Chapter

Role of Nutrient and Energy Sensors in the Development of Type 2 Diabetes

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Abstract

Cell survival depends on the constant challenge to match energy demands with nutrient availability. This process is mediated through a highly conserved network of metabolic fuel sensors that orchestrate both a cellular and whole-body energy balance. A mismatch between cellular energy demand and nutrient availability is a key factor in the development of type 2 diabetes, obesity, metabolic syndrome, and other associated pathologies; thus, understanding the fundamental mechanisms by which cells detect nutrient availability and energy demand may lead to the development of new treatments. This chapter reviews the role of the sensor PASK (protein kinase with PAS domain), analyzing its role in the mechanisms of adaptation to nutrient availability and the metabolic response in different organs (liver, hypothalamus) actively cooperating to control food intake, maintain glycaemia homeostasis, and prevent insulin resistance and weight gain.

Keywords: PASK, mTOR, AMPK, obesity, food intake, fasting/feeding, GLP-1

1. Introduction

Nutrients such as carbohydrates, amino acids, fats, vitamins, minerals, etc. are supplied at regular intervals by food intake and are necessary for the normal functioning of cells and a healthy physiology [1]. They act as metabolic substrates for energy production and as building blocks for the synthesis of macromolecules and cellular components. Accordingly, organisms have developed mechanisms to detect levels of specific nutrients in the extra- and intracellular compartments to ensure rates of growth, proliferation, and function coordinate properly and adjust to nutrient availability.

2. Nutrients and energy sensors: importance in glucose and energy homeostasis

Nutrient-sensing mechanisms are found in all organisms, from yeast through to mammals. Importantly, some of these mechanisms in multicellular organisms have also evolved for regulation by the endocrine system, allowing the coordination of nutrient-sensing activity among different cells/tissues in the body [2].

Nutrient sensors are proteins that detect fluctuations in nutrient levels or products of their metabolism within the physiological range, and induce a cellular response, leading to changes in the nutrient distribution or in feeding behavior [1]. These sensors respond to alterations in nutrient levels through diverse mechanisms, including the activation of phosphorylation cascades, changes in gene transcription, and enzymatic activities, among others [2].

The sensing of a nutrient may involve the direct binding of its molecule to the sensor or an indirect mechanism relying on the detection of a surrogate molecule that reflects nutrient abundance. There are homeostatic responses in multicellular eukaryotes to maintain nutrient levels circulating within a narrow range, such as hormone release, which act as signals to facilitate the coordination of consistent responses in the whole organism [3].

An effective and adequate response to changes in nutrient availability is vital in the human body, and its alteration triggers pathologies such as obesity, metabolic syndrome, and aging-related diseases (e.g. cancer and neurodegeneration).

Some situations in which nutrient sensors are chronically affected by excessive amounts of certain nutrients (e.g. carbohydrates and some fats) lead to the development of common characteristics of obesity and type 2 diabetes mellitus (T2D), such as insulin resistance, oxidative stress, and the dysfunction of organelles including the endoplasmic reticulum and mitochondria [4].

The increasing number of overweight and obese people, and associated diseases such as T2D, is driving research to explore the basic mechanisms that maintain nutrient homeostasis in a healthy state and the molecular mechanisms disrupted in T2D and obesity, as well as the neural and molecular underpinnings of feeding behavior. A central role in these disease-related mechanisms corresponds to nutrient sensing and the regulation of feeding behavior [1].

Glucose is a critical nutrient in mammals, with extracellular and intracellular mechanisms to maintain its levels within a narrow physiological band.

Glucose is an energy substrate, but it is also a key molecule in the control of glucose-dependent insulin secretion by the pancreas. The increase in insulin in the blood as glycaemia facilitates the uptake of glucose by the liver and skeletal muscle, highlighting the cooperation and retro-regulation between glucose and insulin signaling. In short, circulating and intracellular glucose, acting as a signaling molecule, is detected by different glucose sensors that modulate eating behavior and the release of counter-regulatory hormones in response to hypoglycemic states. The answer is therefore to maintain glucose and energy homeostasis [5], avoiding the development of T2D and other diseases. Some of the main nutrient sensors described are the following:

- **Glucokinase (GCK)**: It is an enzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate. GCK is expressed in hepatocytes, pancreatic α and β cells, entero-endocrine cells, and specialized brain cells in humans and most other vertebrates. It is considered a true glucose sensor due to its kinetic properties that ensure that the rate of glucose phosphorylation is proportional to blood glucose levels. For example, pancreatic GCK connects glucose sensing to insulin secretion by the pancreatic β-cell, and so regulates blood glucose homeostasis.

  GCK in the liver is also a glucose sensor (**Figure 1**). Its activity regulates the rate of glycogen accumulation and hepatic glucose production [6].

  Mutations in the GCK gene that increase enzyme activity lead to hypoglycemia due to hyperinsulinism, while mutations that decrease enzyme activity lead to hyperglycemia or diabetes. Due to its importance in glucose homeostasis, this enzyme is one of the main study targets for the development of a new antidiabetic therapy strategy [7].

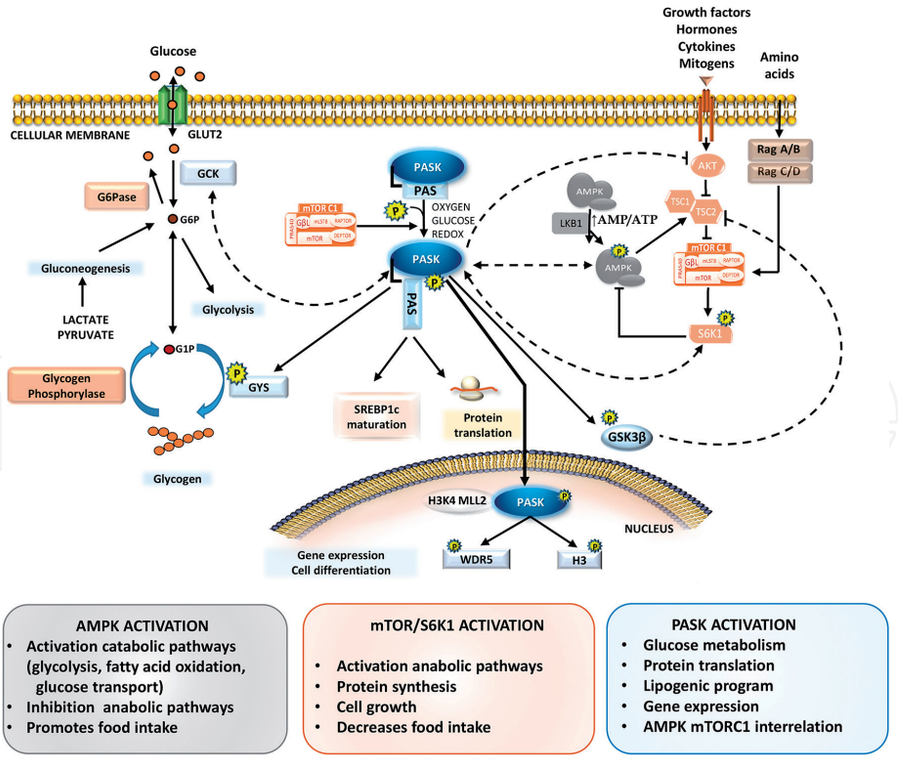

Figure 1.
PASK signaling interaction with other nutrient sensors. PAS domains detect environmental parameters (light, oxygen and redox state). A metabolite or protein binds to the PAS domain after transient activation auto- or transphosphorylation stabilizes and activates PASK. Physiological effects of PASK in other nutrient sensors (glucose transporter (GLUT2), glucokinase (GCK), AMP-activated kinase (AMPK) and mammalian target of rapamycin (mTOR))/S6K pathway due to activation or inhibition (direct: solid lines; indirect: dashed lines).

Additionally, GCK is a key component in glucose-sensing neurons located in the hypothalamus [8–10].

- **Glucose transporter GLUT-2**: It is a glucose transporter that acts as a sensor of changes in extracellular glucose levels. This is due to a high Km (more than other glucose transporters of the same family) and thus a very low affinity for glucose, allowing a rapid equilibrium between the glucose concentration on both sides of the membrane, independently of the action of insulin [11]. GLUT2 is expressed mainly in hepatic cells, pancreatic β cells, basolateral membranes of intestinal, renal epithelial cells, and in specific brain nuclei. In hepatic cells, GLUT2 involves an efficient transport of glucose across the plasmatic membrane only when intra- or extra-cellular glucose is high, being a key controller of glucose homeostasis (**Figure 1**). β-pancreatic cells take up glucose through the GLUT-2 transporter and carry out insulin synthesis and secretion. Glucose transport by GLUT-2 and then GCK facilitates oxidation by increasing intracellular ATP, which leads to signaling via ATP-dependent K^+ channels [1]. Decreased GLUT2 levels in pancreatic β cells have been detected in cases of diabetes in both animals and human patients.
  GLUT2 is also found in neurons located in certain glucose-sensing brain regions involved in controlling feeding behavior [9, 12, 13].

- **AMP activated protein kinase (AMPK):** It is a heterotrimeric complex with a serine/threonine kinase domain [14, 15]. AMPK perceives cellular energy availability by detecting the AMP/ATP ratio (**Figure 1**). This kinase is activated in states of low energy availability or metabolic stress that reduces

ATP production (e.g. heat shock, hypoxia, ischemia and fasting conditions) or accelerates its consumption (muscle contraction). Once active, AMPK acts by inhibiting the anabolic pathways responsible for the synthesis of macromolecules (proteins and glycogen) and lipids (fatty acids, triglycerides and cholesterol), and by activating catabolic pathways, such as the β-oxidation of fatty acids, glucose uptake and glycolysis. The net result of AMPK activity is the restoration of the energy balance, as the main energy sensor [16]. The AMPK pathway at central level integrates peripheral information through nutrients and hormones. Hypothalamic AMPK is involved in feeding behavior, the thermogenesis of brown adipose tissue (BAT) and browning of white adipose tissue (WAT) [17, 18].

- **Mammalian Target of Rapamycin (mTOR)**: The mTORC1 complex is a serine/threonine kinase which forms part of the mTOR/S6K pathway integrating nutrients, hormones, growth factors and cellular energy levels to control protein transcription and synthesis and cell size, growth, metabolism, autophagy and thermogenesis [19]. Growth factors, amino acids, mitogens, and favorable energy states activate the mTORC1/S6K1 pathway, promoting anabolic processes (**Figure 1**), while states of energy depletion and cellular stress such as hypoxia suppress this pathway. The hypothalamic mTORC1 complex is an energy sensor involved in food intake and body weight control [20, 21]. AMPK and mTORC1 act together in food intake regulation, as low nutrient levels during fasting activate AMPK, although the mTORC1 complex remains inactive, while the activity of these sensors is reversed after food ingestion, indicating that AMPK and mTORC1 could have opposite functions in the control of feeding behavior [22].

