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Insulin in the Brain: Recent Advances on its Source and Target - A Review

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ABSTRACT
Insulin is not just a peripheral hormone but it's present in the brain and has its receptors distributed unevenly throughout the brain. It crosses the Blood Brain Barrier-BBB through a saturable transport mechanism. It has independent functions in the periphery and brain & leads parallel lives. It's basically a metabolic regulatory hormone in the periphery but affects feeding, cognition amongst others in the brain. Host of its cerebral functions in the brain is mediated through the Phosphoinositide-3-kinase P1(3)K and Ras/Mitogen activated protein kinase (MAPK) cascades. An impairment in these signal transductions is mostly evident as Alzheimer's disease and obesity. A neuron-based insulin therapy is being proposed as a replacement to insulin beta cells in the pancreas.

INTRODUCTION
Insulin is a peptide hormone belonging to the super family of structurally related protein which includes the insulin–like Growth factors 1&2 (1GF-1&2) [1]
Insulin was discovered almost a century ago[2] . Its secreted by the pancreatic beta cells, generally known for its role in reduction of high blood glucose levels[3]. It does this through stimulation of glucose uptake by liver, adipose tissue and muscle, hence, insufficient insulin action in insulin sensitive tissues leads to increased blood glucose levels. The increased blood glucose leads to more insulin release by beta cells [4], thus initially restoring normal blood concentration(hyper-insulinaemic euglycaemia) but no longer does this when the pancreatic beta cells reach their maximum production capacity (hyper- insulinaemic hyperglycemia) thus a state known as Type 2 Diabetes.

Is Insulin Really Present In The Brain?
Until 3 decades ago, insulin was considered a peripheral hormone only, not affecting the Central Nervous System because of its inability to cross the blood brain barrier (BBB) [5,6]. This assumption was based on the fact that insulin is a protein, hence could not cross the BBB.
Secondly, because the brain metabolizes glucose independent of insulin stimulation [7].
However,this was challenged after the detection of immunoreactive insulin in the cerebrospinal fluid of a dog[8]. In the following years, more studies were carried out to confirm this [9,10]. High concentration of insulin was also reported in both human & experimental animals [11,12] exerting long term tropic effect on the central nervous system neurons.

So How Does It Get There?
High concentration of insulin in the brain has generated questions as to it specific origin. This question remains a debatable aspect of research as regards cerebral insulin.
Two Sources has been proposed, namely;
1. Peripheral source
2. Central source

Peripheral source - This proposes that insulin in the brain is majorly from the pancreatic beta cells and its transported by cerebrospinal fluid (CSF) into the brain [13]. It crosses the BBB via a saturable, regulatable process which is limited by the barrier system formed by tight junctions between endothethial cells [1].

Margolis and Altszuler[8] were the first to show that insulin level in rats CSF was slightly increased after peripheral infusion - inferring that insulin crossed the BBB by saturable transport. It was subsequently confirmed by intravenous administration of insulin in dogs [4]. In addition, acute increase in peripheral insulin levels led to increased CSF insulin level whilst chronic peripheral insulinaemic insulin resistance down regulates insulin receptors at BBB impairing its transport into the brain [5].
The circumventricular regions with leaky BBB allows free diffusion of plasma -
soluble such as insulin into the brain [16]. This transport may be regulated by multiple factors such as Glucocorticoids [17], fasting and refeeding [12], Obesity [18], Diabetes Mellitus and Alzheimer's disease - AD [19].

Central Source (Local Synthesis)
De novo insulin synthesis in the brain have been proposed and widely studied as well. This hypothesis the production of insulin in the brain as an alternative source. It is based on numerous experimental evidences outlined below: -

Detection of insulin MRNA

The presence of Preproinsulin I & II m-RNA in rat fetal brain and cultured neurons [20,21] has been reported using R-Nase-protection & sensitive reverse transcription-polymerase chain Reaction (RT-PCR) assays. These m-RNA was also found in the hypothalamus (Periventricular nucleus), olfactory bulb and hippocampus, suggesting the ancestral insulin gene expression in the rat brain belongs to the pancreatic stage of embryonic development [22,23,12].

