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Insulin: a connection between pancreatic β cells and the hypothalamus

Insulin: pancreas and hypothalamus

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Abstract

Insulin is a hormone secreted by pancreatic β cells. The concentration of glucose in circulation is proportional to the secretion of insulin by these cells. In target cells, insulin binds to its receptors and activates the phosphatidylinositol-3-kinase/protein kinase B, inducing different mechanisms depending on the cell type. In the liver it activates the synthesis of glycogen, in adipose tissue and muscle it allows the capture of glucose, and in the hypothalamus it regulates thermogenesis and appetite. Defects in insulin function [insulin resistance (IR)] are related to the development of neurodegenerative diseases in obese people. Furthermore, in obesity and diabetes, its role as an anorexigenic hormone in the hypothalamus is diminished during IR. Therefore, hyperphagia prevails, which aggravates hyperglycemia and IR further, becoming a vicious circle which, the patient cannot regulate their need to eat. Uncontrolled calorie intake induces an increase in reactive oxygen species, overcoming cellular antioxidant defenses (oxidative stress). Reactive oxygen species activate stress-sensitive kinases, such as c-Jun N-terminal kinase (commonly known as JNK) and p38 mitogen-activated protein kinase (commonly known as p38), that induce phosphorylation in serine residues in the insulin receptor, which blocks the insulin signaling pathway, continuing the mechanism of IR. The brain and pancreas are organs mainly affected by oxidative stress. The use of drugs that regulate food intake and improve glucose metabolism is the conventional therapy to improve the quality of life of these patients. Currently, the use of antioxidants that regulate oxidative stress has given good results because they reduce oxidative stress and inflammatory processes, and they also have fewer side effects than synthetic drugs.

Key Words: Insulin; Pancreas; Hypothalamus; Hyperphagia; Hyperglycemia; Stress

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Core Tip: Insulin is the connection between the β cells of the pancreas and the hypothalamus. Insulin reaches the arcuate nucleus of the hypothalamus and represses the expression of orexigenic neuropeptides to suppress appetite. However, its function decreases when there is damage to the β cells of the pancreas. Its anorexigenic effect decreases and thus increases appetite. The excess of nutrients, specifically carbohydrates, aggravates the damage to β cells and induces obesity and/or diabetes and oxidative damage. The use of antioxidants constitutes a therapeutic approach that has been approached experimentally to regulate the negative effects of alterations in insulin secretion and function.

INTRODUCTION

Insulin is a peptide hormone that plays an important role in glucose homeostasis, cell growth and metabolism[1]. This hormone is synthesized in the β cells of the pancreatic islets; its transcription and translation is regulated in part by nutrients, specifically in response to glucose concentrations[2,3]. The active structure of this hormone is formed by two chains named "chain A" with 21 amino acid residues and "chain B" with 30 amino acid residues linked by three disulfide bonds between both chains[2,4]. The insulin is stored in vesicles to be released into the bloodstream when β cells take up glucose from the extracellular medium[1]; through the bloodstream it will reach all peripheral organs and the brain[5].

Insulin will bind to its receptor in the cell membrane and allow activation of the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) insulin signaling pathway[6]. The effects of the activation of this pathway will depend on the cell lineage. It has an anti-atherogenic effect in the vascular system. In the liver, it promotes energy utilization. In the muscle, insulin promotes glucose metabolism and participates in protein synthesis. In adipose tissue, insulin induces lipogenesis[7,8], and finally, in the brain it will activate thermogenesis and regulate appetite, glucose homeostasis and metabolism[8-10]. When there are alterations in the secretion or function of insulin,

chronic-degenerative pathologies are produced such as hyperphagia, hyperglycemia, insulin resistance (IR) and diabetes mellitus (DM)[11]. A common feature of these pathologies is the formation of reactive oxygen species (ROS), which alter signaling pathways activated by insulin[12-14]. Currently, the use of nutraceuticals has been reported with highly positive effects on the control of ROS, alterations in the secretion and function of insulin at the pancreatic[15] and cerebral levels[16].

