DR GARRON THOMAS DODD (Orcid ID : 0000-0002-7554-4876)

Article type : Review Article

Corresponding author mail id : garron.dodd@monash.edu

Insulin Action in the Brain: Roles in Energy and Glucose Homeostasis

Garron T. Dodd¹ and Tony Tiganis¹

¹Metabolic Disease and Obesity Program, Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Victoria 3800, Australia.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/jne.12513](https://doi.org/10.1111/jne.12513)

This article is protected by copyright. All rights reserved

SUMMARY

A growing body of evidence from research in rodents and humans has identified insulin as an important neuoregulatory peptide in the brain, where it coordinates diverse aspects of energy balance and peripheral glucose homeostasis. This review discusses where and how insulin interacts within the brain and evaluates the physiological and pathophysiological consequences of central insulin signalling in metabolism, obesity and type 2 diabetes.

Insulin targets peripheral tissues, including skeletal muscle, and adipose tissue, where it promotes glucose uptake from the blood, and the liver where it inhibits gluconeogenesis and glycogenolysis and promotes glycogen synthesis to coordinately repress hepatic glucose production. In this way insulin prevents postprandial hyperglycemia and maintains euglycemia. In addition to these peripheral targets, insulin also signals in the brain, but the physiological significance of this interaction has only recently started to emerge. Evidence over the past two decades has provided compelling evidence that central insulin signalling plays pivotal roles in many aspects of energy and glucose homeostasis (**Fig. 1**). Given the epidemics of obesity and type 2 diabetes (T2D) in developed and increasingly developing countries, there is a pressing need to fully understand the mechanisms by which the body coordinates energy and glucose homeostasis. A key hallmark of obesity and T2D is insulin resistance, where defective insulin signalling downstream of the insulin receptor (IR) renders insulin's peripheral target tissues insensitive to insulin action. What is becoming increasingly apparent is that neurons in the brain also become resistant to insulin, however the relative contributions of central insulin resistance to the development of obesity and T2D are poorly understood.

This review discusses insulin's role in the brain in coordinating glucose metabolism with energy expenditure. In particular, we discuss our understanding of the locations and mechanisms by which insulin elicits its effects in the brain, its influence on glucose metabolism and energy expenditure and the potential contributions of central insulin resistance in the development of obesity and the metabolic syndrome.

1. Insulin and the brain

The integral role of the brain in glucose homeostasis was first described by Claude Bernard in 1855, where he found that puncturing the fourth ventricle resulted in marked glycosuria in dogs [1]. Despite these early observations that inferred an important role for the central nervous system (CNS) in the control of glucose homeostasis, the discovery of insulin by Banting and Best in 1921 along with its ability to markedly reduce blood glucose overshadowed and diverted attention away from the role of the brain [2, 3]. Indeed, much of the research that followed insulin's characterisation, particularly during 1960s-1980s ultimately type-casted insulin so that it was understood to act solely through peripheral

tissues to control glucose metabolism, ignoring any involvement of the brain [4]. Despite the IR and its signalling intermediates being widely expressed within mammalian and human brains [5-9], this peripheral-centric view was reaffirmed by early observations indicating the majority of glucose transport into neurons occurred independently of insulin; it is now known that in a subset of hypothalamic neurons, glucose uptake may occur via the insulin-dependent glucose transporter GLUT4 [10, 11]. Nonetheless, evidence emanating from decades of research now supports varied neuromodulatory roles for CNS insulin signalling.

2. Insulin transport.

The primary source of circulating insulin is from pancreatic β -cells [12]. Insulin is secreted into the circulation in response to post-prandial rises in blood glucose and it readily accesses tissues throughout the body, including the brain. Early experiments described high concentrations of insulin in both animal and human brain homogenates [13, 14]. As insulin is a 51-amino acid peptide, passive diffusion into the brain is limited by the blood brain barrier (BBB). The precise mechanisms of how and where insulin enters the brain are unclear, however emerging evidence suggests that insulin's entry into the brain parenchyma can occur directly via the median eminence (ME) (**Fig.2**), or indirectly via the cerebrospinal fluid (CSF).

2.1. CNS entry via the median eminence

The brain is not entirely ensheathed by the BBB. Specialised regions named circumventricular organs lack the BBB but have fenestrated capillaries allowing for direct communication between the circulation and the brain parenchyma [15]. The fenestrated capillary cells of the ME allow the passive and rapid extravasation of most blood-borne nutrients and metabolic hormones to the brain.

The importance of peripheral insulin access to circumventricular organs is inferred from quantitative autoradiography studies showing that peripheral administered [^{125} I]-insulin rapidly penetrates the circumventricular organs, most notably the ME. The ME is a circumventricular organ positioned directly below the mediobasal hypothalamus affording unique access for insulin to communicate with the energy and glucose sensing neurons in the arcuate nucleus (ARC) of the hypothalamus [15-18]. In fact, it is the neurons of the ARC

that rapidly respond with AKT Ser-473 phosphorylation, a surrogate marker of IR activation, and c-Fos, a surrogate marker of neuronal activation, in response to a peripheral bolus of insulin; this effect is attenuated in insulin resistant states such as T2D and obesity [18-23]. The restriction of the capillary fenestration to the ME together with the presence of tight junction complexes between adjacent tanycytes (specialized hypothalamic glia that line the 3rd ventricle and extend processes to contact the capillary plexus) act as a physical barrier controlling the diffusion of hormones such as insulin to the rest of the brain interstitial fluid (ISF) [24-26]. There is some evidence that the capillary loops reaching the ARC can be affected by nutritional state [26, 27]. In addition, studies attempting to measure ARC ISF by microdialysis show that the low levels of fasting concentrations of insulin in the ARC are rapidly increased 30 minutes after feeding or peripheral insulin infusion [28, 29]. As this accumulation of insulin within the ARC occurs so rapidly it likely involves access via the ME.

2.2. CNS entry via the CSF

For most studies CSF has been used as a surrogate for brain ISF, however recent studies show that CSF measured in the cerebral ventricles, lumbar spine or cisterna magna differs in composition from that of the brain ISF [17, 30, 31]. By administering several different tracers into the CSF and tracing their movement over time, recent studies have redefined the relationship between the CSF and the brain ISF. CSF is produced by the choroid plexus in the ventricles and passes through the 3rd and 4th ventricles into the cisterna magna, the subarachnoid space and eventually accesses the Virchow-Robin space [32]. This para-arteriolar pathway brings the CSF into contact with the micro-vasculature, forming an interface facilitating the possible transport of insulin from the blood to the CSF. Insulin can enter the ISF either by passing through the astrocytes that line the Virchow-Robin space or interacting with tanycytes lining the walls of the ventricles [32]. The precise point by which CSF insulin enters the ISF is unknown, however it is important to note that transport of other metabolic hormones such as leptin (secreted from the adipose tissue functioning to signal levels of adiposity to the brain) from the blood and CSF to the ISF has been shown to be facilitated by tanycyte processes, a transport mechanism that is thought to be essential for leptin's central metabolic actions [33]. Peripheral insulin could therefore have a bi-phasic entry into the brain, via the ME and via the CSF, however the relative contribution of either route remains unknown.

3. CNS insulin signalling

3.1. IR

Insulin signals both centrally and peripherally via the IR [5], a heterotetrameric receptor that comprises of two extracellular ligand-binding α -subunits that are linked via disulphide bonds to two membrane-spanning β -subunits that contain the cytoplasmic protein tyrosine kinase (PTK) domains, which signal upon ligand binding (for review see [5]). The IR gene in humans is encoded by a single gene on chromosome 19 and is composed by 22 exons [34]. Alternative splicing of the mature transcript yields two mature IR isoforms designated isoforms A and B [35]. The A isoform is expressed ubiquitously, especially in the brain [36], whereas the B isoform is expressed in liver, muscle, adipocytes and kidney. Isoform A lacks exon 11, which encodes a 12 amino-acid segment from the carboxy terminus of the ligand binding α subunit. The A isoform has a higher affinity for insulin, but also binds insulin-like growth factor II (IGF-II) [36]. The IR was first localised and quantified in the brain by Havrankova *et al* in 1978 and was shown to be expressed throughout all stages of development in the rodent brain [13]. Since then, autoradiography studies have shown that [^{125} I]-insulin binds to homogenates of the hypothalamus to a greater extent than the rest of the brain, along with significant binding being noted in the olfactory areas, limbic regions, neocortex, basil ganglia, hippocampus, cerebellum and choroid plexus, implying a broad neuromodulatory function within the brain [18]. The IR is also widely distributed within the human brain [37] and *In situ* hybridization studies in rodents have shown that IR mRNA parallels insulin binding, with highest IR expression occurring in the ARC [38].

3.2. Insulin-like Growth Factor 1 Receptor

Insulin also binds and signals via the Insulin-like Growth Factor-1 Receptor (IGF-1R), albeit at a lower affinity than the IR [39]. Like IR, the IGF1-R is expressed throughout the brain with abundant expression evident in the cortex, thalamus and hippocampus, and moderate expression in the cerebellum and hypothalamus [40]. Moreover the IR and IGF-1R can form homodimers and heterodimers [41], but IR homodimers exhibit a higher affinity for insulin than IR/IGF-1R heterodimers [39]. In the rabbit brain, IRs exist predominantly as heterodimers, whereas around 50% of IGF-1Rs form heterodimers [42]. The distribution of and relative contribution of IR/IGF-1R heterodimers and IGF-1R homodimers to insulin's

actions in the brain in glucose metabolism and energy homeostasis remain to be determined. This review will hereon focus on the IR.

