BRAIN INSULIN RESISTANCE IN NEURODEVELOPMENTAL AND NEURODEGENERATIVE DISORDERS: MIND THE GAP!

EDITED BY: Eugenio Barone and Mara Dierssen

PUBLISHED IN: Frontiers in Neuroscience and Frontiers in Neurology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-457-6
DOI 10.3389/978-2-88963-457-6

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

BRAIN INSULIN RESISTANCE IN NEURODEVELOPMENTAL AND NEURODEGENERATIVE DISORDERS: MIND THE GAP!

Topic Editors:
Eugenio Barone, Sapienza University of Rome, Italy
Mara Dierssen, Centre for Genomic Regulation (CRG), Spain

The failure of insulin signaling – a condition known as insulin resistance – is a key pathological feature of both type 2 diabetes (T2DM, systemic insulin resistance) and Alzheimer's disease and related dementias (ADRDs, brain insulin resistance) and greatly contribute to their development. Considerable overlap has been identified in the risk factors, comorbidities and putative pathophysiological mechanisms of ADRDs and T2DM, thus proposing AD as type 3 diabetes.

Examination of postmortem AD and amnestic mild cognitive impairment brain uncovered key signs of brain insulin resistance, i.e., reduced insulin receptor (IR) and increased serine phosphorylation (inhibitory) of insulin receptor substrate 1 (IRS1), particularly in the hippocampus, cortex and hypothalamus. Higher levels of insulin resistance markers are associated with poorer performance on cognitive tests of episodic and working memory, independent of the senile plaques and tangles load, thus suggesting a role for insulin signalling in neuronal functions. At the cellular level, these dysfunctions might manifest as the impairment of neuroplasticity, receptor regulation or neurotransmitter release in neurons, or the impairment of processes more directly implicated in insulin metabolism, such as neuronal glucose uptake in neurons expressing GLUT4, or homeostatic or inflammatory responses to insulin. Further, intense research over the last two decades has highlighted the impact of insulin signalling, brain energy balance and their fluctuations on neurogenesis processes both during post-natal and adult life. Indeed, diabetes, obesity, and overweight are prevalent pregnancy complications that predispose offspring to neurodevelopmental disorders. Moreover, impaired neurogenesis compromises hippocampal function and plays a role in cognitive deficits in ADDRs. In those plastic neural tissues, activation of insulin signalling regulates birth, specification, migration, and integration of newly generated neurons, suggesting that alterations of this key signaling transduction pathway may have a role both in neurodevelopmental disorders and adult neurogenesis.

Citation: Barone, E., Dierssen, M., eds. (2021). Brain Insulin Resistance in Neurodevelopmental and Neurodegenerative Disorders: Mind the Gap!. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-457-6

Table of Contents

frontiers in Neuroscience

EDITORIAL
published: 10 August 2021
doi: 10.3389/fnins.2021.730378

Editorial: Brain Insulin Resistance in Neurodevelopmental and Neurodegenerative Disorders: Mind the Gap!

Mara Dierssen[1,2,3,4] *and Eugenio Barone*[5]*

[1] Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain, [2] Department of Experimental Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Spain, [3] Human Pharmacology and Clinical Neurosciences Research Group, Neurosciences Research Program, Hospital Del Mar Medical Research Institute (IMIM), Barcelona, Spain, [4] Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain, [5] Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Rome, Italy

Keywords: insulin resistance, neurodevelopment and brain metabolism, neurodegenerative diseases, learning and memory, brain insulin resistance, Alzheimer's disease, Down syndrome, Parkinson's disease

Editorial on the Research Topic

Brain Insulin Resistance in Neurodevelopmental and Neurodegenerative Disorders: Mind the Gap!

The failure of insulin signaling—a condition known as insulin resistance—is a key pathological feature of both type 2 diabetes (T2DM, systemic insulin resistance) (Arnold et al., 2018; Kellar and Craft, 2020) and brain disorders, such as Alzheimer disease and related dementias (ADRDs, brain insulin resistance) but also other neurodegenerative disorders such as Parkinson's or Huntington's disease (Brás et al., 2019; Hong et al., 2020), neurodevelopmental disorders such as Down syndrome (DS) (Tramutola et al., 2020; Lanzillotta et al., 2021) or autism (Manco et al., 2021) and behavioral disorders (Kleinridders et al., 2015), and greatly contributes to their pathogenesis (Arnold et al., 2018; Kellar and Craft, 2020). Specifically, regarding ADRDs and T2DM, considerable overlap has been found in the risk factors, comorbidities and putative pathophysiological mechanisms, leading to the proposal that AD is type 3 diabetes (Butterfield et al., 2014; de la Monte, 2019). Examination of postmortem AD, amnestic mild cognitive impairment and DS brains, uncovered key signs of brain insulin resistance, i.e., reduced insulin receptor (IR) and increased serine phosphorylation (inhibitory) of insulin receptor substrate 1 (IRS1), as well as reduced activation of pathways downstream from IRS1, particularly in the hippocampus, cortex, and hypothalamus (Talbot et al., 2012; Tramutola et al., 2015, 2020; Sharma et al., 2019). Higher levels of insulin resistance markers are associated with poorer performance on cognitive tests of episodic and working memory, independent of the load of senile plaques and tangles, thus suggesting a role for insulin signaling in neuronal functions (Talbot et al., 2012). At the cellular level, these dysfunctions might manifest as the impairment of neuroplasticity, receptor regulation or neurotransmitter release in neurons (Spinelli et al., 2017; Barone et al., 2019; Franklin et al., 2019; Melo et al., 2020; Lanzillotta et al., 2021), or the impairment of processes more directly implicated in insulin metabolism, such as neuronal glucose uptake in neurons expressing GLUT4, or homeostatic or inflammatory responses to insulin (Bomfim et al., 2012; Lourenco et al., 2013; Barone et al., 2016; Triani et al., 2018; Melo et al., 2020; Lanzillotta et al., 2021). Further, intense research over the last two decades has highlighted the impact of insulin signaling, brain energy balance, and their

