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Brain insulin signalling in metabolic homeostasis and disease

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Abstract | Insulin signalling in the central nervous system regulates energy homeostasis by controlling metabolism in several organs and by coordinating organ crosstalk. Studies performed in rodents, non-human primates and humans over more than five decades using intracerebroventricular, direct hypothalamic or intranasal application of insulin provide evidence that brain insulin action might reduce food intake and, more importantly, regulates energy homeostasis by orchestrating nutrient partitioning. This Review discusses the metabolic pathways that are under the control of brain insulin action and explains how brain insulin resistance contributes to metabolic disease in obesity, the metabolic syndrome and type 2 diabetes mellitus.

Insulin signalling plays a key role in nutrient partitioning. For example, brain insulin signalling is involved in the regulation of systemic and organ-specific metabolism, often in a complementary manner. Overnutrition is a common trigger for the development of obesity, which is the major driver of insulin resistance, prediabetes and type 2 diabetes mellitus (T2DM)[1]. Overnutrition impairs brain insulin signalling and action in rodents and humans within days, suggesting that brain insulin resistance plays a role in obesity and its metabolic sequelae[2–5]. The manipulation of brain insulin signalling through a variety of methods has been shown to regulate and/or disrupt glucose, lipid and amino acid metabolism in several peripheral tissues, in particular by controlling metabolic pathways in the liver and adipose tissue. However, there is a paucity of studies to confirm existing concepts and there is considerable controversy about the role of brain insulin in the regulation of hepatic glucose production. This Review critically evaluates the studies that support and refute a role for brain insulin in systemic nutrient partitioning, first in rodents and dogs and then in humans (BOX 1 shows a condensed overview). The aim is to elucidate the integrated physiology of brain insulin in metabolic control and the contribution of brain insulin resistance to metabolic disease and to assess the therapeutic potential of enhancing or restoring brain insulin signalling in metabolic disease.

Brain insulin signalling

Insulin receptors, which are expressed early during ontogenesis, are found throughout the brain and in most cell types. The highest density of insulin receptors is found in the hypothalamus, olfactory bulb, hippocampus, striatum, cerebral cortex and cerebellum[6–9]. The early expression and widespread distribution of the insulin receptor suggest that it has pivotal and possibly diverse roles in the central nervous system (CNS). Insulin levels in cerebrospinal fluid (CSF) correlate with circulating plasma levels, which is consistent with pancreatic insulin being the primary source for brain insulin[10,11]. Whether or not insulin peptides are produced in the brain in physiologically relevant amounts remains controversial. While mRNA expression has been described in multiple cell types within the CNS by several studies[12–15], it has not been convincingly ruled out whether insulin peptides detected in brain cells do not simply originate from the circulation. Circulating insulin secreted by the endocrine pancreas enters the brain via a saturable transport across the blood–brain barrier (BBB)[16–20]. Importantly, the transport of insulin and the ratio of CSF insulin to plasma insulin levels is reduced in obesity, T2DM and in inflammatory conditions[21]. Further, the CSF to plasma ratio is reduced in humans with systemic insulin resistance[22], in elderly individuals and in patients with neurodegenerative diseases such as Alzheimer disease[23,24]. Hence, decreased transport or a saturated transport capacity of insulin into the brain might contribute to reduced brain insulin signalling and action in these conditions and contribute to brain insulin resistance in metabolic diseases, ageing and neurodegenerative conditions.

Insulin induces its cellular action by binding to the extracellular α-subunits of insulin receptors, which induces the dimerization and autophosphorylation of the intracellular β-subunits, followed by tyrosine phosphorylation of insulin receptor substrate proteins, phosphoinositide 3-kinase (PI3K) activation and AKT phosphorylation as key signalling nodes. Insulin-like growth factor 1 (IGF1) also signals through the insulin receptors, albeit with lower binding affinity, and both insulin and IGF receptors can initiate similar intracellular signalling pathways[25,26] (FIG. 1).

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Key points

- Insulin crosses the blood–brain barrier via a saturable transport to bind to insulin receptors widely expressed throughout the brain; insulin signalling in the brain regulates systemic nutrient partitioning in animal models and humans.
- Brain insulin action controls appetite, adipose tissue lipolysis, hepatic triglyceride secretion and branched-chain amino acid metabolism, protecting the organism from ectopic lipid accumulation and lipotoxicity.
- The role of brain insulin in suppressing hepatic glucose production remains controversial.
- Overnutrition rapidly induces brain insulin resistance even before peripheral insulin signalling is impaired, implicating brain insulin resistance as a key culprit of metabolic disease and diabetes.
- Tools to assess brain insulin action in humans are limited and involve MRI techniques, PET, magnetoencephalography and electroencephalography.
- Pharmacological interventions to improve brain insulin signalling have therapeutic potential for metabolic disease, diabetes and non-alcoholic fatty liver disease; augmenting brain insulin signalling might be particularly beneficial in preventing lipotoxicity with a low risk of hypoglycaemia.

In neurons, insulin plays key roles in neurosynaptic functioning[27–30]. Insulin enhances neurite outgrowth, modulates catecholamine release and uptake, regulates the expression and localization of *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and γ-aminobutyric acid (GABA) receptors, modulates synaptic plasticity[31,32], and promotes neuronal survival by inhibiting apoptosis[33]. The insulin receptor is also expressed in 20–40% of all non-neuronal glia cells in the human brain; these cells provide critical physical and nutritional support to neurons[34,35]. In astrocytes, the insulin receptor regulates glucose uptake across the BBB and participates in brain glucose sensing[36].

Insulin signalling in the CNS regulates energy homeostasis through several mechanisms. Studies performed in rodents and non-human primates provide evidence that insulin decreases food intake and increases satiation in a dose-dependent manner[37–44], although the robustness of these anorexic effects remains controversial[45]. In humans, intranasal insulin administered to preferentially deliver insulin to the CNS and activate brain insulin signalling seemed to reduce food intake after a single dose in men but not in women[46]. Furthermore, chronic intranasal insulin administration only led to a mild reduction in body weight and adipose mass in men but not in women[47], suggesting sex-specific differences in the overall moderate effects of brain insulin in the regulation of appetite. Possibly more important to energy homeostasis are the systemic metabolic effects of brain insulin in orchestrating peripheral nutrient partitioning, in particular in liver and adipose tissue and, in consequence, the contribution of brain insulin resistance to metabolic disease, T2DM and obesity.

Brain insulin resistance

Brain insulin resistance is best defined as the impaired physiological actions of insulin in the brain. It can result from reduced BBB transport of insulin (as mentioned previously[48,49]) and due to impaired molecular signalling originating from the insulin receptor or the IGF receptor. Brain insulin resistance can also be present when cellular insulin signalling emanating from the insulin receptor or the IGF receptor is still intact[50]. Hence, organismal and brain insulin resistance might be present even though cellular insulin signalling is intact because the physiological actions of insulin can be impaired even when intracellular insulin signalling is still intact and vice versa. For example, functional brain insulin resistance can occur when the neuronal transmission of an insulin-induced signal is impaired even though cellular insulin signalling is not impaired as in the setting of increased endocannabinoid tone[50].

Impaired cellular insulin signalling can result from the reduced activation of the intracellular insulin signalling cascade, for example, through the increased expression of phosphatases (such as protein-tyrosine phosphatase 1B (PTP1B)) that dephosphorylate the insulin receptor and insulin receptor substrate proteins at their tyrosine residues and thereby reduce insulin signalling[51,52]. Overfeeding and hypothalamic inflammation have been shown to reduce neuronal insulin signalling[53]. Further, the reduced expression of insulin receptors has been described as a possible mechanism of insulin resistance in peripheral tissues in obesity[54]; however, the mechanisms have not been clearly established for brain insulin resistance.

The physiological consequences of brain insulin resistance can manifest as the impaired ability to regulate central or peripheral metabolism but might also impair mood and cognition. Early triggers of brain insulin resistance remain incompletely understood but can be induced by increasing circulating fatty acids and by the activation of microglia and astrocytes that release inflammatory cytokines, which act upon neurons and impair intracellular insulin signalling[53,55]. Other mechanisms that have been implicated in brain insulin resistance are endoplasmic reticulum stress[56–58] and the increased expression of negative regulators of insulin signalling, such as SOCS3 (REF.[58]), p70-S6 kinase[2] and PKCθ[59], in response to a high caloric intake.

In the following sections, we review the effects of brain insulin action on nutrient partitioning, all of which can be elicited by increasing brain insulin and occur independently of changes in plasma levels of insulin and glucose. Key pathways regulated by brain insulin include lipolysis and de novo lipogenesis in adipose tissue[60–62], hepatic triglyceride secretion[63], hepatic catabolism of branched-chain amino acids (BCAAs)[64], brown adipose tissue (BAT) thermogenesis, and the suppression of hepatic glucose production[65–67]. The latter remains a controversial topic within the scientific community. We do not review the vast literature on the genetic approaches to delete the insulin receptor conditionally in specific neuronal populations as this has been summarized in a series of excellent reviews[68–71]. However, we provide a condensed overview of these studies in BOX 2.