- **PAS kinase (PASK):** It is also called PASKIN, and is defined as the protein kinase that contains an N-terminal Per-Arnt-Sim (PAS) domain and a C-terminal serine/threonine kinase catalytic domain [23]. Like AMPK and mTORC1, it is a nutrient-responsive protein that regulates glucose metabolism and cellular energy, and is also responsive to a variety of intracellular cues, including light, oxygen, and redox state, among many others [24]. In mammals, PASK may be activated by small metabolites, and could regulate glycogen synthesis and protein translation (**Figure 1**), in addition to being involved in the regulation of glucose homeostasis and energy metabolism [25–27], and epigenetics and differentiation [28].

This chapter will focus on the study of this last sensor, and like AMPK and mTORC1, it can be considered a pharmacological target for diseases, such as obesity and diabetes.

2.1 Neuronal and peripheral regulation of homeostasis by nutrient sensing

The key to maintaining homeostatic and energy control is a balanced food intake and energy expenditure, whereas altered regulation leads to obesity and T2D. The regulation of the energy balance is controlled by the hypothalamus, as the central organ that integrates nutrient levels and hormonal changes. The hypothalamic response to regulate glucose and whole-body energy homeostasis is to control food intake and several physiological functions in peripheral organs, such as lipid metabolism and thermogenesis [29, 30]. The brain receives inputs from nutrients, adiposity signals, and hormonal neural and metabolic signaling from the gastrointestinal tract. The gut-brain and gut-brain-liver axes act to regulate energy

and glucose homeostasis, respectively [31–33]. The brain likewise controls energy-consuming processes such as skeletal muscle fatty acid oxidation, thermogenesis, and locomotor activity [34]. Deficient intercommunications between the brain and peripheral organs may contribute to the appearance of obesity and T2D [30, 33].

The hypothalamus is a key brain area for maintaining an energy balance and homeostasis. Hypothalamic areas thereby play a key role in the control of food intake and energy homeostasis. The mid-20th century recorded the first indications that the electrical stimulation of the ventromedial hypothalamus (VMH) suppresses food intake, and that bilateral lesions of these areas induce hyperphagia and obesity. The VMH has therefore been called the satiety center. By contrast, alterations in the lateral hypothalamic area (LH) induce the opposite set of responses, and the LH is hence called the hunger center. Changes in blood glucose levels can be monitored by neuronal cells located in the hypothalamus or the brain stem [35]. They can therefore act as a true glucose sensor in the control of food intake and energy homeostasis. In fact, the first brain glucose sensors were discovered in the VMH and LH nucleus, where circulating glucose concentrations drive changes in neuronal electrical activity [36, 37]. This means glucose would act mainly as an excitatory molecule in certain VMH neurons, and as an inhibitory molecule in those of LH and the nucleus of the tractus solitarius (NTS) [38]. This is due to at least two kinds of glucose sensor neurons: glucose-excited neurons (GE) are located mainly in the VMH (as well as the arcuate nucleus, ARC, and the paraventricular nucleus PVN), and are excited by increased glucose levels in the extracellular space, while glucose-inhibited neurons (GI) are present mainly in the LH, median ARC, and PVN, and are excited by decreases in glucose concentrations [10, 37, 39]. It has been suggested that the activation of the firing rate of GE neurons depends on the closure of the ATP-sensitive K^+_{ATP} channels by increases in extracellular glucose (similar electrophysiological pattern to β-pancreatic cells), whereas GI neurons may increase their firing rate in response to hypoglycemia following the inactivation of the Na^+/K^+-ATPase pump (similar electrophysiological pattern to α-pancreatic cells) [40].

Some of the component molecules responsible for the hypothalamic glucose sensing systems are as follows: GCK, GLUT-2, and the GLP-1 receptor, which are co-expressed in areas involved in energy homeostasis regulation, food intake, and glucose metabolism [9, 12, 41, 42]. The most glucose-sensitive regulator seems to be the GCK, which is present in both GE and GI neurons (albeit to a lesser extent) [43]. However, glucose transporters such as GLUT-2, GLUT-3, the insulin-dependent transporter (GLUT-4), and the sodium-glucose transporter (SGLT) do not seem to have a predominant role in the response by GE and GI neurons to alterations in glucose levels [10]. GE neurons are known to use GLUT-2 for glucose uptake, then GCK mediates the phosphorylation, and the glucose oxidation increases ATP/ADP, leading to the closure of ATP-sensitive K^+_{ATP} channels and depolarization, promoting Ca^{2+} influx and neurotransmitter release [33, 44, 45].

Hypothalamic sensing neurons also use fatty acids (FA) as signaling molecules [46]. Some of these sensing neurons respond to both FA and glucose, whereby these neurons distinguish between fasting and feeding states. When the effect of glucose is excitatory, FA tend to inhibit those neurons [46]. A deficiency of fatty acid translocator/receptor CD36 in VMH neurons stimulates food intake, enhances insulin resistance, and increases body weight and fat mass in lean and obese rats [47]. FA sensing therefore plays a key role in integrating signals for regulating glucose and energy homeostasis and fat deposition.

It has also been reported that FA are oxidized by astrocytes in VMH under a low-fat diet, while under a high-fat diet (HFD) astrocytes in this area generate ketone bodies that can be exported to neurons and signal a decrease in short-term food intake and protect against obesity. However, this effect is lost when besides

HFD there is a resistance to leptin. Animals in these cases remain hyperphagic and exposed to obesity [48].

Additionally, with changes in nutrient concentrations some neurons located in hypothalamic nuclei secrete and respond to the hormones and neuropeptides involved in the control of food intake and energy homeostasis.

For example, the ARC secretes hormones and detects inputs from the peripheral signals involved in the control of feeding behavior. There are two important subpopulations of secretory neurons in ARC: one synthetizes the α-melanocyte-stimulating hormone (α-MSH) derived from pro-opiomelanocortin (POMC), as well as the cocaine- and amphetamine-regulated transcript (CART); both of which are anorexigenic peptides. The second subpopulation of neurons secretes the agouti-related protein (AgRP) and neuropeptide Y (NPY) orexigenic peptides [49]. These peptides are directed by nerve fibers to other important hypothalamic regions, and their synthesis and release coordinate with metabolic sensors to accurately control eating behavior and energy metabolism. Additionally, these two populations and other neurons located in different hypothalamic nuclei have receptors for hormones secreted peripherally, such as leptin, insulin, ghrelin, and other gastrointestinal peptides, such as glucagon like peptide (GLP-1), which in turn are being secreted under the control of changes in nutrient availability.

AMPK is another hypothalamic molecule responsible for energy sensing. It has been reported to act as an "energy integrator", and not only perceives the cellular energy state, but also has a role in the regulatory mechanisms of body energy homeostasis [50, 51]. It has a mainly neuronal distribution [52], highly expressed in ARC, PVN, VMH and LH, with AMPKα2 being the most predominant isoform [53].

Fasting increases and feeding decreases AMPK activity in various hypothalamic nuclei [18, 53]. Several studies have shown that hypothalamic AMPK is regulated by blood glucose levels. Peripheral or central hyperglycemia inhibits AMPK in several hypothalamic nuclei. Furthermore, the anorexigenic neuronal signaling (NPY/AgRP) is AMPK-dependent in the hypothalamus. Thus, AMPK mutants have suppressed the NPY/AgRP response, and therefore food intake, reducing body weight. However, elevated AMPK increases NPY/AgRP expression, food intake and body weight [53, 54].

Fasting and feeding are accompanied by hormonal and nutrient changes both in peripheral tissues and in the CNS, which can lead to variations in AMPK activity. Accordingly, AMPK integrates nutritional information and hormonal signals. Several studies have shown that fasting and orexigenic signals (e.g. ghrelin, adiponectin, cannabinoids and glucocorticoids) increase hypothalamic AMPK activity, contributing to an increase in food intake; by contrast, food and anorectic signals such as leptin, insulin, resistin, GLP-1 and α-MSH decrease this kinase's activity, helping to generate a state of satiety. AMPK activation therefore promotes food intake, while the decrease in its enzymatic activity is associated with hypophagia. This effect is due, at least in part, to an increase in the expression of NPY and AgRP in the arcuate nucleus, and of MCH in the lateral hypothalamus [17, 18, 54].

AMPK activity can induce appetite via the inhibition of malonyl-CoA and the activation of carnitine palmitoyltransferase-1 (CPT-1). The inhibition of malonyl-CoA leads to decreased fatty acid synthesis and increased β-oxidation. Furthermore, increased β-oxidation could induce orexigenic gene expression. In addition, AMPK activation through the sympathetic nerve can reduce thermogenesis and energy expenditure. Additionally, activated hypothalamic AMPK may prompt enhanced glucose production [55].

Besides AMPK, mTORC1 is another hypothalamic metabolic sensor regulator of feeding behavior and body weight [20]. mTORC1 and the downstream target S6K1 are widely distributed in the brain, mainly in the PVN and ARC. Their signaling

responds to nutrient availability and is colocalized with NPY/AgRP and POMC/CART neurons in the ARC [20]. mTORC1 activation decreases food intake and body weight [56]. mTORC1 integrates signals from nutrients, adiposity signals, and gut hormones [21]. Hypothalamic AMPK and mTORC1 respond to nutrient levels in opposite ways [57, 58]. Additionally, mTORC1 is inhibited by AMPK activation via the tuberous sclerosis complex 2 (TSC2) [59, 60]. Moreover, AMPK is also a substrate for the mTOR-S6K1 pathway (**Figure 1**) [22].

In short, the interplay between both hypothalamic pathways plays an important role in regulating food intake and body weight.

Several peripheral signals are involved in controlling food intake and energy homeostasis. Moreover, changes in nutrient levels involving glucose [61] and FA [62, 63] or ketone bodies [48], adiposity signals (leptin, insulin) [64], and gastrointestinal (ghrelin [65], GLP-1 [57, 58, 66]) signals, alter the activity of sensing neurons located in the VMH and other brain areas.

Two peripheral hormones, leptin and insulin, provide the brain with information about the energy stored as adipose tissue [64]. Leptin and insulin levels in plasma correlate with adipose mass and body weight. Insulin levels correlate better with visceral adiposity [67]. Its plasma levels also reflect changes, decreasing during fasting and increasing during feeding; glucose-induced insulin secretion is also dependent on body fat (review by Benoit et al.) [64]. Obesity is frequently related to insulin resistance as higher insulin levels are required to maintain suitable levels of blood glucose. The administration of insulin to the brain reduces food intake and increases energy expenditure [68]. Impaired insulin signaling due to neuronal deletion of the insulin receptor and insulin receptor substrate 2 (IRS2) increases food intake [69]. Leptin is secreted by adipose tissue, and blood levels correlate directly with adiposity [70]. Leptin receptor deficiency has been related to hyperphagia and obesity [71]. However, leptin supplied to the ARC reduces food intake and body weight, and promotes locomotor activity [72].

