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# Neuroendocrine Control of Hepatic Gluconeogenesis

Zhuo Mao and Weizhen Zhang

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## Abstract

Glucose is intricately regulated in human body through a complex network of hormonal and neuronal factors. A series of evidence suggests that the gastrointestinal tract and the central nervous system play prominent roles in the regulation of glucose and energy homeostasis. The gut senses the nutrient supply to co-ordinate the release of hormones that activate neuronal networks in the brain, leading to the subsequent modulation of hepatic glucose output via the gut-brain-liver axis. The gut hormones also act on multiple peripheral tissues to regulate glucose level through an insulin-dependent and/or -independent mechanism. The brain, especially the hypothalamus, could also response to the hormones such as insulin and leptin and different nutrients to modulate the glucose homeostasis. In this chapter, we review the gut-brain-liver axis and the role of this organ interaction in the control of glucose homeostasis. A better understanding of these pathways will provide novel strategies for improved glycaemic control.

**Keywords:** glucose homeostasis, hepatic glucose production, gut-brain-liver axis, gastrointestinal hormones, GLP-1, ghrelin, hypothalamus, insulin, leptin, nutrient sensing, lipid sensing

## 1. Introduction

In human body, glucose production is finely matched to glucose utilisation in order to maintain the glucose level within a relatively narrow range, ~0.8–1.2 mg/dL. This glucose homeostasis is regulated by a network of signals including endocrine, neural and metabolic factors. The various signals monitor the internal energy status and exogenous energy supply to control endogenous glucose production or utilisation.

For a long time, pancreatic insulin and its sensitivity have been the research focus for glucose regulation under normal or diseased condition. Emerging evidence has suggested that the gut

and central nervous system (CNS) are also critical in the control of glucose homeostasis. In this chapter, we will review the recent advances on the gut-brain interaction and its effect on the hepatic glucose production (HGP). We will first introduce the concept of 'gut-brain-liver axis' and its physiological relevance in HGP. Then, we will review the current understanding of gastrointestinal (GI) hormones in the control of glucose homeostasis. Lastly, the role of hypothalamic insulin/leptin signalling and nutrients sensing implicated in glucose homeostasis will be discussed.

## 2. Gut-brain-liver axis

Upon meal ingestion, food enters the digestive tract and triggers a series of mechanical and chemical responses. The tension and stretch receptors, named mechanoreceptors, on the vagal afferent nerves convey these mechanical signals to the brain. On the other hand, the pre-absorbed nutrients and the digested nutrients such as glucose, amino acids and lipids could activate the chemo-receptors on the vagal afferents to regulate glucose homeostasis [1, 2]. Lipids enter the upper intestine in the form of triglycerides (TGs) that are hydrolysed by lipases to form long-chain fatty acids (LCFAs). Intraluminal LCFAs can be further transformed to long chain fatty acyl-co-enzyme A (LCFA-CoA) catalysed by acyl-CoA synthase. Accumulated LCFA-CoAs induce local release of the satiety peptide cholecystokinin (CCK) from the duodenum I cells via a G-protein-coupled receptor GPR40 [3]. Aromatic amino acids could also stimulate the release of CCK via an extracellular calcium-sensing receptor (CaSR) by increasing an intracellular  $\text{Ca}^{2+}$  level [4, 5]. There are two CCK receptors, CCK-A in the periphery tissues and CCK-B in the brain. Secreted CCK binds to CCK-A receptors on the intestinal vagal afferent neurons and increases the neurophysiological activity and conveys neuronal signals to the CNS [6, 7]. These biochemical signals between the gastrointestinal tract and the CNS are critical for the glucose and lipid metabolism in liver. This communication network between the gut, brain and liver is defined as the gut-brain-liver axis.

### 2.1. The gut-DVC-liver axis

The main structures in the CNS that are involved in the control of glucose homeostasis are the dorsal vagal complex (DVC) in brain stem and hypothalamus. DVC is composed of the nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus (DMV) and area postrema. NTS, adjacent to DMV, is the site where the vagal afferent nerves terminate. Activation of vagal afferent signalling increases the NTS neuronal expression of c-Fos, a marker for neuronal activation. Activation of mechanoreceptors in the gut and administration of CCK increases the c-Fos expression in the NTS, suggesting an activation in the gut-DVC signalling [8]. Furthermore, N-methyl D-aspartate (NMDA) channels are located in the NTS neurons. Blocking NTS neuron activity by an NMDA channel blocker abrogates the gut lipid's effect on HGP. This observation further confirms that the NTS neurons relay the signals from the gut to the CNS [2].