3.3. Canonical IR signalling pathways

The binding of insulin to the IR α -subunits induce conformational changes that induce intracellular IR auto-phosphorylation [43]. A comprehensive review of the mechanisms of insulin signalling can be found elsewhere [5, 41, 44], but in brief, IR autophosphorylation on Y1150/Y1151 [45], (Y1162/Y1163 on IR isoform B [45, 46]) fully activates the PTK and results in the phosphorylation of additional IR sites including Y960, Y1146, Y1316 and Y1322 and the phosphorylation of several cellular IR substrates, most notably the insulin receptor substrate (IRS) proteins. IRS-1 and IRS-2 are widely expressed in mammalian tissues and numerous studies have shown that IRS-1 and IRS-2 have both overlapping and distinct physiological functions (for review see, [44]). Both IRS-1- and IRS-2-null mice are glucose intolerant and insulin resistant [47-50], however only IRS-2 null mice exhibit a decreased brain to body ratio [49], implying an important role of IRS-2 in brain development. IRS-2 is more abundantly expressed than IRS-1 within the brain and is particularly abundant in the hypothalamus [51]. Brain-specific deletion of IRS-2 results in increased adiposity, hyperphagia, hypertension, hyperinsulinemia and insulin resistance [52, 53], effects that are mediated, at least in part, by neurons expressing leptin receptors (LepRb) [54]. IRS-4 is also expressed within the hypothalamus [55], however IRS-4-null mice exhibit only a mild metabolic phenotype [56]. Despite this, a recent study found that IRS-4 may cooperate with IRS-2 in the hypothalamus and elicit synergistic effects on energy balance and glucose metabolism [57].

The tyrosine-phosphorylated IRS proteins function as signalling nodes, recruiting adaptor proteins to transduce IR signalling and activate multiple downstream pathways, including the phosphatidylinositol 3 kinase (PI3K)/AKT and the mitogen-activated protein kinase (MAPK) pathways [58] to coordinate neuromodulatory gene transcription and neuronal excitability (**Fig. 2**) to influence energy and glucose homeostasis [59-61].

3.4. Negative Regulators of Central Insulin Signalling

IR signalling is negatively regulated by protein tyrosine phosphatases (PTPs), most notably protein tyrosine phosphatase 1B (PTP1B) and T-cell like protein tyrosine phosphatase (TCPTP), which act to dephosphorylate the IR [46, 59, 62-65]. *Ptpn1*^{-/-} mice that are globally deficient for PTP1B, or mice that lack PTP1B in muscle (*Mck-Cre;Ptpn1*^{fl/fl}) or liver (*Alb-Cre;Ptpn1*^{fl/fl}) exhibit enhanced IR phosphorylation and signalling in muscle and liver and improved whole-body glucose homeostasis [66-69]. PTP1B knockdown in the brain, using antisense oligonucleotides administered intracerebroventricularly also enhances insulin signalling, attenuates body weight and adiposity and improves glucose metabolism [70]. Despite this, the role of PTP1B within the brain may be cell-type dependent, as PTP1B deletion in steroidogenic factor 1 (SF1) neurons located in the ventromedial hypothalamus (VMH) enhances insulin signalling yet promotes adiposity and weight gain [71], whereas conditional PTP1B deletion in proopiomelanocortin (POMC)-expressing neurons in the ARC has no effect on insulin signalling and glucose homeostasis, but enhances leptin signalling [60]. Interestingly, TCPTP deletion in POMC neurons enhances insulin-induced AKT Ser-473 phosphorylation, *Pomc* gene expression and improved whole-body glucose homeostasis consistent with TCPTP but not PTP1B being the principal phosphatase regulating insulin signalling in POMC neurons. PTP1B and TCPTP may therefore differentially regulate central IR signalling in a cell-type and tissue context dependant manner.

Another important negative regulator is suppressor of cytokine signalling 3 (SOCS3) [72]. In the brain SOCS3 has mostly been studied in the context of LepRb signalling, as leptin-induced signalling via Janus-activated kinase-2 (JAK-2)/signal transducer and activator of transcription 3 (STAT-3) drives SOCS3 expression, which in turn negatively regulates JAK-2/STAT-3 signalling as part of a negative feedback loop [72-76]. The elevated circulating leptin levels associated with obesity are thought to increase basal LepRb/STAT3 signalling in ARC neurons [73, 75, 77-79] resulting in chronic SOCS3 expression and the development of cellular leptin resistance [75, 77-83]. The elevated SOCS3 also inhibits IR signalling either directly or by binding and promoting IRS-1 and IRS-2 ubiquitination and degradation [76, 84, 85]. *Socs3*^{+/-} heterozygous mice or the conditional deletion of SOCS3 in neuronal and glial cells or specifically in POMC neurons protects mice against the development of obesity-associated leptin and insulin resistance [80, 86-88]. Conversely SOCS3 overexpression in hypothalamic neurons leads to the development of glucose intolerance and systemic insulin resistance [83] [89]. The obesity-associated hyperleptinemia and/or inflammation also

promote the expression of PTP1B and TCPTP which dephosphorylates JAK2/STAT3 and/or the IR thus contributing to cellular leptin/insulin resistance (**Fig. 2**) [16, 90-94].

4. CNS targets of insulin

Although it is well established that insulin interacts with neurons of the brain, understanding the anatomical location and precise neuronal populations where this interaction occurs is vital to defining the role of insulin signalling in the brain.

4.1. Hypothalamic targets of insulin signalling

Functional *in vivo* imaging studies in both human and rodents all point toward an unequivocal role for insulin signalling in the hypothalamus (**Fig. 1**) [95-97]. In humans, intranasal delivery of insulin modulates functional hypothalamic neuronal activity [98-102], an effect that is attenuated in obesity [98, 101]. In rodents, functional immunohistochemical studies show that several regions of the hypothalamus including the ARC, VMH, dorsomedial hypothalamus (DMH) and paraventricular hypothalamus (PVH) show enhanced functional c-Fos immunoreactivity following peripheral or central insulin administration [22, 103]. A caveat of these functional studies is that they do not ascertain whether insulin directly influences the response of c-Fos positive neurons. By immunohistochemistry, AKT Ser-473 phosphorylation is seen rapidly (<15 minutes) in response to insulin administration and is almost exclusively seen within the hypothalamus in two functionally antagonistic ARC neuronal populations; the anorectic POMC (α -MSH precursor) and the orexigenic AgRP (agouti-related peptide)/NPY (neuropeptide Y)-neuropeptide expressing neurons [104, 105]. AgRP neurons promote feeding, weight gain and repress energy expenditure, whereas POMC neurons attenuate feeding, weight gain and promote energy expenditure [59, 106]. Genetic ablation or pharmacogenetic inhibition of AgRP neurons decreases food intake and body weight [107-109], whereas POMC neuronal activation promotes α -MSH release to agonise melanocortin-4 receptors (MC4Rs) on second order neurons in regions of the brain such as the PVH, VMH and DMH [110]. MC4R activation has been shown to decrease food intake, increase energy expenditure and regulate glucose homeostasis [110]. AgRP/NPY neuronal activation promotes the release of AgRP and GABA that antagonise α -MSH/MC4R interactions and inhibit POMC neurons [106]. Insulin inhibits AgRP/NPY neurons by activating PI3K which subsequently activates ATP-sensitive potassium channels resulting in

membrane hyperpolarization and decreased action-potential frequency [59, 60, 104, 111]. Insulin has long been thought to hyperpolarise and inhibit POMC neurons via the same mechanism described for AgRP neurons. Recent studies by Qiu *et al.* report that insulin depolarises and activates POMC neurons via the activation of transient receptor potential-5 channels [22], whereas Williams *et al.* report the existence of distinct insulin-responsive POMC populations throughout the hypothalamus [21]. Such discrepancies could potentially be explained by the high synaptic plasticity of POMC neurons responding to insulin [112-127]. Indeed, synaptic inputs to POMC neurons are re-modelled in response to feeding, fasting and diet-induced obesity [112, 122, 126, 127].

As the AgRP and POMC neurons in the ARC are positioned proximal to the ME they are ideally positioned to be the first neuronal subsets that interact with peripheral insulin. From here, AgRP and POMC neurons transduce insulin signalling to the rest of the brain via their projections to nuclei such as the PVH, VMH, DMH, lateral hypothalamus (LH), amygdala, bed nucleus of stria terminalis, parabrachial nucleus and the dorsal vagal complex (encompassing the nucleus of the solitary tract) allowing insulin to influence a constellation of CNS processes [106, 128]. It is likely that many of the distinct actions of central insulin signalling in AgRP/POMC neurons (discussed later) are coordinated by projections to these distinct nuclei. For example, a recent neurocircuitry mapping study highlighted discrete AgRP projections to the PVH coordinate feeding behaviour whereas projections to the LH or bed nucleus of the stria terminalis (BNST) regulate peripheral insulin sensitivity [129].

Beyond the ARC, insulin has also recently been shown to act directly on SF1-neurons of the VMH [130]. Conditional excision of the IR in SF-1 cells protects against diet-induced obesity and improves glucose homeostasis by enhancing glutamatergic signalling onto POMC neurons [130]. In addition to neurons of the VHM, insulin has also been shown to act directly on melanin-concentrating-hormone (MCH) neurons in the LH. Interestingly, there are distinct insulin-sensitive subsets of MCH neurons within the LH, each responding electrophysiologically to insulin with either excitation, inhibition or non-responsivity [131]. The precise physiological functions of these distinct subsets remain unclear, however conditional deletion of the IR in MCH neurons modulates locomotor activity and improves insulin sensitivity in diet-induced obese mice [131]. In addition to neuronal insulin

signalling, a recent study showed that insulin signals to ARC glial cells and regulates glucose transport into the ARC ISF, thereby influencing how ARC neurons sense glucose [132].

4.2. Extra- hypothalamic targets of insulin signalling

Beyond the hypothalamus, dopaminergic midbrain neurons of the ventral tegmental area have been shown to be responsive to insulin [133]. Conditional deletion of IR in tyrosine hydroxylase (the rate-limiting enzyme in catecholaminergic biosynthesis) expressing cells resulted in increased body weight, increased fat mass, hyperphagia and altered responses to cocaine under food-restricted conditions [133]. Insulin signalling in dopaminergic neurons may therefore be critical for the integration of signals of food palatability/reward into the overall complex control of energy homeostasis.