INSULIN AND THE PANCREAS

The human pancreas is a retroperitoneal organ in the upper abdomen; weighing between 100-150 g and measuring between 15-25 cm in length, it is connected to other abdominal organs such as the spleen, stomach, duodenum and colon[17]. This organ is surrounded by a fibrous capsule that divides its parenchyma into distinct lobes and lobules[18] separated by connective tissue which divides the pancreas into two structurally distinct components: the exocrine pancreas, which consists mainly of acinar cells and duct cells and the endocrine pancreas, which is the site of islet cells[17,19].

The endocrine portion is composed of groups of cells known as islets of Langerhans, which are attributed with the secretion of several pancreatic peptide hormones for glucose homeostasis, including insulin. There are five major cell types that constitute the islet: α cells, β cells, δ cells, PP cells and ε cells, which are responsible for producing glucagon, insulin, somatostatin, pancreatic polypeptide and ghrelin, respectively[1,17,20]. The most numerous are the β cells that synthesize and secrete insulin. Insulin is a peptide hormone that was discovered in 1922 by surgeon Frederick Grant Banting and physician Charles Herbert Best and purified by biochemist James Bertam Collip[21,22]. This hormone plays an important role in glucose homeostasis, cell growth and metabolism[1]. In humans, it is encoded by the *INS* gene on chromosome 11, in rats (*Rattus norvegicus*) by the *ins1/2* gene on chromosome 1 and in mice (*Mus musculus*) by the *Ins1* (chromosome 19) and *Ins2* (chromosome 7)[23].

The human *INS* gene (1425 bp) is composed of three exons and two introns, as is the rodent *Ins2* gene. However, the rodent *Ins2* gene is composed of only two exons,

with the entire coding sequence contained in the second exon[24,25]. In the insulin gene promoter there are response elements such as the A element, GG box, C1 [rat insulin promoter element (RIPE)3b1/C2 (RIPE3b2) element (RIPE 3b1/2), CRE (cAMP response element), E element, ILRP (insulin-linked polymorphic region), enhancer core and Z region where the negative regulatory element is located. These regulatory elements within the promoter region of the insulin gene either enhance or inhibit transcription of the gene and are located between positions -340 and -91 bp relative to the transcription start site.

Several transcription factors bind in these regions including PDX-1, PAX4/6, MafA, HNF-1α and NeuroD1[2,26,27]. The signal transducer and activator of transcription (STAT) protein also has a very important role in the activation of insulin gene transcription. It has been reported that elevated Ca^{2+} levels activate calpain-1, a protease that cleaves a cytosolic fragment of islet cell autoantigen 512, which promotes transient fusion of the cell membrane with the membrane of insulin-containing granules to release insulin into the extracellular milieu. The free fragment of islet cell autoantigen 512 targets the nucleus and binds to STAT5, which in turn promotes increased transcription of the insulin gene, thus maintaining optimal levels of stored insulin[3].

In addition, there are polypyrimidine tract-binding proteins that positively regulate mRNA translation. Cytosolic polypyrimidine tract-binding protein 1 can bind to pyrimidine, *i.e.* cytosine-uracil-rich, sequences in the 3′ UTR region of insulin mRNA, thereby stabilizing the insulin mRNA strand and increasing its translation[28].

Insulin translation in pancreatic β cells is regulated in part by nutrients, specifically in response to glucose concentrations[2]. Levels between approximately 2 mmol/L and 4 mmol/L glucose are required to promote insulin biosynthesis and levels greater than 5 mmol/L to promote insulin release[29]. Increased glucose concentrations contribute to the activation of protein phosphatase 1, which dephosphorylates eukaryotic translation initiation factor 2a promoting insulin translation. However, pancreatic endoplasmic reticulum (ER) kinase decreases insulin synthesis through phosphorylation of eukaryotic translation initiation factor 2a[2].