NTS neurons are projected to other brain stem nuclei: the nucleus ambiguus (NA) and the DMV. These two nuclei further send out the vagal efferent fibres to form a motor limb of vago-vagal

reflexes innervating multiple peripheral organs. Major organs involved in energy consumption and metabolism include the gut, pancreas and liver. Two distinct populations of DMV pre-ganglionic neurons provide parallel innervations to the gut. The cholinergic-muscarinic (excitatory) pathway enhances GI motility and stimulates gastric acid secretion. The non-adrenergic and non-cholinergic (NANC) (inhibitory) pathway inversely decreases the gastric motility. Activation of the vago-vagal reflexes alters the GI functions in different manner via co-operation of these two parallel pathways [9]. In addition, the vagus influences glucose homeostasis through the innervation of the pancreas and liver. Vagal efferents innervating the pancreas enhance the post-prandial insulin release and compensatory insulin responses during prolonged stimulation of pancreatic  $\beta$  cells. If glucose is administered intragastrically that bypasses the vagal activation, post-prandial glucose levels are higher and insulin levels are blunted [10]. Vagal efferents innervating the liver modulate glucose production through the inhibition of key gluconeogenic enzymes and activation of enzymes promoting glycogen synthesis [2, 11].

## 2.2. The gut-hypothalamus-liver axis

Gastrointestinal signals could be transmitted to the higher centre hypothalamus either through the relay in NTS or via its direct sensing of the gut hormones. Within the hypothalamus, there are three important nuclei, which send out the neural autonomic information to the peripheral organs including the liver. They are ventromedial nucleus (VMN), the lateral hypothalamic area (LHA) and paraventricular nucleus (PVN).

The hypothalamus regulates the hepatic metabolic functions via specific autonomic nerves. Autonomic innervation of liver contains sympathetic and parasympathetic nerves. The pre-ganglionic neurons of sympathetic nerve are located in intermediolateral column of the spinal cord (T7–T12). They convey signals to the post-ganglionic neurons at the celiac and superior mesenteric ganglia and send out the sympathetic splanchnic nerves innervating the liver. The parasympathetic nerves originate from pre-ganglionic neurons in the DMV, while the post-ganglionic neurons have not been clearly identified yet. Neurons in the VMN stimulate liver glycogenolysis (increase liver glucose output) through the activation of the sympathetic system that increases the activity of the liver gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) while suppresses pyruvate kinase (PK) activity. The LHA stimulates liver glucogenogenesis (decrease liver glucose output) by decreasing PEPCK activity via the parasympathetic vagal nerve. PVN is a special area that receives projections from both VMN and LHA or other areas. PVN neurons control hepatic glycogen storage via modulating the sympathetic-parasympathetic balance [11, 12].

## 2.3. Molecular mechanism

The molecular mechanism by which hypothalamic neurons control HGP remains largely unknown. Previous studies have indicated that an intact  $K_{ATP}$  channel in the hypothalamic neurons is required for the proper functioning of this brain control on liver glucose metabolism.  $K_{ATP}$  channel, a member of the inwardly-rectifying  $K^+$  channel family, is located on the plasma membrane of hypothalamic neurons. It is composed of sulfonylurea receptors (SUR)

and inwardly rectifying  $K^+$  channel subunits. Both upper intestinal lipid and central lipid infusion could activate  $K_{ATP}$  channel and hyperpolarise the hypothalamic neurons. Activation of  $K_{ATP}$  channel in the hypothalamus lowers blood glucose through the inhibition of hepatic gluconeogenesis but not glycogenolysis. The hepatic expressions of *G6Pase* and *Pepck* are significantly reduced. Genetic or pharmacological blockade of hypothalamic  $K_{ATP}$  channel, as well as surgical resection of the hepatic vagal nerve, abrogates the gut lipid induced in the inhibition of the HGP [2, 13, 14].

Protein kinase C isoforms also participate in this physiological process. PKC $\theta$  is expressed in the hypothalamic neurons, specifically in the neuropeptide Y/agouti-related protein neurons in the arcuate nucleus and the dorsal medial nucleus. Palmitic acid induces the trans-localisation of PKC $\theta$  to cell membranes and is associated with impaired insulin and leptin signalling. Knocking down PKC $\theta$  in hypothalamic neurons prevented diet-induced obesity and improved insulin signalling [15]. Moreover, activation of PKC in hypothalamus suppresses hepatic glucose production. Inhibition of PKC $\delta$  signalling in hypothalamus will abolish this effect, indicating that PKC $\delta$  also mediates the central role in glucose homeostasis [16]. In addition to the hypothalamus, PKC $\delta$  is expressed in the mucosal layer of duodenum. Activation of duodenal PKC $\delta$  stimulates CCK secretion and the gut-brain-liver neuronal pathway and lowers the glucose production in the liver [6, 17].

### 3. Gut hormones in the regulation of glucose homeostasis

Gastrointestinal tract is the largest endocrine organ in the human body. It consists of a large number of different enteroendocrine cells, which secrete distinct gut hormones. In general, food ingestion acutely stimulates the secretion of gut hormones. These hormones travel in the circulation to the distant tissues, or act locally on vagal afferents and convey signals to CNS. They bind to specific receptors and exert pleiotropic metabolic functions through changes in food intake, gastrointestinal motility and energy expenditure.