Cell-specific neuronal insulin receptor knockout mouse models have provided a wealth of insight into the distributed neuronal network in which brain insulin signalling partakes in metabolic control[68–71]. However, it is important to keep in mind that the cellular effects of insulin vary widely and understanding the complexity of the actions of insulin in different developmental

stages, brain regions and physiological states remains a major challenge for anybody working in this field.

Lifelong Cre-loxP knockout models, which are predominantly used to study the conditional loss of a specific protein in a specific target tissue, cell type or brain region, have several limitations in identifying neurons that mediate the acute effects of brain insulin in energy metabolism. First, during development, the Cre drivers are sometimes expressed in different neuronal populations and the adult phenotype might result from these developmental effects. This point is particularly pertinent when considering agouti-related peptide (AgRP) and/or pro-opiomelanocortin (POMC)-expressing neuronal knockout mouse models[72]. The changes in expression during development might explain why the targeted genetic ablation of the leptin receptor in POMC or AgRP neurons using CRISPR–Cas9 show divergent results compared with conventional genetic ablation using the Cre-loxP system[73].

Second, even if the deletion of insulin receptors in a specific neuronal group blunts the ability of insulin to regulate a given metabolic function, this does not necessarily mean that the particular neuronal population is the main target. For example, it might just mean that the balance within the neuronal network is disturbed and this imbalance renders the brain insensitive to the acute effects of insulin. Hence, in the following sections, we intentionally focus on brain insulin infusion studies in defining brain insulin action. The advantage of these studies is that they define the acute effects of insulin in regulating energy homeostasis. That said, we do acknowledge the limitations of this approach in that it is difficult to ascertain whether the insulin doses are indeed physiological despite the use of doses that should approximate the doubling to tripling of brain insulin concentrations observed during a meal[74,75]. As rat CSF insulin levels have been reported to be in the range of 2–3 μU/ml (REF.[76]), an intracerebroventricular insulin infusion of 5 μU per hour, which was commonly used by us and others[60,63,65,67], should increase CSF insulin levels to a similar range (given the CSF volume of a rat is 0.6 ml (REF.[77]) and the half-life of insulin in CSF in rodents is about 30 minutes[78]). Thus, stereotaxic insulin infusion studies using low insulin doses in the microunits per hour scale simulate brain insulin excursions in the physiological range.

Box 1 | **Key metabolic functions and effects of brain insulin action**

Evidence from animal studies

- Food intake: downregulated in baboons[44], chickens[236], mice[58,237] and marmots[238]; mixed results in rats: lower in some studies[43,239] and unchanged in others[45,240].
- Hepatic glucose production: suppressed after hours of brain insulin infusion in rodent pancreatic clamp studies[67]; no change in plasma levels of glucose for up to 4 hours after initiation of brain insulin infusions in dogs[235]: unaltered hepatic glucose production, lower hepatic gluconeogenic gene expression, higher hepatic glucose uptake and glycogen synthesis leading to reduced net hepatic glucose output.
- Suppressed lipolysis and increased de novo lipogenesis via the suppression of sympathetic nervous signalling outflow to white adipose tissue[60], leading to an anabolic effect on white adipose tissue.
- Higher uptake of fatty acids[118] (CD36 dependent).
- Loss of insulin receptors in the brain leads to lipodystrophy[62].
- Increased hepatic VLDL secretion lowers hepatic lipid content and protects from non-alcoholic fatty liver disease[63].
- Stimulation of hepatic branched-chain amino acid (BCAA) catabolism leads to increased BCAA degradation and circulating BCAAs[64].
- Increased thermogenesis and brown adipose tissue activation[132,134].
- Stimulation of white adipose tissue browning after the co-administration of insulin and leptin to the central nervous system[142].

Evidence from human (intranasal insulin) studies

- Food intake (short term): lower in men[46,177], unchanged in women[46]; increased postprandial satiety, decreased appetite for palatable foods in women[241]. Body weight (long term): decreased in men, unchanged in women[47].
- Decreased endogenous glucose production, independent of peripheral insulin signalling (clamped condition, effect after a few hours following intranasal insulin)[170]; decreased endogenous glucose production during a hyperinsulinaemic clamp[206]; no effect in the fasting state[242]; no acute (within minutes) glucose-lowering effect[174].
- Decreased whole-body lipolysis[61] and circulating free fatty acids[111,170,243].
- Acute reduction in hepatic lipid content after intranasal insulin in men[111].
- No change in hepatic lipid content after 4 weeks of (chronic) intranasal insulin[130].
- Decreased circulating levels of BCAAs after 2 and 4 weeks of intranasal insulin[130].
- Unchanged triglyceride-rich lipid particle production in the constant-fed state or clamp conditions[243]; no data on VLDL secretion in the fasted state in humans.
- Increase in postprandial thermogenesis[138].

Brain insulin and WAT metabolism

White adipose tissue (WAT) plays a critical role in energy homeostasis both as an endocrine organ and as a storage organ of energy-rich triglycerides. Obesity is often associated with dysfunctional WAT[79–81], which is the source of excess free fatty acids and inflammatory mediators that cause and worsen insulin resistance[82]. A key function of WAT is to hydrolyse stored triglycerides through lipolysis and to release glycerol and fatty acids during fasting or energetically demanding states, such as cold exposure, exercise and infection, as well as to provide energy substrates, for example, for thermogenesis and gluconeogenesis[83,84]. In metabolic disease and obesity, lipolysis is inappropriately high in the postprandial state. Unrestrained lipolysis increases gluconeogenesis and thereby hepatic glucose output at a time when it should be low. WAT is also capable of synthesizing fatty acids during de novo lipogenesis. This process enables the conversion of excess non-lipid nutrients into lipids that can be safely stored in adipose tissue and might be the source of important signalling molecules[85–87]. De novo lipogenesis is reduced in the adipose tissue of people with obesity, which is a hallmark of adipose tissue dysfunction[88–91].

Catecholamines, in particular noradrenaline, are key hormones that drive lipolysis in WAT. Noradrenaline is released from sympathetic fibres and terminals that densely innervate WAT. Insulin is considered the major anti-lipolytic[83] and pro-lipogenic regulator[92] in WAT. Insulin infusion into the medial basal hypothalamus (MBH) acutely restrains lipolysis in rats as assessed by the rate of appearance of glycerol measured through the tracer dilution technique, WAT triglyceride hydrolase activity and a hormone-sensitive lipase activation (HSL) state[60], probably by reducing sympathetic outflow to WAT.

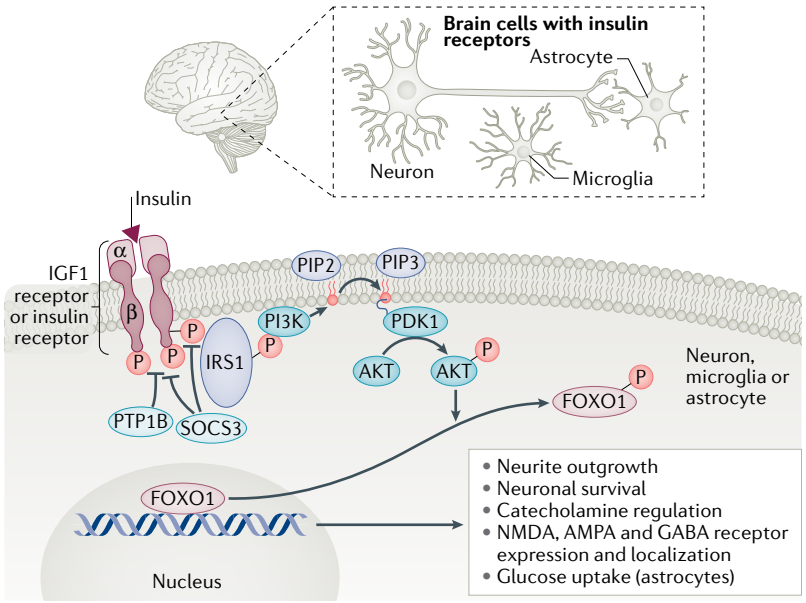

Fig. 1 | **Brain insulin signalling cascade.** Insulin receptors are found in many regions and cell types in the brain[6–9]. Insulin induces its cellular action by binding to the α-subunit of the insulin receptor, which leads to the activation of the insulin receptor tyrosine kinase, causing autophosphorylation of the receptor on tyrosine residues and tyrosine phosphorylation of insulin receptor substrate (IRS) proteins. Conversely, protein-tyrosine phosphatase 1B (PTP1B)[51,52] and suppressor of cytokine signalling 3 (SOCS3)[58] dephosphorylate and thereby inactivate the insulin receptor. Phosphorylated tyrosine sites on IRS proteins enable the binding of phosphatidylinositol 3-kinase (PI3K), which converts phosphatidylinositol (3,4)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the plasma membrane[26]. This process recruits phosphoinositide-dependent protein kinase 1 (PDK1) to phosphorylate AKT as the key signalling node. AKT regulates several target proteins of the insulin signalling pathway, such as the transcription factor FOXO1, which, upon phosphorylation, is excluded from the nucleus[151], resulting in the inhibition of the FOXO1-mediated transcription of genes that regulate several neuronal functions.

