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Insulin Resistance in Brain and Possible Therapeutic Approaches

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Abstract: Although the brain has long been considered an insulin-independent organ, recent research has shown that insulin has significant effects on the brain, where it plays a role in maintaining glucose and energy homeostasis. To avoid peripheral insulin resistance, the brain may act via hypoinsulinemic responses, maintaining glucose metabolism and insulin sensitivity within its own confines; however, brain insulin resistance may develop due to environmental factors. Insulin has two important functions in the brain: controlling food intake and regulating cognitive functions, particularly memory. Notably, defects in insulin signaling in the brain may contribute to neurodegenerative disorders. Insulin resistance may damage the cognitive system and lead to dementia states. Furthermore, inflammatory processes in the hypothalamus, where insulin receptors are expressed at high density, impair local signaling systems and cause glucose and energy metabolism disorders. Excessive caloric intake and high-fat diets initiate insulin and leptin resistance by inducing mitochondrial dysfunction and endoplasmic reticulum stress in the hypothalamus. This may lead to obesity and diabetes mellitus (DM). Exercise can enhance brain and hypothalamic insulin sensitivity, but it is the option least preferred and/or continuously practiced by the general population. Pharmacological treatments that increase brain and hypothalamic insulin sensitivity may provide new insights into the prevention of dementia disorders, obesity, and type 2 DM in the future.

Keywords: Brain, hypothalamus, insulin resistance, leptin.

INTRODUCTION

Insulin resistance is associated with an increase in visceral fat and waist circumference, and leads to central obesity [1]. A number of studies have shown that adipokines, especially tumor necrosis factor (TNF)-α, resistin, interleukin-6, plasminogen activator inhibitor-1, acylation stimulating protein, retinol binding protein 4, secreted from visceral adipose tissue are responsible for insulin resistance [2, 3, 4]. Insulin resistance develops in the liver and muscles. Adipose tissue, liver, and muscles are also the organs studied most in the context of insulin resistance [2]. However, whether these adipokine-mediated pathways are the origin of insulin resistance and whether insulin resistance also develops in the central nervous system (CNS) in addition to the more studied peripheral tissues are the subjects of this review.

It has long been known that the insulin receptor is widely expressed throughout the CNS, particularly high concentrations in the hypothalamus, cerebellum, and cortex [5]. However, the brain has continued to be considered a mostly insulin-independent organ insofar as glucose uptake here is not significantly stimulated by insulin [6]. However, in recent years, evidence has emerged for an important role of insulin in various brain functions. Among other actions, central insulin has been found to be involved in the regulation of body weight and food intake, processing of food-related stimuli, and memory. In this context, studies have shown that the brain is an insulin-sensitive organ whose functions, including energy and glucose homeostasis, neuroendocrine functioning, memory and learning, may be affected by insulin [7]. Central insulin signaling is a major factor controlling peripheral glucose homeostasis. Animal studies suggested the presence of multiple pathways that interconnect the pancreas, liver, and adipose tissue with the CNS [8-11]. In nervous system, insulin and insulin growth factor (IGF) modulate neuronal growth, survival, differentiation, migration, metabolism, gene expression, protein synthesis, synapse formation, and plasticity [12, 13].

Insulin plays a critical role in regulating glucose uptake and utilization by cells as an energy source [14]. Insulin also regulates lipogenesis [15, 16]. Through these functions, which are clearly associated with food and energy metabolism, insulin acts as an anabolic hormone [17]. Besides well-known peripheral effects, the presence of its receptors in CNS of rats were shown decades ago [18] and Woods *et al*. demonstrated that intracerebral infusion of insulin in baboons decreased food intake followed by a decrease in body weight [19].

High amounts of insulin secreted postprandially by the pancreas may function as a satiety signal in the brain, especially in the arcuate nucleus area, inhibiting food intake. Insulin directly suppresses prepro-neuropeptide Y (NPY) mRNA transcription in the arcuate nucleus, leading to a reduction in NPY in the centrally and a decrease in food intake

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[20]. In contrast, partial deregulation of insulin receptor substrate protein (IRS)-2 signaling causes hyperphagia and obesity in animals [21]. Furthermore, genetic variation within the IRS1 locus has been shown to determine the insulin responsiveness of the human brain [22].

INSULIN AND GLUCOSE METABOLISM IN BRAIN

Results of animal and human studies showed that insulin passes from systemic circulation to brain tissue and may have some physiologic roles which are different than peripheral metabolic effects. Insulin in the brain serves two major functions: controlling food intake and regulating cognitive functions, particularly memory; deranged insulin signaling in the brain has also been implicated in neurodegenerative disorders [23].

Data from Animal and *Ex-vivo* Studies

Pardridge *et al*. reported the presence of insulin receptors in endothelial cells constituting the blood-brain barrier (BBB), providing a mechanism for active, receptor-mediated insulin transport into the brain [24]. Although most brain insulin is thought to originate from the systemic circulation, the brain itself also synthesizes insulin in small amounts [25]. Clarke *et al*. reported that insulin is released from cultured rat brain neuronal cells [26]. Autoradiographic examinations in rats have shown that insulin may cross over the BBB and penetrate into the circumventricular organs, including the arcuate and ventromedial hypothalamic nuclei [27]. Following an acute elevation in plasma insulin levels, the concentration of insulin in the cerebrospinal fluid increases, indicating that brain insulin is controlled by circulating insulin [28]. But according to pharmacokinetic studies in mice and murine brain, chronic plasma hyperinsulinemia may not promote parallel increases in central insulin levels [29, 30].

Astrocytes in the brain modulate a variety of neuronal functions and, like neurons, also express insulin receptors; thus, they may also contribute to cerebral insulin actions (Fig. **1**) [31]. Insulin receptors and their mRNA are widely distributed in the rat brain, including in the olfactory bulb, hypothalamus, hippocampus, cerebellum, amygdala, and cerebral cortex [32]. Glucose transporter (GLUT) 4 and GLUT8 have also been detected in brain. Insulin enhances glucose uptake into astrocytes, but not neurons [33].

Insulin-stimulated glycogen formation is important for supplying neurons with energy. Neuronal activity triggers the mobilization of astrocytic glycogen, probably via the release of neurotransmitters [34, 35].