#### 3.1. GLP-1

Glucagon-like peptide-1 (GLP-1) is a peptide hormone secreted mainly from enteroendocrine L cells of the intestine, and to a much lesser extent from pancreatic  $\alpha$  cells and the brain. GLP-1 is cleaved from the precursor proglucagon molecule by protease convertase 1/3. Circulating GLP-1 has two forms, GLP<sub>17-37</sub> and GLP<sub>17-36</sub> amide. About 80% of circulating GLP1 in human is GLP<sub>17-36</sub> amide [18]. Plasma levels of GLP-1 in the basal level range between 5–10 pM and its level could increase to 50 pM after a meal. The half-life of native bioactive GLP-1 is less than 2 min. After secretion from L cells, GLP-1 is quickly excreted by kidney or enzymatically degraded by dipeptidyl peptidase-4 (DPP4) [19]. GLP-1 exerts its biological function through a specific G-protein coupled receptor called GLP-1 receptor (GLP-1r). Using the GLP-1-Cre mice crossed with fluorescent reporter strains, GLP-1 receptor (GLP-1r) is found to be expressed on a variety of tissues including pancreatic  $\beta$  and  $\delta$  cells, enteric neurons, vagal and dorsal root ganglia, vascular smooth muscle, cardiac atrium and gastric antrum/pylorus [20].



The biological function of endogenous GLP-1 has been examined using GLP-1r antagonists, immunoneutralising antisera and Glp-1r deficient mice. Exendin<sub>9-39</sub> (Ex-9) is an antagonist of GLP-1r and is the truncated form of the lizard GLP-1-related peptide exendin-4 (Ex-4). Eliminating GLP-1 activity by antisera or infusion of its antagonist results in impaired glucose tolerance and glucose-induced insulin secretion in rodents and humans [21]. Consistently, genetic deficiency of GLP-1r gene in mice causes glucose intolerance and impaired glucose-stimulated insulin secretion, as well as fasting hyperglycemia [22]. Pancreas, especially the pancreatic islet cells, is the target of GLP-1. *In vitro* studies on pancreatic islets or  $\beta$  cell lines demonstrate that GLP-1 increases insulin biosynthesis through both cAMP/PKA-dependent and independent pathways. GLP-1 also stimulates insulin secretion via modulation of  $K_{ATP}$  channels, which subsequently increases the intracellular  $Ca^{2+}$  level. In addition to the insulinotropic effects, GLP-1 could also stimulate the proliferation of  $\beta$  cell, prevent  $\beta$  cell apoptosis and enhance the differentiation of exocrine-like cells towards a more differentiated  $\beta$  cell phenotype [21]. The signal transduction pathways in which GLP-1 mediates these physiological functions have been extensively studied. Its mitotic effect involves PI3K/AKT, EGFR transactivation, p38 MAPK and PKC $\zeta$  signalling pathways [23]. Pancreatic and duodenal homeobox gene 1 (PDX1), forkhead box protein O1 (FoxO1), AKT and insulin receptor substrate 2 (IRS2) are essential mediators for the anti-apoptosis effect of GLP-1 [21]. GLP-1 receptor agonist could increase the pancreas weight. However, this effect is not caused by the increase in proliferation of islet or ductal cells. Increased protein synthesis in the exocrine acinar cells accounts for the increase of pancreas weight [24]. GLP-1 also inhibits glucagon secretion from pancreatic  $\alpha$  cells after meals to lower the blood glucose. The mechanism by which GLP-1 inhibits glucagon secretion is not completely clear. Recent evidence suggests this effect may be indirectly mediated by another islet hormone, somatostatin [25].

Apart from the pancreas, GLP-1 also acts on CNS to modulate systemic glucose homeostasis. In the brain, only a few neurons in an NTS and area postrema express and secrete GLP-1 [26]. GLP-1 receptors are found in the area postrema, ARC, PVN and VMN [20]. Central administration of GLP-1r antagonist induces glucose intolerance. Under the hyperglycemia condition, activation of central GLP-1 signalling enhances insulin secretion and increases hepatic glycogen storage [27]. Two chronic CNS GLP-1 loss of function mouse models, knocking down GLP-1 gene pre-proglucagon in NTS and chronic ICV infusion of GLP-1r antagonist both caused hyperphagia and glucose intolerance [28]. Therefore, these data suggest that central GLP-1 action plays an important role in systemic glycaemic control.