Of note, and as discussed in more detail later in the Review, in one study, the ability of MBH or intracerebroventricular-infused insulin to suppress lipolysis precedes the suppression of hepatic glucose production and occurred before a clamp was initiated. Furthermore, conditional deletion of the insulin receptor in neurons (that is, neuronal insulin receptor knockout (NIRKO)) of mice results in increased lipolytic rates in the fasted state and the altered suppression of free fatty acid release after refeeding[60]. Conversely, NIRKO mice exhibit suppressed de novo lipogenesis in WAT, which coincides with a decrease in WAT palmitoleate levels, a lipokine with potential anti-inflammatory properties[60,93].

Similar to the NIRKO mouse model, mice that lack insulin receptors on POMC neurons from birth lack the ability to suppress lipolysis during a hyperinsulinaemic clamp as assessed by the glycerol tracer dilution technique[94]. Interestingly, AgRP insulin receptor knockout mice showed normal rates of lipolysis despite a lack of suppression in hepatic glucose production. These data suggest that brain insulin requires the melanocortin pathway to regulate adipose tissue lipolysis but that insulin signalling in the melanocortin system is less important in regulating hepatic glucose production. In keeping with this, while insulin inhibits the electrical activity of POMC neurons[95,96], the infusion of a melanocortin receptor agonist into the third ventricle increases lipolysis and the sympathetic drive to WAT[97]. Therefore, the anti-lipolytic effects of insulin are mediated via direct effects on adipocytes antagonizing adrenergic signalling[98] as well as by indirect effects in the brain. The indirect effects are probably due to a reduction in sympathetic outflow to WAT, which is evidenced by a reduction in HSL activation and perilipin phosphorylation[60]. HSL activation and perilipin phosphorylation have been shown to closely reflect sympathetic input to WAT as they are directly regulated by adrenergic signalling[99,100], although other confirmations, such as nerve recordings or noradrenaline turnover studies or any other method of monitoring sympathetic activity, have not been used to confirm this finding. The loss of insulin signalling in the brain causes WAT dysfunction by unrestraining lipolysis and reducing lipogenic capacity as shown in studies in which a series of complementary approaches to alter brain insulin where utilized[3,60,85].

The 3 day overfed Sprague–Dawley rat, which is a model for common forms of obesity, develops systemic insulin resistance and exhibits severely impaired hypothalamic insulin action, defined as the ability of brain insulin to suppress WAT lipolysis and glucose production[3]. The systemic and adipose tissue insulin resistance in this model is not due to impaired insulin signalling in WAT[3], which indicates that insulin action in WAT can be impaired before peripheral insulin signalling defects become apparent, possibly due to increased sympathetic outflow. Importantly, short-term overfeeding in humans produces similar insulin resistance[4,5,101–103] and, as obesity in men is associated with functional brain insulin resistance[104], it suggests that unrestrained lipolysis and reduced lipogenic capacity in WAT[105] might, to a large part, be explained by impaired hypothalamic insulin action. Whether the restoration of brain insulin is sufficient to prevent adipose tissue insulin resistance in overfeeding-induced insulin resistance remains to be examined.

These data are compatible with a role of brain insulin signalling in the pathogenesis of insulin resistance driving hyperglycaemia and dyslipidaemia in states of hyperalimentation. As WAT lipolysis provides the gluconeogenic precursor glycerol and energy substrates in the form of fatty acids, which drive hepatic gluconeogenesis and therefore hepatic glucose production, impaired brain insulin action might contribute to overfeeding-induced hepatic insulin resistance by unrestraining adipose tissue lipolysis[4,5].

Brain insulin and liver lipids

The secretion of triglycerides in the form of VLDL protects the liver from excessive lipid accumulation and steatosis[106]. The intracerebroventricular infusion of insulin promotes triglyceride export from the liver[63]. This anti-steatotic effect of brain insulin occurs independent of peripheral (and more specifically direct hepatic) insulin signalling and does not induce de novo lipogenesis in the liver, a key contributor to hepatic steatosis[107]. Of note, hepatic insulin signalling suppresses VLDL secretion[108,109] and causes at least a transient increase in hepatic triglyceride content, which is also detected after a meal in humans[110].

In agreement with these findings, an acute intranasal insulin bolus, a mode to preferentially deliver insulin to the CNS and activate brain insulin signalling, reduces hepatic lipid content, while short-term systemic hyperinsulinaemia (during which insulin also acts directly on the liver) leads to an increase in liver fat content in humans[111]. These opposing effects on hepatic VLDL secretion by brain and hepatic insulin signalling is the only scenario that we are aware of where brain and peripheral insulin effects oppose each other instead of complementing and synergizing with each other as is the case for the other metabolic pathways regulated by brain insulin.

Deletion of the insulin receptor in the CNS reduces hepatic lipid export in mice[63]. Conversely, a loss of insulin receptors in peripheral tissues only increases hepatic triglyceride secretion[63]. A plausible explanation for the latter might be that the systemic hyperinsulinaemia (due to reduced hepatic insulin clearance and insulin resistance) and the increased insulin levels enhance brain insulin signalling (which is intact) in this mouse model. Thus, it is conceivable that brain insulin, as a promoter of hepatic triglyceride export, counterbalances the direct insulin effects in the liver. An imbalance between central and hepatic insulin action could potentially lead to steatosis. As a high-caloric diet can rapidly induce brain insulin resistance in rodents (a finding that is also supported in humans) even before signalling defects at the level of the hepatocyte can be detected[2,3,104], the phenomenon of brain insulin resistance provides a potential mechanism through which overnutrition leads to the development of hepatic steatosis as is often seen in the metabolic syndrome.

Insulin is not the only regulator in the brain that can regulate hepatic lipid handling; other regulators include neuropeptide Y (NPY)[112], nutrients such as glucose[113] or lipids[114], and the adipocyte-derived hormone leptin. Brain leptin, like insulin, also has anti-steatotic effects by enhancing hepatic triglyceride secretion and reducing liver de novo lipogenesis independent of changes in body weight. The anti-steatotic effects of brain leptin require vagal efferences originating from the dorsal vagal complex. Similar to brain insulin, brain leptin protects the liver from excessive lipid accumulation even in metabolically challenged obese rats[115], which might explain why treatment with recombinant leptin in patients who are hypoleptinaemic because they have lipodystrophy profoundly improves hepatic steatosis[116,117]. Taken together, these data provide evidence that the brain integrates the sensing of nutrients and peripheral hormones to fine-tune lipid handling in the liver. Brain insulin signalling protects the liver from ectopic lipid accumulation and lipotoxicity twofold — by enhancing triglyceride secretion in the liver[63] and by reducing lipid flux to the liver through its anti-lipolytic effects in WAT[60,63]. As brain insulin can also increase fatty acid uptake into WAT[118], insulin action in the brain promotes lipid flux from the liver into WAT, thus protecting other organs from excessive lipid accumulation (FIG. 2).

In states of aggravating insulin resistance, when insulin levels increase, the saturable transport of insulin across the BBB creates an imbalance between peripheral and central insulin signalling. In other words, the brain is exposed to relative hypoinsulinaemia compared with the periphery[119]. This could be relevant in the development of non-alcoholic fatty liver disease (NAFLD), the classic organ manifestation of the metabolic syndrome in the liver. While insulin signalling in the brain protects from NAFLD[111] by increasing hepatic triglyceride export[63] and blocking lipolysis and hepatic lipid influx[60], insulin signalling at the level of the hepatocyte reduces plasma levels of triglycerides at the cost of increasing hepatic lipid backup (FIG. 3).

Brain insulin and BCAA metabolism

The BCAAs leucine, isoleucine and valine are essential amino acids that are elevated in obesity and T2DM and are sensitive predictors for the future risk of developing T2DM[120–126]. Improvements in insulin sensitivity in response to a dietary and/or behavioural weight loss

Box 2 | The role of brain insulin receptors in regulating systemic insulin action

Findings from the neuronal and glial insulin receptor knockout (NIRKO) mouse
High-fat diet (HFD)-sensitive obesity; increased white adipose tissue mass; mild insulin resistance at 4–6 months of age (increase in fasting insulin levels, no change in glucose)[148]; impaired adipose tissue insulin action (incomplete suppression of lipolysis during hyperinsulinaemic clamp and refeeding)[60]; impaired response to hypoglycaemia[244] and cold exposure[135]; increased anxiety and depression-like behaviour[245]; impaired fertility[148].

Findings from the agouti-related peptide insulin receptor knockout mouse
No change in body weight, food intake, fasting insulin or glucose levels, and glucose tolerance on both regular chow and HFD[162]; impaired hepatic insulin action (fails to fully suppress hepatic glucose production during a hyperinsulinaemic clamp)[94,162]; normal adipose tissue insulin action (unimpaired suppression of lipolysis during a hyperinsulinaemic clamp)[94].