Interestingly and more recently too, Molar and his team [24] reported the detection of insulin MRNA neuroglia form cells in the rat cortex using RT-PCR. The quantity of INS2 gene per cell was greater when glucose was increased extracellularlly[24,25]. This data in the most recent evidence for local production of insulin within the brain, notwithstanding, more investigation is needed to buttress this finding.

Presence of C-peptides

High concentration of C- peptides was also found in the brain of cadavers [26]. C-peptide is a by-product in the synthesis of insulin. The brain tissues were seen to have higher concentration of both insulin & C-peptides than the plasma.

Post mortem investigations of the brain cortex of AD patients showed the presence of c-peptides too [27]. This particular experiment showed a correlation between C-peptides and a decrease in the number of insulin receptors in the brain. In the same vein, there is no report showing that C-peptide can cross the BBB to get access to the brain from the periphery [26].

Insulin Receptors In The Brain

Insulin receptors (IR) were located and quantified in the brain for the first time by Havrankova and his team [28]. Further investigations also indicated the presence of insulin receptors in cell membranes of the brain at all stages of development [29]. The presence of insulin receptors has been confirmed and its uneven distribution equally reported. IRs are highly abundant in the olfactory bulb, choroid plexus, hypothalamus, cerebral cortex and hippocampus [30, 1]. Nerve terminals show enriched densities of insulin receptors too.

Insulin receptors are glycoproteins belonging to the receptor tyrosine kinase super family. Its composed of 2 subunits (α & β) joined to form a homodimer [7]. Insulin-like growth factor receptors (IGF-1R) are also widespread in the brain (in both glial and neuron)[12].

Insulin receptors in the brain are similar to the peripheral insulin receptors in kinetics and pharmacological properties [31] but differ in molecular size, antigenicity and degree of glycosylation [19]. They also differ in their response to excess insulin. Peripheral receptors down regulate in hyperinsulinaemia while central receptors do not [32,33,12].

Effect Of Insulin On Bec & Bbb Function

The blood brain barrier (BBB) is formed by endothelial cells which are exposed to both the tissue and the blood stream thereby receiving signals from both the periphery and central nervous system [34,12]. These brain endothelial cells (BEC's) have insulin binding sites that seemingly has 2 functions:

1. Transport of insulin across the BBB
2. Classic receptors

In other words, BEC insulin transport is insulin receptor dependent but it's done through transcytosis [35].

Sarah *et al* [35] also noted that despite the presence of insulin degrading enzymes (IDE) in the BBB, it is not altered during transcytosis, consistent high feeding also administers the uptake of insulin [34].

Insulin Signal Transduction

Once insulin gets into the brain, it rapidly binds to the extracellular alpha subunit of its receptor inducing the auto phosphorylation of the its intracellular beta-subunit, followed by the phosphorylation of proteins inside the cell known as insulin receptor substrate (IRS). The phosphorylation of IRS activates the signal transduction cascade leading to the recruitment of Phosphoinositide-3-kinase P1(3)K and Ras/Mitogen activated protein kinase (MAPK) pathway [36]. These are the two canonical pathways involved in mediating insulin signaling in the CNS [37]. The activation state of the receptor is regulated by its phosphorylation state. It may be deactivated through dephosphorylation by the actions of phosphotyrosine phosphatase & serine/threonine kinase (causes phosphorylation at serine/threonine residues[36]). Hence, insulin receptor signalling dysfunction maybe caused when tyrosine phosphorylation, and/or when tyrosine dephosphorylation fails, and/or when serine /threonine is increased and maintained at a higher level.

PI (3)K targets multiple downstream pathways - MTORC (mammalian target of rapamycin C), G3K3β (Glycogen Synthase Kinase 3), Fox O (Fork head box protein 01) which are a family of transcription factors [38]. These pathways have been shown to play important roles in the normal function of brain. Amongst such functions are:

(i) Fox O is involved in the controlling of energy homeostasis. [38]

(ii) MTORC1 is necessary for degrading misfolded protein in neurons (autophagy) and synaptic plasticity [39]. Thus, its impairments lead to neurodegenerative diseases and cell death.

Furthermore, activation of P1(3)k pathway triggers glucose transporter 4 (GLUT 4) translocation as in the periphery.