Higher cortical regions have also been shown to be responsive to insulin in both humans and animals. For example, in humans the feeding induced functional activation of the fusiform gyrus and prefrontal cortex is attenuated by insulin administration [134, 135]. Interestingly, the attenuation of functional activity in the prefrontal cortices appears to be limited to lean but not obese individuals [136]. The hippocampus, an area central to declarative memory formation is also insulin sensitive in both rodents and humans [136-139]. Beyond the hypothalamus, the hippocampus represents the only other significant brain region that signals via AKT in response to insulin administration [140]. However, it is important to keep in mind that a lack of AKT signalling does not preclude insulin acting through alternate pathways. In addition, it is possible that the strength of downstream IR coupling could differ between brain regions, as seen with the cannabinoid 1 receptor [141, 142]. Moreover, it is possible that insulin-rich ISF may diffuse to different brain regions at different rates. In human's, insulin administration has been shown to improve higher cognitive function, memory formation and mood, an effect that could be facilitated by either the direct or indirect insulin engagement of such extra hypothalamic regions (**Fig. 1**, for review see [143]).

5. Insulin's actions in the brain

Interest in the actions of insulin in the brain was re-ignited at the turn of the century when mice with a brain-specific knockout of the IR (Nestin-Cre;*Insr*^{fl/fl}: NIRKO) were shown to be

obese, hyperphagic, and develop whole-body insulin resistance with dyslipidaemia and hyperinsulinemia, highlighting the importance of CNS insulin signalling in the coordination of energy and glucose homeostasis [144-146]. In fact, research over the past decade now suggests that central insulin signalling plays sentinel roles in feeding behaviour, energy expenditure, adiposity, whole body glucose homeostasis and peripheral insulin sensitivity (Fig.1).

5.1. Effects on feeding behaviour, adiposity and body weight

The first clues that insulin regulates food intake came from studies administering insulin into the ventricular system of the brain. Studies in rodents and non-human primates following intracerebroventricular (ICV) delivery of insulin showed decreases in food intake and a significant reduction in body weight [82, 147-152]. Despite a plethora of studies recapitulating insulin CNS hypophagic effects under many different conditions, a body of conflicting studies exists showing little to no effect of insulin on food intake and body weight [149, 153-155]. The use of supraphysiological doses of exogenous insulin possibly resulting in attenuated insulin sensitivity could underlie such discrepancies. Indeed, a recent study found that the hypophagia elicited by centrally administered insulin is compromised if mice are pre-administered insulin 2 days but not 7 days prior to the experiment [149]. Furthermore, many studies only determined daily food intake as opposed to assessing feeding behaviour *per se*. Streptozotocin treated mice, with ablated insulin secretion, show no alteration in cumulative food intake, but exhibit a latency in feeding behaviour [90]. Several human studies show that changing feeding behaviour (reduced meal size, enhanced frequency) as opposed to simply reducing daily food intake has a much more dramatic effect on weight loss and glucose homeostasis [156]. Conversely patients with uncontrolled T2D report feelings of excessive hunger, whereas healthy individuals show an attenuated valence towards pictures of food in response to exogenous insulin or heightened endogenous insulin levels [100, 134, 137]. Insulin has also been shown to affect the smelling capacity in lean people, an effect that could regulate feeding behaviour in response to elevated postprandial insulin levels [157, 158]. Although complex, these studies highlight a potential role of CNS insulin signalling in the control of feeding behaviour, however the neuronal populations mediating these responses remain unclear. Substantial evidence points to insulin signalling in the ARC being instrumental for insulin's CNS effects on energy

homeostasis. For example, IR knockdown with antisense oligonucleotides in the ARC results in rapid onset hyperphagia and a marked increase in adiposity [145]. Moreover, intra-nasal insulin delivery into the CNS of healthy patients improves whole-body insulin sensitivity, promotes weight loss and attenuates caloric intake via the activation of neurons within the hypothalamus [159].

It is likely that these effects of hypothalamic insulin signalling on feeding, adiposity and body weight are mediated by AgRP and POMC neurons [111]. However, studies attempting to delineate the specific contributions of IR signalling in AgRP, POMC, Sim1 (PVH) and NKx2.1 (ARC, VMN) neurons of the hypothalamus to energy homeostasis have proved underwhelming [160, 161], as the conditional IR deletion in such subset leads to no overt effects on feeding behaviour or body weight. One possible explanation for this is that IR deletion *in utero* is compensated by alternate pathways. A recent study by Loh *et al* reported that the inducible deletion of IR from NPY expressing cells in adult mice increased body weight and adiposity suggesting that insulin signalling in NPY neurons is important in the regulation of energy homeostasis [162]. Similar inducible approaches may be required to explore the roles of AgRP and POMC IR signalling in energy homeostasis. It is worth while noting that conditional deletion of IRS-2 in the whole brain or specifically in POMC neurons promotes marked increases in adiposity [53]

5.2. Effects on energy expenditure

Feeding behaviour represents only one arm of the energy balance equation, the other being energy expenditure. Whole-body energy expenditure can be accounted for by basal metabolic rate (55-65%), physical activity (25-35%) and adaptive thermogenesis (10%). Research into defining the hormonal influences and neurons coordinating energy expenditure has received much attention over the last 5 years, particularly in understanding the phenomenon of adaptive thermogenesis.

Adaptive thermogenesis refers to the body's ability to increase energy expenditure in response to thermal challenges (cold-induced thermogenesis) and/or nutritional state (diet/feeding-induced thermogenesis) [163]. The body does this by engaging brown adipose tissue (BAT, expressed in the intrascapular regions in rodents and infants and possibly in

different fat depots in adult humans) and by promoting the recruitment and activation of beige adipocytes in white adipose tissue (WAT, notably within the inguinal or interclavicular subcutaneous fat depots in rodents and human respectively) a process referred to as WAT browning [163-167]. Unlike traditional white adipocytes which store energy, brown and beige adipocytes function to expend energy. In response to cold exposure or β -adrenergic stimulation, brown and beige adipocytes become activated and generate heat by expending energy [165]. They do this, in part, by upregulating the mitochondrial uncoupling protein 1 (UCP-1) which acts to uncouple oxidative phosphorylation at the expense of adenosine triphosphate (ATP) [168, 169]. Understanding how adaptive thermogenesis is regulated is of high therapeutic significance, as the promotion of WAT browning and BAT activity has been shown to protect against diet-induced obesity and T2D [169].

Insulin's actions on adaptive thermogenesis were inferred as early as 1983 in studies that found rats treated with diazoxide, a potent blocker of pancreatic β cell insulin secretion, attenuated the thermic response to a carbohydrate meal [170]. Subsequent studies found that insulin administration directly into various regions of the hypothalamus resulted in robust increases in body temperature and whole-body energy expenditure [23, 171, 172]. Consistent with these observations, ICV insulin administration increases sympathetic nervous system (SNS) activity, as assessed by recordings from nerve fibres [172, 173], and intranasal delivery of insulin in humans enhances post-prandial energy expenditure [174].

The neuronal populations by which insulin may influence adaptive thermogenesis remain unclear. The effects of conditional IR deletion in AgRP or POMC neurons on energy expenditure have not yet been directly characterised. However, Lin *et al* found that restoration of insulin signalling specifically in POMC neurons in L1 mice (IR deficient mice expressing IR only in the liver and pancreatic β -cells) using a targeted knock-in approach increased energy expenditure and locomotor activity, however, BAT thermogenesis or WAT browning were not determined. Moreover, a recent study has shown that insulin and leptin can act together on ARC POMC neurons to promote SNS-dependent WAT browning and BAT activity [60] and that enhancing this attenuates the development of diet-induced obesity. Therefore, in part the effects of insulin on adaptive thermogenesis may be mediated by the POMC mediated control of WAT browning and BAT activity.

In addition to adaptive thermogenesis, physical activity accounts for a substantial proportion of total energy expenditure and can be ascribed to exercise and non-exercise activity-dependent thermogenesis (walking, sitting, standing, fidgeting, but excluding voluntary exercise, sleeping, or eating) [175]. Interestingly, NIRKO mice and those lacking TCPTP in neuronal and glial cells in the brain, show not only changes in energy expenditure but in ambulatory activity [91, 144]. As no effects on activity are seen in mice lacking IR or the IR phosphatase TCPTP in POMC neurons alone [60, 161], these effects on activity are likely mediated by other neurons and/or other brain regions such the cerebellum where the IR is abundant [176].

5.3. Effects on peripheral glucose metabolism

A substantial body of data has defined an important role for CNS insulin signalling in the control of peripheral glucose metabolism. In addition to the role of CNS insulin signalling in controlling feeding and energy expenditure, it makes sense that the same neuronal signalling pathways controlling nutrient intake should also control nutrient fluxes within the body.

Insulin administered directly into the hypothalamus or into the ARC attenuates peripheral insulin's ability to suppress hepatic glucose production (HGP), an effect that is lost in NIRKO mice [144, 145, 161]. Consistent with this, ICV infusion of IR antisense oligonucleotides or neutralising insulin antibodies also attenuate peripheral insulin's ability to suppress HGP [145]. These effects appear to be mediated via AgRP neurons as the conditional deletion of the IR in AgRP neurons blunts the ability of both systemic and ICV insulin to attenuate HGP, as assessed in hyperinsulemic euglycemic clamped mice [177]. Although the ablation of IR in POMC neurons has no overt effect on glucose metabolism, combined IR and LepRb deletion in POMC neurons results in profound deregulation of peripheral glucose metabolism [161, 178].