Neuronal insulin receptors are concentrated at synapses and are components of postsynaptic densities [36]. Recently, mice with insulin deficiency were shown to exhibit a defect in the control of neuronal cholesterol biosynthesis; these deficits could be reversed by intracerebroventricular administration of insulin. In addition, insulin may regulate the production of acetylcholine and uptake of norepinephrine in rat locus coeruleus [37-39].

Data from Studies on Humans and Human Tissues

Studies to assess effects of insulin on human brain have technical limitations, such as resolution problems in functional MRI, measurement of metabolic processes in neuronal tissues, or new magnetoencephalography techniques which lack established set of criteria for spike identifications, and insulin effects on brain may be affected by metabolic status of the person, such as differences in lean and obese people [40]. In general, glucose uptake in the human brain is an entirely insulin-independent process [41]. Bingham *et al*. studied the effect of basal insulin on global and regional brain glucose uptake and metabolism in humans using 18-

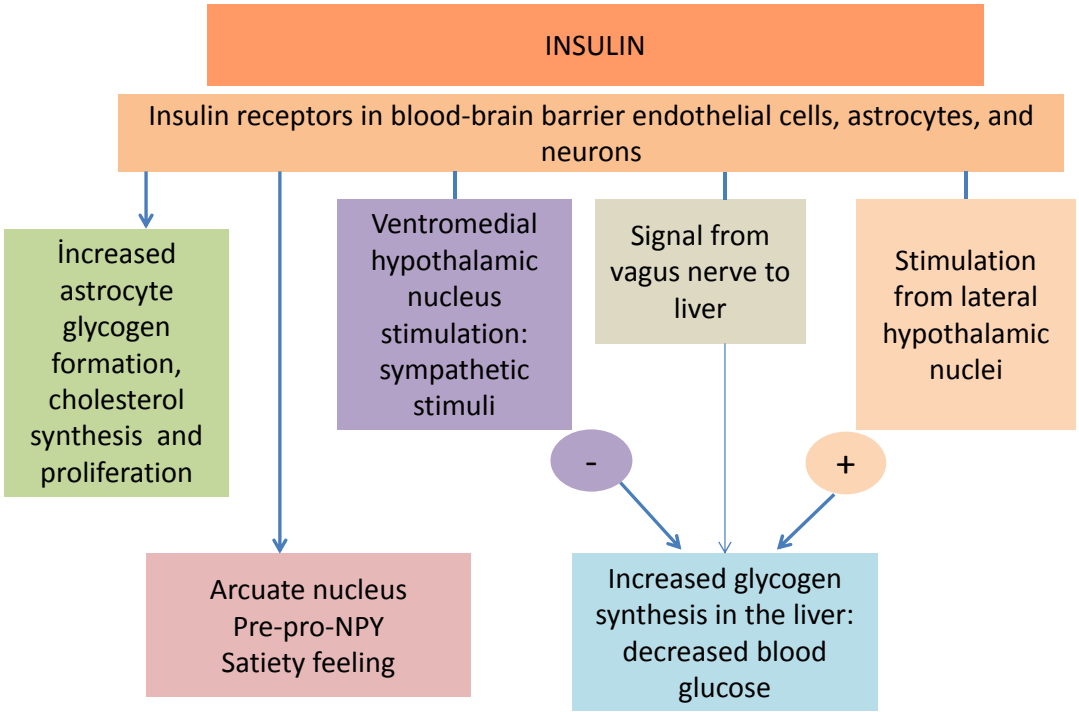

Fig. (1). Insulin actions in brain. NPY: neuropeptide Y.

fluorodeoxyglucose and positron emission tomography. Specifically, they tested the effect of basal insulin replacement during somatostatin infusion, showing that global brain glucose uptake was significantly reduced when circulating insulin levels were reduced below this basal level, indicating that brain glucose uptake was partially insulin sensitive [42]. Consistent with this, brain glucose uptake was increased by 15% following insulin stimulation. In conclusion, these researchers showed that basal insulin has a role in regulating global brain glucose uptake in humans, an effect that was most marked in cortical areas [42].

Heni *et al*. examined the mRNA levels of major insulin signaling molecules in humans, comparing their expression in astrocytes, myotubes, and adipocytes [43]. They found that the levels of insulin receptor transcripts in astrocytes were similar to those in myotubes; approximately two thirds of astrocytic insulin receptors were isoform A and about one third were isoform B. Moreover, expression levels of insulin receptor substrates IRS1 and IRS2 were significantly higher in astrocytes than in myotubes or adipocytes [43]. GLUT1 mRNA was found in significantly greater amounts in astrocytes than in the other analyzed cells, whereas GLUT3 expression was in a similar range in all three cell types. GLUT2 mRNA levels were greater in astrocytes than in adipocytes or myotubes, but were about 70-fold less than those found in HepG2 cells. GLUT4 was barely detectable in astrocytes and myotubes compared with adipocytes. At the protein level, insulin receptor β-subunit, IRS-1, Akt (protein kinase B), and glycogen synthase kinase (GSK) 3β were detected in astrocytes [43].

Heni *et al*. also found that phosphorylation of L-α-phosphatidylinositol (PI) to PI-39-phosphate was increased, indicating increased PI-3 kinase activity following insulin stimulation [43]. This effect of insulin was dose-dependent and could be detected at concentrations as low as 1 nM. Consistent with this, Akt phosphorylation on serine 473 was increased by insulin stimulation, trending higher beginning at a concentration of 1 nM and increasing significantly at an insulin concentration of 50 nM; PI-3 kinase activity was also significantly higher at this latter concentration. Incorporation of labeled glucose into glycogen was also significantly increased following insulin stimulation. Under basal conditions, the PI-3 kinase inhibitor LY294002 significantly decreased glucose incorporation into glycogen (~30%), whereas insulin-stimulated glycogen synthesis was abolished by the addition of LY294002. Thus, basal glycogen synthesis as well as insulin-stimulated glycogen synthesis is PI-3 kinase-dependent [43]. Collectively, these results demonstrate that the insulin signaling cascade is functionally active in human primary astrocytes. In addition to stimulating glycogen formation, insulin also induced cell proliferation, but did not affect glucose uptake or lactate secretion [43].

