.
527 Hippocampal neurogenesis is defective in IGN-deficient mice and stimulated by IGN-
528 activated by nutrients⁶³. Moreover, hippocampal neurogenesis has been identified as a
529 target of leptin in relation with the hormone anti-depressive-like features⁹⁸.

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 IGN⁹⁹. Leptin is widely regarded as a signal to the brain of the evolution of
533 lipid energy reserves^{89,94,95,99}. 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 gut⁸⁸. The presence of food results in decreased hunger and the
539 absence of food in hunger reset⁸⁸. 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 IGN¹⁰⁰ and also exhibit anti-anxiety properties^{101,102}.

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

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570

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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

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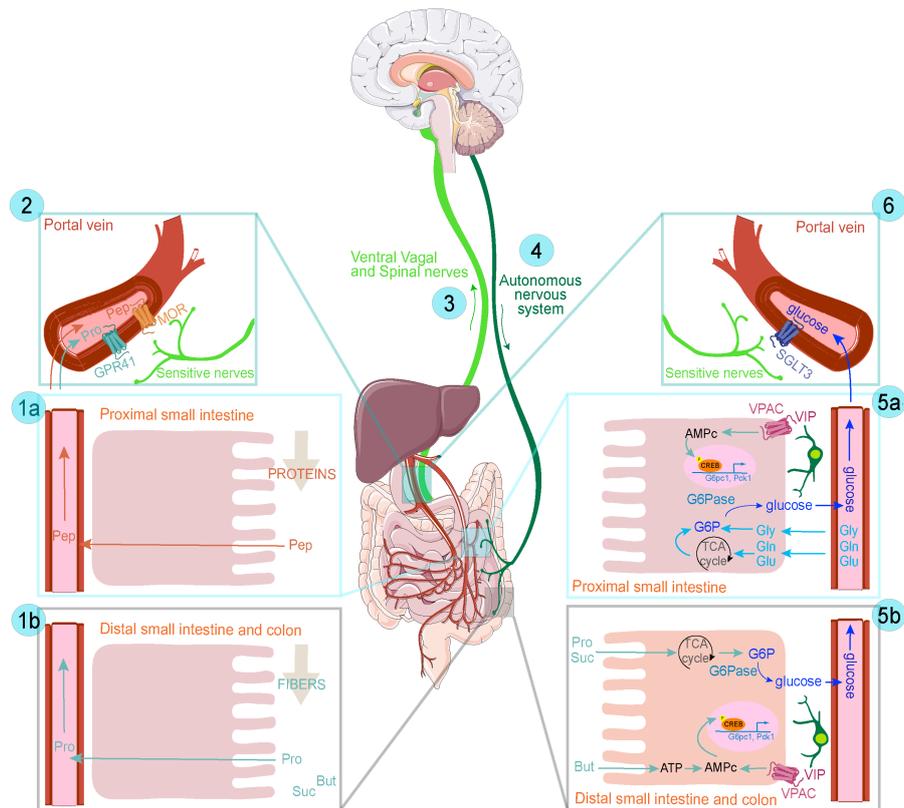
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Figure 1: Sequence from nutrient induction of IGN to glucose detection in the portal vein.