The efferent mechanisms coupling insulin action in the brain to HGP are relatively unknown but involve the vagal efferents to the liver [179]. A recent study shows this brain-liver coupling is mediated by hepatic 7-nicotinic acetylcholine receptors [180]. The current

working model is that central innervation to the liver modulates HGP by activating Kupffer cells to release IL-6, which in turn acts on hepatocytes via signal transducer and activator of transcription 3 (STAT3) to repress the expression of gluconeogenic enzymes, such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase [181]. Neural tracing studies attempting to delineate the efferent projections from the hypothalamus to the liver found that trans-synaptic efferents emerge from POMC, but not AgRP neurons of the hypothalamus, inferring at least one possible route of how central insulin signalling can regulate the liver [182, 183]. Although these studies highlight POMC efferents, future studies using new generation transgene-mediated viral vectors will be essential to provide a more comprehensive understanding of hepatic innervation [183]. In rodents, liver denervation attenuates HGP, whereas in humans (liver transplant) and canines, denervation only blunts the counter regulatory HGP response during hypoglycaemia [184-186]. It is therefore important not to overstate insulin's CNS actions on HGP. A new role of AgRP neurons in the coordination of whole-body insulin sensitivity was recently described by Steculorum *et al* [187]. Here the authors demonstrated that the acute pharmacogenetic activation of AgRP but not POMC neurons induced the expression of myostatin within BAT, robustly impairing whole-body insulin sensitivity by selectively repressing BAT glucose uptake. Using sophisticated optogenetic techniques the authors further identified that distinct AgRP projections to the PVH regulate feeding behaviour whereas projections to the LH or BNST regulate peripheral BAT insulin sensitivity and glucose metabolism [129]. Others have also shown that distinct subpopulations of AgRP neuronal projections (mainly the BNST, PVH and LH) also independently evoke feeding behaviour [188]. These neurocircuitry mapping experiments have provided initial insights into the complex neuronal networks underlying the control of feeding and insulin sensitivity.

Central insulin signalling has also been implicated in the control of WAT lipolysis, as NIRKO mice exhibit unrestrained lipolysis and decreased *de novo* lipogenesis in WAT [144]. Moreover, ARC insulin infusion increases WAT lipogenic protein expression and inactivates WAT hormone-sensitive lipase to repress WAT lipolysis [61, 189, 190]. A recent study has shown that IR signalling in POMC but not AGRP neurons may be responsible for insulin's ability to repress WAT lipolysis [191]. In humans, intranasal insulin delivery suppressed plasma free fatty acid levels, consistent with the attenuation of lipolysis [159, 174]. When

taken together with the CNS effects of insulin on WAT browning, it is seemingly paradoxical that CNS insulin action both promotes (WAT browning) and inhibits catabolic (WAT lipolysis) in the same tissue (**Fig.1**). Although the neuronal correlates mediating insulin's CNS effects on lipolysis are unclear, it will be important to delineate if common or distinct neuronal efferents mediate these opposing metabolic actions.

6. Insulin resistance and metabolic disease

A phenomenon observed since the earliest studies investigating insulin signalling in obesity, is the considerable attenuation of the insulin response [5, 192-194]. As this attenuation of signalling in obesity occurs despite heightened circulating levels of insulin, this phenomenon is referred to as "insulin resistance" [13]. Insulin resistance results from defects in insulin signalling downstream of the IR (**Fig.2**) [59]. This renders insulin's peripheral target tissues and the brain insensitive to insulin action [20, 59, 94, 195-199]. Despite an emerging physiological role of CNS insulin signalling, the relative contributions of CNS insulin resistance to the development of peripheral insulin resistance and T2D is unclear.

The constant availability of highly palatable nutrient-dense foods, together with the trend towards a more sedentary lifestyle in western societies, underpin the obesity and T2D epidemics. Strikingly the consumption of a high fat diet for as short as 72 h is sufficient to reduce hypothalamic insulin sensitivity, independent of changes in body weight and fat mass in rodents [23]. Moreover, consumption of saturated fatty acids in lean mice attenuates the ability of centrally administered insulin to regulate food intake and body weight [23]. At a molecular level, saturated fatty acids such as palmitate or stearate cross the BBB where they are thought to activate local inflammatory signalling within the hypothalamus resulting in central cellular insulin and leptin resistance [200, 201]. The inflammation and the hyperleptinemia in obesity are known to drive reactive gliosis, endoplasmic reticulum (ER) stress and the expression of negative regulators of insulin signalling, such as SOCS3, PTP1B and TCPTP within the hypothalamus [59, 89, 202] (**Fig.2**). The hyperleptinemia seen in obesity is thought to increase the hypothalamic expression of TCPTP, PTP1B and SOCS3, whereas ER stress and the obesity-associated inflammation drives the expression of PTP1B and SOCS3 [91, 92, 203-207]. These negative regulators may be instrumental in the initiation/exacerbation and/or maintenance of cellular leptin and insulin

resistance (**Fig. 2**) [59, 91, 208]. In the case of SOCS3, increased expression is seen as rapidly as two days in AgRP neurons and 2 weeks in POMC neurons [89]. Deletion of TCPTP or SOCS3 in POMC neurons, or SOCS3 in AgRP neurons enhances hypothalamic insulin signalling and whole-body glucose homeostasis in diet-induced obese mice [60, 83, 87, 89, 209, 210]. The inducible constitutive expression of X-box-binding protein 1 (Xbp1, a key regulator of the unfolded protein response to ER stress) within the POMC neurons alone protects against ER stress and diet-induced obesity and improves glucose homeostasis, effects that may be dependant on the expression of SOCS3 and PTP1B [211].

In addition to defective IR signalling, obesity is also associated with a decreased BBB permeability thus potentially restricting access to hormones such as insulin and leptin to the brain (**Fig. 2**) [212, 213]. Insulin CSF concentrations are lower in insulin resistant individuals despite higher circulating levels of insulin, suggesting an impaired delivery into the brain [212]. The relative importance of impaired insulin delivery to the brain in metabolic disease remains to be determined. Furthermore, diet-induced obesity results in reactive gliosis in the ARC in both rodents and humans [126, 214-217]. This gliosis effectively makes POMC and AgRP neurons less accessible to insulin in the surrounding ISF (**Fig. 2**) [126]. Understanding the relative contribution of elevated PTP1B, TCPTP and SOCS3 expression, defective BBB permeability or reactive gliosis in the development of CNS insulin resistance and its influence in obesity and T2D remains to be determined.

7. Conclusions and future prospective

An important function of the brain is to ensure a steady supply of energy substrate to maintain the body's *internal milieu*. To accomplish this task, divergent signals must be integrated, processed and transduced into homeostatic adjustments of food intake, energy expenditure, glucose and lipid metabolism. Insulin signalling in the brain plays several distinct roles in feeding behaviour, energy expenditure, adiposity and peripheral insulin sensitivity, all of which are essential for the sentinel coordination of energy and glucose homeostasis. There is accumulating evidence that an integral component of insulin's CNS actions is coordinated by AgRP and POMC neurons in the ARC. Deconstruction of the neuronal subsets responding to peripheral insulin and the delineation of how these neurons transduce insulin's multifunctional actions throughout the brain represents a future

challenge. Beyond the hypothalamus, recent work has highlighted tantalizing new roles of central insulin signalling in aspects of mood, food preference, olfaction and memory formation [96]. Understanding insulin signalling in this context is still in its infancy, however exploring the molecular mechanisms of how insulin orchestrates such responses will shed light on how energy expenditure and glucose metabolism may be integrated with changes in mood and behaviour. Moreover, defining the relative contribution of CNS insulin resistance to the development of obesity and T2D remains a key unanswered question. What is apparent, however, is that when the brain becomes insulin resistant, the crucial modulatory effects of the brain on energy and glucose homeostasis are impaired. Future therapeutic advances targeting CNS insulin signalling may therefore have far reaching implications for the prevention and treatment of metabolic disease.

Figure legends

Figure 1: Physiological effects of insulin action in the brain. Insulin is released postprandially from the pancreas where it enters the blood circulation. Insulin reaches the brain where it signals to regulate mood (pre-frontal cortex, nucleus accumbens, striatum, amygdala and raphe nucleus), memory (hippocampus) and olfactory capacity (olfactory bulb) [96, 157, 218-221]. Insulin signals to the hypothalamus where it coordinates food intake, peripheral insulin sensitivity, hepatic glucose output, lipolysis and WAT browning [60, 222]. Abbreviations: SNS, sympathetic nervous system; WAT, white adipose tissue.

Figure 2: Mechanisms of central insulin resistance. a) In the lean state, insulin is transported to the ARC/hypothalamus across a fenestrated blood–brain barrier (BBB, dashed blue line) [223]. Insulin crosses the BBB and engages the IR on two opposing neuronal populations in the ARC known as POMC and AgRP/NPY neurons [111]. Insulin signals via PI3K and the protein kinase AKT to regulate metabolic neuropeptide gene transcription and neuronal excitability [5, 60, 224]. Insulin signalling in these neurons is propagated to the rest of the brain and peripheral tissues, such as white adipose tissue, brown adipose tissue, and the liver to coordinate food intake, whole-body energy

expenditure and glucose metabolism. **b)** A key hallmark of diet-induced obesity is defective insulin signalling in the ARC, whereby AgRP and POMC neurons become insensitive to insulin action and are termed 'insulin resistant' [111]. In diet-induced obesity, **b1)** the BBB is less permeable and the access of insulin to the ARC/hypothalamus is decreased (depicted by light grey shading) despite heightened plasma insulin levels [212-214, 223]; **b2)** Increased inflammation and reactive gliosis in the ARC impair insulin's access and the responsiveness of the AgRP and POMC neurons to insulin [126, 200]; **b3)** The enhanced expression of negative regulators of IR signalling, such as TCPTP, PTP1B, SOCS3 within the hypothalamus [59, 89, 91, 223] attenuate insulin signalling [5, 59]. The decreased insulin signalling diminishes insulin's ability to repress food intake, increase whole-body energy expenditure, repress hepatic glucose production and promote glucose storage and influence lipid flux to maintain metabolic homeostasis. Abbreviations: AgRP, agouti-related peptide; ARC, arcuate nucleus; CSF, cerebrospinal fluid; IR, insulin receptor; ME, median eminence; PI3K, phosphatidylinositol 3-kinase; POMC, pro-opiomelanocortin; NPY, neuropeptide Y; SOCS3, suppressor of cytokine signalling 3.