In β cells, insulin is translated as a 110 amino acid pre-proinsulin in the cytosol. Pre-proinsulin contains a 24 amino acid, nuclear transport signal peptide (Ala-Ala-Ala-Ala-Pro-Asp-Pro-Gly-Trp-Leu-Ala-Leu-Leu-Leu-Ala-Leu-Leu-Leu-Pro-Leu-Leu-Leu-Arg-Met-Trp-Leu-Ala-Met)[30], which guides pre-proinsulin to the rough endoplasmic reticulum (RER) membrane for translocation to the RER cisternae *via* two mechanisms: (1) A signal recognition particle (SRP)-dependent cotranslational translocation mechanism where SRP recognizes and binds to the signal peptide of pre-proinsulin arising from ribosomes, forming a complex that interacts with the SRP receptor (TRAP) on the RER membrane, thereby directing nascent pre-proinsulin to the Sec61 translocon[31]; and (2) An SRP-independent post-translational translocation mechanism where in addition to the Sec61 translocon several RER (BIP) and cytosolic molecular chaperones are involved, including Hsp70, TRC40, calmodulin and Sec62/63[31].

During translocation, the pre-proinsulin signal peptide must be correctly oriented within the Sec61 translocon so that the N-terminal end of the signal peptide faces the cytosolic side of the RER. This orientation allows the signal peptide cleavage site to be exposed to signal peptidase on the luminal side of the RER membrane[31], generating pro-insulin, a chain of 86 amino acids that folds and stabilizes in its three-dimensional configuration by linking peptide chains A and B through the formation of three disulfide bonds *via* chaperones such as thiol reductase. The first bond is between amino acids CysA6 and CysA11, the second is between amino acids CysA7 and CysB7, and the third bridge is between amino acids CysB19 and CysA20[23,32].

After acquiring three-dimensional folding, pro-insulin is transferred from the RER to the Golgi *via* vesicles where pro-insulin is converted to insulin as these immature vesicles acidify and mature[31] (Figure 1). In the secretory granules there are two endoproteases involved in the conversion of proinsulin to insulin called prohormone convertases PC2 and PC1/3. The former hydrolyzes between the basic amino acids Arg33-Gly1 at the C-peptide and A-chain junction, and the latter hydrolyzes between the dipeptide Thr30-Arg31 at the B-chain and C-peptide junction[33]. Subsequently, carboxypeptidase E hydrolyzes between the Gln31-Lys32

amino acids as well as between Arg32 and Glu1 basic C-termini of the resulting peptide chains, producing a mature insulin protein of 51 amino acids[23] (Figure 2).

Insulin in its monomeric form tends to form dimers as insulin concentration increases. In the presence of zinc and pH optima (10 mmol/L Zn^{2+}, pH 6.0), the hydrophobic amino acids in the dimeric structures interact and assemble into higher order conformations called hexamers, useful for insulin storage[2]. Once the hexamers are secreted into the circulation by exocytosis, they diffuse into the blood in favor of their concentration gradient. A combination of electrostatic repulsion and decrease in insulin concentration favors the dissociation of insulin into its monomeric form, releasing active insulin and an equimolar proportion of C-peptide[2,33].

This active structure is formed by two chains named "chain A" with 21 amino acid residues and "chain B" with 30 amino acid residues linked by three disulfide bonds between both chains (CysA7-CysB7, CysA20-CysB19 and CysA7-CysA11) (Figure 2). The secondary structure of the A chain contains two antiparallel α-helices connected near the two ends of the A chain. The secondary structure of the B chain contains α-helices and β-strands. This chain can generate two distinct conformations. In a taut state, there is a central α-helix from SerB9 to CysB19, as well as a β-twist from GlyB20-GlyB23 generating a "V" folding. This twist also allows the formation of a β-sheet with Phe24 and Tyr26 in contact with Leu11 and Leu15 of the α-helix of the B-chain. In a resting state, there is a continuous alpha helix from PheB1-CysB19. Disulfide bonds between residues CysA7-CysB7 and CysA20-CysB19 contribute to the stability of the native insulin structure[2,4]. The overall tertiary structure of the protein is highly organized and stabilized by specific interactions involving residues CysA6-CysA11 and LeuA11, PheB1 and LeuB15, IleA2, PheB24, ValA3, IleA13, ValB18 and ValB12 generating a hydrophobic core[2].