Liver is another action site of GLP-1. After being secreted from enteroendocrine cells, GLP-1 enters the mesenteric venous and the hepatic portal circulation. Due to the rapid degradation of GLP-1, the close anatomical distance makes the liver as an important target organ for GLP-1. Glucose sensor in the hepatoportal area can sense the circulating glucose level and regulate the hepatic glucose disposal. Basal fasting levels of GLP-1 are sufficient to activate the receptor and maintain its glucoregulatory ability [29]. GLP-1 receptor is present in nodose ganglia and nerve terminals innervating the portal vein, suggesting a possible link of GLP-1 action through the hepatic neuronal network with other peripheral organs. Intraportal infusion of GLP-1 can increase the firing frequency of the vagal afferents and promote glucose disposal in non-hepatic organs [30]. Consistently, administration of the GLP-1r antagonist to

block hepatic GLP-1 signalling results in glucose intolerance [31]. *In vitro* study in cultured rat hepatocyte has showed that GLP-1 promotes glycogen accumulation by increasing the activity of glycogen synthase-A while decreasing the activity of glycogen phosphorylase-A [32]. Whether the GLP-1 acts directly on hepatocytes or indirectly through neuronal networks remains to be determined.

Taken together, GLP-1 acts on a variety of distant organs, including pancreas, CNS and liver tissue, to co-ordinate the overall glucose homeostasis.

### 3.2. Ghrelin

Ghrelin is a 28-amino acids peptide hormone, which is predominantly produced by the enteroendocrine X/A-like cells in the gastric oxyntic mucosa. Ghrelin exerts strong growth hormone (GH)-releasing effects, as well as lactotroph and corticotroph function. It also influences a diverse of biological processes, including food intake, sleep and behaviour, gastrointestinal tract motility and secretion, as well as glucose and energy homeostasis. In this section, we will focus on its role in glucose homeostasis.

Ghrelin is octanoylated at its serine residue catalysed by ghrelin O-acyl transferase (GOAT). This acylation is essential for ghrelin binding to its receptor, GH secretagogue (GHS) receptor 1a (GHS-R1a), to exert its biological function. The GHS-R1a is abundantly expressed in the hypothalamus-pituitary region, in other brain areas and peripheral endocrine and non-endocrine tissues. Binding of GHS-R1a initiates a series of intracellular events, including activation of the phospholipase C signalling, leading to an increased inositol phosphate turnover, inhibition of  $K^+$  channel and increased concentration of intracellular  $Ca^{2+}$ .

The first evidence illustrating the relationship of ghrelin to glucose homeostasis is that injection of ghrelin induces a prompt increase of blood glucose, which precedes a transient decrease of insulin secretion in humans [33]. This result suggests that ghrelin-induced blood glucose rise is caused by increased hepatic glucose production and/or peripheral glucose disposal instead of suppressed insulin secretion. Infusion of ghrelin in healthy young men increases plasma glucose and free fatty acid levels. Exogenous ghrelin suppresses glucose-stimulated insulin secretion but demonstrates no effect on basal levels of serum insulin. These observations suggest that ghrelin impairs insulin sensitivity [34, 35]. Evidence from rodent models also consistently demonstrates that exogenous ghrelin administration induces hyperglycaemia and worsens glycaemic control under a high-fat diet condition [36, 37]. Another evidence for the link of ghrelin and glucose homeostasis comes from the clinical assessment of the diurnal variations of ghrelin, glucose and insulin. Fasting ghrelin levels correlates with insulin levels [38]. The ghrelin levels surge before meals and decline after meals. Post-prandial glucose levels are correlated with acyl-ghrelin, independently of insulin. And the ghrelin is lower during sleep than during wake [39].

Since both ghrelin and GHS-R1a are expressed in pancreas, ghrelin synthesised by the islets or elsewhere may regulate the glucose-induced insulin released in autocrine and paracrine manners. Most studies have reported that ghrelin reduces glucose-induced insulin release, while a few studies have found that under certain conditions acyl-ghrelin could amplify insulin

secretion [40]. This is partly due to an attenuation of  $\text{Ca}^{2+}$  signalling [41]. Over-expressing ghrelin in the stomach and brain induces glucose intolerance and attenuates glucose stimulated insulin secretion [42]. Transgenic mice with over-expression of pre-proghrelin gene driven by the rat insulin II promoter-driven (RIP-G Tg) in cells display impaired glucose stimulated insulin secretion. In contrast, over-expression of pre-proghrelin gene driven by rat glucagon promoter-driven (RGP-G Tg) in  $\alpha$  cells shows no significant changes in the insulin secretion and glucose metabolism [43]. Ghrelin null mice have no alterations in basal plasma insulin levels but enhanced glucose induced insulin release [44, 45]. High-fat diet-induced glucose intolerance is prevented in ghrelin knockout mice [45]. Isolated ghrelin-deficient islets have increased glucose-induced insulin release but demonstrated no changes in islet density, size and insulin content, suggesting that ghrelin augments insulin secretion through enhanced insulin vesicle exocytosis capacity/efficiency [45]. Detection of ghrelin in the leptin-deficient ob/ob mice also shows enhanced glucose-induced insulin secretion and increased peripheral insulin sensitivity [46].