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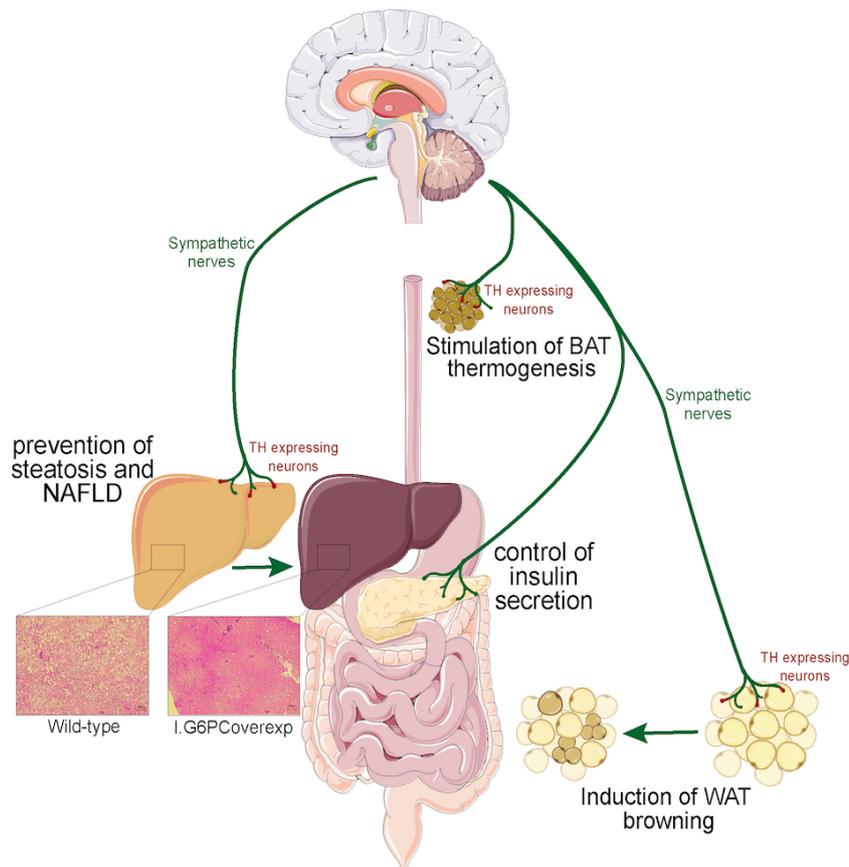
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Proteins are digested in peptides (Pep) in the proximal small intestine and a fraction traverses the mucosa to reach the portal blood via peptide transporters (1a). In the portal vein, Pep act as antagonists on the μ -opioid receptor (MOR) to signal 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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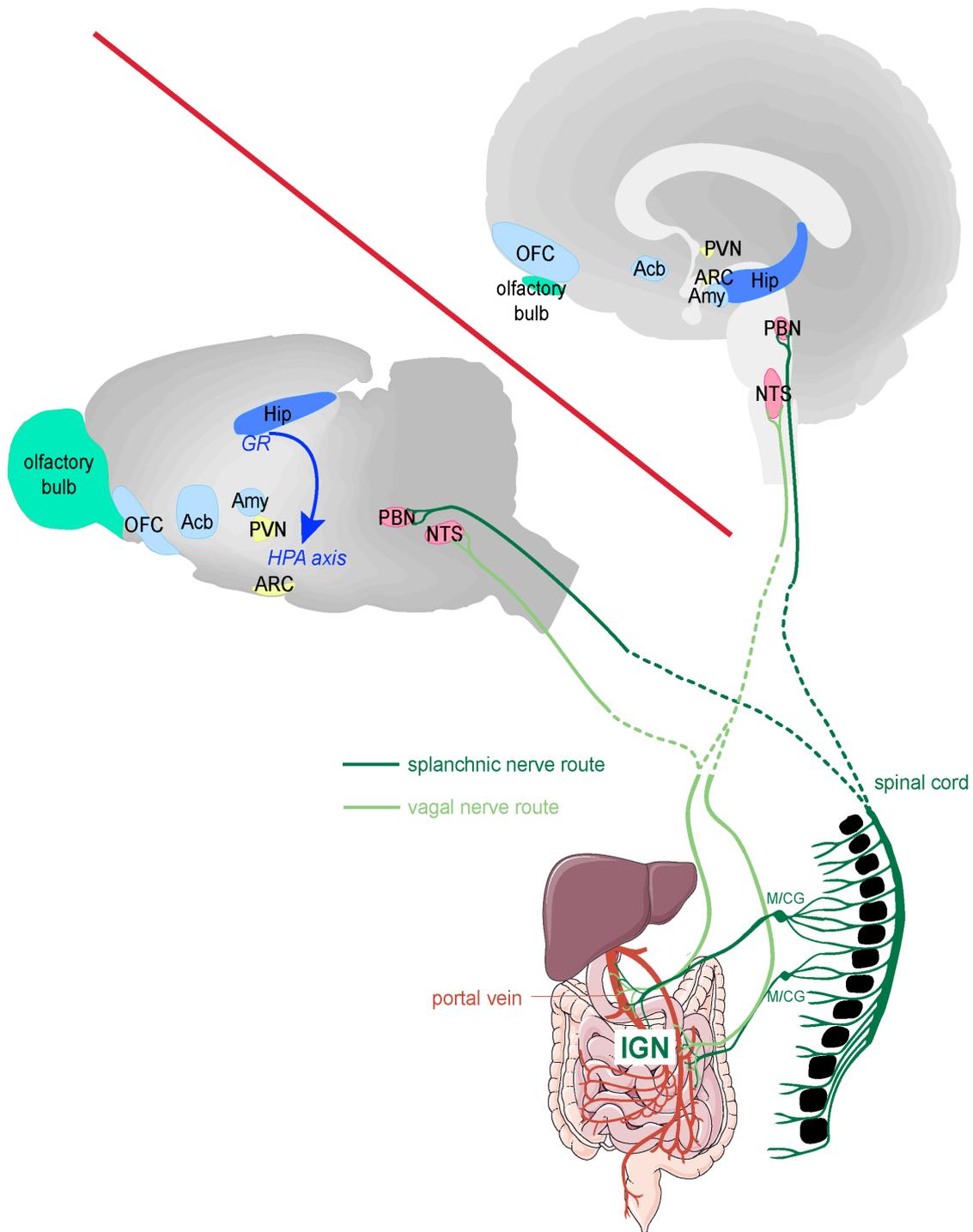
868 **Figure 2: Metabolic processes target of IGN.**