THE BRAIN AND CENTRAL CONTROL OF PANCREATIC ISLET CELL FUNCTIONS

Corticotropin-releasing factor (CRF) is a major neuroregulatory factor in stress responses. Its receptors, CRFR-1 and CRFR-2, mediate CRF actions centrally and peripherally [44]. Although CRFR-2 is the predominant peripheral form [44], animal studies have shown that CRFR-1 is present in pancreatic β-cells and potentiates glucose-stimulated insulin secretion, an action similar to that of incretins [44]. In anterior pituitary corticotropes, CRF activation of CRFR-1 results in the secretion of glucocorticoids that antagonize the actions of insulin. Thus, the effects of CRF on CRFR-1 in the pituitary may oppose the actions of CRF in the pancreas Acute stress and glucocorticoids inhibit islet cell CRFR-1 and incretin-receptor expression, decreasing insulin output and increasing plasma glucose levels [44-46]. It has been shown that chronic glucocorticoid increase, as in obesity or Cushing's disease, probably suppresses this system [46]. Synergistic stimulation of CRFR-1 and CRFR-2 also decreases β-cell apoptosis [43]. Therefore, β-cell functions may be controlled centrally as well as by hormonal control of β-cell secretory function and survival via CRFR signaling.

INSULIN SIGNALING BETWEEN THE BRAIN AND THE LIVER

Insulin controls nutrient and metabolic homeostasis via effects on the liver, muscle, and adipose tissue. After a meal, insulin acts on the liver to inhibit net hepatic glucose output. Ramnanan *et al*. demonstrated that arterial infusion of insulin into the brains of dogs reduced net hepatic glucose output without altering endogenous glucose production [11]. Many studies in rodents support a role for insulin action in the brain as a regulator of peripheral glucose homeostasis (Fig. **2**) [47]. The central effects of insulin were thought to signal the liver via the vagus nerve. Moreover, administration of insulin receptor antisense or antagonists via the third cerebral ventricle blunts the inhibitory effect of insulin on hepatic glucose production [9]. Stimulation of lateral hypothalamic nuclei increases parasympathetic nervous system activation and has been shown to decrease blood glucose levels by increasing glycogen synthesis in the liver [9, 13, 48]. In contrast, stimulation of ventromedial hypothalamic nuclei results in activation of the sympathetic nervous system and a rise in blood glucose level that is mediated by hepatic glycogenolysis. These studies support the idea that the brain plays a key role in hepatic glucose modulation [48].

Obesity, type 2 diabetes mellitus (T2DM), and non-alcoholic steatohepatitis (NASH) can be complicated by cognitive impairment and neurodegeneration. Experimentally, high fat diet (HFD)-induced obesity with T2DM causes mild neurodegeneration in association with brain insulin resistance in C57Bl/6 mice [49]. Progressive brain insulin resistance and pancreatic insulin deficiency are observed in Alzheimer's disease (AD), and at the same time, T2DM is a significant risk factor for developing AD [50-54].

Ceramides are considered to have an impact on this interaction among T2DM, NASH and AD, for which a common pathogenesis is suspected [55]. Ceramides comprise a family of lipids generated from fatty acids and sphingosine that are widely distributed in cell membranes. In addition to their structural functions, ceramides have key roles in intracellular signaling, and serve to regulate growth, proliferation, cell migration, adhesion, growth arrest, differentiation, senescence, and apoptosis [55]. Ceramides are generated either by *de novo* biosynthesis or sphingolipid degradation. For *de novo* synthesis, ceramides are produced from sphingosine or sphinganine plus fatty acyl-CoA through the actions of ce-

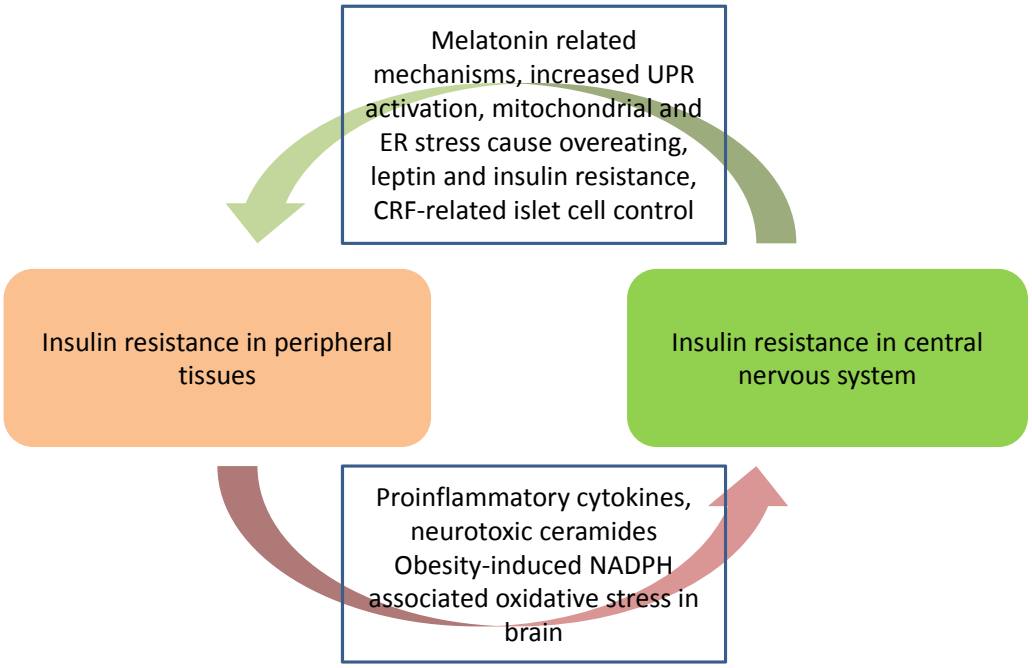

Fig. (2). Interrelationship between peripheral and central insulin resistance. CRF: corticotropin-releasing factor, ER: endoplasmic reticulum; NADPH: reduced nicotinamide adenine dinucleotide phosphate; UPR: unfolded protein response.

ramide syntheses. Activation of serine palmitoyl transferase or dihydroceramide synthase increases ceramide production in the endoplasmic reticulum (ER) via dihydroceramide desaturase. Glycosphingolipids are composed of ceramide plus an oligosaccharide, and are generated enzymatically in the Golgi. UDP glucoceramide glycosyltransferase mediates the first step in transferring glucose from UDP-glucose to ceramide. Ceramides also can be generated by hydrolysis of sphingomyelin by sphingomyelinase or degradation of complex sphingolipids and glycosphingolipids localized in late endosomes and lysosomes. Potential roles for ceramides in diabetes, obesity, inflammation, NASH, and neurodegeneration have already been suggested [55]. Notably, proinflammatory cytokines, such as TNF-α, can induce ceramide synthesis in animal studies, and TNF-α levels are increased in both T2DM and NASH [55, 56].