Despite that ghrelin receptor is not detected, acylated ghrelin increases glucose output in the primary porcine hepatocytes while unacylated ghrelin completely reverses this effect. This result suggests that an acyl-ghrelin probably acts on the primary hepatocytes through a different receptor other than the GHS-R1a and unacylated ghrelin can counteract the acyl-ghrelin's biological function under certain conditions [47]. Ghrelin administration induces a lipogenic and glucogenic pattern of gene expression in the liver and increases hepatic triglyceride content [48]. Also, ghrelin attenuates liver AKT signalling, which may contribute to the blood glucose increments [49].

In CNS, GHS-R1a expression is largely confined to ARC of hypothalamus and somatotroph pituitary cells, both of which are responsible for appetite control and GH release, respectively. Ghrelin is also locally produced by the hypothalamic neurons. These neurons project to NPY/AgRP and POMC neurons, representing a regulatory circuit controlling energy intake [50]. In addition, GH is an important counter-regulatory factor of insulin [51]. Ghrelin may affect glucose homeostasis partly through the GH releasing regulation.

The ghrelin receptors have been detected in the epididymal and pericardial adipose tissue deposits. Ghrelin administration in the mice causes increased adiposity in white adipose tissue and up-regulates UCP1 mRNA expression in brown adipose tissue [52]. *In vitro* study has showed that ghrelin promotes adipogenesis, inhibits lipolysis and glucose uptake into the adipocytes. In addition, ghrelin increases proliferation and differentiation in 3T3-L1 pre-adipocytes. Ghrelin also prevents adipocyte apoptosis caused by serum starvation. Ghrelin activates MAPK and PI3K/AKT pathways in 3T3-L1 pre-adipocytes and adipocytes, whereas inhibition of these pathways blocks the effects of ghrelin on these cells [53, 54].

### 3.3. Other gut hormones

#### 3.3.1. Peptide YY

Peptide YY (PYY), also known as peptide tyrosine, is a gut peptide hormone primarily released from the enteroendocrine L cells of the colon mucosa. It is also expressed in pancreatic



$\alpha$  cells. Similar to GLP-1, PYY has two forms, the full-length PYY1-36 and a truncated form PYY3-36, which is cleaved by the enzyme DPP IV. The major role of PYY is its inhibition on gut functions, including gastrointestinal tract motility and secretion, gallbladder emptying and food intake [55]. Recent evidence reveals an emerging role of PYY involved in glucose homeostasis. *In vitro* study has showed that PYY inhibits glucose stimulated insulin secretion from the isolated islets by inhibiting the accumulation and action of cAMP [56]. *In vivo* evidence also suggests that deficiency of PYY in mice significantly increases body weight and fat mass. These mice exhibit elevated fasting insulin and glucose stimulated insulin level, suggesting that PYY regulates glucose homeostasis via its stimulation of insulin secretion [57]. Another form of PYY, PYY3-36, elicits no effect on glucose homeostasis under basal or hyperglycaemic conditions. Transgenic mice over-expressing PYY are protected against diet-induced obesity due to increased energy expenditure. Moreover, over-expressing PYY in ob/ob mice significantly reduces adiposity and serum triglyceride levels and improves glucose tolerance [58].

### 3.3.2. Nesfatin-1

Nesfatin-1, an 82-amino acids peptide, is pre-dominantly secreted from gastric X/A-like cells. It was initially identified in the hypothalamus as a satiety molecule [59]. Later, it is also found in pancreatic  $\beta$  cells. Studies using mouse insulinoma cell line MIN6 cells and isolated murine islets have revealed that nesfatin-1 directly enhances the glucose stimulated insulin released by increasing the intracellular  $\text{Ca}^{2+}$  level through the voltage-dependent L type  $\text{Ca}^{2+}$  channel [60, 61]. Infusion of nesfatin-1 in the third cerebral ventricle potently inhibits hepatic glucose production and promotes muscle glucose uptake. Inhibition of central nesfatin-1 activity using the adenoviral-mediated RNA interference method increases hepatic glucose flux and decreases glucose uptake in the peripheral tissue. This effect is caused by an alteration of hepatic PEPCK/InsR/IRS-1/AMPK/AKT/mTOR/STAT3 pathway [62, 63]. In addition to the action in CNS, peripheral infusion of nesfatin-1 also significantly improves insulin sensitivity in the skeletal muscle, adipose tissue and liver under normal or high-fat diet condition, via altering AKT phosphorylation and GLUT4 membrane translocation [64].

## 4. Hypothalamic regulation of glucose homeostasis

Three major hypothalamic nuclei, such as ARC, VMN and PVN, actively participate in the regulation of glucose homeostasis [65]. In 1854, French physiologist Claude Bernard showed that brain lesion in dogs causes hyperglycemia and first proposed that the CNS could be involved in the control of energy and glucose homeostasis. Till date, it has been widely accepted that CNS especially hypothalamus receives and integrates hormonal and nutritional signals from periphery and actively regulates systemic glucose homeostasis. Here, we will focus on how the hypothalamus integrates the critical glucoregulatory hormones, insulin and leptin, as well as the diet nutrients to modulate the intricate regulation of glucose homeostasis.