Role Of Insulin In The Brain

Glucose metabolism

It's a known that glucose is the main fuel the brain utilizes but in times of starvation, ketone bodies acts as substitutes [12]. While the systemic circulation is dependent on insulin for its glucose uptake and metabolism, the brain is not.

GLUT 4 is the main glucose transporter found in the periphery and it is insulin dependent [40]. Glut 1 and 3 are the glucose transporters responsible for glucose uptake in the brain (Neurons and Glial) and both are insulin independent [41]. Glut 2 is also expressed in some neuronal population such as those in the hypothalamus [42].

Before now, the brain was thought to be an insulin insensitive organ because its glucose transporters did not depend on insulin for glucose transport to the brain from the periphery but surprisingly, Glut 4 has been found to be present at low levels in the hippocampus, hypothalamus and cerebral cortex however, it does not also seem to influence glucose uptake much[40].

Given the presence of insulin, its receptors and signal transduction in the brain, what then is the role of insulin in the brain especially as it relates to glucose metabolism?

Glut 4 was seen in both the plasma membrane and cytoplasm of the neurons of the hippocampus, hypothalamus etc. suggesting the likelihood of translocation of Glut 4 to the surface as seen in the periphery[43,44].

More evidence shows the trafficking of Glut 4 to cortex of neurons under conditions that increased plasma insulin levels, such as peripheral glucose administration[45]. The dependence of brain glucose metabolism on insulin is being revisited and researched upon in recent times[46,47].

The medial temporal lobe was seen to effectively stimulate glucose uptake through GLUT 4 during intense neuronal activity[48]. The presence of Glut 4 especially in the hippocampus and neo-cortex (areas demanding high glucose because of high frequency firing during memory formation/cognitive tasks) acts as an alternative route of glucose supply[48]. Its proposed that the glucose supplied by Glut 1 and 3 are sufficient mainly for resting brain activity only[48].

Glucose Homeostasis

Insulin also plays an important role in glucose homeostasis.

In 1853, Claude Bernard[49] through his experimental studies hypothesized that puncturing the 4th cerebral ventricle produced glycosuria in mice, thus suggesting the involvement of brain in glucose homeostasis. Recently, more voices have given support to this finding.

It's now becoming accepted that hypothalamic insulin regulates hepatic glucose production by modulating hepatic sensitivity to circulating peripheral insulin[51,50]. Hypothalamic insulin blocks the production of liver glucose through its own receptors in the liver and hypothalamus thus eliciting a CNS-liver axis response [52, 51]

Experimental inhibition of insulin action in the hypothalamus induced a decrease in insulins' ability to block glycogenolysis/gluconeogenesis[52] in the liver. The signal from the neuron is transmitted to the motor nucleus of the vagus nerve carrying information to the liver, thus producing appropriate response[53].

Furthermore, in confirmation to above, another animal study reported that a reduction or impairment in insulin sensitivity in the hypothalamus (arcuate nucleus) led to a subsequent decrease in efficiency of insulin hormone in blocking glucose formation in the liver[52,7,12]. This response may contribute to hyperglycemia seen in diabetic patients[54]. Resection of the livers branch of vagus nerve induced a reduction in insulin's inhibiting ability on hepatic glucose production[51,12,53,30].

Appetite modulation

Woods and his team in the late 70's generated evidence that intra-cerebral infusion of insulin in Baboons reduced food intake and thus body weight[55]. This finding sparked an intense research as to the role of insulin in the brain.[53] The precise mechanism underlying this hypothesis is now being unraveled. It appears to involve a major integration of nutritional status and peripheral hormonal signals regulated by a group of neurons in the arcuate nucleus of the hypothalamus also called glucose sensing neurons[53]. They generate co-ordinated responses to received stimuli related to fuel storage[12].These neurons contain Orexigenic and Anorexigenic molecules, the Orexigenic Neuropeptides being Neuro-peptide Y(NPY) and agouti-related protein (AgRP) while the Anorexigenic are proopiomelanocortin(POMC) and cocaine- and amphetamine regulated transcript (CART)[57]. The presence of insulin in the hypothalamus activates its (insulin) receptors which directly suppresses prepro-NPY mRNA transcription in the arcuate nucleus thereby leading to a reduction in NPY thus, reducing food intake[53]. on the other hand, insulin increases POMC expression, thus decreasing appetite and food intake as well.[58] These peptides increase the activity of X-melanocytes stimulating hormone in neurons of the Periventricular nucleus in the hypothalamus.[59] Insulin's action in the brain in catabolic while its anabolic in the periphery.[60]