Hormones secreted in the gut after feeding as cholecystokinin and GLP-1 promote satiety when administered centrally and peripherally [66]. By contrast, ghrelin released under fasting conditions by the stomach acts as an orexigenic signal inducing food intake [31].

The liver plays a vital role in regulating whole-body glucose and lipid homeostasis. It is the main site for the synthesis, metabolism, storage and redistribution of carbohydrates, proteins and lipids, especially during the adjustment periods in fasting and feeding. In postprandial states, the liver is exposed to more ingested nutrients and to higher levels than other tissues. The liver is especially responsible for much of glucose uptake when hyperinsulinemia and hyperglycemia coincide, storing it as a glycogen and associated to a reduction in muscle glucose uptake [73]. The efficiency of hepatic glucose uptake is coordinated neurally, depending also on diet components and high-fat and high-fructose decreases in glycogen storage. Glucose transport is facilitated by GLUT2, with the intrahepatic glucose concentration being similar to that of blood glucose. Its metabolism therefore depends on GCK activity, which in part determines glycogen synthesis [74]. By contrast, when blood glucose drops, and other organs require energy, the liver produces glucose by glycogenolysis and/or gluconeogenesis. Gluconeogenesis is responsible for half of the total glucose produced by the liver during an overnight fast, so this contribution is essential for glucose homeostasis [75]. Therefore, hepatic metabolism is critical for proper glucose homeostasis in response to insulin and for preventing diabetes [73, 75]. Insulin in the hypothalamic nuclei regulates hepatic glucose production [76, 77]. Insulin acts on the brain (hypothalamus and brain stem), also modulating pancreatic insulin and glucagon secretion [78]. The close coordination between the brain and peripheral organs helps to maintain whole-body glucose and energy homeostasis.

In turn, there is a close relationship between the appearance of insulin resistance in the liver and the development of T2D [79, 80]. Decreased hepatic insulin sensitivity contributes to postprandial hyperglycemia and enhances hepatic glucose production, leading to exacerbated hyperglycemia and chronic hyperinsulinemia in diabetics [81]. There is evidence to suggest that impairing insulin hypothalamic signaling [82] or an HFD [83] contributes to the appearance of diabetes.

3. PASK: a new nutrient sensor

PASK is an evolutionarily conserved nutrient-responsive protein kinase that regulates glucose homeostasis, senses a cell's energy or nutrient status, and suitably regulates cellular metabolism. PASK responds to glucose availability and regulates glucose homeostasis in yeast, rodents and mammals. Despite this pivotal role, the molecular mechanisms of PASK regulation and function are largely unknown [84].

PAS domains (see Section 2) are versatile sensors designed to detect environmental parameters, such as light, oxygen and redox state [24]. These domains are often regulated by the binding of a diverse group of small ligands, including ATP, heme or flavins, within the hydrophobic pocket at the core of the domain (review by Henry et al.) [85]. As with other PAS domains, the PASK adopts this characteristic fold and binds small organic molecules within its hydrophobic core [86]. Unlike other PAS domains, however, the physiological ligand(s) for PASK remain unknown. *In vitro* experiments performed indicate that this domain should inhibit kinase activity [84].

In a hypothetical activation model, a metabolite or protein activates PASK by binding to the PAS domain and relieving PAS domain inhibition. This transient activation may subsequently be stabilized through auto- or transphosphorylation (**Figure 1**). PASK can then phosphorylate several substrates (**Figure 2**) [23, 27, 86, 87].

PASK is known to be a physiological regulator of glucose metabolism, functioning in pancreatic islet cells regulating glucagon and insulin secretion [88, 89]; several translation factors and glycogen synthase are PASK substrates [90, 91], suggesting its implication in the control of protein synthesis and glycogen metabolism.

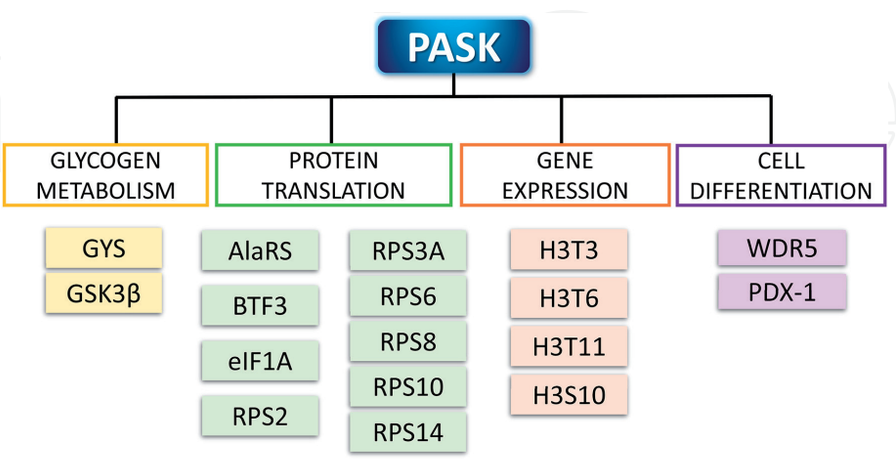

Figure 2.
PAS Kinase substrates in mammals. Cellular process regulated by PAS kinase and in vitro *or* in vivo *substrates identified in mammals. Glycogen metabolism: Glycogen synthase (GYS), Glycogen synthase kinase 3 beta (GSK3β). Protein translation: Alanine-tRNA ligase (AlaRS); Basic transcription factor 3 (BTF3); Eukaryotic translation elongation factor (eIF1A); Ribosomal proteins S2, S3A, S6, S8, S10 and S14 (RPS2), (RPS3A), (RPS6), (RPS8), (RPS10) and (RPS14). Gene expression: Histone H3 tails residues threonine 3, 6, 11 and serine 10 (H3T3), (H3T6), (H3T11) and (H3S10); Cell differentiation: WD repeat-containing protein 5 (WDR5) and Pancreatic Duodenal Homeobox 1 (PDX1).*

Katschinski et al. [92] have been the first to inactivate mouse gene coding to PASK. These PASK-deficient mice ($PASK^{-/-}$) recorded normal development, growth and reproduction. It was subsequently found that $PASK^{-/-}$ male mice are resistant to weight gain, hepatic triglyceride accumulation, and insulin resistance when placed on an HFD [93]. Without a change in food intake or exercise, these $PASK^{-/-}$ male mice also record a hypermetabolic phenotype, giving off more CO_2 and taking in more O_2. PASK is involved in the proteolytic maturation of the sterol regulatory binding protein (SREBP1c), the main lipogenic transcription factor [94, 95]. SREBP1c activity and target genes decreased in $PASK^{-/-}$ mice, with an associated decrease in hepatic lipid deposits [96].

Lipids are important substances that store energy for oxidation and metabolism. As the main cause of imbalanced lipid metabolism, excessive lipid accumulation in the liver has been involved in the development of metabolic syndromes, such as T2D, obesity, hepatic adipose infiltration and unpredicted morbidity. It is therefore extremely important to maintain a balance between lipid synthesis and catabolism. PASK has been reported to regulate many of the phenotypes.

PASK deficiency decreases insulin production, insulin resistance, body weight and hepatic triglyceride accumulation, while leading to increased glycogen storage, as well as metabolic rate [97]. Some of the effects observed in $PASK^{-/-}$ have also been confirmed using PASK pharmacologic inhibitors [98].

Our studies have been based on this mouse model. $PASK^{-/-}$ mice have been described by Hao et al. [93], and generously donated to us by Dr. Roland H. Wegner (Veterinary Department of the canton of Zurich).

New PASK functions have recently been described, including the unexpected role it has in promoting the differentiation of myogenic progenitor cells, embryonic stem cells, and adipogenic progenitor cells. This PASK function is dependent upon its ability to phosphorylate WD repeat-containing protein 5 (WDR5), which is a member of several protein complexes, including those that catalyze histone H3 Lysine 4 trimethylation (H3K4me3) during transcriptional activation. Thus, as an upstream kinase of WDR5, PASK integrates signaling cues with the transcriptional network to regulate the differentiation of progenitor cells [99]. In addition, the phosphorylation of PASK by mTORC1 is required for the activation of myogenin transcription, exiting from self-renewal, and the induction of the myogenesis program. mTORC1-PASK signaling is required for increasing myogenin-positive committed myoblasts (early stage of myogenesis) [100].

Moreover, it has been confirmed that the metabolic sensor PASK could affect both the phosphorylation and the methylation of histone H3 tails. It contributes to the methylation of H3 lysine 4 (H3K4) di- and tri-methylation through its association with the H3K4 MLL2 methyltransferase complex and to the phosphorylation of several threonine residues (T3, T6 and T11) and serine (S10) on H3 as a histone kinase [101]. The methylation of histone H3 lysine 4 H3K4 has been linked to transcriptional activation.

4. PASK hypothalamic function in food intake and energy homeostasis

The hypothalamus is the key to controlling food intake. The identification of hypothalamic glucose sensing systems and neuronal populations expressing and responding to orexigenic and anorexigenic peptides (see Section 2.1) has focused the studies on the hypothalamic nuclei. They have been specifically directed toward identifying the mechanisms involved in controlling nutrient sensing, feeding behavior and its relationship with insulin actions in the central nervous system in order to regulate energy and glucose homeostasis. Hypothalamic metabolic sensors

respond in opposite ways to changes in nutrients and orexigenic or anorexigenic peptides, and their activation/inhibition regulates food intake. For example, the hypothalamic AMPK is activated by fasting and inhibited by refeeding [53, 57, 102], and the mTORC1/S6K pathway is activated by glucose and amino acids, inhibiting food intake [20, 57, 103]. Both pathways are involved in controlling feeding and regulating the energy balance.