Findings from the POMC insulin receptor knockout mouse
No change in body weight, fat mass[161,162], food intake, fasting insulin or glucose levels, and glucose tolerance on both regular chow and HFD[162]; normal hepatic insulin action[94,162]; impaired adipose tissue insulin action (incomplete suppression of lipolysis during hyperinsulinaemic clamp and refeeding)[94]; lower respiratory exchange ratio as a sign for increased lipid utilization[94]; mice develop a fatty liver on HFD[94].

Findings from the L1 mouse (>90% reduction in ARC and PVN insulin receptors)
Unchanged plasma lipids, fasting glucose and body weight; increased fasting insulin; impaired hepatic insulin action[246].

Findings from targeted insulin receptor knock-ins in L1 mice
Insulin receptor knock-in agouti-related peptide normalizes hepatic insulin action[163]. Insulin receptor knock-in POMC leads to increased hepatic glucose production, locomotor activity and energy expenditure[163].

Findings from the SF1 insulin receptor knockout mouse (VMH)
Under regular chow, no change in food intake and body weight. HFD challenge resulted in protection from obesity and insulin resistance and improved leptin sensitivity[247].

Findings from the sensory neuronal insulin receptor knockout (SNIRKO) mouse
No change in body weight and circulating glucose; increased insulin levels and lower glucose tolerance[248].

Findings from the insulin receptor and IGF1R double knockout mouse (hippocampus or amygdala)
Lower glucose tolerance and cognition; enhanced depression-like behaviour[137].

Findings from the inducible NPY insulin receptor knockout mouse
Increased body weight, food intake and fat mass; lower energy expenditure[249].

Findings from inducible insulin receptor knockout in astrocytes
No change in body weight or food intake on HFD and regular chow; altered dynamic feeding behaviour in response to acute fasting and hyperglycaemia; lower glucose tolerance, insulin secretion and insulin sensitivity[36].

intervention or to bariatric surgery are associated with a reduction in circulating levels of BCAAs[122]. Insulin dose-dependently reduces plasma levels of BCAA through the induction of hepatic protein expression and activity of branched-chain α-keto acid dehydrogenase (BCKDH), the rate-limiting enzyme in the BCAA degradation pathway[127]. Hypothalamic insulin infusion in rats and genetic modulation of brain insulin receptors in mice demonstrated that brain insulin signalling is a key regulator of BCAA metabolism by inducing hepatic BCKDH[64].

In rats, short-term overfeeding impairs the ability of brain insulin to reduce BCAAs, while high-fat feeding in non-human primates and obesity and/or T2DM in humans are associated with reduced levels of BCKDH protein in the liver[64]. In addition, BCAAs are a major substrate for de novo lipogenesis in adipose tissue[128] that, as described earlier, is under the control of brain insulin[60]. Of note, in adipose tissue, BCAAs are major substrates for the synthesis of branched-chain fatty acids, a class of lipids that can exhibit insulin-sensitizing properties[87]. Hence, brain insulin infusions induce BCAA metabolism by increasing hepatic BCAA catabolism and by increasing the utilization of BCAA for de novo lipogenesis in adipose tissue, which is suggestive of a key role for brain insulin action in BCAA homeostasis and is consistent with the notion that brain insulin resistance might be a culprit for the elevated BCAA levels observed in obesity[64]. Furthermore, plasma levels of BCAA might be a marker of brain insulin action as supported by the finding that, in mouse models of Alzheimer disease, brain insulin signalling is impaired and BCAA levels are increased[129]. In humans, the long-term intranasal administration of insulin reduced the plasma levels of BCAA, indicating that plasma levels of BCAA might also serve as markers of brain insulin action in humans[130].

Brain insulin and thermogenesis

BAT is a key organ in energy homeostasis owing to its role in thermogenesis and the high energetic demands that are required for this process[131]. There is evidence for a role of brain insulin action in thermogenesis and energy expenditure. For example, intracerebroventricular insulin infusion in fed but not in fasted rats promotes BAT activation[132]. Furthermore, mice injected with insulin into the preoptic area (a region of the hypothalamus involved in core body temperature regulation and where sympathetic outflow to BAT originates[133]) display hyperthermia in a dose-dependent manner. The observed increase in body temperature is probably mediated by the activation of BAT thermogenesis as demonstrated by an increase in ^{18}F-FDG uptake into BAT[134]. Conversely, NIRKO mice, which lack the insulin receptor in all neurons, are intolerant to the cold and show a rapid drop in body temperature in response to a 4 °C cold exposure challenge, which is consistent with a defect in thermogenesis[135]. Similar to insulin, IGF1 infusion into the preoptic area of male C57BL/6J mice also induces BAT thermogenesis and hyperthermia, which at least partially relied on intact neuronal insulin receptor expression[136]. Furthermore, analogous to the NIRKO mouse model, the genetic ablation of both insulin and IGF1 receptors in the amygdala of mice results in a drop in body temperature in response to cold stress but without significant changes in BAT activation as assessed by the expression of uncoupling protein 1 (UCP1)[137]. Hence, these studies suggest that brain insulin signalling is required for proper thermoregulation, at least in mice.

Human data on brain insulin action and thermogenesis are scarce. Intranasal insulin administration in humans moderately increases postprandial energy expenditure in one study using indirect calorimetry[138]. However, the postprandial increase in energy expenditure

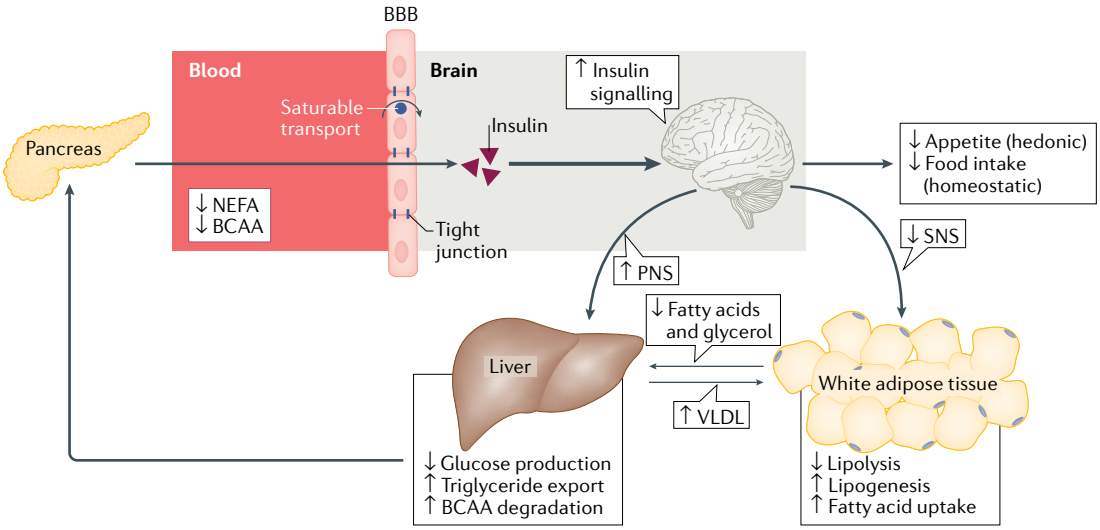

Fig. 2 | **Physiological functions of brain insulin action.** Insulin secreted from the pancreas crosses the blood–brain barrier (BBB) to bind to insulin receptors expressed throughout the brain[16–20]. Insulin might act as an anorexic signal[37–44] but, more importantly, it controls nutrient partitioning in peripheral organs such as the adipose tissue or the liver via the autonomic nervous system. Brain insulin action thereby regulates lipolysis and lipogenesis[60], fatty acid uptake[118], hepatic triglyceride secretion[63], branch-chain amino acid (BCAA) metabolism[64,130], thermogenesis[132,134], and hepatic glucose production[67,157] and, through these actions, it prevents lipotoxicity. NEFA, non-esterified fatty acid; PNS, peripheral nervous system; SNS, sympathetic nervous system.