Insulin works in concert with leptin in the hypothalamus[7]. Like leptin, insulin can be said to be a signal of nutrient abundance [53] because leptin also decreases food intake. Both hormones share the same signaling transduction pathway which is P1(3)k as

recently deciphered by Niswender and colleagues[61].

Learning and memory

Based on the heterogeneous distribution of insulin receptors in the brain, It is involved in several other functions like maintenance of excitatory/inhibitory synapse and development of dendritic arborization[62]. It's been reported that the peripheral and central administration of insulin by ICV or intra-hippocampal routes to experimental animals have positive effects on cognitive processes[65]. Insulin receptor signaling plays a role in synaptic plasticity in the hippocampus. It does this by modulating the activity of excitatory and inhibitory receptors thereby affecting cognitive functions [66]. Insulin is seen to bind to its receptors during learning thus activating its receptors leading to signal transduction via P1(3)k. This may be involved in memory formation through potentiation of glutamatergic NMDA(N- methyl-D-aspartate) receptors channel activity enhancing an increase in calcium ion influx (LTP). It may be involved in long term depression by internalization of glutamatergic AMPA (Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid) receptors [68] or through the recruitment of GABA

(gamma-amino-butyric acid) receptors on the post synaptic membrane[69]. The aforementioned functions in conjunction with long term potentiation (LTP) and long term depression (LTD) are essentials for memory and learning. LTP, a molecular model of learning is modulated by insulin. It occurs when a presynaptic neuron stimulates and excites the postsynaptic neuron in a repetitive and prolonged manner, thereby causing prolonged depolarization[64].This also increases the influx of calcium ions into the post synaptic neuron, reinforcing synaptic communication between the neurons. Long term depression (a compensatory effect) facilitates a decrease in transmission efficiency, allowing the cells activity return to its previous excitatory level, invariably getting ready to store new information[12].

Nitric oxide has also been reported to be involved in insulin-induced memory improvement [70].

Insulin also fields neurotrophic effects.[19,71] its shown to be neuroprotective against a wide range of insults such as oxidative stress, ischemia, beta amyloid toxicity and apoptosis.[72,73]

When Things Go Wrong

Insulin resistance at the level of insulin receptors in the brain is considered as Type 3 Diabetes like its counterpart in the periphery (Type 2 Diabetes). Its suggested as an alternative term for Alzheimer's disease.

Studies done show less response or activation of neuronal insulin receptors and signaling mechanisms when exposed to insulin.[54]

This is supported by the reports on age and AD-related decrease in insulin mRNA and protein levels, IR expression, IRS-132 levels and markers of tyrosine kinase activity such as P1(3)k and IRS-1.[78,79,80]. The consequence of this is manifested mainly as distorted APP/Aβ metabolism and tau-protein hyper phosphorylation, cognitive deficits in learning and memory and altered feeding behavior. Insulin resistance is also associated with aging, obesity and T2D.[81,82]'

Neuronal insulin Therapy

Glibenclamide administration was seen to trigger the release of neuronal insulin[24]. Autologous graft of insulin expressing neurons or neural progenitor cells are being considered as potential replacement of lost beta cells in the pancreas[74,4].There uncertainty as to whether the insulin produced in the brain is able to cross down to the periphery through the BBB since intranasal insulin delivery does not seem to impact peripheral insulin.[4,34,76]. The hypothesis of Autologous graft by Kuwabara and his team[74] suggests that neural stem cells could be isolated from either human or rodent olfactory bulbs and transplanted into the pancreas of people living diabetes with the hope of reprogramming via the WNT signaling transduction in order to enable them express insulin.

