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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        **INTESTINAL GLUCONEOGENESIS: METABOLIC BENEFITS MAKE SENSE IN THE LIGHT OF** 2        **EVOLUTION**

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## 13       **ABSTRACT**

14       The intestine, like the liver and kidney, is able in various vertebrates and humans to carry out  
15       gluconeogenesis and release glucose in blood. In fasting, it contributes to the maintenance  
16       of plasma glucose. In the fed post-absorptive state, intestinal glucose is sensed by the  
17       gastrointestinal nervous system. This initiates a signal to the brain regions controlling energy  
18       homeostasis and anxiety/stress-related behavior, such as the parabrachial nucleus, the  
19       hypothalamus, the central amygdala and the hippocampus. Intestinal gluconeogenesis (IGN)  
20       is activated by several complementary mechanisms in particular nutritional situations: when  
21       food is enriched in protein or fermentable fibre and after gastric bypass surgery of obesity.  
22       In these situations, IGN promotes several metabolic (anti-obesity and anti-diabetes) and  
23       behavioral benefits (anti-anxiety) in the context of high calorie feeding. Since IGN is  
24       activated by nutrients capable of fuelling systemic gluconeogenesis, IGN could be a signal to  
25       the brain that food previously ingested is suitable to maintain plasma glucose for a while  
26       following meal assimilation. This may account for the metabolic and behavioral benefits  
27       observed. Finally, we discuss how the metabolic benefits provided by IGN in both fasting and  
28       fed states could explain that IGN emerged and was maintained in vertebrates by a process of  
29       natural selection.  
30

## 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 neuroglucopenia 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

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

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

## 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<sup>19,20</sup>. 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 rat<sup>21</sup>. It was shown that the expression of the regulatory genes of gluconeogenesis (*G6pc1* and *Pck1*) is increased on PED in the kidney<sup>10</sup> and intestine<sup>21</sup>, 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 intake<sup>21</sup>. 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 place<sup>21</sup>. 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 transporters<sup>22</sup>. Thus, it was shown that under PED, peptides released in blood bind to and antagonize  $\mu$ -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 inactivated<sup>23</sup>. 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 PED<sup>24</sup> or of infused peptides<sup>23</sup>.

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

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

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

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

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

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

### *c. Gastric bypass surgery*

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

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

## **2.2. Metabolic benefits**

### *a. Management of body weight*

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

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

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

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

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

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



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

### *c. Prevention of NAFLD*

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

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

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

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

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

### 3. NEURAL MECHANISMS

#### 3.1 Gastrointestinal glucose sensing

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

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

#### 3.2 Hypothalamic mechanisms

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

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

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

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

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

## 4. MODULATION OF BEHAVIOR

### 4.1 Brain targets of IGN

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

### 4.2 IGN and anxiety-related behavior

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

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

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

## 5. ATTEMPT AT AN EVOLUTIONARY PERSPECTIVE

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

### 5.1. EGP and IGN in evolution

There exist 2 isoforms of G6PC1, the catalytic subunit of G6Pase, carrying out EGP and expressed in the liver, kidneys and intestine specifically<sup>1</sup>. G6PC2 is mainly expressed in the beta-cell of the islets of Langerhans (insulin-secreting cells) and G6PC3 is ubiquitous. It is not the matter to discuss their function here; the important observation is that both of these isoforms have no gluconeogenic activity, i.e. no capacity to lead to glucose release in the 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.