#### 4.1. Hypothalamic insulin signalling

In addition to the classical insulin target tissues (liver, skeletal muscle and adipose tissue), insulin receptors are also expressed in the CNS, with various densities in different regions [66]. Infusion of insulin in the cerebral ventricle suppresses glucose production independent of circulating insulin and other glucoregulatory hormones [67]. Blunting central insulin signalling by the insulin receptor antisense oligonucleotides also suppresses HGP during hyperinsulinemic clamp studies in rats [68]. Neuron specific knockdown of insulin receptor in whole brain leads to obesity, mild insulin resistance and hyperleptinemia [69]. These results suggest that insulin signalling in hypothalamus controls peripheral glucose production and/or utilisation.

ARC is located at the mediobasal hypothalamus adjacent to the third ventricle and the median eminence, where the blood-brain barrier (BBB) is weakly formed. Insulin or other hormone can easily cross the BBB and functions there. L1 mouse has insulin receptor deficiency in hypothalamus while maintaining insulin receptors in liver and pancreatic  $\beta$  cells. Lack of hypothalamic insulin signalling in L1 mice attenuates the inhibitory effect of insulin on HGP. ARC mainly contains two populations of neurons, the proopiomelanocortin (POMC): expressing neurons and the agouti-related peptide (AgRP) and neuropeptide Y (NPY): co-expressing neurons. To further dissect the role of different groups of neurons, Lin et al. used targeted knock-ins to restore insulin receptor expression in AgRP/NPY or POMC neurons of L1 mice. They have found that re-expression of insulin receptor in AgRP/NPY neurons could normalise insulin's effects on HGP. In contrast, knock-in insulin receptors in POMC neurons increases HGP. These results indicate that these two different types of neurons mediate distinct insulin functions on peripheral glucose and energy homeostasis [70]. Later study has also confirmed that insulin signalling in AgRP neurons is crucial for HGP, expression of G6Pase and insulin-induced expression of interleukin-6 [71].

At the cellular level, the IRS-PI3K pathway mediates the hypothalamic insulin effects on glucose metabolism. Inhibition of PI3K signalling by infusing PI3K inhibitor into the third cerebral ventricle attenuates insulin-induced glucose lowering effects, whereas over-expression of IRS-2 or PI3K substrate AKT enhances the glycaemic response [72].

FoxO1 is a transcriptional repressor of POMC and the transcriptional activator of AgRP. Insulin increases FoxO1 phosphorylation through an InsR/IRS1/PI3K pathway and promotes its exit from the nucleus to the cytoplasm. This translocation dis-inhibits its repression of POMC transcription, allowing for STAT3 binding to the promoter, thus increasing *Pomc* gene expression. Meanwhile, the nuclear export of FoxO1 decreases *Agrp* gene expression since STAT3 binds to the *Agrp* promoter and inhibits its expression [73]. FoxO1 deletion in AgRP neurons of mice leads to the improved glucose homeostasis and increased sensitivity to insulin and leptin.

$K_{ATP}$  channels are also involved in the hormonal control of glucose metabolism. Activation of  $K_{ATP}$  channels in the hypothalamus is sufficient to decrease blood glucose levels by inhibition of HGP. ICV or systemic administration of insulin causes an inhibition of HGP. These effects are prevented by blockade of  $K_{ATP}$  channel activity or genetic deletion of SUR1 subunits [14]. Thus, the insulin action in the CNS to regulate glucose level requires intact  $K_{ATP}$  channels.

In summary, data from rodents have confirmed that hypothalamic insulin reduces blood glucose concentrations by suppressing gluconeogenesis through inhibition of the expression of key gluconeogenic enzymes. It is worth noting that the role of insulin signalling in glucose homeostasis may vary among species and different physiological conditions. Most experiments have been performed on rodent models. However, it is not exactly the same in other animals. For example, blunting CNS insulin signalling in dogs does not suppress glucose production or gluconeogenesis; instead it augments hepatic glycogen synthesis [74].

#### 4.2. Hypothalamic leptin

Leptin is a hormone produced by an adipose tissue, which regulates pleiotropic metabolic functions, such as satiety, body weight gain and glucose homeostasis. Systemic leptin receptor (Lep-r) deficient ob/ob mice are obese, hyperglycemia and hyperinsulinemia. Neuron-specific deletion of Lep-r in mice also exhibits obesity and elevated plasma levels of leptin, glucose and insulin, suggesting the brain is a direct target for leptin and responsible for its role in systemic metabolic effects [75]. Lipodystrophy is a disease characterised by the complete or partial absence of adipose tissue, leptin deficiency, systemic insulin resistance and fatty liver. Central infusion of leptin in the lipodystrophy mice corrects the insulin resistance and improves glucose homeostasis [76]. Overfeeding rapidly induces hepatic, leptin and insulin resistance. Central administration of leptin in overfeeding conscious rats could rescue the hepatic insulin resistance. Leptin inhibits HGP by decreasing glycogenolysis with reduced hepatic expression of G6Pase and PEPCK [77].