In 2013, PASK was identified in the hypothalamic areas involved in feeding behavior, and its expression was regulated under fasting/refeeding conditions [104, 105]. It was proposed as a hypothalamic and liver nutrient sensor and a general regulator of glucose metabolism and cellular energy. Moreover, $PASK^{-/-}$ mice resist diet-induced obesity [93]; it might therefore be understood that PASK could control the hypothalamic function related to intake control. For example, elevated glucose levels decrease mRNA coding to PASK in VMH and LH areas in hypothalamic organotypic cultures and in neuroblastoma N2A cells [104]. The PASK expression is also regulated *in vivo* in response to fasting/refeeding conditions. This effect is clearer in LH: mRNA coding to PASK is lower under fasting conditions and increases in response to refeeding conditions [105]. The effect observed after refeeding *in vivo* is the opposite to the glucose effect found in VMH and LH in hypothalamic organotypic cultures and neuroblastoma N2A cells. However, the effect is similar to those produced in the presence of both glucose and the anorexigenic peptide GLP-1 (an incretin release from intestinal L-cells in response to feeding) [106, 107]. The role of PASK in the hypothalamus would be similar to other well-known metabolic sensors, such as AMPK and mTORC1. The activation of AMPK and mTORC1 is coordinated and antagonistic. While AMPK is activated by a fall in energy, mTORC1 is activated by its increase. Hypothalamic metabolic sensors, such as AMPK and mTORC1, therefore play an important role in feeding behavior, body weight homeostasis, and energy balance (see Section 2.1). These sensors respond to changes in nutrient levels in the VMH and LH (hypothalamic areas involved in feeding behavior) and in neuroblastoma N2A cells, and those effects are modulated by the GLP-1 in lean and obese rats [57].

Studies in $PASK^{-/-}$ mice have indicated that PASK-deficiency involves a downregulation of mRNA levels coding to AMPKα2 in VMH, and slightly so in LH [105], while impairing the coordination of the AMPK and mTORC1/S6K1 pathways. Thus, both the AMPK and mTORC1/S6K1 pathways are surprisingly activated at the same time under fasting and feeding conditions in $PASK^{-/-}$ mice [105]. This finding could mean that the inhibition of mTORC1/S6K through AMPK activation requires the coordinated phosphorylation of TSC2 by Glycogen synthase kinase 3β (GSK3β) [59], which is a PASK substrate *in vitro,* and PASK deficiency could therefore alter hypothalamic GSK3β activity [108]. Additionally, this study has found that the exendin-4 regulatory effect on metabolic sensor activity is lost in $PASK^{-/-}$ mice, and the anorexigenic properties of exendin-4 significantly reduced, suggesting that PASK could be a mediator in the brain GLP-1 signaling pathway. Some of the antidiabetogenic effects of exendin-4 might be modulated through these processes. This means that hypothalamic PASK, interacting with AMPK and mTORC1 pathways, and in coordination with anorexigenic/orexigenic peptides, could be a key enzyme in food intake control, and in peripheral tissue functions, such as brown adipose tissue thermogenesis, pancreatic insulin secretion, etc. However, more studies are needed to clarify this hypothesis and the putative molecular mechanism of PASK actions in whole-body physiology.

The downregulation of mRNA coding to AMPKα2 and the modulation of the GLP-1 effects in hypothalamus in $PASK^{-/-}$ mice suggest that both effects may also be regulating thermogenesis in BAT and the browning of white fat, as both processes are mediated by the inhibition of hypothalamic AMPK [109, 110].

As well as acting in hypothalamic functions, PASK also has key functions in the peripheral tissues. For example, diabetes and PASK have been linked, as a human mutation of the *PASK* gene has been correlated with maturity-onset diabetes of the young (MODY). This mutation increases kinase activity and decreases glucose-stimulated insulin secretion by the pancreas [111]. In addition, decreased PASK expression in pancreatic islets has been reported in human T2D [88]. The PASK function in peripheral tissues could be crucial for maintaining metabolic and energy homeostasis.

5. PASK contribution to hepatic adaptation to fasting/feeding

The liver maintains metabolic homeostasis, and it is especially essential in the proper control of glucose during fasting and feeding periods. In particular, the liver is one of the main insulin-responsive organs, so it records a greater glucose uptake when glycaemia rises, storing it as glycogen (see Section 2.1). By contrast, when blood glucose falls, and other organs require energy, the liver produces glucose by glycogenolysis and gluconeogenesis. Therefore, the correct hepatic response to insulin and hepatic metabolism are critical for maintaining glycaemia within physiological ranges, and therefore for the proper control of diabetes.

Studies with PASK$^{-/-}$ mice have reported the critical role PASK plays in hepatic adaptation to fasting/feeding periods, especially under an HFD [93, 112]. It is interesting that PASK expression is regulated in the liver by fasting/feeding, with fasting downregulating it [112]. Moreover, Perez-Garcia et al. [113] have found that PASK deficiency alters the complex hepatic response to fasting/feeding. The expression of the transcription factors and key enzymes that regulate gluconeogenesis and mitochondrial fatty acid transport under fasting conditions is altered in PASK$^{-/-}$ mice, with lower forkhead box protein O1 (*Foxo 1*) and carnitine palmitoyltransferase 1A (*Cpt1a*) and higher peroxisome proliferator-activated receptor alpha (*Ppara*). Similarly, PASK deficiency modifies the activity of the protein kinase B (AKT) overactivated under fasting and the stability of phosphoenolpyruvate carboxykinase (PEPCK) [113], while no detectable changes have been observed in the maintenance of blood glucose homeostasis during prolonged fasting periods [105].

A good example of PASK deficiency effects under feeding involves the changes recorded in GCK, which is a critical enzyme in the hepatic function. GCK is an enzyme involved in hepatic glucose sensing (see Section 2). This enzyme is activated by the increase in blood glucose which occurs in feeding periods. It therefore adjusts hepatic glucose phosphorylation to blood glucose levels, acting as a glucose sensor. The importance of GCK in maintaining glucose homeostasis is evidenced by the severe impacts caused by mutations in the GCK gene. The loss of GCK function in the human body causes maturity-onset diabetes of the young type 2 (MODY2) [114]. By contrast, activating mutations generate persistent hyperinsulinemia [115]. Many liver functions are controlled by GCK, which acts together with insulin in the maintenance of blood glucose homeostasis [116], and the activation of glycolytic and lipogenic gene expression. GCK is also involved in glycogen synthesis and storage in the liver [117]. The enzymatic activity of GCK is controlled by transcriptional and posttranscriptional mechanisms. While the transcriptional regulation of the GCK gene is basically insulin-dependent [118], the posttranscriptional mechanisms of regulation involve interaction with other proteins, highlighting the glucokinase regulatory protein (GCKR). GCKR modulates GCK activity when glucose levels decline by binding and sequestering it in the nucleus, and thus avoiding its function in the cytoplasm [116, 119].

Studies relating the role of PASK to the GCK function [113] have revealed that GCK activity is reduced in PASK$^{-/-}$ mice for two reasons: on the one hand, the lower protein expression, and on the other, its mainly nuclear location. It cannot be ruled out that the decrease in GCK may be partly due to the blocking of lipogenesis that characterizes PASK$^{-/-}$ mice. In addition, the conversion of excess carbohydrates into lipids might also be limited by the low levels of acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS), although the gene coding to ACC and liver pyruvate kinase (LPK) is overexpressed under non-fasted conditions in PASK$^{-/-}$ mice. Additionally, glycogen metabolism could also be modified because glycogen synthase is a PASK substrate [90].

The hepatic PASK role in the control of hepatic adaptation to fasting/feeding becomes more important under an HFD because it dysregulates hepatic metabolic responses to fasting and feeding, leading to a non-alcoholic fatty liver, obesity, insulin resistance, diabetes, and associated cardiovascular problems. Some studies have evidenced that PASK$^{-/-}$ mice fed with a HFD resist the development of obesity and hepatic steatosis, with improved insulin sensitivity [93, 96, 112]. A consequence of an HFD for the liver is that it alters the downregulation of the *Pask* expression produced by fasting, as normally happens in a standard-fat diet [112]. Interestingly, PASK$^{-/-}$ mice with an HFD record improved parameters for the following: body weight, glucose tolerance, insulin resistance (see Section 6.1.), and serum lipid parameters [112]. Some of the PASK effects are due to changes in the proteolytic maturation of SREBP1c, others by regulating transcription factors and enzymes that play a key role in the hepatic response to fasting/feeding. Thus, PASK deficiency compensates for gene expression altered by an HFD. For example, it decreases the expression of genes overexpressed in HFD-fed mice (transcription factors involved in the regulation of gluconeogenic enzymes, the transport of fatty acid into mitochondria, β-oxidation, and *de novo* lipogenesis). PASK also modifies the expression of the short noncoding RNAs involved in lipid metabolism and glucose homeostasis. Such is the case of miR-33a and miR-143, whose expression in HFD-fed mice is controlled by PASK. Thus, PASK deficiency improves the hepatic adaptation to feeding/fasting, especially under pathogenic situations such as an HFD, through a highly regulated molecular mechanism that controls the expression and function of the transcription factors, enzymes and miRNAs involved in glucose and insulin signaling.

PASK deficiency also improves oxidative metabolism and mitochondrial biogenesis [120], increasing the ROS-detoxifying enzymes and the expression of *FoxO3a* and PTEN-induced kinase 1 (PINK1) involved in cell survival and mitophagy, respectively. All of these are interesting effects of PASK deficiency for states that increase oxidative stress, such as aging, diabetes, and obesity.

In sum, there are several results that highlight PASK's role in the control of the key genes and proteins that lead to hepatic metabolic adaptation to fasting or feeding situations.

Accordingly, PASK has been proposed as one of the possible targets for the treatment of the metabolic syndrome.

6. PASK and insulin resistance

The growing interest in the PASK function began with the finding that its deficiency prevents many of the deleterious effects of HFDs [93, 112], with a highlight being the insulin resistance that accompanies these diets, and which has been widely associated with the development of T2D [121, 122].

The same level of PASK is expressed in pancreatic α and β cells, and it is involved in insulin and glucagon secretion [88]. PASK promotes insulin expression [108], while PASK deficiency decreases the expression of preproinsulin at high glucose concentrations [123]. As for glucagon, PASK regulates its secretion by glucose [88].

PASK is also important in the development of pancreatic α and β cells, reflecting its key role in diabetes [89]. Thus, even though PASK deletion does not affect glucose-stimulated insulin secretion or insulin levels in response to fasting and feedback, it does decrease pancreatic β-cell mass in specific KO animals. By contrast, the deletion of PASK in pancreatic α cells improves glucagon secretion both *in vivo* and *in vitro*. PASK therefore plays a clear role in glucagon secretion and in the development of β-cell precursors.

There are two determining factors that promote the onset of T2D, dysfunctions in insulin secretion and peripheral resistance to the action of insulin. We cannot speak of a single class of T2D because there is considerable heterogeneity. In general, obesity prompts the metabolic syndrome [124], which in addition to obesity includes other pathologies such as hypertension, hypertriglyceridemia, elevated fasting glucose levels, and dyslipidemia. T2D is associated with insulin resistance (**Figure 3**), and over long periods it can lead to hyperinsulinemia. Finally, the pancreas fails and hypoinsulinemia sets in.