References

1. Bernard, C., *Leçons de physiologie expérimentale appliquées à la médecine*. J. B. Baillière et fils, 1855.
2. Banting, F.G., Best, C.H., Collip, J.B., Campbell, W.R., Fletcher, A.A., Macleod, Noble, E.C., *The effect produced on diabetes by extracts of pancreas*. Trans Assoc Am Physicians, 1922. **37**: p. 337–347.
3. Banting, F.G.a.B., C.H. , *The internal secretion of the pancreas*. J Lab Clin Med, 1922. **7**: p. 251–266.
4. Quianzon, C.C. and I. Cheikh, *History of insulin*. J Community Hosp Intern Med Perspect, 2012. **2**(2).
5. Boucher, J., A. Kleinridders, and C.R. Kahn, *Insulin receptor signaling in normal and insulin-resistant states*. Cold Spring Harb Perspect Biol, 2014. **6**(1).
6. Zhao, W., et al., *Brain insulin receptors and spatial memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats*. J Biol Chem, 1999. **274**(49): p. 34893-902.
7. Adamo, M., M.K. Raizada, and D. LeRoith, *Insulin and insulin-like growth factor receptors in the nervous system*. Mol Neurobiol, 1989. **3**(1-2): p. 71-100.
8. Havrankova, J., J. Roth, and M. Brownstein, *Insulin receptors are widely distributed in the central nervous system of the rat*. Nature, 1978. **272**(5656): p. 827-9.
9. Werther, G.A., et al., *Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry*. Endocrinology, 1987. **121**(4): p. 1562-70.
10. McEwen, B.S. and L.P. Reagan, *Glucose transporter expression in the central nervous system: relationship to synaptic function*. Eur J Pharmacol, 2004. **490**(1-3): p. 13-24.
11. Ren, H., et al., *Glut4 expression defines an insulin-sensitive hypothalamic neuronal population*. Mol Metab, 2014. **3**(4): p. 452-9.
12. Fu, Z., E.R. Gilbert, and D. Liu, *Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes*. Curr Diabetes Rev, 2013. **9**(1): p. 25-53.
13. Havrankova, J., et al., *Identification of insulin in rat brain*. Proc Natl Acad Sci U S A, 1978. **75**(11): p. 5737-41.
14. Watanabe, M., et al., *Histologic distribution of insulin and glucagon receptors*. Braz J Med Biol Res, 1998. **31**(2): p. 243-56.

15. Siso, S., M. Jeffrey, and L. Gonzalez, *Sensory circumventricular organs in health and disease*. Acta Neuropathol, 2010. **120**(6): p. 689-705.
16. Corp, E.S., et al., *Localization of 125I-insulin binding sites in the rat hypothalamus by quantitative autoradiography*. Neurosci Lett, 1986. **70**(1): p. 17-22.
17. Schwartz, M.W., et al., *Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid*. Am J Physiol, 1990. **259**(3 Pt 1): p. E378-83.
18. Hill, J.M., et al., *Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas*. Neuroscience, 1986. **17**(4): p. 1127-38.
19. Olson, B.R., et al., *c-Fos Expression in Rat Brain and Brainstem Nuclei in Response to Treatments That Alter Food Intake and Gastric Motility*. Mol Cell Neurosci, 1993. **4**(1): p. 93-106.
20. Koch, L., et al., *Central insulin action regulates peripheral glucose and fat metabolism in mice*. J Clin Invest, 2008. **118**(6): p. 2132-47.
21. Williams, K.W., et al., *Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons*. J Neurosci, 2010. **30**(7): p. 2472-9.
22. Qiu, J., et al., *Insulin excites anorexigenic proopiomelanocortin neurons via activation of canonical transient receptor potential channels*. Cell Metab, 2014. **19**(4): p. 682-93.
23. Clegg, D.J., et al., *Consumption of a high-fat diet induces central insulin resistance independent of adiposity*. Physiol Behav, 2011. **103**(1): p. 10-6.
24. Prevot, V., F. Langlet, and B. Dehouck, *Flipping the tanycyte switch: how circulating signals gain direct access to the metabolic brain*. Aging (Albany NY), 2013. **5**(5): p. 332-4.
25. Myers, M.G., Jr., *How is the hungry brain like a sieve?* Cell Metab, 2013. **17**(4): p. 467-8.
26. Langlet, F., et al., *Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting*. Cell Metab, 2013. **17**(4): p. 607-17.
27. Devaskar, S.U., et al., *Insulin gene expression and insulin synthesis in mammalian neuronal cells*. J Biol Chem, 1994. **269**(11): p. 8445-54.

28. Gerozissis, K., et al., *Insulin responses to a fat meal in hypothalamic microdialysates and plasma*. *Physiol Behav*, 1997. **62**(4): p. 767-72.
29. Orosco, M., et al., *Feeding-related immunoreactive insulin changes in the PVN-VMH revealed by microdialysis*. *Brain Res*, 1995. **671**(1): p. 149-58.
30. Brinker, T., et al., *A new look at cerebrospinal fluid circulation*. *Fluids Barriers CNS*, 2014. **11**: p. 10.
31. Veening, J.G. and H.P. Barendregt, *The regulation of brain states by neuroactive substances distributed via the cerebrospinal fluid; a review*. *Cerebrospinal Fluid Res*, 2010. **7**: p. 1.
32. Iliff, J.J., et al., *A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta*. *Sci Transl Med*, 2012. **4**(147): p. 147ra111.
33. Balland, E., et al., *Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain*. *Cell Metab*, 2014. **19**(2): p. 293-301.
34. Kosaki, A., J. Nelson, and N.J. Webster, *Identification of intron and exon sequences involved in alternative splicing of insulin receptor pre-mRNA*. *J Biol Chem*, 1998. **273**(17): p. 10331-7.
35. Mosthaf, L., et al., *Functionally distinct insulin receptors generated by tissue-specific alternative splicing*. *EMBO J*, 1990. **9**(8): p. 2409-13.
36. Frasca, F., et al., *Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells*. *Mol Cell Biol*, 1999. **19**(5): p. 3278-88.
37. Hopkins, D.F. and G. Williams, *Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity*. *Diabet Med*, 1997. **14**(12): p. 1044-50.
38. Marks, J.L., et al., *Localization of insulin receptor mRNA in rat brain by in situ hybridization*. *Endocrinology*, 1990. **127**(6): p. 3234-6.
39. Belfiore, A., et al., *Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease*. *Endocr Rev*, 2009. **30**(6): p. 586-623.
40. Fernandez, A.M. and I. Torres-Aleman, *The many faces of insulin-like peptide signalling in the brain*. *Nat Rev Neurosci*, 2012. **13**(4): p. 225-39.

41. Siddle, K., *Signalling by insulin and IGF receptors: supporting acts and new players*. J Mol Endocrinol, 2011. **47**(1): p. R1-10.
42. Bailyes, E.M., et al., *Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting*. Biochem J, 1997. **327 (Pt 1)**: p. 209-15.
43. Shemer, J., et al., *Insulin and IGF-I stimulate phosphorylation of their respective receptors in intact neuronal and glial cells in primary culture*. J Mol Neurosci, 1989. **1**(1): p. 3-8.
44. Kleinridders, A., *Deciphering Brain Insulin Receptor and Insulin-Like Growth Factor 1 Receptor Signalling*. J Neuroendocrinol, 2016. **28**(11).
45. White, M.F., S. Takayama, and C.R. Kahn, *Differences in the sites of phosphorylation of the insulin receptor in vivo and in vitro*. J Biol Chem, 1985. **260**(16): p. 9470-8.
46. Galic, S., et al., *Coordinated regulation of insulin signaling by the protein tyrosine phosphatases PTP1B and TCPTP*. Mol Cell Biol, 2005. **25**(2): p. 819-29.
47. Araki, E., et al., *Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene*. Nature, 1994. **372**(6502): p. 186-90.
48. Tamemoto, H., et al., *Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1*. Nature, 1994. **372**(6502): p. 182-6.
49. Schubert, M., et al., *Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation*. J Neurosci, 2003. **23**(18): p. 7084-92.
50. Withers, D.J., et al., *Disruption of IRS-2 causes type 2 diabetes in mice*. Nature, 1998. **391**(6670): p. 900-4.
51. Torsoni, M.A., et al., *Molecular and functional resistance to insulin in hypothalamus of rats exposed to cold*. Am J Physiol Endocrinol Metab, 2003. **285**(1): p. E216-23.
52. Taguchi, A., L.M. Wartschow, and M.F. White, *Brain IRS2 signaling coordinates life span and nutrient homeostasis*. Science, 2007. **317**(5836): p. 369-72.
53. do Carmo, J.M., et al., *Regulation of Blood Pressure, Appetite, and Glucose by Leptin After Inactivation of Insulin Receptor Substrate 2 Signaling in the Entire Brain or in Proopiomelanocortin Neurons*. Hypertension, 2016. **67**(2): p. 378-86.
54. Sadagurski, M., et al., *IRS2 signaling in LepR-b neurons suppresses FoxO1 to control energy balance independently of leptin action*. Cell Metab, 2012. **15**(5): p. 703-12.