Following food intake, glucose is transported into pancreatic β cells *via* the glucose transporter (GLUT) 2 in humans and mice[30,33]. Once pancreatic β cells have internalized glucose and its degradation through glycolysis and the Krebs cycle is initiated, intracellular ATP levels increase, which generates the closure of K^+ channels,

causing a change in membrane permeability opening Ca^{2+} channels. This induces the remodeling of the cytoskeleton and the translocation of insulin granules to the plasma membrane to subsequently release the hormone, which through the bloodstream will reach all peripheral organs and the brain[5,30,33] (Figure 1).

Levels between approximately 2 mmol/L and 4 mmol/L glucose are required to promote insulin biosynthesis and levels greater than 5 mmol/L to promote insulin release[29]. Once insulin synthesis is stimulated in the β cells of the pancreas, it is exported through the portal vein to the liver. During this process, more than 50% of the insulin is eliminated by hepatocytes from the liver. The remaining insulin exits through the hepatic vein until it reaches the heart to be distributed through the arterial circulation to the rest of the body to fulfill its various functions. Finally, the remaining circulating insulin is degraded in the kidney[23].

In the peripheral organs that depend on insulin to bring glucose into the cells, the hormone will bind to its receptor and allow activation of the PI3K/AKT insulin signaling pathway. This will generate translocation of GLUT4 to the cell membrane thus allowing glucose to enter the cell. Therefore, insulin, through anabolic pathways, regulates blood glucose concentrations[6]. Whereas, the counter-regulatory hormone, glucagon, regulates glucose concentrations through catabolic pathways[1].

Within the positive regulators of insulin, in addition to glucose, are amino acids, glucagon, glucagon-like peptide 1 (GLP-1), growth hormone, secretin, gastrin, glucose-dependent insulinotropic peptide and cholecystokinin. Among the major negative regulators of insulin are adrenocorticosteroids, somatostatin, adrenaline, norepinephrine, neuropeptide Y and calcitonin gene-related peptide[33].

INSULIN SECRETORY DYSFUNCTION

Insulin-secreting β cell dysfunction, defined as the loss of the ability of pancreatic β cells to produce and release insulin in concentrations sufficient to maintain euglycemia, occurs when high and prolonged insulin secretion in response to environmental insults leads to exhaustion of pancreatic β cells[34]. β cells can suffer from insulin secretory

dysfunction due to multiple factors. The most common causes are overnutrition (excess nutrients such as glucose and fatty acids), increased body weight, a sedentary lifestyle and aging, which will lead to pathological conditions such as obesity and type 2 DM (T2DM)[34-36]. Other causes of β cell dysfunction, accounting for less than 5% of cases, include diseases that destroy the pancreas, such as acute pancreatitis, chronic pancreatitis and cystic fibrosis[37-39], that specifically inhibit insulin secretion (genetic β cell defects) or that alter counterregulatory hormones (Cushing's syndrome, obesity)[34]. The clinical presentations in these cases depend on the exact nature of the process.

The most common causes of β cell dysfunction share the formation of ROS and cellular oxidative stress as the initiation mechanism[40-42]. Pancreatic β cells are especially vulnerable to stress and oxidative damage[38] due to the low expression of classical antioxidant enzymes such as catalases, glutathione peroxidases and superoxide dismutases compared to other cell types[43,44]. The main antioxidant system of β cells consists of peroxiredoxins, thioredoxins and thioredoxin reductase. This system has been shown to be sufficient to protect β cells against short-term oxidative stress and hypothetically provides a signaling role required for glucose-stimulated insulin secretion in both rodent and human cells[45]. However, long-term glycolipotoxic conditions compromise β cell metabolism and ATP production through glycolytic dysfunction and reduced activation of glyceraldehyde 3-phosphate dehydrogenase, which reduces the generation of pyruvate and promotes β-oxidation.