Lascelles *et al.* investigated the potential role of ceramides as mediators of neurodegeneration in the HFD obesity/T2DM model [57]. They pair-fed C57BL/6 mice with a HFD or control diet for 4–20 weeks and examined proceramide gene expression in liver and brain and neurodegeneration in the temporal lobe. HFD feeding gradually increased body weight, but after 16 weeks, liver weight surged due to lipid (triglyceride) accumulation and brain weight declined. HFD feeding increased ceramide synthase, serine palmitoyl transferase, and sphingomyelinase expression in the liver, but not in the brain. In HFD-fed mice, temporal lobe levels of ubiquitin and 4-hydroxynonenal increased, and tau, β-actin, and choline acetyltransferase levels decreased in association with the development of NASH. Therefore, if ceramides have a role in mediating brain insulin resistance, neurodegeneration, and cognitive impairment in obesity, T2DM and NASH, the source is not likely the CNS, and instead is probably the liver and possibly adipocytes. In obesity, T2DM and/or NASH, neurodegeneration with brain insulin resistance may be mediated by excess hepatic production of neurotoxic ceramides, which readily cross the BBB [57].

Also studies in humans have demonstrated a role for the hormone melatonin in brain–liver interactions. Melatonin secreted into the bloodstream and cerebrospinal fluid by the pineal gland in the brain helps the body adjust to day–night differences [58]. Melatonin is formed by transformation of the essential amino acid tryptophan via the intermediate serotonin. Melatonin function is under the control of the suprachiasmatic nucleus of the hypothalamus, which enables circadian secretion of melatonin from the pineal gland [58]. Melatonin is secreted during the night phase, increasing as the night progresses and decreasing in daylight [58]. This temporal pattern of melatonin release, in turn, impacts the circadian rhythm, facilitating sleep at night and waking in the morning [59, 60]. In insomnia, melatonin secretion is impaired [58]. Those who have regular sleep patterns are less likely to have impaired glucose tolerance and diabetes [60]. Recent study of Byberg *et al.* showed that an additional hour of sleep is reported to cause a 0.3% decrease in glycated hemoglobin A1c [61]. The operation of 10% of our genes relies on the light–dark cycle the diurnal rhythm [58]. The synthesis of proteins encoded by these genes shows some variability throughout the day. The autonomous nervous system, operating based on the diurnal rhythm, determines how the body will function based on the light–dark information obtained from the retina. Metabolic regulation depends on a system that originates in the retina and ends at the pineal gland, which secretes melatonin. This system, known as the photoneuroendocrine system, autonomously prepares the human body for day and night. Plasma melatonin concentrations decline with age. With dementia, melatonin levels may decline to an even greater extent than occurs in normal aging. However, melatonin does not appear to have a beneficial

effect on cognition in patients with dementia [62]. The effect of melatonin on non-cognitive symptoms, such as sleep and agitation, is unclear.

Increased glycemia and reduced melatonin levels have been recently shown to coexist in diabetic patients at the end of the night period [62]. In parallel, pinealectomy is known to cause glucose intolerance with increased basal glycemia exclusively at the end of the night. Nogueira *et al.* demonstrated that pinealectomized rats show nighttime hepatic insulin resistance characterized by reduced insulin-stimulated Akt1 phosphorylation and increased phosphoenolpyruvate carboxykinase expression [62]. The nighttime hepatic insulin resistance and increased gluconeogenesis in these rats was associated with activation of the nocturnal unfolded protein response (UPR). UPR activation has been reported to generate insulin resistance in skeletal muscle and adipose tissue [62].

Brain and Hypothalamic Insulin Resistance

Neuronal-specific insulin receptor-knockout (NIRKO) mice generated using nestin Cre-mediated ablation show normal brain development and neuronal survival [63]. However, these mice exhibit hyperphagia, mild insulin resistance, and enhanced sensitivity to diet-induced obesity [63]. The counter-regulatory response to hypoglycemia is impaired in the NIRKO model, manifesting as a reduced sympathoadrenal response [63]. More recently, NIRKO mice were reported to have a deficit in IGF-1-induced hyperthermia, a response mediated by the preoptic area of the hypothalamus that activates brown adipose tissue [64]. Insulin infusion into the mediobasal hypothalamus was shown to suppress lipolysis while increasing white adipose tissue lipogenic protein expression in normal rats, but not in NIRKO mice [65].

Brain oxidative stress could play an important role in the pathogenesis of metabolic diseases, given that the brain, and particularly the hypothalamus, is the central regulator of whole-body energy and metabolic homeostasis. Dietary obesity was found to induce NADPH oxidase-associated oxidative stress in rat brains [66]. Mitochondrial dysfunction in hypothalamic proopiomelanocortin (POMC) neurons causes impaired central glucose sensing, and brain mitochondrial dysfunction induced by genetic deletion of peroxisome proliferator-activated receptor (PPAR) coactivator 1α disrupts central regulation of energy homeostasis. Unresolved ER stress can induce apoptosis, which forms the pathogenic basis for neurodegeneration, diabetic islet cell death, atherosclerosis, myocardial infarction, and stroke. Recent studies have causally linked brain ER stress to the development of metabolic syndrome and related disorders, such as overeating, obesity, leptin resistance, insulin resistance, β-cell dysfunction and hypertension, under conditions of overnutrition [66]. Prolonged oxidative stress and ER stress can cause intracellular accumulation of dysfunctional mitochondria, and ER and cytosolic proteins, leading to increased autophagy stress and autophagic defects. Autophagy is an evolutionarily conserved lysosomal degradation pathway that plays an essential role in maintaining cellular homeostasis and promoting cell survival, growth, and differentiation under adverse conditions. To maintain a healthy and functional intracellular environment, cells must eliminate defective proteins. Environmental stresses, such as nutrient deprivation or hypoxia, induce autophagy, which serves to break down macromolecules into reusable amino acids and fatty acids and enhance survival [67, 68]. Autophagy defects in the CNS have been implicated in a number of neurodegenerative diseases, including AD, Parkinson's disease, Huntington's disease, and transmissible spongiform encephalopathies [69].