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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.**

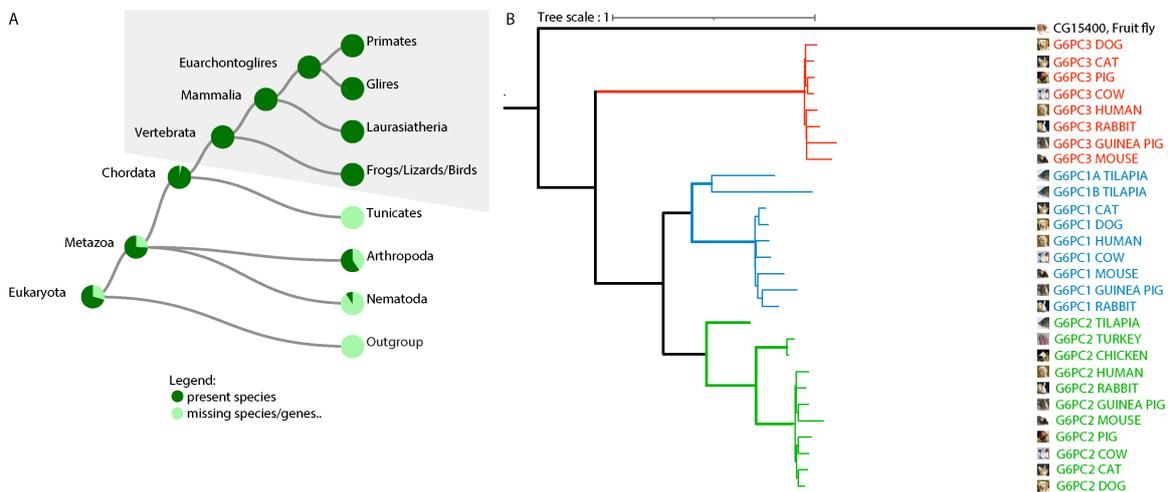


879 Intestinal gluconeogenesis and portal glucose signal activate several brain nuclei involved in
 880 the regulation of energy homeostasis (*in yellow*, the hypothalamic Arcuate (ARC),
 881