55. Numan, S. and D.S. Russell, *Discrete expression of insulin receptor substrate-4 mRNA in adult rat brain*. Brain Res Mol Brain Res, 1999. **72**(1): p. 97-102.
56. Fantin, V.R., et al., *Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis*. Am J Physiol Endocrinol Metab, 2000. **278**(1): p. E127-33.
57. Sadagurski, M., et al., *Irs2 and Irs4 synergize in non-LepRb neurons to control energy balance and glucose homeostasis*. Mol Metab, 2014. **3**(1): p. 55-63.
58. Rojas, F.A., A.E. Hirata, and M.J. Saad, *Regulation of insulin receptor substrate-2 tyrosine phosphorylation in animal models of insulin resistance*. Endocrine, 2003. **21**(2): p. 115-22.
59. Zhang, Z.Y., G.T. Dodd, and T. Tiganis, *Protein Tyrosine Phosphatases in Hypothalamic Insulin and Leptin Signaling*. Trends Pharmacol Sci, 2015. **36**(10): p. 661-74.
60. Dodd, G.T., et al., *Leptin and insulin act on POMC neurons to promote the browning of white fat*. Cell, 2015. **160**(1-2): p. 88-104.
61. Mayer, C.M. and D.D. Belsham, *Insulin directly regulates NPY and AgRP gene expression via the MAPK MEK/ERK signal transduction pathway in mHypoE-46 hypothalamic neurons*. Mol Cell Endocrinol, 2009. **307**(1-2): p. 99-108.
62. Tiganis, T. and A.M. Bennett, *Protein tyrosine phosphatase function: the substrate perspective*. Biochem J, 2007. **402**(1): p. 1-15.
63. Galic, S., et al., *Regulation of insulin receptor signaling by the protein tyrosine phosphatase TCPTP*. Mol Cell Biol, 2003. **23**(6): p. 2096-108.
64. Meng, T.C., et al., *Regulation of insulin signaling through reversible oxidation of the protein-tyrosine phosphatases TC45 and PTP1B*. J Biol Chem, 2004. **279**(36): p. 37716-25.
65. Fukushima, A., et al., *T-cell protein tyrosine phosphatase attenuates STAT3 and insulin signaling in the liver to regulate gluconeogenesis*. Diabetes, 2010. **59**(8): p. 1906-14.
66. Bence, K.K., et al., *Neuronal PTP1B regulates body weight, adiposity and leptin action*. Nat Med, 2006. **12**(8): p. 917-24.
67. Elchebly, M., et al., *Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene*. Science, 1999. **283**(5407): p. 1544-8.

68. Klamann, L.D., et al., *Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice*. Mol Cell Biol, 2000. **20**(15): p. 5479-89.
69. Delibegovic, M., et al., *Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress*. Diabetes, 2009. **58**(3): p. 590-9.
70. Picardi, P.K., et al., *Reduction of hypothalamic protein tyrosine phosphatase improves insulin and leptin resistance in diet-induced obese rats*. Endocrinology, 2008. **149**(8): p. 3870-80.
71. Chiappini, F., et al., *Ventromedial hypothalamus-specific Ptpn1 deletion exacerbates diet-induced obesity in female mice*. J Clin Invest, 2014. **124**(9): p. 3781-92.
72. Wunderlich, C.M., N. Hovelmeyer, and F.T. Wunderlich, *Mechanisms of chronic JAK-STAT3-SOCS3 signaling in obesity*. JAKSTAT, 2013. **2**(2): p. e23878.
73. Lubis, A.R., et al., *The role of SOCS-3 protein in leptin resistance and obesity*. Acta Med Indones, 2008. **40**(2): p. 89-95.
74. Eyckerman, S., et al., *Identification of the Y985 and Y1077 motifs as SOCS3 recruitment sites in the murine leptin receptor*. FEBS Lett, 2000. **486**(1): p. 33-7.
75. Bjorbaek, C., et al., *Identification of SOCS-3 as a potential mediator of central leptin resistance*. Mol Cell, 1998. **1**(4): p. 619-25.
76. Shi, H., et al., *Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling*. J Biol Chem, 2004. **279**(33): p. 34733-40.
77. Bjorbaek, C., et al., *The role of SOCS-3 in leptin signaling and leptin resistance*. J Biol Chem, 1999. **274**(42): p. 30059-65.
78. Bjorbaek, C., et al., *SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985*. J Biol Chem, 2000. **275**(51): p. 40649-57.
79. Munzberg, H. and M.G. Myers, Jr., *Molecular and anatomical determinants of central leptin resistance*. Nat Neurosci, 2005. **8**(5): p. 566-70.
80. Howard, J.K., et al., *Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3*. Nat Med, 2004. **10**(7): p. 734-8.
81. Shi, H., et al., *Overexpression of suppressor of cytokine signaling 3 in adipose tissue causes local but not systemic insulin resistance*. Diabetes, 2006. **55**(3): p. 699-707.

82. Plata-Salaman, C.R., Y. Oomura, and N. Shimizu, *Dependence of food intake on acute and chronic ventricular administration of insulin*. *Physiol Behav*, 1986. **37**(5): p. 717-34.
83. Reed, A.S., et al., *Functional role of suppressor of cytokine signaling 3 upregulation in hypothalamic leptin resistance and long-term energy homeostasis*. *Diabetes*, 2010. **59**(4): p. 894-906.
84. Ueki, K., T. Kondo, and C.R. Kahn, *Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms*. *Mol Cell Biol*, 2004. **24**(12): p. 5434-46.
85. Rui, L., et al., *SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2*. *J Biol Chem*, 2002. **277**(44): p. 42394-8.
86. Mori, H., et al., *Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity*. *Nat Med*, 2004. **10**(7): p. 739-43.
87. Kievit, P., et al., *Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells*. *Cell Metab*, 2006. **4**(2): p. 123-32.
88. Pedroso, J.A., et al., *Inactivation of SOCS3 in leptin receptor-expressing cells protects mice from diet-induced insulin resistance but does not prevent obesity*. *Mol Metab*, 2014. **3**(6): p. 608-18.
89. Olofsson, L.E., et al., *Modulation of AgRP-neuronal function by SOCS3 as an initiating event in diet-induced hypothalamic leptin resistance*. *Proc Natl Acad Sci U S A*, 2013. **110**(8): p. E697-706.
90. Davidson, A.J., et al., *Food-anticipatory activity and liver per1-luc activity in diabetic transgenic rats*. *Physiol Behav*, 2002. **76**(1): p. 21-6.
91. Loh, K., et al., *Elevated hypothalamic TCPTP in obesity contributes to cellular leptin resistance*. *Cell Metab*, 2011. **14**(5): p. 684-99.
92. Zabolotny, J.M., et al., *Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo*. *J Biol Chem*, 2008. **283**(21): p. 14230-41.
93. Gamber, K.M., et al., *Over-expression of leptin receptors in hypothalamic POMC neurons increases susceptibility to diet-induced obesity*. *PLoS One*, 2012. **7**(1): p. e30485.

94. Williams, K.W., et al., *Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis*. Cell Metab, 2014. **20**(3): p. 471-82.
95. Dodd, G.T., S.R. Williams, and S.M. Luckman, *Functional magnetic resonance imaging and c-Fos mapping in rats following a glucoprivic dose of 2-deoxy-D-glucose*. J Neurochem, 2010. **113**(5): p. 1123-32.
96. Heni, M., et al., *Impaired insulin action in the human brain: causes and metabolic consequences*. Nat Rev Endocrinol, 2015. **11**(12): p. 701-11.
97. Kullmann, S., et al., *Insulin action in the human brain: evidence from neuroimaging studies*. J Neuroendocrinol, 2015. **27**(6): p. 419-23.
98. Matsuda, M., et al., *Altered hypothalamic function in response to glucose ingestion in obese humans*. Diabetes, 1999. **48**(9): p. 1801-6.
99. Little, T.J., et al., *Mapping glucose-mediated gut-to-brain signalling pathways in humans*. Neuroimage, 2014. **96**: p. 1-11.
100. Heni, M., et al., *Differential effect of glucose ingestion on the neural processing of food stimuli in lean and overweight adults*. Hum Brain Mapp, 2014. **35**(3): p. 918-28.
101. Smeets, P.A., et al., *Functional MRI of human hypothalamic responses following glucose ingestion*. Neuroimage, 2005. **24**(2): p. 363-8.
102. Page, K.A., et al., *Effects of fructose vs glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways*. JAMA, 2013. **309**(1): p. 63-70.
103. Porter, J.P. and H.S. Bokil, *Effect of intracerebroventricular and intravenous insulin on Fos-immunoreactivity in the rat brain*. Neurosci Lett, 1997. **224**(3): p. 161-4.
104. Varela, L. and T.L. Horvath, *Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis*. EMBO Rep, 2012. **13**(12): p. 1079-86.
105. Yang, L. and G.S. McKnight, *Hypothalamic PKA regulates leptin sensitivity and adiposity*. Nat Commun, 2015. **6**: p. 8237.
106. Xu, B. and X. Xie, *Neurotrophic factor control of satiety and body weight*. Nat Rev Neurosci, 2016. **17**(5): p. 282-92.
107. Luquet, S., et al., *NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates*. Science, 2005. **310**(5748): p. 683-5.

108. Gropp, E., et al., *Agouti-related peptide-expressing neurons are mandatory for feeding*. Nat Neurosci, 2005. **8**(10): p. 1289-91.
109. Krashes, M.J., et al., *Rapid, reversible activation of AgRP neurons drives feeding behavior in mice*. J Clin Invest, 2011. **121**(4): p. 1424-8.
110. Millington, G.W., *The role of proopiomelanocortin (POMC) neurones in feeding behaviour*. Nutr Metab (Lond), 2007. **4**: p. 18.
111. Roh, E., K. Song do, and M.S. Kim, *Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism*. Exp Mol Med, 2016. **48**: p. e216.
112. Zeltser, L.M., R.J. Seeley, and M.H. Tschop, *Synaptic plasticity in neuronal circuits regulating energy balance*. Nat Neurosci, 2012. **15**(10): p. 1336-42.
113. Pinto, S., et al., *Rapid rewiring of arcuate nucleus feeding circuits by leptin*. Science, 2004. **304**(5667): p. 110-5.
114. Cone, R.D., et al., *The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis*. Int J Obes Relat Metab Disord, 2001. **25 Suppl 5**: p. S63-7.
115. Mizuno, T.M., et al., *Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and [corrected] in ob/ob and db/db mice, but is stimulated by leptin*. Diabetes, 1998. **47**(2): p. 294-7.
116. Cowley, M.A., et al., *Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus*. Nature, 2001. **411**(6836): p. 480-4.
117. Cowley, M.A., et al., *Electrophysiological actions of peripheral hormones on melanocortin neurons*. Ann N Y Acad Sci, 2003. **994**: p. 175-86.
118. Takahashi, K.A. and R.D. Cone, *Fasting induces a large, leptin-dependent increase in the intrinsic action potential frequency of orexigenic arcuate nucleus neuropeptide Y/Agouti-related protein neurons*. Endocrinology, 2005. **146**(3): p. 1043-7.
119. Horvath, T.L., et al., *Neuropeptide-Y innervation of beta-endorphin-containing cells in the rat mediobasal hypothalamus: a light and electron microscopic double immunostaining analysis*. Endocrinology, 1992. **131**(5): p. 2461-7.
120. Baskin, D.G., et al., *Leptin binding in the arcuate nucleus is increased during fasting*. Brain Res, 1999. **828**(1-2): p. 154-8.
121. Gao, Q., et al., *Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals*. Nat Med, 2007. **13**(1): p. 89-94.