As a result of metabolic dysfunction, the generation of superoxide and hydrogen peroxide by the mitochondrial electron transport chain is increased[46], increasing cellular ROS concentrations. Excess ROS are capable of oxidizing DNA (mainly mitochondrial DNA), proteins and lipids and function as effector and signaling molecules in cell membranes that mediate signal transduction and inflammation pathways[46,47]. In addition, inflammation, which is also present in the aforementioned pathologies, aggravates the damage and functions as a feedback for stress and oxidative damage because polymorphonuclear neutrophils at the site of inflammation release large amounts of ROS as an immune defense response, causing tissue damage and

endothelial dysfunction[48]. Oxidative stress can induce and maintain a proinflammatory environment through the activation of proinflammatory pathways regulated by the transcription factor NF-kB and JNK and the production of inflammatory cytokines such as IL-1β[34,38,40,49]. This improves polymorphonuclear neutrophils recruitment, which further stimulates the pro-inflammatory condition in the tissue, thus generating a feedback process oxidative stress-inflammation-oxidative stress[46].

Persistent inflammation of the pancreas causes ER stress, progressive atrophy and/or replacement with fibrotic tissue, pain, exocrine pancreatic insufficiency, trypsin activation leading to pancreatic autodigestion, loss of functional β cell mass and consequently the reduced ability of β cells to secrete insulin (Figure 3). This pathology is known as pancreatic endocrine dysfunction or DM[50,51].

DM is a complex and heterogeneous disorder defined by the presence of hyperglycemia[11] and can lead to life-threatening complications such as severe hypoglycemia or chronic micro- and macroangiopathic complications[52]. There are several types of diabetes, although type 1 DM (T1DM) and T2DM are the most common. The American Diabetes Association defines T1DM as the autoimmune destruction of β cells, usually leading to absolute insulin deficiency, and T2DM as the progressive loss of insulin action in target tissues as well as a decrease in their secretion from β cells[53]. All cellular events are summarized in Figure 3.

INSULIN AND APPETITE REGULATION

The hypothalamus is the specific area of the brain where eating behavior is regulated, which is directly related to glucose homeostasis[54]. The hypothalamus is located around the third ventricle, below the thalamus and above the median eminence, one of the circumventricular organs in which the blood brain barrier is slightly modified with semi-permeable capillaries that allow selective exchange between molecules of the blood and cerebrospinal flow with the neurons of the hypothalamus[55,56]. This region is divided into several nuclei, among which the arcuate nucleus (ARC), paraventricular

nucleus, ventromedial nucleus, dorsomedial nucleus and lateral area nucleus stand out[57,58]. The ARC is located very close to the median eminence. It is made up of first-order neurons that first receive signals from peripheral organs such as the stomach, adipose tissue and the pancreas[56,59].

Insulin is the signal derived from the pancreas in response to the presence of nutrients (glucose) in the bloodstream[54]. After being secreted from pancreatic β cells, insulin *via* the bloodstream reaches the hypothalamus crossing the median eminence or crossing the vascular endothelium *via* transport proteins or *via* the insulin receptor itself, which is assumed to also act as its transporter (mechanism not fully defined)[60,61].

Insulin reaches the ARC and binds to its receptor in the first-order neurons. Once insulin binds to its receptor in the hypothalamus, it leads to rapid autophosphorylation of the insulin receptor, followed by tyrosine phosphorylation of insulin receptor substrates, which induces the activation of the PI3K/AKT and the MAPK cascades[61]. The PI3K/AKT pathway promotes the activation of the mammalian target of rapamycin complex 1/p70-S6 kinase[61,62], which is capable of phosphorylating AMP-activated protein kinase (AMPK) at serine 485/491 sites[63], reducing the ability of CaMKKII to phosphorylate AMPK in the threonine 172 residue and resulting in the low expression of genes related to appetite induction (orexigenic), such as neuropeptide Y (*NPY*) and agouti-related protein (*AgRP*) in the ARC, the paraventricular nucleus and the lateral area nucleus, which decreases appetite[56,63,64].

Moreover, AKT induces the phosphorylation of the transcription factor forkhead box protein O1. When forkhead box protein O1 is phosphorylated it leaves the nucleus and therefore decreases the expression of genes that are activated by this factor, such as *NPY* and *AgRP*[56,64]. Therefore, insulin and the activation of its signaling pathway promotes an anorexigenic effect, by inducing a decrease in the expression of the neuropeptides that induce appetite (*NPY*/*AgRP*).