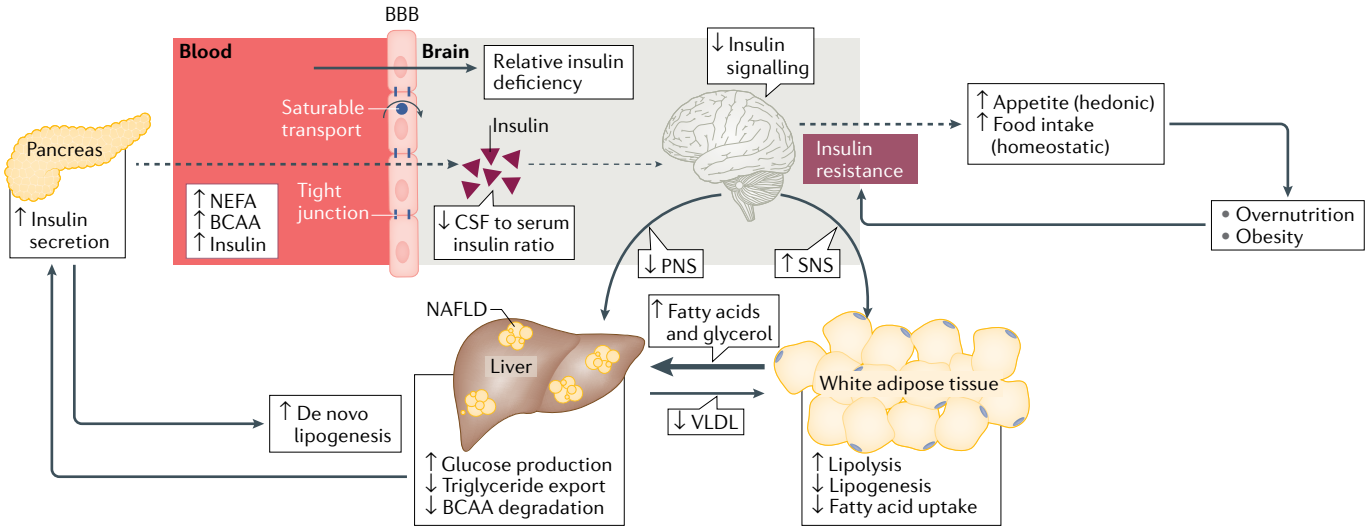

Fig. 3 | **Brain insulin resistance and its metabolic sequelae.** Overnutrition and obesity induce brain insulin resistance and systemic hyperinsulinaemia, in part owing to the reduced and saturable transport of insulin across the blood–brain barrier (BBB). Impaired brain insulin action leads to unrestrained lipolysis in white adipose tissue, driving lipotoxicity. Hepatic triglyceride export is inadequate to compensate for the increased lipid influx from white adipose tissue due to the preponderance of peripheral insulin action at the level of the liver. The imbalance between peripheral and central insulin signalling promotes de novo lipogenesis and reduces hepatic triglyceride secretion, while VLDL secretion that is facilitated by brain insulin signalling is reduced. Hence, brain insulin resistance plays an important role in overnutrition-induced hepatic steatosis and adipose tissue dysfunction, which in turn disrupt glucose homeostasis. Dashed lines indicate that the insulin effect is impaired. BCAA, branched-chain amino acid; CSF, cerebrospinal fluid; NAFLD, non-alcoholic fatty liver disease; NEFA, non-esterified fatty acid; PNS, peripheral nervous system; SNS, sympathetic nervous system.

is not entirely accounted for by increases in thermogenesis but consists of the sum of both heat generation in BAT and the energy required for nutrient digestion, metabolism and storage[139]. Thus, it is unclear to what extent thermogenic effects and BAT activation in humans are controlled by brain insulin signalling.

In addition to classic BAT, brown-like (or beige) adipocytes embedded in WAT depots also contain multilocular lipid droplets and express UCP1 and therefore have thermogenic properties[140]. Beige adipocytes can switch from energy storage to expenditure upon environmental stimuli such as cold, a process referred to as WAT browning. Although the relative contribution of brown versus beige adipocytes to whole energy expenditure is not well defined, both cell types can significantly improve energy metabolism and insulin sensitivity in mammals[141]. Notably, brain insulin action can affect WAT browning as the co-infusion of insulin and leptin into the CNS using a stereotaxic intracerebroventricular cannula in mice synergistically promotes WAT browning and energy expenditure, resulting in a lean phenotype[142]. The effects were partially inhibited by sympathetic denervation of inguinal adipose depots or pharmacological inhibition of the PI3K pathway in the arcuate nucleus, which suggests that insulin and leptin act via a central mechanism. In keeping with a role of brain insulin in WAT browning, the differential hypothalamic expression of T cell protein-tyrosine phosphatase (TCPTP; an insulin receptor phosphatase and negative regulator of insulin signalling) that is induced by fasting and repressed by feeding modulates insulin sensitivity in AgRP and/or NPY neurons, thereby coordinating the feeding-induced increase in energy expenditure by promoting WAT browning[143]. Notably, this programme was disrupted in obese mice while hypothalamic TCPTP deletion restored feeding-induced WAT browning.

In summary, brain insulin action, at least in rodents, can modulate BAT function and WAT browning, which can affect energy metabolism and body weight. In addition, the neuronal insulin receptor seems to be required for proper thermoregulation in cold stress and feeding-induced thermogenesis, which is pivotal for maintaining energy balance. However, it remains unclear whether the cold intolerance observed in the neuronal insulin receptor knockout models is due to impaired BAT function or to other mechanisms, such as impaired shivering thermogenesis. Further studies are required to define the role of brain insulin action in BAT function and WAT browning in humans.

Control of pancreatic insulin secretion

The CNS can regulate pancreatic insulin secretion. Early evidence for this phenomenon came from Woods et al. in the late 1960s and early 1970s showing that pancreatic insulin secretion can be conditioned using Pavlovian behavioural paradigms[144]. There is evidence that brain insulin action could have a role in this effect as insulin delivery into the CSF triggers pancreatic insulin secretion[145,146]. However, the physiological relevance of this effect remains unclear. No major effect of brain-infused insulin on plasma insulin emerged from our studies where insulin was infused intracerebroventricularly and into the MBH in rodents. MBH-delivered insulin sometimes tended to suppress peripheral insulin levels[60], while intracerebroventricularly delivered insulin did not[60,63]. However, we did not observe this consistently[3]

and hence the effect of brain insulin on regulating insulin secretion from the β-cell is probably minor.

Hepatic glucose production

Rodents. Unrestrained hepatic glucose production is an important contributor to fasting hyperglycaemia in patients with T2DM[147]. The role of brain insulin signalling in the regulation of hepatic glucose metabolism was first suggested in studies that reported the intracerebroventricular infusion of insulin-suppressed hepatic glucose production in rats[67]. This finding was congruent with an insulin-resistant phenotype of a lifelong knockout mouse model that lacked the brain insulin receptor[148] and with the observation that the deletion of the hepatic insulin receptor was able to suppress hepatic glucose production[149]. Two studies using mice with a liver-specific double knockout of the insulin receptor and its downstream target FOXO1, which therefore lacked hepatic insulin signalling, had normal suppression of glucose production by insulin in vivo[150,151]. This finding supports the notion that extrahepatic insulin action plays a key role in the regulation of hepatic glucose production. However, the concept that brain insulin is a key site for the rapid and acute effect of insulin to alter glucose fluxes and, in particular, hepatic glucose production remains controversial[152]. Some of the controversy is probably due to the differing experimental protocols that were used to study insulin action; we will hence review this in more detail.

The experimental protocol used in the above rat studies consisted of the intracerebroventricular infusion of insulin for 6 hours. During the last 2 hours of the intracerebroventricular insulin infusion, a euglycaemic clamp study was performed in which systemic insulin levels were kept low (achieved through the infusion of somatostatin and a low dose of replacement insulin)[67]. The purpose of the basal (low insulin) clamp study was to restrict hyperinsulinaemia to the brain while circulating levels of insulin were maintained at low fasting levels; hence, peripheral tissues like the liver did not experience an increase in insulin signalling. The key finding was that, at the end of the 6-hour, intracerebroventricular or MBH insulin infusions and the 2-hour clamps, hepatic glucose production was suppressed by >50%. The suppression of hepatic glucose production resulted from the suppression of hepatic gluconeogenesis and the reduction of gluconeogenic mRNA expression (such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PCK1)); however, peripheral glucose utilization (mostly accounted for by muscle and adipose tissue) was not altered by brain insulin. It is important to note that 4 hours of intracerebroventricular insulin infusion did not suppress hepatic glucose production until the euglycaemic clamp was initiated although, once it was, hepatic glucose production fell rapidly.

If indeed postprandial insulin is supposed to suppress hepatic glucose production via the brain as a major site of action, one would expect this to occur rapidly after the induction of brain insulin signalling. Therefore, it remains somewhat unclear why hepatic glucose production was not suppressed after 4 hours of central insulin infusion but was suppressed only during the clamp period (after 5–6 hours) when somatostatin was infused to suppress endogenous insulin secretion. On the other hand, fewer than 4 hours of brain insulin infusion resulted in markedly reduced lipolysis[60], which was not dependent on the performance of a glucose clamp during which somatostatin was infused, suggesting that brain insulin exerts stronger control over adipose tissue lipolysis rather than having the ability to suppress hepatic glucose production. It is also important to emphasize that the ability of intracerebroventricular or MBH-infused insulin to suppress hepatic glucose production seems to depend on the infusion of somatostatin as that is the key variable that is altered between the basal and the clamp period in this protocol (insulin is intentionally just replaced to maintain and not to change the low (basal) insulin levels and glucose levels are kept constant). Another caveat of this protocol is that it creates relative hypoinsulinaemia in the portal circulation, as has been pointed out[152]. Under physiological conditions, the pancreas secretes insulin into the portal vein, resulting in portal insulin levels that are approximately three times greater than in the systemic circulation, a gradient that is lost when pancreatic insulin secretion is suppressed through somatostatin and when insulin is replaced via a peripheral vein[153].