It has been reported that feeding a 20-week HFD increases reactive oxygen species (ROS) and prostaglandin E2 production along with up-regulation of nuclear factor-kappaB (NF-κB) signaling in the rat cerebral cortex [70]. De Souza *et al.* demonstrated that the immune-related, proinflammatory cytokines IL-1β, TNFα, and IL-6 represent the largest class of genes with altered hypothalamic expression after 16 weeks of HFD [71]. Underlying these responses in rodent models of diet-induced obesity (DIO) are activation of both JNK (C-Jun N-terminal kinase) and the IKKβ (inhibitor of κB kinase-β)/NF-κB pathway as well as induction of ER stress over a time frame that parallels the onset of reduced hypothalamic leptin sensitivity. The association of DIO with both higher serum levels of insulin and leptin and increased activation of inflammatory signaling pathways raises the possibility that these two alterations are causally linked. However, animals lacking leptin signaling are obese and hyperphagic and manifest an even greater degree of inflammatory changes in the periphery and hypothalamus than do DIO animals [72].

The saturated fatty acid palmitate induces NF-κB signaling through a Toll-like receptor 4 (TLR4)-dependent mechanism when added to neuronal cell culture or infused into the mice brain, and induces leptin and insulin resistance [73, 74].

HFD feeding increases expression of SOCS3 (suppressor of cytokine signaling 3) specifically within the arcuate nucleus of the hypothalamus, coincident with the onset of leptin resistance selectively in this brain area [75]. Hypothalamic inflammation is linked to leptin and insulin resistance via up-regulation of SOCS3, which inhibits insulin and leptin signaling both by binding directly to their respective receptors and by targeting IRS proteins for proteasomal degradation [76].

Protein tyrosine phosphatase (PTP)-1B is a signal termination molecule that inhibits both leptin and insulin signaling. Feeding a HFD increases PTP1B expression in the hypothalamus [77, 78]. Pan-neuronal and POMC neuron-specific PTP1B-knockout mice are resistant to DIO owing to enhanced hypothalamic leptin and insulin sensitivity [79, 80].

Overnutrition constitutes an environmental stimulus that can activate TLR pathways and thereby induce the development of metabolic syndrome-related disorders, such as obesity, insulin resistance, T2DM and atherosclerotic cardiovascular diseases, in rodents [66, 81]. TLRs are an important class of membrane-bound pattern-recognition receptors involved in classical innate immune defense. Upon binding non-self molecules such as pathogens, TLRs promote the synthesis and secretion of immune-response molecules. Most hypothalamic cell types, including neurons and glia, express TLRs [82]. A central lipid excess, produced by direct intrabrain lipid administration or HFD feeding, activates hypotha-

lamic TLR4s and downstream inflammatory signals in animal models [83].

Based on these animal data, we can say that overnutrition, in the form of high circulating levels of glucose, free fatty acids and/or amino acids, is the predominant pathogenic inducer of central metabolic inflammation. Excessive nutrients transported into cells can impose severe stresses on cellular metabolism and affect organelles. Depending on diet composition, cytokines are expressed in the hypothalamus, contributing to the activation of intracellular inflammatory signal transduction. These phenomena, as well as activation of hypothalamic IKKβ/NF-κβ signaling through elevated ER stress in the hypothalamus are associated with central insulin and leptin resistance, hyperphagia, and body-weight gain.

THE ROLE OF BRAIN INSULIN RESISTANCE IN COGNITIVE FUNCTION AND AD

Recent studies on mouse brain have demonstrated that the degenerative plaque formation observed in AD is associated with insulin resistance [84]. The plaques formed in the brain due to AD contain β-amyloid (Aβ), which is an abnormally structured, neurotoxic protein. High insulin resistance triggers the formation of Aβ. Besides animal studies, *ex vivo* studies on human brain tissue also showed that glucose metabolism is initially impaired in areas where brain tissue damage is observed due to AD [85]. In addition to AD, cognitive dysfunctions and dementia have also been definitively linked with hyperinsulinemia in Rotterdam Study group [86].

AD is the most prevalent form of dementia. It is characterized by cognitive insufficiencies and behavioral changes that impact memory and learning abilities, daily functioning and quality of life, even in its early stages [87]. AD is a progressive and irreversible neurodegenerative disease that lacks a well-known cause or pathogenesis. Approximately 6-8% of individuals over 65 years of age have AD, and this risk increases with age; and it is more prevalent among women (~1.5-fold) than men [88]. The brains of AD patients exhibit characteristic histopathological, molecular, and biochemical abnormalities, including cell loss, abundant neurofibrillary tangles, dystrophic neurites, amyloid precursor protein amyloid-β (APP-Aβ) deposits, increased activation of prodeath genes and signaling pathways, impaired energy metabolism, mitochondrial dysfunction, chronic oxidative stress, and DNA damage [88]. Several hypotheses have been proposed to explain the pathogenesis of AD, including senile plaque and neurofibrillary tangle formation, increased oxidative stress, and cell-cycle abnormalities [88]. Emerging evidence points to an association of insulin resistance with increased oxidative stress. Hyperinsulinemia, hyperglycemia, and hyperleptinemia are considered important components of insulin resistance. Thus, it is likely that pathological changes in insulin signaling pathways play very important roles in this process. Peripheral insulin resistance is associated with hyperinsulinemia, which may be associated with the brain insulin deficiency characteristic of sporadic AD [89]. Oxidative insult, which is the result of an insulin-associated disordering of brain energy metabolism, is a significant early event in the pathological cascade of sporadic AD. Aggregation of disease-specific proteins, such as Aβ and tau, may act as a compensatory response to the oxidative insult at the early periods. In later stages, oxidative stress stimulates JNK activation. The deficiency in insulin signaling is ultimately linked to the Akt pathway and subsequently to GSK3 and forkhead transcription factors (FOXO) [89]. It has been postulated that peripheral insulin resistance-related, intense interactions among JNK, GSK3, FOXO factors, and p53 may lead to apoptotic neuronal death [89].