Similar to insulin, another anorexigenic signaling pathway is activated by leptin[56,64]. Leptin is secreted from adipocytes in proportion to levels of body fat stores[66]. Through the bloodstream it reaches first-order neurons, binds to its receptors

and activates the Janus tyrosine kinase pathway and STAT3 pathway. STAT3 is a transcription factor that stimulates the expression of the precursor neuropeptide of α-melanocyte-stimulating hormone, named POMC, and the transcript regulated by cocaine and amphetamines (CART). These neuropeptides exert an anorexigenic effect[56,64]. Leptin and insulin signaling converge in the activation of PI3K/AKT, thus the anorexigenic effect is enhanced since the expression of NPY/AgRP is decreased by insulin and leptin, while POMC/CART expression is increased by leptin[56,64,65].

POMC/CART are the main anorexigenic neuropeptides expressed in neurons of the first-order (named neurons POMC/CART). These neurons release multiple cleavage products of POMC, including α-melanocyte-stimulating hormone, that bind in the second-order neurons located in the paraventricular nucleus, dorsomedial nucleus, ventromedial nucleus and lateral area nucleus to activate downstream melanocortin receptors (MC3R/MC4R) to promote satiety and control eating behavior, glucose homeostasis and body weight[54,58,64,66].

In periods of fasting, when glucose decreases, the release of insulin in the pancreas also decreases, and consequently the expression of POMC and CART decreases along with the satiety effect[56]. Meanwhile, the concentrations of ghrelin, a hormone secreted in the stomach during periods of starvation, increase[67]. This hormone reaches ARC through the bloodstream to activate the growth hormone receptor 1a, a G protein-coupled receptor, for the release of the α subunit from the βγ subunits of G protein. The α subunit activates phospholipase C. Phospholipase C induces the production of diacyl glycerol and phosphoinositol triphosphate. Phosphoinositol triphosphate is a second messenger that binds to its receptor in the ER and causes the release of Ca^{2+} into the cytosol[68]. Increasing Ca^{2+} activates the CaMKKII, which phosphorylates AMPK in the threonine 172 residue. AMPK activates transcription factors such as the cAMP-response element binding protein and forkhead box protein O1, which act on the promoter region of the *NPY* and *AgRP* genes, promoting their expression and inducing appetite[14,56].

NPY exerts its orexigenic effect on second-order neurons through stimulation of the Gi-coupled NPY family of receptors[66,69], mediating the inhibition of adenylate cyclase, decreased levels of cAMP[57,70] and the activation of MAPK[61,70]. AgRP is a biased agonist of melanocortin receptors (MC3R/MC4R) and prevents the binding of α-melanocyte-stimulating hormone to these receptors, blocking the induction of satiety and driving sustained increase in food intake[66]. This constitutes an orexigenic signal.

Therefore, under normal physiological conditions, the release of the specific signal (inducing or inhibiting appetite) in the peripheral organs will depend on the metabolic state of the organism and will induce a response in the form of orexigenic or anorexigenic neurotransmitters in the hypothalamus[9,56,64,66]. The strict regulation of these afferent and nutrient-related hormonal signals is necessary to avoid alterations in the regulation of appetite since an uncontrolled increase in POMC/CART would cause anorexia, but the uncontrolled increase in the expression of NPY /AgRP will generate hyperphagia, which due to excessive consumption of hypercaloric diets has been related to weight gain and obesity (characteristics that are linked to IR and T2DM)[9].

In studies in experimental models of hyperphagia and DM induced with streptozotocin, NPY/AgRP neurons are more active and the expression level of NPY and AgRP is increased, while POMC/CART neurons are less active and the expression level of POMC and CART is decreased. This change is explained in part to the inefficiency and/or deficiency of insulin[71,72] and leptin[73] and increased levels of circulating ghrelin[74,75].

During diabetic hyperphagia, high glucose intake will induce a proportional release of insulin from pancreatic b cells (hyperinsulinemia). The high concentration insulin will induce the constant activation of the receptor at the cerebral and peripheral levels, which generates molecular and cellular regulation mechanisms such as: (1) Internalization of the receptor by clathrin-mediated endocytosis[76]; (2) Dephosphorylation in tyrosine residues of the insulin receptor by protein- tyrosine phosphatase 1B, which is a nontransmembrane tyrosine phosphatase that acts as a potent negative modulator of insulin signaling by reversing insulin- induced

phosphorylation in tyrosine residues and impairs insulin signal transduction); and (3) Phosphorylation on serine residues by serine–threonine kinases, such as JNK and p38 MAPK[12,13]. This will generate a lack of response to the presence of the hormone (*i.e.* IR). At the level of the hypothalamus, this will decrease the activity of one of the pathways that induce satiety.