Similar to insulin, hypothalamic ARC area is the prominent action site for leptin in regulation of glucose homeostasis. Leptin injection specifically into the ARC increases glucose uptake in BAT [78]. Restoration of Lep-r in the ARC could normalise insulin and blood glucose level without significantly altering the body weight and food intake [79]. Koletsky (fa(k)/fa(k)) rat, in which Lep-r is deficient, is characterised by insulin resistance and hyperglycemia. Re-expression of Lep-r specifically in the ARC region significantly improves peripheral insulin sensitivity and suppresses HGP in this animal. This effect was mediated through CNS-autonomic system-dependent suppression of hepatic expression of the gluconeogenic enzyme, G6Pase and PEPCK [80].

Neuroanatomical and electrophysiological studies have identified POMC neurons as the neuronal target of leptin. Approximately 25–40% of POMC neurons express functional Lep-r. These neurons are distinct from those expressing insulin receptors [81]. Mice lacking leptin signalling in POMC neurons are hyperleptinemic [82]. Simultaneous deletion of insulin and leptin receptors in POMC neurons causes a pronounced insulin resistance [83]. Reconstitution of Lep-r in POMC neurons of the systemic Lep-r deficient mice is sufficient to normalise the hyperglycemia, improve hepatic insulin sensitivity and dyslipidemia [84]. All these results indicate that leptin signalling in POMC neurons plays a key role in regulating glucose homeostasis via control of HGP. The role of leptin in AgRP neurons remains to be investigated.

Lep-r has six splicing variants (Lep-r-a to Lep-r-f). The long-form Lep-r-b is highly expressed and functionally active in the hypothalamus. Leptin binds to the Lep-r-b, recruits and activates the

Janus kinase (JAK). JAK in turn phosphorylates Lepr-b and the signal transducer and activator of transcription 3 (STAT3). Phospho-STAT3 traffics into the nucleus and activates the transcription of several genes including suppressor of cytokine signalling 3 (SOCS3). SOCS3 is a negative regulator of leptin signalling. Neuronal deletion of SOCS3 in mice improves leptin sensitivity, with a greater body weight loss and less food intake. These mice are resistant to high-fat diet obesity and hyperleptinemia. The insulin sensitivity is also retained [85]. Lentivirus-mediated knock-down of SOCS3 in hypothalamus significantly increases STAT3 activation, decreases body weight gain and improves metabolic parameters in mice fed with a high-fat diet [86].

Leptin and insulin can engage similar hypothalamic intracellular signalling pathway. Leptin could activate PI3K-PDK1-AKT pathway and influences the neurotransmitter's expression and neuronal activity. Leptin can down-regulate NPY's gene expression and enhance POMC's expression [87, 88]. In addition, leptin causes depolarisation and increased excitability of POMC neurons, while hyperpolarises and inhibits the AgRP neurons [89, 90].

In addition to ARC area, Lep-r is also abundantly expressed in the VMN. Microinjection of leptin in VMN increases glucose uptake in brown adipose tissue, heart, skeletal muscles and spleen through the VMH-sympathetic nervous network [91]. A specific group of neurons in VMN expresses transcription factor steroidogenic factor-1 (SF-1), called SF-1 neurons. Leptin can directly activate SF-1 neurons and leads to body weight loss. Specific deletion of Lep-r in SF-1 neurons in mice results in increased fat mass, hyperinsulinemia and diet-induced obesity, highlighting the importance of VMN in the control of energy homeostasis [92, 93]. SOCS3 in SF1 neurons also negatively regulates leptin signalling, mediating central leptin resistance and glucose dysfunction [93].

### 4.3. Nutrients sensing and glucose metabolism

#### 4.3.1. Fatty acid/lipid sensing

The brain not only reacts to the circulating hormones but also senses and responds to different nutrients such as lipid, glucose and amino acids. Nutrients sensing in the hypothalamus exert a negative feedback regulation on glucose metabolism and food intake. The most studied example is fatty acid. ICV administration of long-chain fatty acid (LCFA) oleic acid, but not the short-chain fatty acid octanoic acid, could markedly inhibit glucose production and food intake [94]. Fatty acid synthase (FAS) is the rate limiting enzyme for de novo lipogenesis and mediates the synthesis of LCFA. Deletion of FAS in hypothalamus and  $\beta$  cells results in reduced LCFA's level, hypophagic and hypermetabolic in mice fed with a standard or high fat diet [95, 96].