## 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

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

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

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

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

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

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

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

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

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

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



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

## 5.5. IGN through human evolution

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

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

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

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

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

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

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

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

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

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

## 6. CONCLUSION

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

## ACKNOWLEDGEMENTS:

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

## COMPETING INTERESTS:

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

## KEY POINTS:

- Like the liver and kidney, the intestine is able to carry out gluconeogenesis and release glucose in blood in the fasting state to contribute to the maintenance of plasma glucose.
- In the fed post-absorptive state, intestinal glucose is sensed by gastrointestinal nerves and initiates a signal to the brain regions controlling energy homeostasis and anxiety/stress-related behavior.
- The activation of intestinal gluconeogenesis promotes anti-obesity and anti-diabetic effects associated with minimization of anxiety/stress-related behavior.
- Intestinal gluconeogenesis is activated after gastric bypass surgery in rodents and in obese insulin-resistant humans and was shown in obese mice to account for the metabolic benefits observed.
- Intestinal gluconeogenesis is activated by macronutrients capable of fuelling systemic gluconeogenesis and to maintain plasma glucose for a while, thereby delaying the need to return to foraging and store energy.
- Intestinal gluconeogenesis might be a mid-term signage related to plasma glucose, between short-term signage related to food, by gastrointestinal hormones, and long-term signage related to energy stores, by leptin.

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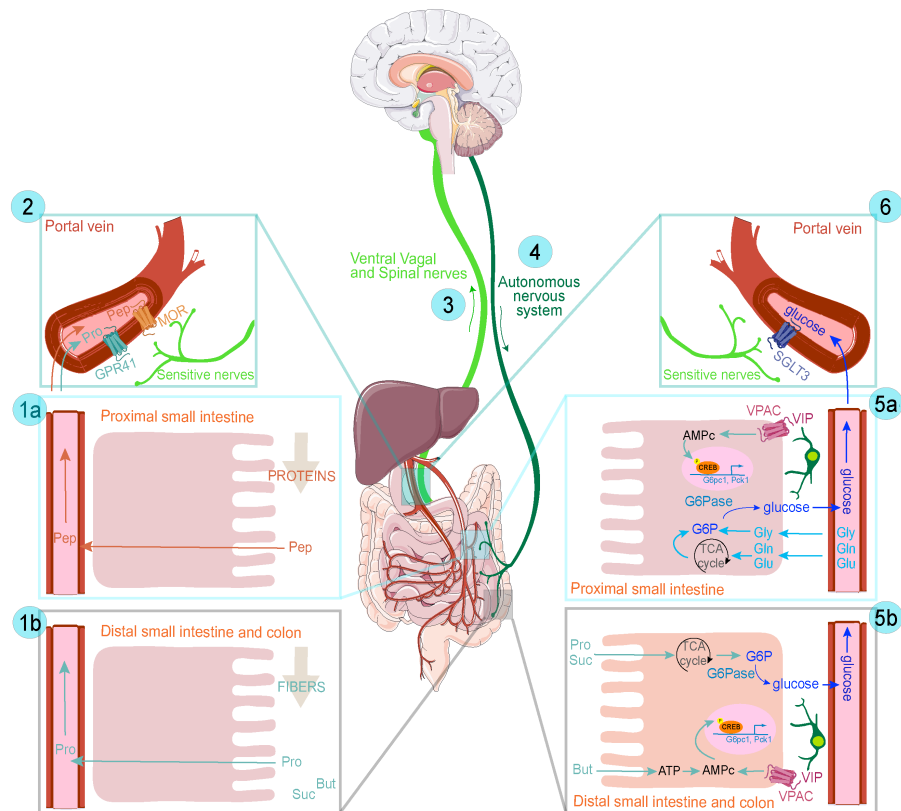
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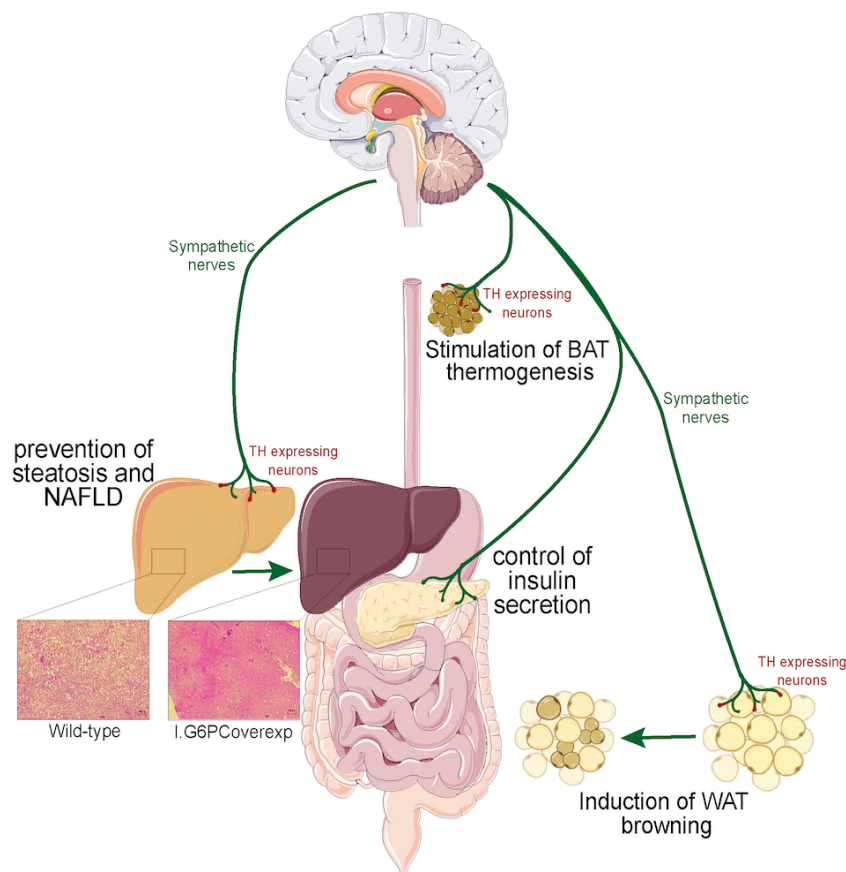
**Figure 1: Sequence from nutrient induction of IGN to glucose detection in the portal vein.**