On the other hand, hyperphagia is often associated with the accumulation of visceral fat[77] and consequently elevated plasma leptin concentrations. This situation will induce the failure to respond to the hormone at central and peripheral levels, named leptin resistance[78,79]. In this way, there will be a decrease in the two central signals that induce satiety, favoring the persistence of hyperphagia and the onset of resistance to both hormones. This becomes a vicious circle: hyperphagia-hyperglycemia-hyperinsulinemia/hyperleptinemia-insulin/Leptin resistance-hyperphagia.

In addition, excessive consumption of carbohydrates (glucose and/or fructose), coupled with a lack of physical activity, will generate an increase in glucose uptake in all cells but mainly in cells that have glucose transporters that act independently of the presence or absence of insulin[9,13,14] such as GLUT1 and GLUT3, transporters mainly present in the brain[80]. With excessive intake of carbohydrates, glycolysis will increase, and therefore the release of ROS (species produced normally in glycolysis) will increase progressively until they overcome antioxidant barriers and oxidative stress develops[13,14,81].

It has been reported that during oxidative stress there is the activation of stress-sensitive kinases (JNK, P38 MAPK) that induce phosphorylation of serine residues in the insulin receptor and in the insulin receptor substrates, which blocks the pathway of insulin signaling aggravating the condition of IR[12,13]. In addition, studies carried out in rat models fed with fructose and subjected to an environmental stress protocol revealed that stress decreased body mass, adiposity and blood leptin level, decreased expression of leptin receptor and POMC in the hypothalamus and led to marked increase of AgRP, associated with AMPK phosphorylation and reduced Akt activity[14]. In parallel studies

undertaken in normal rats, chronic blockade of hypothalamic insulin receptors caused hyperphagia and IR[82]. Furthermore, it has been reported that stimulation of hypothalamic insulin signaling would be sufficient to inhibit the glucose production in the liver through the intracerebroventricular administration of agonists and antagonists of insulin signaling[83], combined with evidence that mice with neuron-specific insulin receptor deletion are overweight, insulin-resistant and glucose-intolerant. These data demonstrate that neuronal insulin signaling is required for intact control of both body fat mass and glucose homeostasis[9]. Consequently, chronic stress can dysregulate the hypothalamus-adipose tissue[14,84] and hypothalamus-pancreas[64] axis over time, which affects glucose metabolism, promotes IR and influences multiple appetite-related hormones in the hypothalamus[64,84].

On the other hand, the effect of insulin has not only been studied in the hypothalamus at the level of glucose homeostasis. It has also been shown that the administration of insulin into the hippocampus of rats promotes Akt-dependent translocation of GLUT4[85]. Furthermore, hippocampal-specific suppression of insulin signaling reduces long-term potentiation in the hippocampus and significantly impairs memory and learning ability[86]. In hypothalamic neurons they have an important effect on body thermoregulation by signaling with brown adipose tissue[87]. Therefore, the effect of insulin at the brain level has been fully established. All cellular and molecular events are summarized in Figure 4.

THERAPEUTIC CONSIDERATIONS

Medical therapy is the first step to achieve adequate control of complications related to alterations in insulin secretion. Considering that DM is the main pathology related to this alteration, therapeutic treatments are focused on reducing hyperglycemia as well as stimulating the production and secretion of insulin in the b cells of the pancreas and its signaling in the different tissues.