122. Gyengesi, E., et al., *Corticosterone regulates synaptic input organization of POMC and NPY/AgRP neurons in adult mice*. Endocrinology, 2010. **151**(11): p. 5395-402.
123. Liu, T., et al., *Fasting activation of AgRP neurons requires NMDA receptors and involves spinogenesis and increased excitatory tone*. Neuron, 2012. **73**(3): p. 511-22.
124. Yang, Y., et al., *Hunger states switch a flip-flop memory circuit via a synaptic AMPK-dependent positive feedback loop*. Cell, 2011. **146**(6): p. 992-1003.
125. Dietrich, M.O., et al., *Agrp neurons mediate Sirt1's action on the melanocortin system and energy balance: roles for Sirt1 in neuronal firing and synaptic plasticity*. J Neurosci, 2010. **30**(35): p. 11815-25.
126. Horvath, T.L., et al., *Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity*. Proc Natl Acad Sci U S A, 2010. **107**(33): p. 14875-80.
127. Sternson, S.M., G.M. Shepherd, and J.M. Friedman, *Topographic mapping of VMH --> arcuate nucleus microcircuits and their reorganization by fasting*. Nat Neurosci, 2005. **8**(10): p. 1356-63.
128. Wang, D., et al., *Whole-brain mapping of the direct inputs and axonal projections of POMC and AgRP neurons*. Front Neuroanat, 2015. **9**: p. 40.
129. Hill, J.W., et al., *Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice*. J Clin Invest, 2008. **118**(5): p. 1796-805.
130. Klockener, T., et al., *High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons*. Nat Neurosci, 2011. **14**(7): p. 911-8.
131. Hausen, A.C., et al., *Insulin-Dependent Activation of MCH Neurons Impairs Locomotor Activity and Insulin Sensitivity in Obesity*. Cell Rep, 2016. **17**(10): p. 2512-2521.
132. Garcia-Caceres, C., et al., *Astrocytic Insulin Signaling Couples Brain Glucose Uptake with Nutrient Availability*. Cell, 2016. **166**(4): p. 867-80.
133. Konner, A.C., et al., *Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis*. Cell Metab, 2011. **13**(6): p. 720-8.
134. Guthoff, M., et al., *The insulin-mediated modulation of visually evoked magnetic fields is reduced in obese subjects*. PLoS One, 2011. **6**(5): p. e19482.
135. Guthoff, M., et al., *Insulin modulates food-related activity in the central nervous system*. J Clin Endocrinol Metab, 2010. **95**(2): p. 748-55.

136. Kullmann, S., et al., *Selective insulin resistance in homeostatic and cognitive control brain areas in overweight and obese adults*. Diabetes Care, 2015. **38**(6): p. 1044-50.
137. Kroemer, N.B., et al., *(Still) longing for food: insulin reactivity modulates response to food pictures*. Hum Brain Mapp, 2013. **34**(10): p. 2367-80.
138. Zhang, H., et al., *Intranasal insulin enhanced resting-state functional connectivity of hippocampal regions in type 2 diabetes*. Diabetes, 2015. **64**(3): p. 1025-34.
139. Winocur, G., M. Moscovitch, and B. Bontempi, *Memory formation and long-term retention in humans and animals: convergence towards a transformation account of hippocampal-neocortical interactions*. Neuropsychologia, 2010. **48**(8): p. 2339-56.
140. Grillo, C.A., et al., *Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent*. Brain Res, 2009. **1296**: p. 35-45.
141. Breivogel, C.S., L.J. Sim, and S.R. Childers, *Regional differences in cannabinoid receptor/G-protein coupling in rat brain*. J Pharmacol Exp Ther, 1997. **282**(3): p. 1632-42.
142. Dodd, G.T., et al., *Central cannabinoid signaling mediating food intake: a pharmacological-challenge magnetic resonance imaging and functional histology study in rat*. Neuroscience, 2009. **163**(4): p. 1192-200.
143. Ott, V., et al., *Intranasal administration of insulin to the brain impacts cognitive function and peripheral metabolism*. Diabetes Obes Metab, 2012. **14**(3): p. 214-21.
144. Bruning, J.C., et al., *Role of brain insulin receptor in control of body weight and reproduction*. Science, 2000. **289**(5487): p. 2122-5.
145. Obici, S., et al., *Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats*. Nat Neurosci, 2002. **5**(6): p. 566-72.
146. Obici, S., et al., *Hypothalamic insulin signaling is required for inhibition of glucose production*. Nat Med, 2002. **8**(12): p. 1376-82.
147. Woods, S.C., et al., *Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons*. Nature, 1979. **282**(5738): p. 503-5.
148. Foster, L.A., N.K. Ames, and R.S. Emery, *Food intake and serum insulin responses to intraventricular infusions of insulin and IGF-I*. Physiol Behav, 1991. **50**(4): p. 745-9.
149. Mc Allister, E., et al., *Inconsistencies in the hypophagic action of intracerebroventricular insulin in mice*. Physiol Behav, 2015. **151**: p. 623-8.

150. Brown, L.M., et al., *Intraventricular insulin and leptin reduce food intake and body weight in C57BL/6J mice*. *Physiol Behav*, 2006. **89**(5): p. 687-91.
151. Jaillard, T., et al., *Hypothalamic reactive oxygen species are required for insulin-induced food intake inhibition: an NADPH oxidase-dependent mechanism*. *Diabetes*, 2009. **58**(7): p. 1544-9.
152. Air, E.L., et al., *Acute third ventricular administration of insulin decreases food intake in two paradigms*. *Pharmacol Biochem Behav*, 2002. **72**(1-2): p. 423-9.
153. Woods, S.C. and W. Langhans, *Inconsistencies in the assessment of food intake*. *Am J Physiol Endocrinol Metab*, 2012. **303**(12): p. E1408-18.
154. Jessen, L., D.J. Clegg, and S.D. Bouman, *Evaluation of the lack of anorectic effect of intracerebroventricular insulin in rats*. *Am J Physiol Regul Integr Comp Physiol*, 2010. **298**(1): p. R43-50.
155. Nagaraja, T.N., et al., *In normal rat, intraventricularly administered insulin-like growth factor-1 is rapidly cleared from CSF with limited distribution into brain*. *Cerebrospinal Fluid Res*, 2005. **2**: p. 5.
156. Louis-Sylvestre, J., et al., *Highlighting the positive impact of increasing feeding frequency on metabolism and weight management*. *Forum Nutr*, 2003. **56**: p. 126-8.
157. Ketterer, C., et al., *Acute, short-term hyperinsulinemia increases olfactory threshold in healthy subjects*. *Int J Obes (Lond)*, 2011. **35**(8): p. 1135-8.
158. Brunner, Y.F., C. Benedict, and J. Freiherr, *Intranasal insulin reduces olfactory sensitivity in normosmic humans*. *J Clin Endocrinol Metab*, 2013. **98**(10): p. E1626-30.
159. Heni, M., et al., *Central insulin administration improves whole-body insulin sensitivity via hypothalamus and parasympathetic outputs in men*. *Diabetes*, 2014. **63**(12): p. 4083-8.
160. Chong, A.C., et al., *Central insulin signaling modulates hypothalamus-pituitary-adrenal axis responsiveness*. *Mol Metab*, 2015. **4**(2): p. 83-92.
161. Konner, A.C., et al., *Insulin Action in AgRP-Expressing Neurons Is Required for Suppression of Hepatic Glucose Production*. *Cell Metab*, 2007. **5**(6): p. 438-449.
162. Loh, K., et al., *Insulin controls food intake and energy balance via NPY neurons*. *Molecular Metabolism*. **6**(6): p. 574-584.
163. Wu, J., P. Cohen, and B.M. Spiegelman, *Adaptive thermogenesis in adipocytes: is beige the new brown?* *Genes Dev*, 2013. **27**(3): p. 234-50.

164. Betz, M.J. and S. Enerback, *Human Brown Adipose Tissue: What We Have Learned So Far*. Diabetes, 2015. **64**(7): p. 2352-60.
165. Kajimura, S., B.M. Spiegelman, and P. Seale, *Brown and Beige Fat: Physiological Roles beyond Heat Generation*. Cell Metab, 2015. **22**(4): p. 546-59.
166. Cohen, P. and B.M. Spiegelman, *Brown and Beige Fat: Molecular Parts of a Thermogenic Machine*. Diabetes, 2015. **64**(7): p. 2346-51.
167. Rosen, E.D. and B.M. Spiegelman, *What we talk about when we talk about fat*. Cell, 2014. **156**(1-2): p. 20-44.
168. Cedikova, M., et al., *Mitochondria in White, Brown, and Beige Adipocytes*. Stem Cells Int, 2016. **2016**: p. 6067349.
169. Harms, M. and P. Seale, *Brown and beige fat: development, function and therapeutic potential*. Nat Med, 2013. **19**(10): p. 1252-63.
170. Rothwell, N.J., M.E. Saville, and M.J. Stock, *Role of insulin in thermogenic responses to refeeding in 3-day-fasted rats*. Am J Physiol, 1983. **245**(2): p. E160-5.
171. Menendez, J.A. and D.M. Atrens, *Insulin and the paraventricular hypothalamus: modulation of energy balance*. Brain Res, 1991. **555**(2): p. 193-201.
172. Morgan, D.A. and K. Rahmouni, *Differential effects of insulin on sympathetic nerve activity in agouti obese mice*. J Hypertens, 2010. **28**(9): p. 1913-9.
173. Muntzel, M.S., et al., *Mechanisms of insulin action on sympathetic nerve activity*. Clin Exp Hypertens, 1995. **17**(1-2): p. 39-50.
174. Benedict, C., et al., *Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men*. Diabetes, 2011. **60**(1): p. 114-8.
175. Munzberg, H., et al., *Neural Control of Energy Expenditure*. Handb Exp Pharmacol, 2016. **233**: p. 173-94.
176. Pisotta, I. and M. Molinari, *Cerebellar contribution to feedforward control of locomotion*. Front Hum Neurosci, 2014. **8**: p. 475.
177. Konner, A.C., et al., *Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production*. Cell Metab, 2007. **5**(6): p. 438-49.
178. Hill, J.W., et al., *Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility*. Cell Metab, 2010. **11**(4): p. 286-97.