Insulin signaling activates the PI3K/AKT pathway that controls most metabolic effects (**Figure 3**) [125]. Insulin stimulates glucose uptake in muscle and adipose tissue, promoting glucose transporter type 4 (GLUT4) expression and translocation to the cell membrane [126, 127]. In turn, insulin decreases lipolysis in adipose tissue,

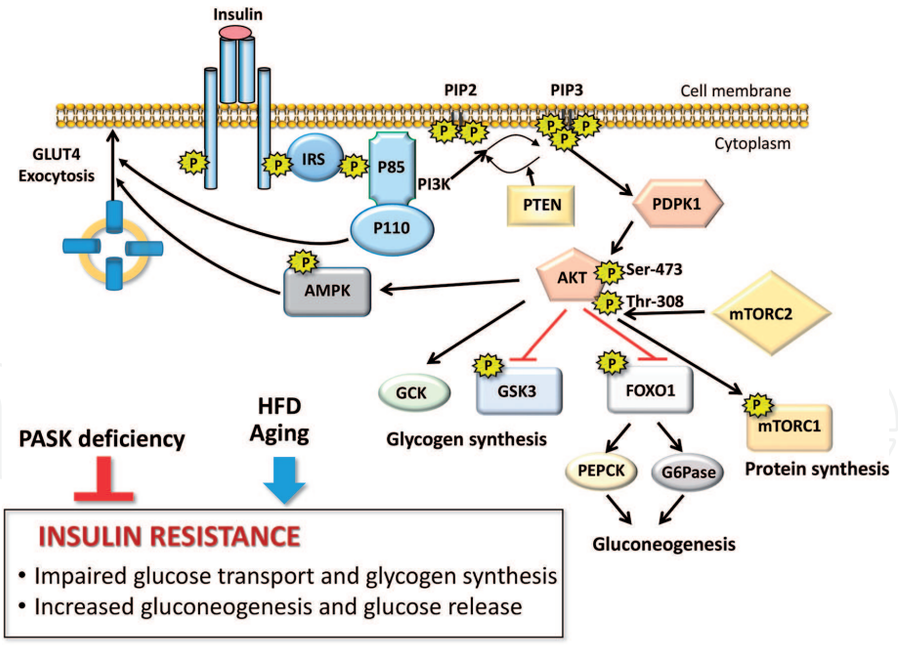

Figure 3.
Insulin signaling and insulin resistance. Insulin signaling recruit insulin receptor substrates (IRS) activates the phosphoinositide 3-kinase (PI3K)/AKT pathway that controls most metabolic effects. In muscle and adipose tissue this pathway activates AMP-activated protein kinase (AMPK) promoting glucose transporter type 4 (GLUT4) expression and translocation to the cell membrane. In the liver this pathway activates glucokinase (GCK) and glycogen synthase kinase (GSK) inducing glycogen synthesis and suppresses gluconeogenesis inhibiting the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). Arrow stimulates, T bar red inhibits. Insulin resistance impairs the activation of PI3K/Akt pathway. In the skeletal muscle and adipose tissue impairs glucose uptake decreasing GLUT4 expression and translocation. In the liver insulin resistance suppresses glycogen synthesis and promotes gluconeogenesis through the expression of PEPCK and G6P genes.

reducing the level of circulating free FA [128]. Insulin in the liver induces glycogen synthesis and suppresses gluconeogenesis [75].

The loss of sensitivity to insulin, generally called insulin resistance, affects this hormone's main peripheral target organs [125]. Insulin resistance could increase the concentration of free FA in the blood [129], decrease glucose uptake in skeletal muscle, and stimulate gluconeogenesis, increasing glucose release in the liver, thereby contributing to the metabolic syndrome [130, 131]. Abdominal obesity releases an excess of free FA, and the associated inflammation may interfere with insulin signaling [132].

6.1 Obesity, insulin resistance and PASK

Obesity in humans is defined as a body mass index (BMI = weight/height^2) of more than 30 kg/m^2. There are numerous studies linking fat intake and the onset of T2D, but it has not been possible to establish a consensus on the relationship between fat intake and obesity. A positive correlation has nonetheless been established between fat consumption and the BMI by Nagao et al. [121]. Moreover, the effect of consuming animal fats that contain high amounts of saturated fatty acids (SFAs) has also been compared with the consumption of fat with monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA). PUFA intake is reported to reduce the risk of T2D. The variability detected also depends on the genetic factors that contribute 40% to the development of T2D [121].

Genetic variability is also considered in studies on the effect of an HFD in mice [121, 133]. In sum, a diet containing high amounts of animal or vegetable fat rich in saturated or unsaturated FA ω6/ω9 increases body weight and resistance to insulin appears, and to compensate there is an increase in insulin levels. These effects are not observed when using oils rich in unsaturated FA ω3 [134].

The liver's key function of maintaining metabolic homeostasis in both fasting and a postprandial state makes it one of the main organs affected by an HFD. It has been posited that a long-term HFD induces lipid (triglycerides) accumulation in hepatocytes as a result of insulin resistance. Nonalcoholic fatty liver disease (NAFLD) is the first step toward the onset of a chronic condition. This first step is followed by oxidative stress and impairment of the mitochondrial function, which triggers associated inflammation, hepatic damage and fibrosis [135, 136].

PASK$^{-/-}$ mice are protected against hepatic steatosis and the insulin resistance induced by an HFD [93]. We have reported that a long-term HFD severely alters the liver response needed to maintain metabolic homeostasis during fasting and feeding periods [112]. Firstly, the hepatic *Pask* expression is stimulated by feeding [96]. By contrast, HFD-fed mice have similar levels of hepatic *Pask* gene expression under fasting and feeding conditions [112]. Our results suggest that this effect might be responsible for some of the metabolic changes associated with this diet.

An HFD has a drastic effect on the expression of transcription factors that regulate the adaptation to fasting/feeding conditions. For instance, the transcription factors (FOXO1, peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) and PPARα) stimulated under fasting to promote the expression of the gluconeogenic enzymes (PEPCK and glucose 6-phosphatase (G6Pase)) and the genes that support fatty acid transport into mitochondria and β-oxidation are significantly overstimulated in HFD-fed mice. However, PASK deficiency blocks or diminishes the expression of all these genes under an HFD.

Similarly, the adaptation to a postprandial state is regulated by glucose and insulin through the expression of transcriptional factors (carbohydrate-responsive element-binding protein (CHREBP), liver X receptor alpha (LXRα) and *SREBP1*) [137, 138]. They promote the expression of lipogenic genes (*Acc1*, *Fas* and

stearoyl-CoA desaturase-1 (*Scd1*)), stimulating the conversion of excess carbohydrates into FA and triglycerides. An HFD also overexpresses both *Lxr*α and peroxisome proliferator-activated receptor gamma (*Ppar*γ) but this effect is diminished in PASK$^{-/-}$ mice [112]. The overexpression of *Lxr*α [139] and *Ppar*γ [140, 141] has previously been associated with liver steatosis in human and mouse models of obesity and diabetes.

The effects of an HFD cause multiple changes in transcription factors and the key enzymes of the main hepatic metabolic pathways that allow metabolic adaptation. Genes promoting de novo lipogenesis that are expressed only under feeding conditions with a standard diet can also be overexpressed under fasting conditions with an HFD, which induces the overexpression of transcription factors and metabolic enzyme genes controlling de novo lipogenesis (*Chrebp*, *Lxr*α, *Srebp1c*, *Acc1* and *Scd1*) under a fasted state [112].

PASK deficiency eliminates many harmful effects that HFDs cause in the liver (**Figure 3**), also decreasing the lipid depots that over time can develop hepatic steatosis. Thus PASK$^{-/-}$ mice under an HFD have lower blood glucose levels, improve their sensitivity to the action of insulin, preventing the appearance of insulin resistance, which in turn is correlated with smaller increases in body weight and improved lipid profile [112]. PASK pharmacologic inhibition likewise confirms its key role for restoring insulin sensitivity, and for reducing hepatic fat content and the fibrosis caused by an HFD [98].

6.2 Aging, insulin resistance and PASK

The risk of T2D increases in aging due to the many imbalances that characterize this stage of life, which are often associated with overweight, impaired glucose metabolism, hypertension and dyslipidemia [142].

The aging process is characterized metabolically by the following: the development of insulin resistance (**Figure 3**), changes in body composition and mitochondrial dysfunction. In addition, hyperinsulinemia and glucose intolerance develop during aging [143, 144]. There are numerous metabolic changes in peripheral tissues that affect the uncontrolled gluconeogenesis of the liver, accompanied by an increase in lipogenesis in adipose tissue, and by defects in glycogen synthesis and glucose uptake in skeletal muscle [144]. Glucose metabolic dysfunction is closely correlated with oxidative stress, as occurs in diabetic or obese patients [145, 146].

Aging is normally accompanied by an increase in visceral fat, which is one of the main contributors to insulin resistance and the development of T2D. Likewise, it leads to an increase in proinflammatory cytokines, which interfere with insulin activity [144].

Another consequence of aging is the progressive loss of mitochondrial function in various tissues such as the liver or skeletal muscle. Thus, certain studies affirm that there is an association in aging between insulin resistance and glucose intolerance, together with a reduction in oxidative activity and mitochondrial ATP synthesis [144]. With aging, the liver undergoes molecular changes such as an increased inflammatory response, dysregulation of the genetic expression of antioxidant enzymes, and mitochondrial dysfunction, significantly altering redox homeostasis. It is also accompanied by the liver's reduced capacity for regeneration, which greatly affects liver function [147].

The nutrient sensing mechanisms needed to detect and respond to variations in their levels are dysregulated by aging [148]. So both nutrient sensor pathways, AMPK and mTORC1, are involved in a lifespan [149]. PASK senses intracellular oxygen, redox state and various metabolites [100]. Additionally, PASK regulates both AMPK and mTORC1 pathways [104, 105]. The aging process at cellular level is

regulated by insulin/IGF signaling and both AMPK and mTORC1 pathways, which are in turn regulated by nutrient levels, whose signals converge on several targets: FOXO, nuclear factor: erythroid-derived 2-like 2 (NRF2), tumor protein p53, and sirtuins (SIRT) in order to control metabolic homeostasis, oxidative stress, and quality cellular housekeeping [150].

PASK$^{-/-}$ mice may avoid several of the deleterious defects induced by the aging process [151]. Aged PASK$^{-/-}$ mice maintain both low blood glucose values and insulin concentrations similar to young WT mice. They do not develop glucose intolerance or insulin resistance, as confirmed by a normal HOMA-IR index. These effects correlate with a high expression of the longevity gene *FoxO3a* and the transcription factor NRF2, as the main regulator of the redox balance [151]. Signaling through the system NRF2/KEAP1: kelch-like ECH-associated protein 1 regulates the transcription of enzymes that protect cells against oxidative stress [152]. An elevated expression of glutamate-cysteine ligase modifier subunit (GCLm) and heme oxygenase-1 (HO1) have been found in aged PASK$^{-/-}$ mice under fasted conditions. The efficiency of this redox system decreases in step with aging in WT mice, significantly diminishing the antioxidant response [153]. Likewise, PASK deficiency prevents the drastically age-related decrease in the expression of several antioxidant enzymes under basal conditions, such as catalase (CAT) and glutathione peroxidase (GPx) [151].