Overfeeding for just 1 day rapidly disrupts central insulin action and with it the ability of brain insulin to suppress hepatic glucose production in rats[2], again consistent with the notion that brain insulin resistance might account for the impaired hepatic insulin action in obesity and T2DM.

Intriguingly, brain insulin also requires intact ATP-sensitive potassium channel (K_{ATP} channel) activity in the hypothalamus as the infusion of a K_{ATP} channel blocker within the MBH obliterated the ability of brain insulin to suppress hepatic glucose production while the central activation of K_{ATP} channels in the MBH was sufficient to lower blood levels of glucose through the inhibition of hepatic gluconeogenesis[65]. Of note, analogous to insulin, glucose and fatty acids infused intracerebroventricularly or into the MBH similarly suppressed hepatic glucose production, possibly due to the confluency of the insulin and nutrient-sensing pathway in regulating long-chain acetyl CoA levels in the hypothalamus[154,155]. While it is still not clear how insulin activates the K_{ATP} channel, it might not be surprising that the K_{ATP} channel plays a critical role in brain insulin action as it is a terminal regulator of neuronal membrane potential. The intracerebroventricular injection of the K_{ATP} channel opener diazoxide suppressed hepatic glucose production in rats[65,67]. Attempts to directly open the K_{ATP} channel in the MBH using diazoxide given orally to humans to suppress hepatic glucose production seem promising[156], yet a major challenge of this approach will be achieving cell specificity as the K_{ATP} channel is not only expressed in neurons that express the insulin receptor.

Finally, the surgical dissection of the efferent hepatic branch of the vagus nerve negated the effects of intracerebroventricular insulin and reduced the ability of systemic insulin to suppress hepatic glucose production, suggesting that the vagus mediates the effects of hypothalamic and CNS insulin control of hepatic glucose production[157].

Yet, when muscarinic acetylcholine receptor 3, which seems to be the major muscarinic receptor in the liver, was genetically deleted in hepatocytes of mice[158], glucose homeostasis was unaltered, which was surprising. Whether the lack of change in glucose homeostasis means that muscarinic receptors in non-parenchymal liver cells induce paracrine signalling that then acts upon hepatocytes still needs to be examined. Alternatively, the involvement of non-cholinergic neurotransmitters of vagal postganglionic neurons could offer an explanation. However, as of yet, classic postganglionic neurons have not been located within the liver[159].

Another caveat of surgical denervation studies is that surgical dissection of the vagus might alter sympathetic outflow to the liver or adipose tissue. Hence, the failure of brain insulin to suppress hepatic glucose production after a vagotomy could be indirect due to unrestrained sympathetic nervous system outflow to the liver. As sympathetic input and adrenergic signalling in the liver were not monitored in these studies, the role of the sympathetic nervous system in mediating the control of hepatic glucose production in the liver cannot be ruled out.

In summary, these rodent studies provide evidence that brain insulin via a PI3K pathway that eventually regulates K_{ATP} channel activity results in a change in vagal outflow to the liver that suppresses hepatic glucose production. These studies agree with a vast number of mouse studies in which, through genetic manipulation, the insulin receptor was conditionally deleted in specific neuronal populations[160–163] or in which insulin signalling molecules were obliterated[148,164], supporting the notion that CNS insulin action plays a role in the suppression of hepatic glucose production. However, it remains challenging to work out the specific metabolic pathways that account for the glucose phenotype in these lifelong and conditional knockout models.

Dogs. A series of carefully performed studies in dogs failed to confirm a similar role of brain insulin signalling in controlling hepatic glucose production. In part, these discrepancies are due to experimental differences in the respective studies. As has been pointed out, in the above-described rodent studies, insulin was being replaced in a peripheral vein, which abrogates the physiological 3:1 gradient of insulin and glucagon that exists between the liver and the rest of the body[152]. This gradient is maintained by the secretion of these pancreatic hormones into the portal vein and their marked first-pass effect in the liver[165,166]. Hence, during a basal insulin clamp, this results in hepatic insulin deficiency, which further enhances the ability of non-insulin signals, such as sympathetic or parasympathetic inputs to the liver, to regulate hepatic glucose production. In other words, this basal clamp protocol increases the sensitivity of the liver to autonomic signals owing to relative portal hypoinsulinaemia, which is unphysiological.

In dogs, however, it is possible to infuse insulin and glucagon intraportally during a clamp and, when this was done during an intracerebroventricular insulin infusion, hepatic glucose production was not altered during the 4-hour clamp period[167]. Further, when insulin was infused bilaterally into the carotid and vertebral arteries to increase CNS insulin levels 10-fold (a more physiological delivery than an intracerebroventricular infusion) while maintaining the liver-periphery insulin and glucagon gradient[167], brain hyperinsulinaemia was unable to alter hepatic glucose production, gluconeogenesis or glycogenolysis but was able to reduce net hepatic glucose balance as a result of an altered net hepatic glycogen metabolism during the fourth hour of the clamp[167]. However, the brain hyperinsulinaemia did increase the molecular markers of brain insulin action in the liver that had been observed in the rodent studies, such as increased STAT3 activation[168], suggesting that such transcriptional regulators are insufficient to suppress hepatic glucose production. These studies suggest that brain insulin is not as potent at suppressing hepatic glucose production as is systemic insulin when insulin is replaced in a physiological fashion into the portal vein unless one postulates that the difference is mostly due to species differences.

Humans. It is challenging to study brain insulin action in humans as one cannot infuse insulin intracerebroventricularly and even carotid artery cannulation for head delivery involves medical risks. However, intranasal insulin administration is believed to result in insulin being taken up by olfactory and trigeminal nerve fibres ending in the nasal cavity and in increases in CSF concentrations of insulin and is hence considered a brain-specific delivery mode of insulin[169]. Insulin has been administered intranasally at a high dose of 40 IU during a pancreatic clamp using somatostatin with insulin and glucagon infused into a peripheral vein to clamp levels at basal arterial values[170]. This protocol results in a relative hepatic deficiency of insulin and glucagon as previously discussed[153]. Intranasal insulin administration resulted in a modest suppression of hepatic glucose production relative to the control group, in which the leakage of intranasally delivered insulin into the circulation was matched by infusing insulin into a peripheral vein. The use of somatostatin, as we pointed out already, might also affect stress-related outflow pathways[171] within the brain, which might exaggerate the effects of brain insulin on hepatic glucose production.

Similar studies using an even higher dose of intranasal insulin (160 IU) found that intranasal insulin increased the glucose infusion rate required to maintain euglycaemia during a hyperinsulinaemic clamp[172] within 15 minutes. The authors understood this finding to suggest that peripheral insulin sensitivity is rapidly increased in humans by intranasal insulin. However, this study did not carefully examine and match the potential peripheral spillover of intranasal insulin into the circulation via the nasal mucosa that can be observed after an intranasal dose of insulin[170]. Given that the dose was more than three times that of daily endogenous insulin release in humans[173], it is quite possible that such a spillover occurred, which could explain the increase in the glucose infusion rate that was required to prevent hypoglycaemia and maintain euglycaemia, rather than a brain insulin effect.

The intranasal application of 10 IU or 20 IU of insulin every 15 minutes over 6 hours (resulting in 210 IU or

420 IU of insulin in total) led to a minimal decrease of ~5 mg/dl in plasma levels of glucose; a similar lowering occurred when intranasal insulin spillover was matched by peripheral insulin infusion in a control group. Of note, during these studies, no glucose clamp was performed and neither glucose nor somatostatin were infused[174]. In addition, several studies of intranasal insulin administration have not demonstrated changes in arterial insulin, C-peptide or glucose concentrations[175–178]; others have shown at most a 5% decrease in plasma levels of glucose[138,179]. Chronic treatment with intranasal insulin (4 × 40 IU per day for 8 weeks) also did not affect plasma concentrations of insulin or glucose in healthy, lean particiants[47].

In summary, while some studies demonstrate that intranasal insulin administration can inhibit hepatic glucose production in humans, the slow onset of the effect (>3 hours) supports the notion that brain insulin action is insufficient to suppress hepatic glucose production anywhere near to what systemic insulin is able to accomplish within minutes as assessed by hyperinsulinaemic clamps in several species[180–182].

Aside from insulin, in one study, the oral administration of the K_{ATP} channel activator diazoxide was conducted to mimic insulin action in the hypothalamus. This study found that diazoxide administered 3 hours prior to a 4-hour peripheral insulin clamp did not alter hepatic glucose production for 5 hours and only decreased hepatic glucose production by 30% 6–7 hours after the diazoxide administration. While this study carefully controlled circulating levels of insulin, somatostatin was required to do so and with its use come the abovementioned caveats. Further, other sites of diazoxide action besides the hypothalamus cannot be ruled out as diazoxide was given orally[156].