SUMMARY/CONCLUSION

Insulin signaling has faced a novel and increased interest in neuroscience research .Insulin now known to be beyond a peripheral hormone, is present in the CNS with its receptors in abundance as well. It gets to the brain majorly by crossing the BBB however, its production within certain neurons is now coming to the fore.

Within the CNS, insulin is involved in glucose metabolism & homeostasis, appetite, modulation learning and memory amongst others. An impairment in cerebral insulin signal transduction leads to neurodegenerative diseases such as Alzheimer's. This makes insulin a very important hormone with therapeutic benefits within the CNS and beyond.

It will also be exciting to see the neuron based insulin therapy become a success in human trials.

List Of Abbreviations

Insulin receptors (IR)
Insulin degrading enzymes (IDE)
Insulin receptor substrate (IRS)
Mammalian target of rapamycin Complex (MTORC)
Glycogen Synthase Kinase 3 Beta (G3K3β)
Fork head box protein 01(Fox O)
Phosphoinositide-3-kinase P1(3)K
Ras/Mitogen activated protein kinase (MAPK)
Cerebrospinal fluid (CSF)
Alzheimer's disease - AD
sensitive reverse transcription-polymerase chain Reaction (RT-PCR)
Brain Endothelial Cells (BEC's)

N- methyl-D-aspartate NMDA
Long Term Potentiation (LTP)
Long Term Depression (LTD)

REFERENCES

1. Schulingkamp RJ, Pagano TC, Hung D, Raffa RB. Insulin receptors and insulin action in the brain: review and clinical implications. *Neuroscience and* Biobehavioral *Reviews* 2000; 24(8):855–872.
2. Banting FG, Best CH. The internal secretion of the pancreas. *Journal of Laboratory and Clinical Medicine*1922; 7:251–266.
3. Skyler JS. Diabetes mellitus: pathogenesis and treatment strategies. *Journal of Medical Chemistry*2004; 47: 4113-4117
4. Éva AC, Gábor T. Cerebral cortex: a target and source of insulin? *Diabetologia*2016; 59(8):1609–1615
5. Erol A. An integrated and unifying hypothesis for the metabolic basis of sporadic Alzheimer's disease. *Journal of Alzheimer's Disease*2008; 13(3): 241–253.
6. Laron Z. Insulin and the brain. *Archives of Physiology and Biochemistry*2009; 115(2):112–116.
7. Ana ID, Paula IM, Catarina RO. Insulin in Central Nervous System: More than Just a Peripheral Hormone. *Journal of Aging Research*2012; 2012: Article ID 384017, 21 pages.
8. Margolis RU, Altszuler U. Insulin in the cerebrospinal fluid. *Nature*1967; 215(5108):1375–1376.
9. LeRoith D. CNS regulation of carbohydrate metabolism. *Advances in Metabolic Disorders, Academic Press* 1983; 10: 304–340
10. Schechter RJ, Whitmire L, Holtzclaw M, George R, Harlow, Devaskar SU. Developmental regulation of insulin in the mammalian central nervous system. *Brain Research*1992; 582(1): 27–37
11. Dorn A, Bernstein HG, Rinne A, Ziegler M, Hahn HJ, Ansorge S. Insulin and glucagon like peptides in the brain. *Anatomical Records* 1983; 207(1):69–7710.
12. Enrique B , Esther V, Verónica HC, Juan MA. Insulin in the Brain: Its Pathophysiological Implications for States Related with Central Insulin Resistance, Type 2 Diabetes and Alzheimer's Disease. *Frontiers in Endocrinology(Lausanne)* 2014; 5:161.
13. Banks WA. The source of cerebral insulin. European Journal of Pharmacology 2004; 490 (1–3): 5–12.
14. Woods SC, Porte D Jr. Relationship between plasma and cerebrospinal fluid insulin levels of dogs . *American Journal of Physiology* (1977); 233(4): 331–4.
15. Moreira PI, Duarte AI, Santos MS, Rego AC, Oliveira CR. An integrative view of the role of oxidative stress, mitochondria and insulin in Alzheimer's disease. *Journal of Alzheimer's Disease* 2009; 16(4): 741–761.
16. Porte D, Seeley RJ, Woods SC, Baskin DG, Figlewicz FP, Schwartz MW. Obesity, diabetes and the central nervous system," *Diabetologia*1998; 41(8): 863–881.
17. Baura GD, Foster DM, Kaiyala K, Porte D Jr, Kahn SE, Schwartz MW. Insulin transport from plasma into the central nervous system is inhibited by dexamethasone in dogs. *Diabetes* (1996); 45(1): 86–90.
18. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes* 2000; 49(9):1525–33.
19. Ghasemi R, Haeri A, Dargahi L, Mohamed Z, Ahmadiani A. Insulin in the brain: sources, localization and functions. *Molecuolar Neurobiology* 2013; 47(1): 145–71.
20. Schechter R, Sadiq HF, Devaskar SU. Insulin and insulin mRNA are detected in neuronal cell cultures maintained in an insulin-free/serum-free medium. *Journal of Histochemistry and Cytochemistry* 1990; 38 (6): 829–836.
21. Schechter D, Beju T, Gaffney, Schaefer F, Whetsell L. Preproinsulin I and II mRNAs and insulin electron microscopic immunoreaction are present within the rat fetal nervous system. *Brain Research* 1996; 736 (1-2): 16–27.
22. Devaskar SU, Singh BS, Carnaghi LR, Rajakumar PA , Giddings SJ. Insulin II gene expression in rat central nervous system. *Regul Pept* (1993) 48(1–2):55–63. doi:10.1016/0167-0115(93)90335-6
23. Schechter R, Whitmire J, Wheet GS, Beju D, Jackson KW, Harlow R, et al. Immunohistochemical and in situ hybridization study of an insulin-like substance in fetal neuron cell cultures . *Brain Research* 1994; 636 (1): 9–2710.1016/0006-8993(94)90170-8
24. Molnár G, Faragó N, Kocsis AK. GABAergic neurogliaform cells represent local sources of insulin in the cerebral cortex. *Journal of Neuroscience*2014; 34:1133–1137
25. Williams KW, Margatho, Lisandra O, Lee S, Charlotte EL Joel K et al. . Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *Journal of Neuroscience*2010; 30:2472–2479
26. Frolich L, Blum-Degen D, Bernstein HG, Engelsberger S, Humrich J, Laufer S, et al. Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *Journal of Neural Transmission*1998; 105(4–5):423–38.
27. Jezova D, Vigas M, Sadlon J. C-peptide-like material in rat brain: response to fasting and glucose ingestion. *Endocrinology Experiments*1985; 19(4):261–70
28. Havrankova J, Roth J, Brownstein M. Insulin receptors are widely distributed in the central