In relation to the maintenance of the mitochondrial function and energy homeostasis, we have confirmed that the expression of the several transcription factors and nuclear receptors needed to maintain mitochondrial biogenesis (*Ppargc1a, Sirt1* and *Nrf2)* are affected in fasted aged WT mice [151] in accordance with the previous literature that relates aging to a decrease in cellular energy input [154], an increase in oxidative stress [155], and the mitochondrial dysfunction of cellular redox homeostasis [156, 157]. However, the expression of *Nrf2, Ppargc1a, Pparγ* and *Sirt1* increases under fasting in aged PASK$^{-/-}$ mice. This means they maintain lower levels of ROS/RNS, while aged WT mice record a lower expression of antioxidant enzymes and increased levels of ROS/RNS [151].

We might therefore contend that some of the dysfunctions produced during aging in PASK$^{-/-}$ mice could be related to hormetic responses. Slight toxic effects can generate beneficial actions that compensate for the initial damage and even improve cellular health [158]. Aging decreases *Pask* expression in the liver of WT mice perhaps as a compensatory mechanism. PASK$^{-/-}$ mice maintain the same blood glucose values as young WT mice, and do not develop insulin resistance.

7. Conclusions

PASK function could be critical for preserving the nutrient effect on hypothalamic AMPK and mTORC1/S6K1 pathways and maintain the regulatory role of GLP1/exendin-4 in food intake.

PASK regulates the hepatic glucose sensor GCK and AKT, an insulin signaling intermediators, and glucose and lipidic metabolism through the regulation of the key genes and proteins required during hepatic fasting/feeding adaptation. Moreover, PASK deficiency improves mitochondrial biogenesis and antioxidant mechanisms.

HFDs alter the adaptive response of *Pask* gene expression to fasting/feeding. PASK deficiency eliminates many of the harmful effects HFDs have on the liver, thereby decreasing the lipid depots. PASK deficiency decreases the expression of several transcription factors stimulated under fasted conditions to promote the expression of the gluconeogenic enzymes and those promoting the expression of

lipogenic genes after feeding that are significantly overstimulated in wild type HFD-fed mice.

PASK deficiency avoids insulin resistance and glucose intolerance during aging, preventing an age-related decrease in the expression of several antioxidant enzymes and improving mitochondrial function.

All these actions make PASK a significant pharmacological target for diseases, such as obesity and diabetes, and for preventing some of the more harmful effects of aging.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Miyamoto T, Wright G, Amrein H. Nutrient sensors. Curr Biol. 2013;23(9):R369-73.

[2] Chantranupong L, Wolfson RL, Sabatini DM. Nutrient-sensing mechanisms across evolution. Cell. 2015;161(1):67-83.

[3] Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. Nature. 2015;517(7534):302-10.

[4] Yamashita AS, Belchior T, Lira FS, Bishop NC, Wessner B, Rosa JC, et al. Regulation of Metabolic Disease-Associated Inflammation by Nutrient Sensors. Mediators Inflamm. 2018;2018:8261432.

[5] Ruud J, Steculorum SM, Bruning JC. Neuronal control of peripheral insulin sensitivity and glucose metabolism. Nat Commun. 2017;8:15259.

[6] Matschinsky FM, Magnuson MA, Zelent D, Jetton TL, Doliba N, Han Y, et al. The network of glucokinase-expressing cells in glucose homeostasis and the potential of glucokinase activators for diabetes therapy. Diabetes. 2006;55(1):1-12.

[7] Osbak KK, Colclough K, Saint-Martin C, Beer NL, Bellanne-Chantelot C, Ellard S, et al. Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia. Hum Mutat. 2009;30(11):1512-26.

[8] Roncero I, Alvarez E, Vazquez P, Blazquez E. Functional glucokinase isoforms are expressed in rat brain. J Neurochem. 2000;74(5):1848-57.

[9] Alvarez E, Martinez MD, Roncero I, Chowen JA, Garcia-Cuartero B, Gispert JD, et al. The expression of GLP-1 receptor mRNA and protein allows the effect of GLP-1 on glucose metabolism in the human hypothalamus and brainstem. J Neurochem. 2005;92(4):798-806.

[10] Levin BE, Routh VH, Kang L, Sanders NM, Dunn-Meynell AA. Neuronal glucosensing: what do we know after 50 years? Diabetes. 2004;53(10):2521-8.

[11] Williams TF, Exton JH, Park CR, Regen DM. Stereospecific transport of glucose in the perfused rat liver. Am J Physiol. 1968;215(5):1200-9.

[12] Roncero I, Alvarez E, Chowen JA, Sanz C, Rabano A, Vazquez P, et al. Expression of glucose transporter isoform GLUT-2 and glucokinase genes in human brain. J Neurochem. 2004;88(5):1203-10.

[13] Jurcovicova J. Glucose transport in brain - effect of inflammation. Endocr Regul. 2014;48(1):35-48.

[14] Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012;13(4):251-62.

[15] Garcia D, Shaw RJ. AMPK: Mechanisms of Cellular Energy Sensing and Restoration of Metabolic Balance. Mol Cell. 2017;66(6):789-800.

[16] Hardie DG. AMP-activated protein kinase: maintaining energy homeostasis at the cellular and whole-body levels. Annu Rev Nutr. 2014;34:31-55.

[17] Lopez M. Hypothalamic AMPK and energy balance. Eur J Clin Invest. 2018;48(9):e12996.

[18] Andersson U, Filipsson K, Abbott CR, Woods A, Smith K, Bloom SR, et al. AMP-activated protein kinase plays a role in the control

of food intake. J Biol Chem. 2004;279(13):12005-8.

[19] Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. Cell. 2017;168(6):960-76.

[20] Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, et al. Hypothalamic mTOR signaling regulates food intake. Science. 2006;312(5775):927-30.

[21] Hu F, Xu Y, Liu F. Hypothalamic roles of mTOR complex I: integration of nutrient and hormone signals to regulate energy homeostasis. Am J Physiol Endocrinol Metab. 2016;310(11):E994-E1002.

[22] Dagon Y, Hur E, Zheng B, Wellenstein K, Cantley LC, Kahn BB. p70S6 Kinase Phosphorylates AMPK on Serine 491 to Mediate Leptin's Effect on Food Intake. Cell Metab. 2012;16(1):104-12.

[23] Kikani CK, Antonysamy SA, Bonanno JB, Romero R, Zhang FF, Russell M, et al. Structural bases of PAS domain-regulated kinase (PASK) activation in the absence of activation loop phosphorylation. J Biol Chem. 2010;285(52):41034-43.

[24] Moglich A, Ayers RA, Moffat K. Structure and signaling mechanism of Per-ARNT-Sim domains. Structure. 2009;17(10):1282-94.

[25] DeMille D, Grose JH. PAS kinase: a nutrient sensing regulator of glucose homeostasis. IUBMB Life. 2013;65(11):921-9.

[26] Zhang DD, Zhang JG, Wang YZ, Liu Y, Liu GL, Li XY. Per-Arnt-Sim Kinase (PASK): An Emerging Regulator of Mammalian Glucose and Lipid Metabolism. Nutrients. 2015;7(9):7437-50.

[27] Grose JH, Rutter J. The role of PAS kinase in PASsing the glucose signal. Sensors (Basel). 2010;10(6):5668-82.

[28] Hurtado-Carneiro V, Perez-Garcia A, Alvarez E, Sanz, C. PAS kinase: A nutrient and energy sensor "master key" in the response to fasting/feeding conditions. Front Endocrinol. 2020; 11(999).

[29] Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature. 2006;443(7109):289-95.

[30] Lam TK. Neuronal regulation of homeostasis by nutrient sensing. Nat Med. 2010;16(4):392-5.

[31] Cummings DE, Overduin J. Gastrointestinal regulation of food intake. J Clin Invest. 2007;117(1):13-23.

[32] Pena-Leon V, Perez-Lois R, Seoane LM. mTOR Pathway is Involved in Energy Homeostasis Regulation as a Part of the Gut-Brain Axis. Int J Mol Sci. 2020;21(16).

[33] Roh E, Song DK, Kim MS. Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. Exp Mol Med. 2016;48:e216.

[34] Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. Cell. 2001;104(4):531-43.

[35] Anand BK, Chhina GS, Sharma KN, Dua S, Singh B. Activity of Single Neurons in the Hypothalamic Feeding Centers: Effect of Glucose. Am J Physiol. 1964;207:1146-54.

[36] Ashford ML, Boden PR, Treherne JM. Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K+ channels. Pflugers Arch. 1990;415(4):479-83.

[37] Song Z, Levin BE, McArdle JJ, Bakhos N, Routh VH. Convergence of pre- and postsynaptic influences

on glucosensing neurons in the ventromedial hypothalamic nucleus. Diabetes. 2001;50(12):2673-81.

[38] Mizuno Y, Oomura Y. Glucose responding neurons in the nucleus tractus solitarius of the rat: in vitro study. Brain Res. 1984;307(1-2):109-16.

[39] Oomura Y, Yoshimatsu H. Neural network of glucose monitoring system. J Auton Nerv Syst. 1984;10(3-4):359-72.

[40] Silver IA, Erecinska M. Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. J Neurophysiol. 1998;79(4):1733-45.

[41] Alvarez E, Roncero I, Chowen JA, Thorens B, Blazquez E. Expression of the glucagon-like peptide-1 receptor gene in rat brain. J Neurochem. 1996;66(3):920-7.

[42] Navarro M, Rodriquez de Fonseca F, Alvarez E, Chowen JA, Zueco JA, Gomez R, et al. Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. J Neurochem. 1996;67(5):1982-91.

[43] Dunn-Meynell AA, Rawson NE, Levin BE. Distribution and phenotype of neurons containing the ATP-sensitive K+ channel in rat brain. Brain Res. 1998;814(1-2):41-54.

[44] Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes. 2001;50(1):1-11.

[45] Marty N, Dallaporta M, Thorens B. Brain Glucose Sensing, Counterregulation, and Energy Homeostasis. Physiology. 2007;22(4):241-51.

[46] Le Foll C, Irani BG, Magnan C, Dunn-Meynell AA, Levin BE. Characteristics and mechanisms of hypothalamic neuronal fatty acid sensing. Am J Physiol Regul Integr Comp Physiol. 2009;297(3):R655-64.

[47] Le Foll C, Dunn-Meynell AA, Levin BE. Role of FAT/CD36 in fatty acid sensing, energy, and glucose homeostasis regulation in DIO and DR rats. Am J Physiol Regul Integr Comp Physiol. 2015;308(3):R188-98.