For T1DM, characterized by the destruction of the b cells of the pancreas by autoantibodies as well as a decrease in the production and secretion of insulin, the first-

line treatment is the administration of non-endogenous insulin[88]. Regarding T2DM, there are various therapeutic approaches, starting with improving eating habits[89] and increasing physical activity, which results in improving insulin sensitivity and helps control blood glucose[90]. When the above does not help control hyperglycemia, the therapeutic approach is based on the use of conventional drugs such as sulfonylureas (inducing insulin release from b cells of the pancreas), biguanides (inducing glucose uptake by cells not -insulin-dependent and reducing hepatic glucose production) and alpha-glucosidase inhibitors (blocking the absorption of glucose in the intestine)[91].

Currently, the use of incretin-based therapy has been implemented. Incretins are enteroendocrine hormones released after nutrient intake that stimulate glucose-dependent insulin secretion from β cells. To date, two incretins have been identified: glucose-dependent insulinotropic polypeptide (GIP) and GLP-1. In mice, deficiencies in GIP and GLP-1 secretion are associated with decreased insulin response and impaired glucose tolerance. In this context, the overexpression of GIP or GLP-1 improves β cell function and glucose tolerance, and enhances insulin sensitivity. However, GIP also has an obesogenic effect, at least in animal models. Therefore, investigations have focused on GLP-1, specifically on its receptor (GLP-1R). Agonists for GLP-1R activation have recently been used; these include liraglutide, albiglutide, dulaglutide, and semaglutide, and the results have been favorable for the management of DM[92].

On the other hand, the importance of finding new therapies that help improve disease control and the use of nutraceuticals has been increasing in recent years[93]. A positive effect has been reported in compounds such as: melatonin[94], aloe vera extract[95] and hibiscus sabdariffa leaf extract[96]. They have regenerated pancreatic β cells and enhanced insulin secretion in streptozotocin-induced diabetic animal models. In patients with metabolic syndrome, a nutraceutical diet composed of barberine, policosanol, red yeast rice or tocotrienols significantly reduced the Homeostatic Model Assessment for IR index, leading to the conclusion that they have beneficial effects on IR[97,98].

Resveratrol, a polyphenol, found in many types of red fruits, has beneficial effects both *in vivo* and *in vitro*, showing great antioxidant capacity while improving insulin sensitivity[99,100]. Resveratrol is capable of activating the AKT pathway to stimulate insulin action[15]. The activation of SIRT1/AMPK has also been reported[101], which has a positive impact on mitochondrial biogenesis, inhibition of lipogenesis and fatty acid oxidation[102] and improves insulin sensitivity in diabetes[103,104].

Another antioxidant compound that has been less studied than resveratrol but with positive effects in models of obesity[105] and diabetes[106] has been curcumin, a non-flavonoid polyphenol[107]. In diabetic animal models, curcumin improves insulin sensitivity and increases glucose uptake. This mechanism is mediated by the LKB1-AMPK pathway. Adding curcumin induced an increase in fatty acid oxidation, an event that improves insulin sensitivity[108]. At the brain level, curcumin increases glucose metabolism and improves the insulin signaling pathway, improving learning and memory[16] both under non-pathological conditions and in Alzheimer's disease[109]. Currently there are several studies on the use and beneficial effects of a wide variety of nutraceuticals, which are described in Table 1.

CONCLUSION

Insulin is a peptide hormone that plays an important role in various organs: in pancreas it participates in glucose homeostasis; in muscle it promotes glucose metabolism for energy generation and storage; in the vascular system it exerts an anti-atherogenic effect and participates in bone formation; in liver it decreases gluconeogenesis and favors glucose storage through glycogenesis; in adipose tissue it induces lipogenesis; and in brain it activates thermogenesis, regulates appetite, participates in glucose homeostasis and metabolism, reduces long-term potentiation and impairs memory and learning ability. Alterations in secretion or function of insulin considerably alter the cellular events regulated by the activation of its signaling pathway. Obesity and DM are pathologies associated with alterations in the function and secretion of insulin. In these pathologies, oxidative stress plays an important role since the uncontrolled increase in

ROS derived from the increase in glycolysis due to the constant entry of glucose into the cells overcomes the antioxidant defenses. ROS induces alterations in insulin signaling and triggers a cascade of cellular alterations in various organs. Specifically in the hypothalamus, it can be the inducer of hyperphagia, which aggravates the diabetic condition and obesity. The use of antioxidants can be a complementary strategy to conventional treatment of DM.

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