There has been a rapid proliferation of literature reports implicating insulin deficiency and insulin resistance as mediators of AD-type neurodegeneration [90, 91]. But this surge of new information is riddled with conflicting and unresolved concepts regarding the potential contributions of T2DM, metabolic syndrome, and obesity to AD pathogenesis [90]. T2DM causes brain insulin resistance, oxidative stress and cognitive impairment, but its aggregate effects fall far short of mimicking AD [90, 91]. Extensive disturbances in brain insulin and IGF signaling mechanisms represent early and progressive abnormalities, and thus could potentially account for the majority of molecular, biochemical, and histopathological lesions in AD [92]. Experimental brain diabetes produced by intracerebral administration of streptozotocin shares many features with AD, including cognitive impairment and disturbances in acetylcholine homeostasis [92]. Experimental brain diabetes is treatable with insulin-sensitizing agents-drugs currently used to treat T2DM. The term "type 3 diabetes" has been proposed as an appropriate descriptor of AD that selectively involves the brain and has molecular and biochemical features that overlap with both T1DM and T2DM [90, 93].

Emerging evidence demonstrates pivotal roles for brain insulin resistance and insulin deficiency as mediators of cognitive impairment and neurodegeneration, particularly in AD. Insulin and IGFs regulate neuronal survival, energy metabolism and plasticity, which are required for learning and memory. Hence, endogenous brain-specific impairments in insulin and IGF signaling account for the majority of AD-associated abnormalities. However, a second major mechanism of cognitive impairment has been linked to obesity and T2DM. Human and experimental animal studies have revealed that neurodegeneration associated with peripheral insulin resistance is likely effectuated by a liver–brain axis whereby toxic lipids, including ceramides, cross the BBB and cause brain insulin resistance, oxidative stress, neuroinflammation, and cell death [57, 94]. In essence, dual mechanisms of brain insulin resistance lead to AD-type neurodegeneration: one is centrally mediated by endogenous CNS factors, and the other is a peripheral mechanism associated with excess cytotoxic ceramide production [94].

Diet plays a critical role in the prevention of AD, which still lacks an effective treatment. Studies have shown that AD patients are deficient in a series of macro- and micronutrients, and the lack of these nutrients is associated with a higher risk of AD [95]. If these nutrients are not provided in the diet, the disease may progress more rapidly. Signal transmission among neurons makes the brain one of the highest energy-requiring organs. Glucose provides a majority of the brain's energy needs. Research shows that individuals with a diet high in cholesterol, saturated fat and energy, and low in fruits and vegetables are at higher risk of developing

AD. Such diets increase the accumulation in the brain of Aβ proteins [94, 95], which damage neurons and have been implicated in the pathogenesis of AD. Oxidative stress and the accumulation of released radicals are other factors of the disease pathophysiology. Large amounts of free radicals cause lipid peroxidation, which accelerates neuronal degeneration. In addition to certain nutrient deficiencies, excessive energy can also increase the risk of AD. Research has shown that obesity, and high calorie and saturated fat intake increase AD risk; in individuals with a body mass index greater than 30, this increase is approximately 35% [95]. Evidence from these studies suggests that reducing energy intake can delay degenerative pathologies related to age, especially degenerative brain diseases, by reducing oxidative stress and free radical production. Increased levels of insulin in the blood due to insulin resistance increase inflammation and oxidative stress, both of which are responsible for mediating the damage caused by AD [95].

Sixty percent of the dry weight of the brain is fat, and dietary fats have a direct impact on the brain structure and cell membrane composition. About 20% of the fats in the brain are the essential omega-3 and omega-6 fatty acids. Polyunsaturated fatty acids are structural components of the phospholipids that form the basic structure of neuronal membranes. These fatty acids are essential in that they cannot be produced in the body and must be provided in the diet. Changes in cell membrane composition that were produced by incorporation of polyunsaturated fatty acids increase membrane fluidity. In contrast, the accumulation of saturated fatty acids in neuron membranes can have a negative impact by increasing membrane stiffness and hardness [95, 96]. Plasma omega-3 fatty acid is known to be associated with cognitive functions and its levels are low in AD; it has also been shown that AD risk is lower among individuals who consume high levels of omega-3 [97, 98]. Omega-3 has been shown to increase the utilization of nutrients necessary for the brain by improving blood flow to the brain, carrying capacity, and BBB integrity [96].

The brain is the organ with the highest concentration of cholesterol, containing 25% of the cholesterol in the entire body. Cholesterol is found in neuronal membranes and plays a role in the formation and continuity of synaptic connections. However, a diet high in both cholesterol and fat negatively affects the structure and fluidity of the neuronal membrane. Age-related increases in the amount of cholesterol in the membrane could lead to a loss of neuronal function. Genetic polymorphism in apolipoprotein E that decreases cholesterol transport across the BBB is strongly associated with AD [97].

The Mediterranean diet, which prioritizes polyunsaturated fatty acids and unrefined carbohydrates (whole-grain) with low amounts of animal protein and saturated fats, has been shown to be effective in preventing and treating AD [98, 99]. Individuals with AD on such diets have a lower mortality rate [98]. Wine with such diets, in moderate amounts, has also proved beneficial [98, 99].