179. Scarlett, J.M. and M.W. Schwartz, *Gut-brain mechanisms controlling glucose homeostasis*. F1000Prime Rep, 2015. **7**: p. 12.
180. Kimura, K., et al., *Central Insulin Action Activates Kupffer Cells by Suppressing Hepatic Vagal Activation via the Nicotinic Alpha 7 Acetylcholine Receptor*. Cell Rep, 2016. **14**(10): p. 2362-74.
181. Inoue, H., *Central insulin-mediated regulation of hepatic glucose production [Review]*. Endocr J, 2016. **63**(1): p. 1-7.
182. Stanley, S., et al., *Identification of neuronal subpopulations that project from hypothalamus to both liver and adipose tissue polysynaptically*. Proc Natl Acad Sci U S A, 2010. **107**(15): p. 7024-9.
183. Ginger, M., et al., *Revealing the secrets of neuronal circuits with recombinant rabies virus technology*. Front Neural Circuits, 2013. **7**: p. 2.
184. Perseghin, G., et al., *Regulation of glucose homeostasis in humans with denervated livers*. J Clin Invest, 1997. **100**(4): p. 931-41.
185. Yi, C.X., et al., *The role of the autonomic nervous liver innervation in the control of energy metabolism*. Biochim Biophys Acta, 2010. **1802**(4): p. 416-31.
186. Wada, M., et al., *Hepatic denervation does not significantly change the response of the liver to glucagon in conscious dogs*. Am J Physiol, 1995. **268**(2 Pt 1): p. E194-203.
187. Steculorum, S.M., et al., *AgRP Neurons Control Systemic Insulin Sensitivity via Myostatin Expression in Brown Adipose Tissue*. Cell, 2016. **165**(1): p. 125-38.
188. Betley, J.N., et al., *Parallel, redundant circuit organization for homeostatic control of feeding behavior*. Cell, 2013. **155**(6): p. 1337-50.
189. Scherer, T., et al., *Brain insulin controls adipose tissue lipolysis and lipogenesis*. Cell Metab, 2011. **13**(2): p. 183-94.
190. Coomans, C.P., et al., *Circulating insulin stimulates fatty acid retention in white adipose tissue via KATP channel activation in the central nervous system only in insulin-sensitive mice*. J Lipid Res, 2011. **52**(9): p. 1712-22.
191. Shin, A.C., et al., *Insulin Receptor Signaling in Pomc, but not Agrp, Neurons Controls Adipose Tissue Insulin Action*. Diabetes, 2017.
192. Abdul-Ghani, M.A. and R.A. DeFronzo, *Pathogenesis of insulin resistance in skeletal muscle*. J Biomed Biotechnol, 2010. **2010**: p. 476279.

193. DeFronzo, R.A., *Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis*. Neth J Med, 1997. **50**(5): p. 191-7.
194. DeFronzo, R.A., et al., *Type 2 diabetes mellitus*. Nat Rev Dis Primers, 2015. **1**: p. 15019.
195. Wang, J., et al., *Overfeeding rapidly induces leptin and insulin resistance*. Diabetes, 2001. **50**(12): p. 2786-91.
196. Smith, U., *Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance--is insulin resistance initiated in the adipose tissue?* Int J Obes Relat Metab Disord, 2002. **26**(7): p. 897-904.
197. Aronne, L.J. and K.R. Segal, *Adiposity and fat distribution outcome measures: assessment and clinical implications*. Obes Res, 2002. **10 Suppl 1**: p. 14S-21S.
198. Cline, G.W., et al., *Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes*. N Engl J Med, 1999. **341**(4): p. 240-6.
199. Samuel, V.T. and G.I. Shulman, *Mechanisms for insulin resistance: common threads and missing links*. Cell, 2012. **148**(5): p. 852-71.
200. Kleinridders, A., et al., *MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity*. Cell Metab, 2009. **10**(4): p. 249-59.
201. Dalvi, P.S., et al., *High fat induces acute and chronic inflammation in the hypothalamus: effect of high-fat diet, palmitate and TNF-alpha on appetite-regulating NPY neurons*. Int J Obes (Lond), 2017. **41**(1): p. 149-158.
202. Panzhinskiy, E., J. Ren, and S. Nair, *Protein tyrosine phosphatase 1B and insulin resistance: role of endoplasmic reticulum stress/reactive oxygen species/nuclear factor kappa B axis*. PLoS One, 2013. **8**(10): p. e77228.
203. Tsunekawa, T., et al., *Deficiency of PTP1B Attenuates Hypothalamic Inflammation via Activation of the JAK2-STAT3 Pathway in Microglia*. EBioMedicine, 2017. **16**: p. 172-183.
204. Song, G.J., et al., *A novel role for protein tyrosine phosphatase 1B as a positive regulator of neuroinflammation*. J Neuroinflammation, 2016. **13**(1): p. 86.

205. Munzberg, H., J.S. Flier, and C. Bjorbaek, *Region-specific leptin resistance within the hypothalamus of diet-induced obese mice*. Endocrinology, 2004. **145**(11): p. 4880-9.
206. Zabolotny, J.M., et al., *PTP1B regulates leptin signal transduction in vivo*. Dev Cell, 2002. **2**(4): p. 489-95.
207. Tsou, R.C., et al., *Deficiency of PTP1B in leptin receptor-expressing neurons leads to decreased body weight and adiposity in mice*. Endocrinology, 2012. **153**(9): p. 4227-37.
208. Scarpace, P.J. and Y. Zhang, *Leptin resistance: a predisposing factor for diet-induced obesity*. Am J Physiol Regul Integr Comp Physiol, 2009. **296**(3): p. R493-500.
209. Banno, R., et al., *PTP1B and SHP2 in POMC neurons reciprocally regulate energy balance in mice*. J Clin Invest, 2010. **120**(3): p. 720-34.
210. Bruder-Nascimento, T., et al., *Ptp1b deletion in pro-opiomelanocortin neurons increases energy expenditure and impairs endothelial function via TNF-alpha dependent mechanisms*. Clin Sci (Lond), 2016. **130**(11): p. 881-93.
211. Ozcan, L., et al., *Endoplasmic reticulum stress plays a central role in development of leptin resistance*. Cell Metab, 2009. **9**(1): p. 35-51.
212. Kern, W., et al., *Low cerebrospinal fluid insulin levels in obese humans*. Diabetologia, 2006. **49**(11): p. 2790-2.
213. Hsu, T.M. and S.E. Kanoski, *Blood-brain barrier disruption: mechanistic links between Western diet consumption and dementia*. Front Aging Neurosci, 2014. **6**: p. 88.
214. Schur, E.A., et al., *Radiologic evidence that hypothalamic gliosis is associated with obesity and insulin resistance in humans*. Obesity (Silver Spring), 2015. **23**(11): p. 2142-8.
215. Dorfman, M.D. and J.P. Thaler, *Hypothalamic inflammation and gliosis in obesity*. Curr Opin Endocrinol Diabetes Obes, 2015. **22**(5): p. 325-30.
216. Thaler, J.P., et al., *Obesity is associated with hypothalamic injury in rodents and humans*. J Clin Invest, 2012. **122**(1): p. 153-62.
217. Berkseth, K.E., et al., *Hypothalamic gliosis associated with high-fat diet feeding is reversible in mice: a combined immunohistochemical and magnetic resonance imaging study*. Endocrinology, 2014. **155**(8): p. 2858-67.
218. Kleinridders, A., et al., *Insulin action in brain regulates systemic metabolism and brain function*. Diabetes, 2014. **63**(7): p. 2232-43.

219. Biessels, G.J. and L.P. Reagan, *Hippocampal insulin resistance and cognitive dysfunction*. Nat Rev Neurosci, 2015. **16**(11): p. 660-71.
220. McNay, E.C., et al., *Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance*. Neurobiol Learn Mem, 2010. **93**(4): p. 546-53.
221. Aime, P., et al., *A physiological increase of insulin in the olfactory bulb decreases detection of a learned aversive odor and abolishes food odor-induced sniffing behavior in rats*. PLoS One, 2012. **7**(12): p. e51227.
222. Blazquez, E., et al., *Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease*. Front Endocrinol (Lausanne), 2014. **5**: p. 161.
223. Bolborea, M. and N. Dale, *Hypothalamic tanycytes: potential roles in the control of feeding and energy balance*. Trends Neurosci, 2013. **36**(2): p. 91-100.
224. Spanswick, D., et al., *Insulin activates ATP-sensitive K⁺ channels in hypothalamic neurons of lean, but not obese rats*. Nat Neurosci, 2000. **3**(8): p. 757-8.

a) Lean**b) Diet-induced obesity**

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Dodd, GT;Tiganis, T

Title:

Insulin action in the brain: Roles in energy and glucose homeostasis

Date:

2017-10-01

Citation:

Dodd, G. T. & Tiganis, T. (2017). Insulin action in the brain: Roles in energy and glucose homeostasis. JOURNAL OF NEUROENDOCRINOLOGY, 29 (10), <https://doi.org/10.1111/jne.12513>.

Persistent Link:

<http://hdl.handle.net/11343/293533>