nervous system of the rat. *Nature* 272:827–829

29. WIlliam L, Lowe Jr, Frederick T, Boyd, Derryl W, Clarke et al. Development of brain insulin receptors: structural and functional studies of insulin receptors from whole brain and primary cell cultures. Endocrinology1986; 119(1):25-35
30. Plum L, Markus S, Jens. The role of insulin receptor signaling in the brain. *Trends in endocrinology and metabolism*2005; 16(2):59-65
31. Heindreich KA, Zahniser NR, Berhanu P, Brandenburg D, Olefsky JM. Structural differences between insulin receptors in the brain and peripheral target tissues. Journal of Biology and Chemistry1983; 258: 8527-8530.
32. . Boyd FT, Raizada MK .Effects of insulin and tunicamycin on neuronal insulin receptors in culture. American Journal of Physiology1983; 245: 283-287
33. Moloney AM, Griffin RJ, Timmons, O'Connor R, Ravid R, O'Neill C. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiology and Aging* 2010; 31(2):224-43.
34. Banks WA, Owen JB, Erickson MA. Insulin in the brain: there and back again. Pharmacology and Therapeutics (2012) ; 136(1):82–93
35. Sarah MG, Kevin WA, Eugene JB. Unravelling the regulation of insulin transport across the brain endothelial cell . *Diabetologia*2017; 60(8)1512–1521
36. Johnstone AM, Pirola L, Van OE. Molecular mechanisms of insulin receptor substrate protein-mediated modulation of insulin signalling. FEBS2003; Letters 546: 32-36
37. Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG, Jr et al. Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes*2003; 52:227–231
38. André K, Heather AF, Weikang C, Ronald KC.