A physically active lifestyle has benefits that extend beyond cardiac health [100]. Exercise increases blood flow to the brain and activates certain cellular activities that can enhance brain functions. Both animal and human studies have demonstrated that physical and mental functions can be developed with aerobic fitness. Among individuals aged 58-78 enrolled in a 6-month brisk walking program and sedentary controls, those who walked were better able to concentrate and pay attention, determined by measuring pre- and post-program brain activities, than those who were physically inactive. Multimodal exercise can also enhance cognitive functions in AD [101].

Metformin, an indispensable T2DM drug that has shown the greatest impact on insulin resistance [102], has not been studied with respect to its effects on brain insulin resistance. However, recent work has suggested that it can cross the BBB and regulate tau phosphorylation in a mouse model of AD [103]. Conversely, metformin has been linked to enhanced amyloid production in cells [104]. However, its insulin-sensitizing properties make it an ideal tool for establishing a potential mechanistic link between insulin resistance and dementia, including AD. A retrospective Taiwanese study provided the first epidemiological evidence that intervention with metformin could reduce the incidence of dementia in people with diabetes [105]. At the time of this writing, several such several clinical trials are underway.

Insulin plays a critical role in the proper functioning of the CNS. The importance of insulin for healthy brain functioning was reinforced by the discovery that impaired insulin signal transmission contributes to the pathophysiology of AD. In this context, CNS insulin levels and activity were found to be low in AD. Craft *et al*. evaluated the impact of insulin treatment on cognitive and functional parameters, and cerebral and cerebrospinal fluid biomarkers of glucose metabolism among adults with mild cognitive impairment or AD [106]. Patients were divided into three groups and given 20 IU of insulin (n=36), 40 IU insulin (n=38), or placebo (n=30) intranasally once daily for 4 months. Patients were scored using the Dementia Severity Rating Scale (DSRS), and the impact of the treatment on delayed recall (ability to recite a story shortly after being told) was evaluated. Compared to the placebo group, the group receiving 20 IU of insulin showed improvement, although the group receiving the higher dose (40 IU) did not. Neither of the insulin groups showed a change in DSRS scores compared with the placebo group. However, both insulin doses protected general cognitive functions in young patients, as assessed by the Alzheimer's Disease Assessment Scale–Cognitive subscale (ADAS-Cog), and in adults, as measured according to the Alzheimer's Disease Cooperative Study–Activities of Daily Living (ADCS-ADL) scale. In contrast, neither of the treatments affected outcome in amnesic patients with mild cognitive impairment, and the cognitive functions of patients in the placebo group actually showed a small decrease. Collectively, the results of this pilot study demonstrate that intranasal insulin stabilizes or heals cognitive functioning and cerebral glucose metabolism among amnesic patients with mild cognitive impairment or patients with AD.

It has been demonstrated that a HFD can cause not only peripheral insulin resistance, but also neuronal insulin resistance; it has also been shown to cause mitochondrial dysfunction in both skeletal muscle and liver. Rosiglitazone, a PPAR-γ ligand, is used to treat T2DM. Studies have reported that rosiglitazone improves learning and memory in both

human and animal models [107, 108]. In one such study, rats fed either a HFD or normal diet for 12 weeks, were given rosiglitazone (5 mg/kg/d) for 14 days. At the end of this treatment, all animals were euthanized, and their brains were removed and examined for insulin-induced long-term depression, neuronal insulin signaling, and brain mitochondrial function. It was found that rosiglitazone significantly improved peripheral insulin resistance and insulin-induced long-term depression, and increased serine phosphorylation of neuronal Akt in response to insulin. Furthermore, rosiglitazone prevented brain mitochondrial conformational changes and attenuated brain mitochondrial swelling, brain mitochondrial membrane potential changes, and brain mitochondrial ROS production. These data suggest that neuronal insulin resistance and the impairment of brain mitochondria caused by 12-week HFD consumption can be reversed by rosiglitazone [109]. Induction of PPAR-γ activity may also reduce both Aβ accumulation and neuroinflammation [106, 110]. Therefore, PPAR-γ agonists have the capacity to improve several molecular pathologies associated with both T2DM and AD, making them potential therapeutics for the treatment of mild cognitive impairment associated with insulin resistance and neuroinflammation. A 6-months treatment with rosiglitazone improved attention and preserved memory in patients with amnesic, mild cognitive impairment and early AD patients [108].

Glucagon-like peptide-1 and gastric inhibitory peptide are peptides made in the gut that induce insulin secretion from pancreatic β-cells in a glucose-dependent manner. Therefore, they are technically insulin secretagogues rather than insulin sensitizers. Drugs that prevent degradation of these peptides (gliptins) and more stable forms of these peptides (exenatide and liraglutide) are now in clinical use as adjunct therapies in diabetes. Receptors for both peptides have been found in other areas of the body, including the brain, and additional biological actions are being discovered. Recently, liraglutide and exenatide were found to antagonize processes linked to neurodegeneration and AD progression in mouse models, even in the absence of diabetes [111, 112]. These incretins prevented the damaging effect of Aβ oligomers on CNS insulin signaling. This raises the exciting possibility that these peptides could be a novel treatment for dementia, irrespective of the presence of diabetes.

POSSIBLE THERAPEUTIC APPROACHES IN BRAIN INSULIN RESISTANCE

Energy intake in excess of energy requirements leads to a state of chronic nutrient excess that causes cellular inflammation in both peripheral tissues and the hypothalamus. The resulting activation of inflammatory pathways generates insulin and leptin resistance, ultimately promoting obesity and diabetes. Therapies that prevent hypothalamic inflammation may disrupt these interlinked vicious cycles with consequent improvements in energy and glucose homeostasis [76].