[48] Le Foll C, Levin BE. Fatty acid-induced astrocyte ketone production and the control of food intake. Am J Physiol Regul Integr Comp Physiol. 2016;310(11):R1186-92.

[49] Cone RD, Cowley MA, Butler AA, Fan W, Marks DL, Low MJ. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int J Obes Relat Metab Disord. 2001;25 Suppl 5:S63-7.

[50] Blanco Martinez de Morentin P, Gonzalez CR, Saha AK, Martins L, Dieguez C, Vidal-Puig A, et al. Hypothalamic AMP-activated protein kinase as a mediator of whole body energy balance. Rev Endocr Metab Disord. 2011;12(3):127-40.

[51] Ramamurthy S, Ronnett G. AMP-Activated Protein Kinase (AMPK) and Energy-Sensing in the Brain. Exp Neurobiol. 2012;21(2):52-60.

[52] Turnley AM, Stapleton D, Mann RJ, Witters LA, Kemp BE, Bartlett PF. Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. J Neurochem. 1999;72(4):1707-16.

[53] Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, et al. AMP-kinase regulates food intake by responding to hormonal and nutrient

signals in the hypothalamus. Nature. 2004;428(6982):569-74.

[54] Claret M, Smith MA, Batterham RL, Selman C, Choudhury AI, Fryer LG, et al. AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J Clin Invest. 2007;117(8):2325-36.

[55] Huynh MK, Kinyua AW, Yang DJ, Kim KW. Hypothalamic AMPK as a Regulator of Energy Homeostasis. Neural Plast. 2016;2016:2754078.

[56] Blouet C, Ono H, Schwartz GJ. Mediobasal hypothalamic p70 S6 kinase 1 modulates the control of energy homeostasis. Cell Metab. 2008;8(6):459-67.

[57] Hurtado-Carneiro V, Sanz C, Roncero I, Vazquez P, Blazquez E, Alvarez E. Glucagon-like peptide 1 (GLP-1) can reverse AMP-activated protein kinase (AMPK) and S6 kinase (P70S6K) activities induced by fluctuations in glucose levels in hypothalamic areas involved in feeding behaviour. Mol Neurobiol. 2012;45(2):348-61.

[58] Hurtado-Carneiro V, Roncero, I., Blazquez, E., Alvarez, E., Sanz, C. Glucagon-like peptide-1 and its implications in obesity. HOT TOPICS IN ENDOCRINE AND ENDOCRINE-RELATED DISEASES. 2013(Chap 7):165-95.

[59] Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003;115(5):577-90.

[60] Byfield MP, Murray JT, Backer JM. hVps34 is a nutrient-regulated lipid kinase required for activation of p70 S6 kinase. J Biol Chem. 2005;280(38):33076-82.

[61] Blouet C, Schwartz GJ. Hypothalamic nutrient sensing in the control of energy homeostasis. Behav Brain Res. 2010;209(1):1-12.

[62] Le Foll C, Dunn-Meynell AA, Miziorko HM, Levin BE. Regulation of hypothalamic neuronal sensing and food intake by ketone bodies and fatty acids. Diabetes. 2014;63(4):1259-69.

[63] Migrenne S, Magnan C, Cruciani-Guglielmacci C. Fatty acid sensing and nervous control of energy homeostasis. Diabetes Metab. 2007;33(3):177-82.

[64] Benoit SC, Clegg DJ, Seeley RJ, Woods SC. Insulin and leptin as adiposity signals. Recent Prog Horm Res. 2004;59:267-85.

[65] Chen X, Ge YL, Jiang ZY, Liu CQ, Depoortere I, Peeters TL. Effects of ghrelin on hypothalamic glucose responding neurons in rats. Brain Res. 2005;1055(1-2):131-6.

[66] Rodriquez de Fonseca F, Navarro M, Alvarez E, Roncero I, Chowen JA, Maestre O, et al. Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. Metabolism. 2000;49(6):709-17.

[67] Woods SC, Seeley RJ, Porte D, Jr., Schwartz MW. Signals that regulate food intake and energy homeostasis. Science. 1998;280(5368):1378-83.

[68] Air EL, Benoit SC, Blake Smith KA, Clegg DJ, Woods SC. Acute third ventricular administration of insulin decreases food intake in two paradigms. Pharmacol Biochem Behav. 2002;72(1-2):423-9.

[69] Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. Science. 2000;289(5487):2122-5.

[70] Considine RV, Sinha MK, Heiman ML, Kriauciunas A,

Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334(5):292-5.

[71] Coppari R, Ichinose M, Lee CE, Pullen AE, Kenny CD, McGovern RA, et al. The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity. Cell Metab. 2005;1(1):63-72.

[72] Oswal A, Yeo G. Leptin and the control of body weight: a review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. Obesity (Silver Spring). 2010;18(2):221-9.

[73] Moore MC, Coate KC, Winnick JJ, An Z, Cherrington AD. Regulation of hepatic glucose uptake and storage in vivo. Adv Nutr. 2012;3(3):286-94.

[74] Gomis RR, Ferrer JC, Guinovart JJ. Shared control of hepatic glycogen synthesis by glycogen synthase and glucokinase. Biochem J. 2000;351 Pt 3:811-6.

[75] Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. Nat Rev Endocrinol. 2017;13(10):572-87.

[76] Schwartz MW, Seeley RJ, Tschop MH, Woods SC, Morton GJ, Myers MG, et al. Cooperation between brain and islet in glucose homeostasis and diabetes. Nature. 2013;503(7474):59-66.

[77] Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. Nat Med. 2002;8(12):1376-82.

[78] Thorens B. Brain glucose sensing and neural regulation of insulin and glucagon secretion. Diabetes Obes Metab. 2011;13 Suppl 1:82-8.

[79] Fisher SJ, Kahn CR. Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. J Clin Invest. 2003;111(4):463-8.

[80] Tripathy D, Eriksson KF, Orho-Melander M, Fredriksson J, Ahlqvist G, Groop L. Parallel manifestation of insulin resistance and beta cell decompensation is compatible with a common defect in Type 2 diabetes. Diabetologia. 2004;47(5):782-93.

[81] Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, et al. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell. 2000;6(1):87-97.

[82] Gelling RW, Morton GJ, Morrison CD, Niswender KD, Myers MG, Jr., Rhodes CJ, et al. Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. Cell Metab. 2006;3(1):67-73.

[83] Ono H, Pocai A, Wang Y, Sakoda H, Asano T, Backer JM, et al. Activation of hypothalamic S6 kinase mediates diet-induced hepatic insulin resistance in rats. J Clin Invest. 2008;118(8):2959-68.

[84] Rutter J, Michnoff CH, Harper SM, Gardner KH, McKnight SL. PAS kinase: an evolutionarily conserved PAS domain-regulated serine/threonine kinase. Proc Natl Acad Sci U S A. 2001;98(16):8991-6.

[85] Henry JT, Crosson S. Ligand-binding PAS domains in a genomic, cellular, and structural context. Annu Rev Microbiol. 2011;65:261-86.

[86] Amezcua CA, Harper SM, Rutter J, Gardner KH. Structure and interactions of PAS kinase N-terminal PAS domain: model for intramolecular kinase regulation. Structure. 2002;10(10):1349-61.

[87] Schlafli P, Troger J, Eckhardt K, Borter E, Spielmann P, Wenger RH. Substrate preference and phosphatidylinositol monophosphate inhibition of the catalytic domain of the Per-Arnt-Sim domain kinase PASKIN. FEBS J. 2011;278(10):1757-68.

[88] da Silva Xavier G, Farhan H, Kim H, Caxaria S, Johnson P, Hughes S, et al. Per-arnt-sim (PAS) domain-containing protein kinase is downregulated in human islets in type 2 diabetes and regulates glucagon secretion. Diabetologia. 2011;54(4):819-27.

[89] Semplici F, Mondragon A, Macintyre B, Madeyski-Bengston K, Persson-Kry A, Barr S, et al. Cell type-specific deletion in mice reveals roles for PAS kinase in insulin and glucagon production. Diabetologia. 2016;59(9):1938-47.

[90] Wilson WA, Skurat AV, Probst B, de Paoli-Roach A, Roach PJ, Rutter J. Control of mammalian glycogen synthase by PAS kinase. Proc Natl Acad Sci U S A. 2005;102(46):16596-601.

[91] Rutter J, Probst BL, McKnight SL. Coordinate regulation of sugar flux and translation by PAS kinase. Cell. 2002;111(1):17-28.

[92] Katschinski DM, Marti HH, Wagner KF, Shibata J, Eckhardt K, Martin F, et al. Targeted disruption of the mouse PAS domain serine/threonine kinase PASKIN. Mol Cell Biol. 2003;23(19):6780-9.

[93] Hao HX, Cardon CM, Swiatek W, Cooksey RC, Smith TL, Wilde J, et al. PAS kinase is required for normal cellular energy balance. Proc Natl Acad Sci U S A. 2007;104(39):15466-71.

[94] Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. Genes Dev. 2000;14(11):1293-307.

[95] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002;109(9):1125-31.

[96] Wu X, Romero D, Swiatek WI, Dorweiler I, Kikani CK, Sabic H, et al. PAS kinase drives lipogenesis through SREBP-1 maturation. Cell Rep. 2014;8(1):242-55.

[97] Pape JA, Newey CR, Burrell HR, Workman A, Perry K, Bikman BT, et al. Per-Arnt-Sim Kinase (PASK) Deficiency Increases Cellular Respiration on a Standard Diet and Decreases Liver Triglyceride Accumulation on a Western High-Fat High-Sugar Diet. Nutrients. 2018;10(12).

[98] Swiatek W, Parnell KM, Nickols GA, Scharschmidt BF, Rutter J. Validation of PAS Kinase, a Regulator of Hepatic Fatty Acid and Triglyceride Synthesis, as a Therapeutic Target for Nonalcoholic Steatohepatitis. Hepatol Commun. 2020;4(5):696-707.

[99] Kikani CK, Wu X, Paul L, Sabic H, Shen Z, Shakya A, et al. Pask integrates hormonal signaling with histone modification via Wdr5 phosphorylation to drive myogenesis. Elife. 2016;5.

[100] Kikani CK, Wu X, Fogarty S, Kang SAW, Dephoure N, Gygi SP, et al. Activation of PASK by mTORC1 is required for the onset of the terminal differentiation program. Proc Natl Acad Sci U S A. 2019;116(21):10382-91.

[101] Karakkat JV, Kaimala S, Sreedharan SP, Jayaprakash P, Adeghate EA, Ansari SA, et al. The metabolic sensor PASK is a histone 3 kinase that also regulates H3K4 methylation by associating with H3K4 MLL2 methyltransferase complex. Nucleic Acids Res. 2019;47(19):10086-103.