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 autonomous 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

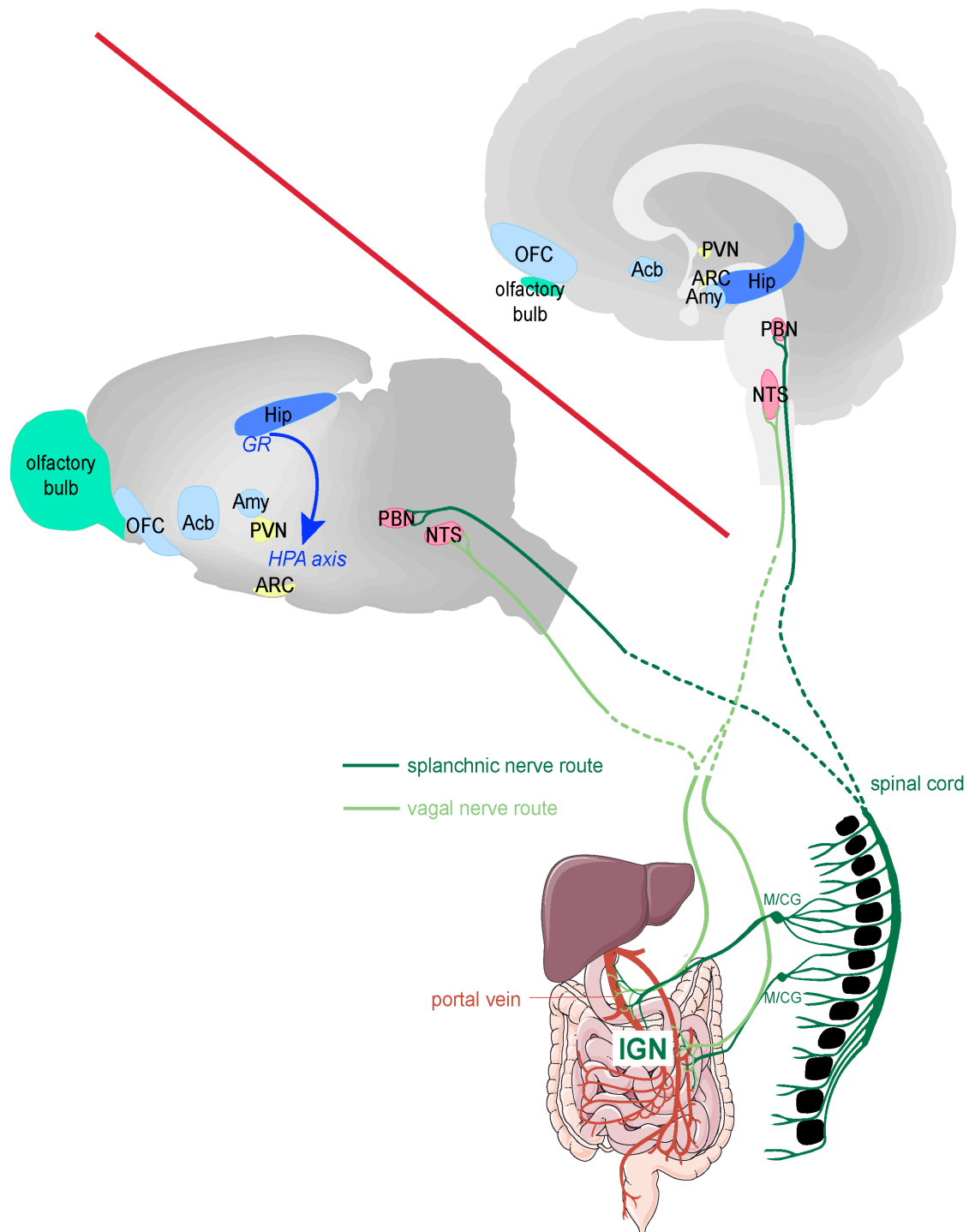
receptor (VIPR) induces IGN gene expression via cAMP. Additionally, But increases cAMP *via* ATP production (5b). Finally, Pro and Suc serve as substrates of IGN (5b). The glucose produced is sensed by SGLT3 in the portal area and signals to the brain *via* spinal nerves (6).