As rodent studies suggested that the vagus mediates the signal initiated by brain insulin, if brain insulin is indeed key to mediating insulin's ability to suppress hepatic glucose production, complete hepatic denervation (as is present after a liver transplant) should lead to profound differences in insulin action. However, denervation had no discernible effect on hepatic insulin action, the caveat being that these patients also receive drugs that might impair insulin action[183–185]. Lastly, two studies examined whether either vagal stimulation or blockade using devices affects glucose homeostasis in humans. Both studies were unable to identify any notable effect on glucose homeostasis, although this might be due to the failure of these devices to actually activate and/or inhibit the vagus as claimed[186,187].

In summary, these studies suggest that the roles of brain insulin and/or its downstream mediators are of only moderate potency in regulating hepatic glucose production but, admittedly, this might still be important in the long-term regulation of glucose homeostasis. Further, intranasal insulin administration is not an ideal tool to restore brain insulin action as brain hyperinsulinaemia might beget hypothalamic insulin resistance due to receptor downregulation on the cell surface of neurons. However, the somewhat limited potency of brain insulin in regulating hepatic glucose production might represent a therapeutic opportunity as one feared adverse effect of insulin treatment — hypoglycaemia — has, to the best of our knowledge, never been reported after intranasal insulin administration[188].

Therapeutic implications

There are several reasons why intranasal insulin administration might not be an ideal and brain-specific mode of insulin delivery. The best evidence that indeed insulin delivered intranasally reaches the brain is the finding that CSF levels of insulin rapidly increase after intranasal insulin administration[176]. Yet, CSF is not just interstitial fluid but is now thought to also carry waste products of the brain[189]; it is therefore conceivable that intranasally applied insulin is preferentially partitioned to the CSF as a way to neutralize a peptide that is administered in a non-physiological way. Secondly, even if intranasal insulin administration leads to increased brain tissue levels of insulin, chronic hyperinsulinaemia in the CNS can promote insulin resistance. The latter concern would also apply to a brain preferential insulin analogue that is administered systemically. For these reasons, it might be more promising to improve insulin signalling in the CNS through insulin sensitizers. Particularly promising targets are the tyrosine phosphatases PTP1B and TCPTP as they dephosphorylate the insulin receptor and are known to play a key role in brain insulin and leptin signalling[52,190]. Furthermore, mice that lack PTP1B in neurons and the CNS are metabolically protected[191]. Importantly, inhibitors of PTP1B and TCPTP administered intracerebroventricularly or intranasally have been shown to improve metabolic control in rodents[192].

We believe that it is important to consider the biology of the opposing effects of brain and liver insulin signalling on hepatic lipid metabolism when developing novel insulin analogues. Pegylated insulin analogues, such as LY2605541, that preferentially target the liver[193,194] were designed with the intention to reduce rates of nocturnal hypoglycaemia and weight gain but were shown to cause hepatic steatosis and to elevate liver function tests during a phase III study[195,196]. One might predict that an insulin analogue that shows reduced brain and adipose tissue insulin signalling increases fatty acid flux to the liver and decreases hepatic triglyceride export, which would increase the risk of hepatic steatosis. Furthermore, subcutaneous insulin analogues vary in their brain bioavailability[197], which might explain differences in their patterns of systemic insulin action. For example, switching patients to insulin detemir results in more favourable weight-gain profiles[198] and even in weight loss[199] compared with other long-acting insulins. The favourable weight effects of detemir could be due to the more stable pharmacokinetics of detemir compared with other insulin analogues but might also be due to the higher brain bioavailability and action[197,200].

Clamp studies in humans show that insulin detemir reduces food intake more potently than regular insulin while having similar glucose-lowering effects[201]. Insulin detemir can modulate cerebrocortical activity recorded by electroencephalography (EEG) and magnetoencephalography (MEG), which suggests that insulin detemir can alter human brain function[201,202]. Taken together, these data suggest that the right balance

between central and peripheral insulin action might be clinically important and relevant. Therefore, insulin analogues that preferentially exert peripheral insulin action might lack critical effects on food intake, energy expenditure, hepatic lipid export, and anti-lipolytic effects and hence have unwanted adverse effects such as an increased incidence of NAFLD. A lack of balance between central and peripheral insulin action might explain the liver steatosis observed in the abovementioned liver-preferential basal insulin, which led to its clinical development being stopped[203].

Human brain insulin action

Assessing brain insulin signalling and/or action in humans is a major challenge. Functional MRI (fMRI) detects changes in cerebral blood flow within a millimetre spatial resolution and is widely used to study neuronal activity[204]. fMRI assumes that cerebral blood flow reflects neuronal and/or glial activity (that is, neurovascular coupling). Thus, the fMRI signal is a surrogate parameter that represents the integrated activity of neurons and cannot distinguish between excitation and inhibition[205]. Despite these shortcomings, fMRI is currently a widely used technique to non-invasively assess human brain function.

Studies combining fMRI with euglycaemic clamps and intranasal insulin administration have been used to assess brain insulin signalling. Intranasal insulin administration decreases regional blood flow in the hypothalamus but also in the striatum and the caudate nucleus, which correlates with its ability to suppress endogenous glucose production[172,206]. Notably, these effects occur roughly an hour after intranasal insulin administration[172,206], seem to be dose dependent[207] and are only observed in lean participants but not in participants with obesity[172,206]. The reduced hypothalamic response to intranasal insulin correlates with visceral adiposity and insulin resistance[208]. In addition, there seems to be a divergent response in prefrontal cortex blood flow after intranasal insulin application in lean individuals versus in those with obesity, whereby lean participants have a reduced cerebral blood flow that correlates with increased insulin sensitivity and reduced food craving[208].

The prefrontal cortex and hypothalamus are, respectively, involved in reward-based decision-making and homeostatic control of body weight[209]. Therefore, neuronal insulin resistance in these key brain regions probably promotes obesity. This idea is supported by the notion that individuals with overweight show a reduced response to intranasal insulin's inhibitory effects on food palatability[210]. fMRI data further suggest that brain insulin modulates food preferences via mesolimbic pathways in such a way that intranasal insulin administration inhibits forward projections from the ventral tegmentum to the nucleus accumbens in insulin-sensitive but not in insulin-resistant individuals[210,211]. Surprisingly, in studies that focused on neuropsychological changes rather than on food intake and glucose metabolism, intranasal insulin administration improved cognitive performance[212] and functional connectivity[213] irrespective of their diabetic or BMI status[212], suggesting that systemic insulin resistance is not necessarily a limiting factor for brain insulin to take effect.

Some select areas of the brain preserve their insulin sensitivity while others become insulin resistant. The fMRI signals triggered by intranasal insulin in the insular cortex[212,214], the mesolimibic system[210,211] and the hypothalamus[172,179,206,208,215] seem to be experimentally reproducible and therefore represent possible therapeutic target regions modulating neurocognition, food reward, appetite and other metabolic effects of brain insulin signalling. However, many of these intranasal insulin studies did not control for a systemic spillover of intranasal insulin via the nasal mucosa. Thus, it cannot entirely be ruled out that changes in circulating insulin and/or metabolites, such as amino acids and fatty acids, could be regulated by brain or peripheral insulin signalling contributing to the observed fMRI signals in response to intranasal insulin.

Other emerging magnetic resonance techniques include pseudo-continuous arterial spin labelling, which uses magnetically labelled water molecules within the arterial blood as an endogenous tracer to quantify regional blood flow, and ^{31}P magnetic resonance spectroscopy, which can assess cerebral energy metabolism (that is, ATP and phosphocreatine)[177,216]. Using these techniques, studies showed that intranasal insulin administration can acutely modulate blood flow[216] and brain energy levels[177].

MEG, a neuroimaging technique that records brain surface activity by measuring neuronal magnetic fields, is another modality used to evaluate brain insulin action in humans[104,217–220]. The main advantage of this technique is its high temporal resolution (millisecond range) as MEG measures neuronal activity directly and does not rely on neurovascular coupling[221]. This advantage comes at the price of lower spatial resolution, especially in subcortical areas, which can be improved to some extent by computational modelling[222,223]. MEG studies on brain insulin action suggest that both systemic hyperinsulinaemia and intranasal insulin administration stimulate surface signals and hence increase brain activity. The insulin-stimulated brain activity recorded with MEG is dampened in individuals with obesity and correlates with insulin sensitivity[104,218]. Notably, insulin-stimulated brain activity measured with MEG and interpreted by the authors as 'cerebral insulin sensitivity' even predicted responsiveness to a lifestyle intervention[220].

EEG, which records the electrical activity of large cortical neuronal groups via electrodes attached to the scalp, has also been used to assess the effects of systemic hyperinsulinaemia and intranasal insulin administration on human brain activity[201,224]. Similar to MEG, EEG has a high temporal resolution, with clear deficits in spatial resolution. Studies show that both systemic hyperinsulinaemia and intranasal insulin administration can lead to changes in electrophysiological activity recorded with EEG within minutes[201,224].