Similar to the gut lipid sensing mechanism, LCFA-CoA is the sensing molecule in the CNS. Malonyl co-enzyme A, a co-enzyme A derivative of malonic acid, plays an important role in chain elongation in LCFA-CoAs biosynthesis. Malonyl-coenzyme A decarboxylase (MCD) mediates the degradation of malonyl co-enzyme A. Thus, over-expression of MCD reduces the abundance of LCFA-CoAs, hence attenuating the lipid sensing ability in the CNS. Indeed, injection of adenovirus expressing MCD into the mediobasal hypothalamus of rats leads to an



increase in food intake and weight gain despite increased circulating leptin and insulin [97]. LCFA-CoAs are oxidised in the mitochondria, which is catalysed by two carnitine-dependent long-chain acyl-transferases, known as carnitine palmitoyl transferases (CPT1 and CPT2). CPT1 has two isoforms, liver form of CPT1 (CPT 1A) and muscle form of CPT1 (CPT 1B). Down-regulation of CPT1A expression or pharmacological inhibition of CPT1 activity within the ARC causes an increase in the concentration of LCFA-CoAs and a decrease in the liver glucose output [98]. These central lipid-sensing effects also require intact neuronal  $K_{ATP}$  channels and increased hepatic vagal outflow [13], suggesting a mechanism dependent on the brain-liver communication.

#### 4.3.2. Glucose sensing

Our brain is able to directly sense the glucose level [99]. A subset of neurons in hypothalamic nuclei is sensitive to the changes of extracellular glucose level. Some neurons are excited by increased glucose level with more firing frequency while others are inhibited with less firing by same stimuli. The glucose sensitive neurons are located in the VMN, ARC, PVN, as well as the dorsal vagal complex in the brain stem [65]. Central glucose sensing also impacts systemic glucose metabolism. Glucose is converted to lactate, enters the pyruvate metabolism and activates  $K_{ATP}$  channels in hypothalamus, leading to an HGP reduction [100].  $K_{ATP}$  channels are able to sense the changes of glucose level and couple membrane excitability to cellular metabolism. High glucose concentrations activate  $K_{ATP}$  channel. The intracellular  $K^+$  fluxes out and hyperpolarises the plasma membrane, leading to the subsequent suppression of neurotransmitter release and inhibition of neuronal excitability [101, 102]. Up to date, the identity of neurons that can directly regulate peripheral glucose production is still not known.

#### 4.3.3. Amino acids sensing

Leucine is an essential branched-chain amino acid (BCAA). Recently, it has been demonstrated that leucine gains access to the CNS. It is metabolised to acetyl- and malonyl-CoA in the hypothalamus, which subsequently controls HGP [103]. Another amino acid proline also exerts similar effects on HGP. Proline is a gluconeogenic amino acid, which is metabolised to pyruvate in astrocytes. Pyruvate is eventually metabolised to acetyl- and malonyl-CoA, which in turn regulates HGP. Indeed, increase in either central or systemic proline level acutely lowers blood glucose by decreasing hepatic glycogenolysis as well as gluconeogenesis [104].

#### 4.3.4. Astrocytes in hypothalamus

In addition to neurons, other cell types in the brain, such as astrocytes, contribute to the intricate regulation of central and systemic glucose homeostasis. Circulating glucose is uptake into the CNS via glucose transporter 1 (GLUT-1). GLUT-1 is highly expressed in astrocytes and endothelial cells along the BBB. Recently, astrocytes have been proved to participate



in the regulation of hypothalamic glucose sensing and systemic glucose homeostasis. The metabolism and activity of astrocytes are regulated by local hormones and metabolic status. Leptin or nutrients-deprived condition causes glutamate and GLUT-1 changes in the astrocytes, affecting synaptic metabolism, glucose uptake and ultimately neuronal activities [105]. Disruption of insulin signalling in astrocytes affects glucose uptake crossing the blood brain barrier (BBB). It also reduces glucose-induced activation of POMC neurons and impairs hypothalamic mediated suppression of systemic blood glucose [106].

## 5. Conclusion

Glucose is the basic energy provider for the organisms. Mammals have redundant systems to control glucose production. Insulin-independent mechanisms account for approximately 50% of overall glucose disposal. Recently, Schwartz et al. have elegantly proposed a two-compartment model which highlights the importance of both pancreatic islet and the brain in glucose homeostasis and development of diabetes [107]. Emerging evidence suggests that the gastrointestinal tract is also a major player in this intricate regulatory process. The best supporting evidence is the pronounced effects of bariatric surgery on obesity and diabetes patients. Although the exact mechanism for the post-operational rapid normalization of glucose homeostasis is still not clear, it is highly possible that alteration in the gut neuroendocrine restores both insulin-dependent and insulin-independent mechanisms [108]. Understanding the gut-brain-liver axis and its control on glucose homeostasis will provide an alternative strategy for the intervention of diabetes.

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## Author details

Zhuo Mao and Weizhen Zhang\*

\*Address all correspondence to: [weizhenz@med.umich.edu](mailto:weizhenz@med.umich.edu)

Department of Physiology, Center for Diabetes, Obesity and Metabolism, Shenzhen University Health Science Center, Shenzhen, China

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