[102] Lee K, Li B, Xi X, Suh Y, Martin RJ. Role of neuronal energy status in the regulation of adenosine 5'-monophosphate-activated protein kinase, orexigenic neuropeptides expression, and feeding behavior. Endocrinology. 2005;146(1):3-10.

[103] Cota D, Matter EK, Woods SC, Seeley RJ. The role of hypothalamic mammalian target of rapamycin complex 1 signaling in diet-induced obesity. J Neurosci. 2008;28(28):7202-8.

[104] Hurtado-Carneiro V, Roncero I, Blazquez E, Alvarez E, Sanz C. PAS kinase as a nutrient sensor in neuroblastoma and hypothalamic cells required for the normal expression and activity of other cellular nutrient and energy sensors. Mol Neurobiol. 2013;48(3):904-20.

[105] Hurtado-Carneiro V, Roncero I, Egger SS, Wenger RH, Blazquez E, Sanz C, et al. PAS kinase is a nutrient and energy sensor in hypothalamic areas required for the normal function of AMPK and mTOR/S6K1. Mol Neurobiol. 2014;50(2):314-26.

[106] Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet. 2006;368(9548):1696-705.

[107] Malik J, Roohi N. GLP-1, a powerful physiological incretin: an update. J Biol Regul Homeost Agents. 2018;32(5):1171-6.

[108] Semache M, Zarrouki B, Fontes G, Fogarty S, Kikani C, Chawki MB, et al. Per-Arnt-Sim kinase regulates pancreatic duodenal homeobox-1 protein stability via phosphorylation of glycogen synthase kinase 3beta in pancreatic beta-cells. J Biol Chem. 2013;288(34):24825-33.

[109] Beiroa D, Imbernon M, Gallego R, Senra A, Herranz D, Villarroya F, et al. GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. Diabetes. 2014;63(10):3346-58.

[110] Lopez M, Dieguez C, Nogueiras R. Hypothalamic GLP-1: the control of BAT thermogenesis and browning of white fat. Adipocyte. 2015;4(2):141-5.

[111] Semplici F, Vaxillaire M, Fogarty S, Semache M, Bonnefond A, Fontes G, et al. Human mutation within Per-Arnt-Sim (PAS) domain-containing protein kinase (PASK) causes basal insulin hypersecretion. J Biol Chem. 2011;286(51):44005-14.

[112] Perez-Garcia A, Dongil P, Hurtado-Carneiro V, Blazquez E, Sanz C, Alvarez E. High-fat diet alters PAS kinase regulation by fasting and feeding in liver. J Nutr Biochem. 2018;57:14-25.

[113] Perez-Garcia A, Dongil P, Hurtado-Carneiro V, Blazquez E, Sanz C, Alvarez E. PAS Kinase deficiency alters the glucokinase function and hepatic metabolism. Sci Rep. 2018;8(1):11091.

[114] Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, et al. Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. Nature. 1992;356(6371):721-2.

[115] Christesen HB, Jacobsen BB, Odili S, Buettger C, Cuesta-Munoz A, Hansen T, et al. The second activating glucokinase mutation (A456V): implications for glucose homeostasis and diabetes therapy. Diabetes. 2002;51(4):1240-6.

[116] Iynedjian PB. Molecular physiology of mammalian glucokinase. Cell Mol Life Sci. 2009;66(1):27-42.

[117] Dentin R, Pegorier JP, Benhamed F, Foufelle F, Ferre P, Fauveau V, et al. Hepatic glucokinase is required for the synergistic action of ChREBP and SREBP-1c on glycolytic and lipogenic gene expression. J Biol Chem. 2004;279(19):20314-26.

[118] Magnuson MA, Andreone TL, Printz RL, Koch S, Granner DK. Rat glucokinase gene: structure and regulation by insulin. Proc Natl Acad Sci U S A. 1989;86(13):4838-42.

[119] Agius L. Glucokinase and molecular aspects of liver glycogen metabolism. Biochem J. 2008;414(1):1-18.

[120] Dongil P, Perez-Garcia A, Hurtado-Carneiro V, Herrero-de-Dios C, Blazquez E, Alvarez E, et al. Pas Kinase Deficiency Triggers Antioxidant Mechanisms in the Liver. Sci Rep. 2018;8(1):13810.

[121] Nagao M, Asai A, Sugihara H, Oikawa S. Fat intake and the development of type 2 diabetes. Endocr J. 2015;62(7):561-72.

[122] Thanopoulou AC, Karamanos BG, Angelico FV, Assaad-Khalil SH, Barbato AF, Del Ben MP, et al. Dietary fat intake as risk factor for the development of diabetes: multinational, multicenter study of the Mediterranean Group for the Study of Diabetes (MGSD). Diabetes care. 2003;26(2):302-7.

[123] da Silva Xavier G, Rutter J, Rutter GA. Involvement of Per-Arnt-Sim (PAS) kinase in the stimulation of preproinsulin and pancreatic duodenum homeobox 1 gene expression by glucose. Proc Natl Acad Sci U S A. 2004;101(22):8319-24.

[124] Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. Annu Rev Nutr. 2005;25:391-406.

[125] Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harb Perspect Biol. 2014;6(1).

[126] Bogan JS. Regulation of glucose transporter translocation in health and diabetes. Annu Rev Biochem. 2012;81:507-32.

[127] Leto D, Saltiel AR. Regulation of glucose transport by insulin: traffic control of GLUT4. Nat Rev Mol Cell Biol. 2012;13(6):383-96.

[128] Haemmerle G, Zimmermann R, Strauss JG, Kratky D, Riederer M, Knipping G, et al. Hormone-sensitive lipase deficiency in mice changes the plasma lipid profile by affecting the tissue-specific expression pattern of lipoprotein lipase in adipose tissue and muscle. J Biol Chem. 2002;277(15):12946-52.

[129] Bederman IR, Previs SF. Hormonal regulation of intracellular lipolysis in C57BL/6J mice: effect of diet-induced adiposity and data normalization. Metabolism. 2008;57(10):1405-13.

[130] Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. Lancet. 2005;366(9491):1059-62.

[131] Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415-28.

[132] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112(12):1821-30.

[133] Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A, Thorens B. Heterogeneous

metabolic adaptation of C57BL/6J mice to high-fat diet. Am J Physiol Endocrinol Metab. 2002;282(4):E834-42.

[134] Buettner R, Scholmerich J, Bollheimer LC. High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity (Silver Spring). 2007;15(4):798-808.

[135] Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology. 2010;52(5):1836-46.

[136] Yilmaz Y. Review article: is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? Aliment Pharmacol Ther. 2012;36(9):815-23.

[137] Hegarty BD, Bobard A, Hainault I, Ferre P, Bossard P, Foufelle F. Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c. Proc Natl Acad Sci U S A. 2005;102(3):791-6.

[138] Ono H, Shimano H, Katagiri H, Yahagi N, Sakoda H, Onishi Y, et al. Hepatic Akt activation induces marked hypoglycemia, hepatomegaly, and hypertriglyceridemia with sterol regulatory element binding protein involvement. Diabetes. 2003;52(12):2905-13.

[139] Lima-Cabello E, Garcia-Mediavilla MV, Miquilena-Colina ME, Vargas-Castrillon J, Lozano-Rodriguez T, Fernandez-Bermejo M, et al. Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C. Clin Sci (Lond). 2011;120(6):239-50.

[140] Rahimian R, Masih-Khan E, Lo M, van Breemen C, McManus BM, Dube GP. Hepatic over-expression of peroxisome proliferator activated receptor gamma2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. Mol Cell Biochem. 2001;224(1-2):29-37.

[141] Bedoucha M, Atzpodien E, Boelsterli UA. Diabetic KKAy mice exhibit increased hepatic PPARgamma1 gene expression and develop hepatic steatosis upon chronic treatment with antidiabetic thiazolidinediones. J Hepatol. 2001;35(1):17-23.

[142] Lechleitner M. Obesity and the metabolic syndrome in the elderly--a mini-review. Gerontology. 2008;54(5):253-9.

[143] Catalano KJ, Bergman RN, Ader M. Increased susceptibility to insulin resistance associated with abdominal obesity in aging rats. Obes Res. 2005;13(1):11-20.

[144] Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. Diabetes. 2012;61(6):1315-22.

[145] Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, et al. Oxidative damage to DNA in diabetes mellitus. Lancet. 1996;347(8999):444-5.

[146] Goodarzi MT, Navidi AA, Rezaei M, Babahmadi-Rezaei H. Oxidative damage to DNA and lipids: correlation with protein glycation in patients with type 1 diabetes. J Clin Lab Anal. 2010;24(2):72-6.

[147] Kim IH, Kisseleva T, Brenner DA. Aging and liver disease. Curr Opin Gastroenterol. 2015;31(3):184-91.

[148] Kenyon C. The plasticity of aging: insights from long-lived mutants. Cell. 2005;120(4):449-60.

[149] Solon-Biet SM, Mitchell SJ, de Cabo R, Raubenheimer D, Le Couteur DG, Simpson SJ. Macronutrients and caloric intake in health and longevity. J Endocrinol. 2015;226(1):R17-28.

[150] Salminen A, Kaarniranta K. AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. Ageing Res Rev. 2012;11(2):230-41.

[151] Dongil P, Perez-Garcia A, Hurtado-Carneiro V, Herrero-de-Dios C, Alvarez E, Sanz C. PAS kinase deficiency reduces aging effects in mice. Aging (Albany NY). 2020;12.

[152] Taguchi K, Motohashi H, Yamamoto M. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. Genes Cells. 2011;16(2):123-40.

[153] Zhang H, Davies KJA, Forman HJ. Oxidative stress response and Nrf2 signaling in aging. Free Radic Biol Med. 2015;88(Pt B):314-36.

[154] Ziegler DV, Wiley CD, Velarde MC. Mitochondrial effectors of cellular senescence: beyond the free radical theory of aging. Aging Cell. 2015;14(1):1-7.

[155] Cui H, Kong Y, Zhang H. Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct. 2012;2012:646354.

[156] Colantoni A, Idilman R, de Maria N, Duffner LA, Van Thiel DH, Witte PL, et al. Evidence of oxidative injury during aging of the liver in a mouse model. J Am Aging Assoc. 2001;24(2):51-7.

[157] Castro Mdel R, Suarez E, Kraiselburd E, Isidro A, Paz J, Ferder L, et al. Aging increases mitochondrial DNA damage and oxidative stress in liver of rhesus monkeys. Exp Gerontol. 2012;47(1):29-37.

[158] Calabrese EJ, Dhawan G, Kapoor R, Iavicoli I, Calabrese V. What is hormesis and its relevance to healthy aging and longevity? Biogerontology. 2015;16(6):693-707.