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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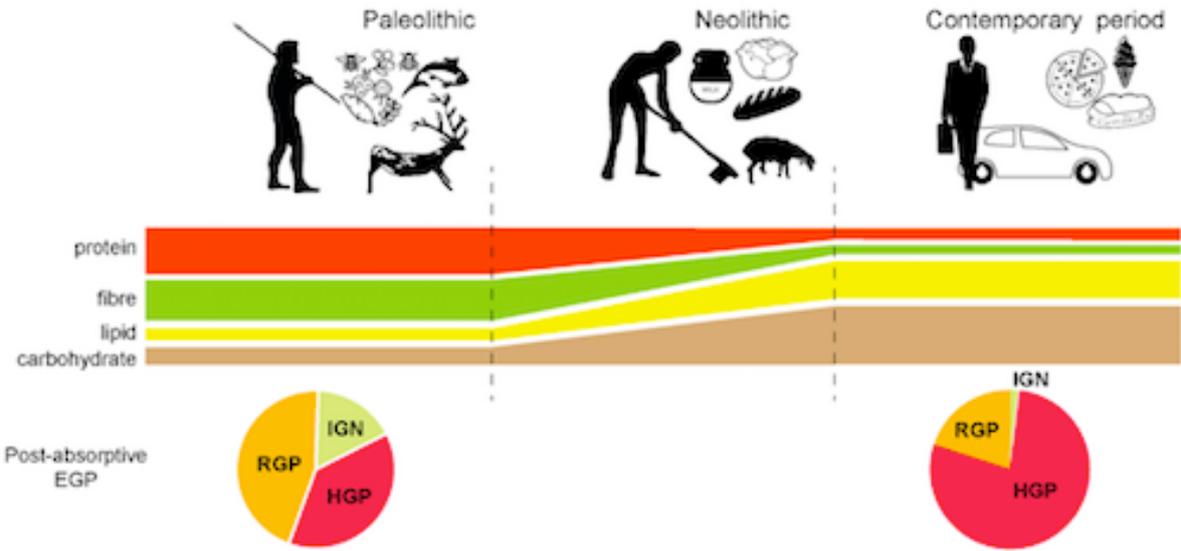
892 **A.** Summary of the evolutionary history of G6PC. Among the predicted orthologous
 893 sequences, species having an annotated G6PC gene are represented in dark green and
 894 predicted orthologous sequence, which are still not annotated are represented in light
 895 green. **B.** Phylogenetic tree 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. TreeBest¹⁰³ was used to build a gene tree and
902 reconciles it with the species tree. TreeBest build 5 source trees using nj and Phylml¹⁰⁴. 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 iTOL¹⁰⁵.

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

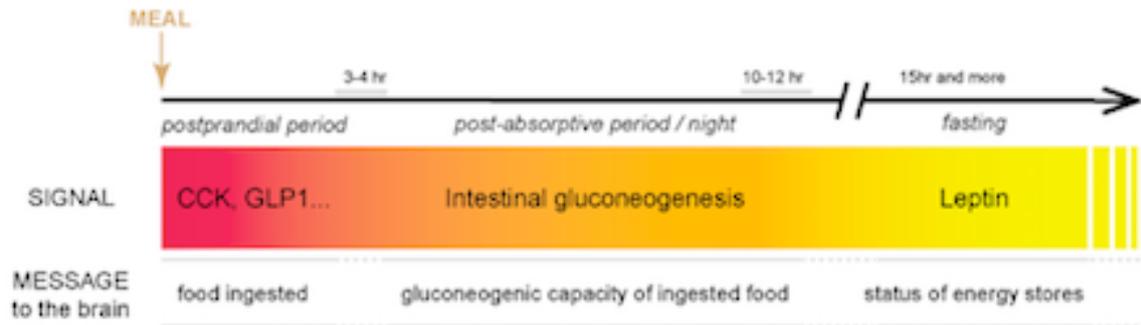


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The proportions of the various macronutrients and their evolution over time are only indicative. The distribution of glucose production between gluconeogenic organs to illustrate the Paleolithic period is derived from our measurements in the post-absorptive period in rats fed a high protein diet¹⁰. 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.