High dietary fructose consumption leads to an increase in the insulin resistance index and insulin and triglyceride levels-characteristic of the metabolic syndrome. Rats fed an omega-3-deficient diet exhibited memory deficits in the Barnes maze that were further exacerbated by fructose intake. In this study by Agrawal and Gomez-Pinilla [113], an omega-3–deficient diet with high fructose was shown to disrupt insulin receptor signaling in the hippocampus, as evidenced by a decrease in phosphorylation of the insulin receptor and its downstream effector Akt. These authors further showed that high fructose consumption with an omega-3–deficient diet increased the omega-6/omega-3 fatty acid ratio and levels of 4-hydroxynonenal (4-HNE), a marker of lipid peroxidation, indicating that this diet disrupts membrane homeostasis. Disturbances in brain energy metabolism due to omega-3 deficiency and a high-fructose diet were revealed by a significant decrease in the phosphorylation of AMPK (AMP-activated protein kinase) and its upstream modulator LKB1 (liver kinase B1), as well as a decrease in Sir2 (sirtuin 2) levels. Levels of phosphorylated CREB (cAMP responsive element binding protein), synapsin I, and synaptophysin were also decreased by omega-3 deficiency and fructose, demonstrating the impact of metabolic dysfunction on synaptic plasticity. Thus, dietary omega-3 fatty acid deficiency and fructose increased the vulnerability to metabolic dysfunction and impaired cognitive function by modulating insulin receptor signaling and synaptic plasticity [113].

Besides probable importance of nutritional life-style changes, augmented hypothalamic inflammation has role in predisposition to HFD-induced obesity, as discussed previously within this text [76]. Neuronal expression of a constitutively active IKKβ isoform increases food intake [77]. Infusion of the Th2 cytokine IL-4 directly into the brain of rats fed a HFD exerts a paradoxically proinflammatory effect on the hypothalamus that exacerbates weight gain in an IKKβ-dependent manner [114]. Intracerebroventricular administration of an IKKβ inhibitor reverses HFD-induced hypothalamic insulin resistance [115]. These animal data collectively suggest that hypothalamic inflammation is both necessary and sufficient for initial and sustained weight gain during HFD feeding and thus represents an important new target for obesity therapeutics. Interestingly, a recent retrospective case-control study showed that the use of anti-inflammatory therapy (statin or aspirin) was associated with a 2-fold increase in the likelihood of weight loss in patients with T2DM at 1-year follow-up [116]. The UPR, an important inflammation-associated mechanism induced by ER stress, may also contribute to HFD-induced hypothalamic inflammation and associated leptin and insulin resistance [72]. Inhibitors of ER stress restore leptin sensitivity to HFD-fed mice and reduce food intake and body weight in obese animals [117].

The adipose tissue-derived hormone, leptin, is involved in glucose homeostasis. A leptin deficiency results in the development of severe T2DM in both humans and experimental animals [118]. In addition, leptin resistance contributes to insulin resistance and impaired glucose tolerance. Leptin is more potent at regulating blood glucose levels than it is at suppressing appetite. Leptin effects on glucose homeostasis are likely mediated centrally via activation of a specific neuronal subpopulation in the hypothalamus [119]. Koch *et al.* showed that leptin administration rapidly improves glucose tolerance in food-restricted $Lep^{ob/ob}$ mice by increasing hypothalamic insulin sensitivity, changes that occur well before (within 15 minutes) any change in body fat mass [120]. Their data suggest that the anti-diabetic effect of leptin occurs independently of phosphorylated Akt, a major downstream target of PI3K (phosphoinositide 3-kinase), and

Table 1. Possible therapeutic targets and treatment approaches for insulin resistance in brain

-	**Nutritional and life-style changes**
	Less fructose, more omega-3 consumption [113], weight loss, exercise and prevention of metabolic syndrome and peripheral hyperinsulinism
-	**Anti-inflammatory treatments**
	Aspirin+statin [116], exercise [122]
-	**Leptin**
	Leptin administration [120], brain-specific inhibition of TLR4 signaling [121]
-	**Liraglutide and exenatide in Alzheimer's disease [111, 112]**
-	**Endoplasmic reticulum oxidative stress inhibitors [117]**

References are given within brackets.

instead involves enhanced sensitivity of the hypothalamus to insulin action upstream of PI3K through modulation of IRS1 phosphorylation.

Overnutrition-induced metabolic derangements, such as central leptin resistance, systemic insulin resistance and weight gain, can be prevented in mice by brain-specific inhibition of TLR4 signaling. Kleinridders *et al.* have characterized mice deficient for the TLR adaptor molecule MyD88 in the CNS [121]. Compared to control mice, these MyD88(DeltaCNS) mice were protected from HFD-induced weight gain, development of HFD-induced leptin resistance, and induction of leptin resistance by acute central application of palmitate. Moreover, CNS-restricted MyD88 deletion protected against HFD- and palmitate-induced impairment of peripheral glucose metabolism. Thus, these authors established neuronal MyD88-dependent signaling as a key regulator of diet-induced leptin and insulin resistance *in vivo*.

Overnutrition caused by overeating is associated with insulin and leptin resistance through IKKβ activation and ER stress in the hypothalamus [72]. Ropelle *et al*. showed that physical exercise suppresses hyperphagia and associated hypothalamic IKKβ/NF-κB activation by a mechanism dependent upon the proinflammatory cytokine IL-6 [122]. The disruption of hypothalamic-specific IL-6 action blocked the beneficial effects of exercise on the re-balance of food intake and insulin and leptin resistance. This molecular mechanism, mediated by physical activity, involves the anti-inflammatory protein IL-10, a core inhibitor of IKKβ/NF-κB signaling and ER stress. IL-10 expression is required in conjunction with exercise and recombinant IL-6 treatment to suppress hyperphagia-related obesity. Moreover, exercise failed to reverse the pharmacological activation of IKKβ and ER stress in C3H/HeJ mice deficient in hypothalamic IL-6 and IL-10 signaling. Hence, inflammatory signaling in the hypothalamus links beneficial physiological effects of exercise to the central action of insulin and leptin.

Recent data on insulin resistance in brain is mostly based on laboratory or animal data while human studies are gradually increasing in number. Therefore, absolute physiology related with insulin action in human brain, its pathophysiological roles in human diseases and possible therapeutic targets is still open to debate (Table **1**).

CONCLUSION

Insulin resistance in the brain is a new topic of interest that has become the recent focus of considerable research. Central insulin resistance clearly contributes to peripheral insulin resistance. However, it is not clear which precedes the other. Peripheral insulin resistance stems mainly from visceral adipose tissue in the liver. On the other hand, the origin of brain insulin resistance, and the potential solution to this problem, lies in the hypothalamus. Further research in this field will provide insight into the pathogenesis and treatment of obesity, diabetes, and even dementia-related diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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