As an alternative to fMRI, cerebral blood flow can also be measured with [^{15}O]H_2O PET. However, owing to the low spatial and temporal resolution compared with fMRI[225] and radiation exposure, PET is not widely used to assess brain insulin action. One study reported no

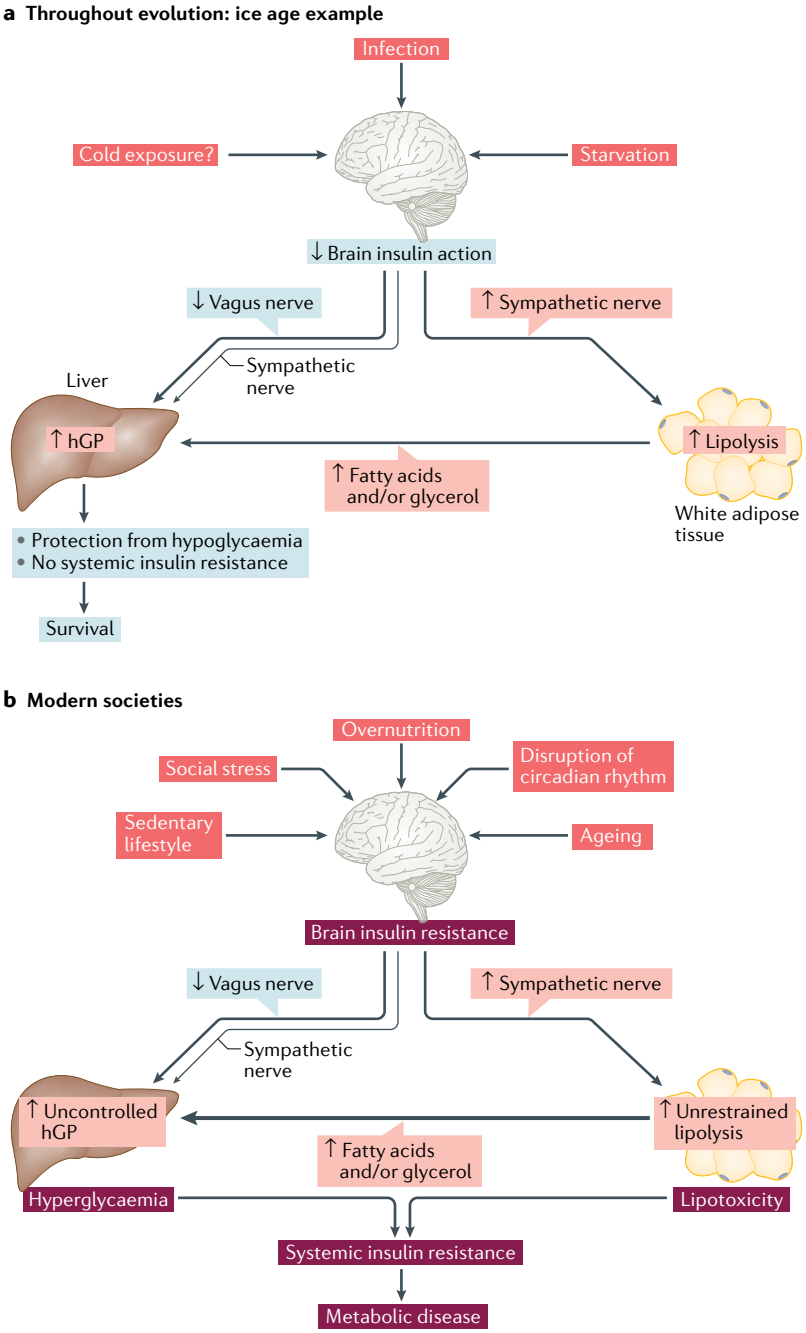

Fig. 4 | **An evolutionary advantage for brain insulin resistance? a** | Throughout mammalian evolution and particularly during the ice ages, cold exposure, starvation and infection were common stressors and a major cause of mortality. The prevention of hypoglycaemia was key to survival as the brain critically depends on glucose as an energy substrate. Brain insulin resistance can be understood as a physiological adaptation to maintain euglycaemia by increasing lipolysis in adipose tissue and augmenting hepatic glucose production (hGP), a process that was probably critical for survival when nutrients were scarce. **b** | In modern societies, overnutrition and an increasingly sedentary lifestyle as well as, possibly, social stress, disruption of circadian rhythmicity and ageing, promote brain insulin resistance as a maladaptive response, which results in the dysregulation of the autonomic nervous system and ultimately ectopic lipid deposition and glucolipotoxicity that further fuels systemic insulin resistance.

changes in cerebral blood flow in response to systemic hyperinsulinaemia[226].

Taken together, the imaging and neurophysiological modalities to non-invasively assess insulin action in humans are manifold. They differ in spatial and temporal resolution and have several limitations. fMRI clearly has the edge but it lacks specificity as a voxel harbours up to 5.5 million neurons[205]. Nevertheless, when combined with intranasal insulin administration under controlled conditions, such as a pancreatic clamp, fMRI offers an additional layer of information that can localize and pinpoint neuronal activity to certain brain regions that can be compared to findings from animal studies. This approach enables researchers to identify brain centres where insulin action occurs and to correlate brain insulin responses with systemic metabolic effects and how they are altered in patients with metabolic diseases.

An evolutionary advantage

The question of why brain insulin resistance develops is still unanswered. This Review has focused on delineating how brain insulin resistance contributes to metabolic disease to elucidate the mechanism through which obesity and overnutrition lead to T2DM. We should, however, consider evolutionary pressures (FIG. 4) to survival that have shaped our biology to explain why hypothalamic and brain insulin might be advantageous in certain circumstances[101,103]. Throughout evolution, but in particular during the ice ages, humans consumed a diet that was low in carbohydrates and needed to endure cold exposure and periodic starvation[227] in addition to infection[228–230]. It is noteworthy that cold exposure[231], starvation[232] and infection[233,234] are conditions characterized by brain insulin resistance. Yet, cold exposure does not lead to systemic insulin resistance, quite the opposite. This finding demonstrates that brain insulin resistance does not necessarily induce systemic insulin resistance as is commonly assumed. In the setting of cold exposure, the increase in substrate mobilization through increased lipolysis might simply represent an advantageous response to the increased metabolic needs imposed by the increased thermogenesis augmented by increased lipolysis and hepatic glucose production. In this scenario, increased substrate mobilization is balanced by increased substrate utilization and hence does not lead to metabolic disease.

Key to survival during cold exposure, starvation and infection is the prevention of hypoglycaemia as the brain critically depends on glucose as an energy substrate. One could speculate that brain insulin resistance represents a physiological adaptation to maintain euglycaemia by increasing lipolysis in adipose tissue and augmenting hepatic glucose production. This biology was probably critical throughout evolution but is much less so today as the threats imposed by cold exposure, starvation and infection have been tamed. Instead, today it seems that primarily overnutrition and an increasingly sedentary lifestyle but possibly also social stress, disruption of circadian rhythms and other lifestyle issues induce brain insulin resistance as a maladaptive response. Hence, we propose that a biology that is best understood through evolutionary pressures allowed mammals to survive the cold, starvation and infection through the transient development of brain insulin resistance but now, in the setting of overnutrition, brain insulin resistance results in glucolipotoxicity

that fuels systemic insulin resistance and is probably a major driver of the T2DM epidemic.

Conclusions

Insulin secreted from pancreatic islets crosses the BBB and binds to insulin receptors expressed throughout the CNS. Brain insulin signalling engages in a complex inter-organ crosstalk, orchestrating nutrient partitioning by regulating appetite, lipolysis, triglyceride secretion and uptake, BCAA metabolism, thermogenesis and hepatic glucose production, all of which ultimately protect the organism from ectopic lipid deposition, lipotoxicity and hyperglycaemia[44,60,63,64,130,142,235]. Overnutrition rapidly promotes brain insulin resistance, well before insulin signalling in peripheral organs is impaired, which suggests that brain insulin resistance represents an early and important mechanism for dyslipidaemia and hyperglycaemia in states of hyperalimentation. Therefore, targeting the brain to prevent CNS insulin resistance and/or to improve insulin action is a promising pharmacological option with a low risk of hypoglycaemia that could reverse the lipotoxicity of metabolic disease.

However, there are considerable roadblocks and additional studies are needed. The main questions that need to be resolved are: does the restoration of insulin signalling ameliorate overnutrition and obesity-related metabolic disease in humans? What is the best pharmacological approach to restore or normalize brain insulin signalling and what is the optimal timing? For example, should we aim for the direct intranasal delivery of insulin or for brain-preferential insulin analogues with an improved ability to cross the BBB even though this local hyperinsulinaemia might induce brain insulin resistance in the long run?

Augmenting brain insulin signalling through brain insulin sensitizers, such as PTP1B and/or TCPTP inhibitors, might avoid the associated risks of local hyperinsulinaemia such as receptor desensitization, which, in our opinion, makes brain insulin sensitizers more promising drug targets.

Another unanswered question is whether existing insulin-sensitizing drugs for T2DM, such as thiazolidinediones or metformin, affect brain insulin signalling. Finally, we propose that brain insulin resistance should be understood from an evolutionary standpoint where it might have represented an adaptive response to enable survival in the setting of metabolic stress. In modern times and in the setting of overnutrition, brain insulin signalling represents a maladaptive response that contributes to the epidemic of metabolic disease we are now facing.

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