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



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

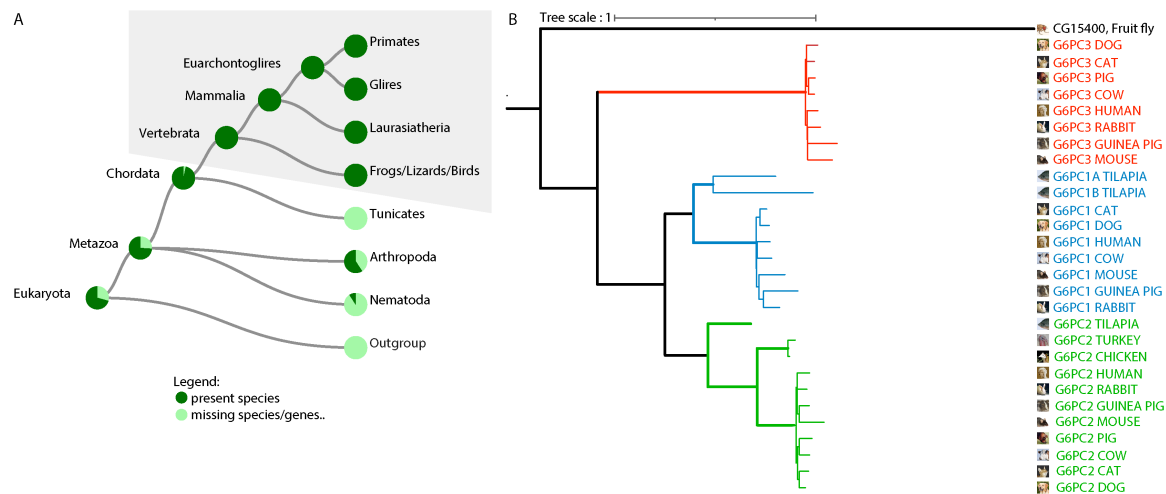
**Figure 3: Brain targets of IGN.**



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

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

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

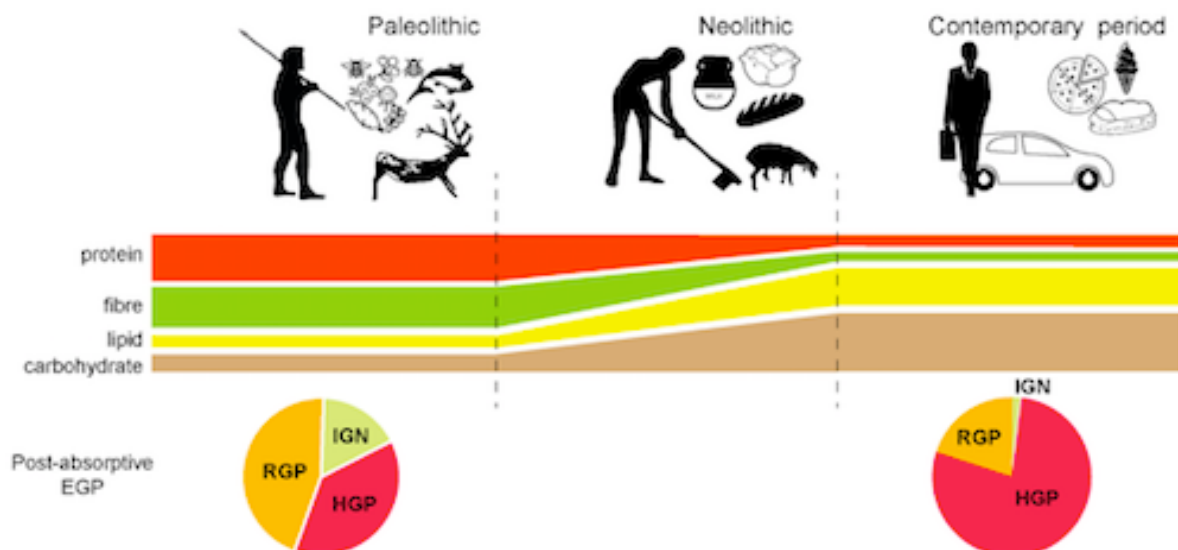


**A.** Summary of the evolutionary history of G6PC. Among the predicted orthologous sequences, species having an annotated G6PC gene are represented in dark green and predicted orthologous sequence, which are still not annotated are represented in light green. **B.** Phylogenetic tree indicating the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

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

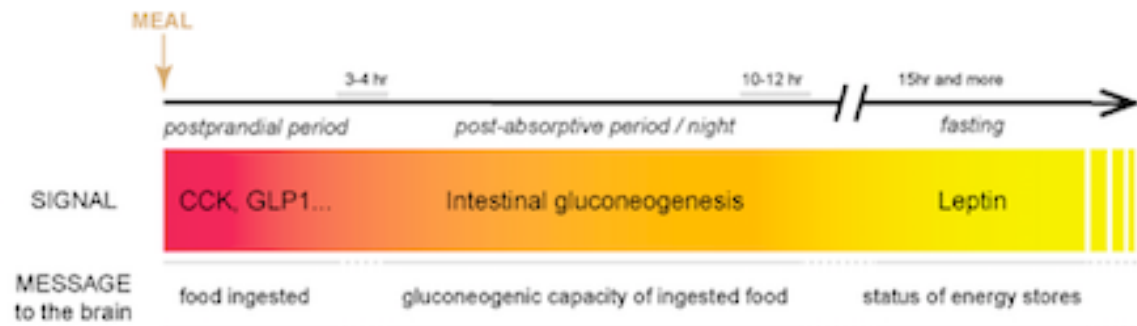
Genomes, JGI and Wormbase) were aligned using MCoffee. The final alignment was 1447 AA long and on average 44% conserved. TreeBest<sup>103</sup> was used to build a gene tree and reconciles it with the species tree. TreeBest build 5 source trees using nj and PhymI<sup>104</sup>. It then merges them into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

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



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.

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



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