OPEN ACCESS

Edited and reviewed by:
Einar M. Sigurdsson, New York University, United States

***Correspondence:**
Eugenio Barone
eugenio.barone@uniroma1.it

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *24 June 2021*
Accepted: *19 July 2021*
Published: *10 August 2021*

Citation:
Dierssen M and Barone E (2021) Editorial: Brain Insulin Resistance in Neurodevelopmental and Neurodegenerative Disorders: Mind the Gap! Front. Neurosci. 15:730378. doi: 10.3389/fnins.2021.730378

fluctuations on neurogenesis processes both during post-natal and adult life (Barone et al., 2014; Arnold et al., 2018; Kellar and Craft, 2020). Indeed, diabetes, obesity, and overweight are prevalent pregnancy complications that predispose offspring to neurodevelopmental disorders (Fusco et al., 2019; Dearden et al., 2020). Moreover, impaired neurogenesis compromises hippocampal function and plays a role in cognitive deficits in ADDRs (Arnold et al., 2018). In those plastic neural tissues, activation of insulin signaling regulates birth, specification, migration, and integration of newly generated neurons, suggesting that alterations of this key signaling transduction pathway may have a role both in neurodevelopmental disorders and adult neurogenesis (Arnold et al., 2018; Kellar and Craft, 2020).

The aim of this Research Topic is to outline the major needs and challenges in the comprehension of the role of insulin signaling and its alterations in the brain and how such alterations contribute to the development of neurodevelopmental and neurodegenerative disorders.

Recently, several studies evaluated the effects of nutrient-related signals on both brain development and cognitive functions. A key finding was the discovery that, other than hypothalamus, a number of brain regions express receptors for hormones known to regulate metabolic processes. In particular, insulin signaling was found to impact on molecular pathways underlying hippocampal plasticity, learning, and memory. Spinelli et al. discuss evidence linking the altered insulin sensitivity in the hippocampus with defects of both adult neurogenesis and synaptic plasticity. These authors also review epidemiological studies and the observations collected in experimental models focusing the attention on the critical role of brain insulin resistance at the crossroad between metabolic and neurodegenerative diseases.

One of the key questions regarding the effects of insulin within the brain, has to do with insulin uptake. Indeed, two sources for insulin in the brain have been identified: (1) circulating insulin produced by the pancreas enters into the brain through a receptor-mediated uptake at the level of blood brain barrier (BBB) (Rhea and Banks; Rhea et al., 2020); or (2) *in situ* synthesis by specific neuronal populations (Nakabeppu, 2019). While the second hypothesis is still under debate, the first one is the most accepted and validated by several studies. Rhea and Banks provide a comprehensive review about the role of the different cell types found at the BBB in regulating insulin uptake. Furthermore, they discuss how alterations of BBB favor the development of brain insulin resistance by stressing the role for the IR at the BBB, that goes beyond its canonical role in mediating the activation of the signal. Indeed, IR at the BBB is the main transporter for insulin within the brain. In this context, the review also addresses the effects of intranasal insulin administration and weight loss-associated improved insulin sensitivity, that are two validated strategies to increase brain insulin uptake and promoting neuroprotective effects.

From a molecular point of view, the activation of the insulin signaling requires IR-mediated tyrosine (Tyr) phosphorylation of IRS1. Following IRS1 activation, two main signaling cascades are activated downstream from IRS1: the mitogen-activated protein kinase (MAPK), and the phosphatidyl-inositol 3-kinase (PI3K)/protein kinase B (Akt). In the brain, MAPK are involved in the regulation of genes controlling synapse growth, neuronal maintenance, and repair processes while the PI3K/Akt pathway is involved in the maintenance of synaptic plasticity, stress response and neuronal metabolism and autophagy (Tramutola et al., 2016; Arnold et al., 2018). Gabbouji et al. highlight the role for the PI3K/Akt axis in the brain, showing how similar alterations were observed both in T2DM and AD within this pathway. Such molecular alterations drive the impairment of glucose uptake and metabolism as well as an increase of inflammatory processes within the brain and likely suggest that PI3K/Akt axis impairment could be a common denominator in those diseases.

Interestingly, the alteration of the PI3K/Akt axis in AD may result from accumulation of cholesterol oxidation products in the brain called oxysterols. Some oxysterols (e.g., 27-OHC, 7β-OHC, and 7-KC) deriving from cholesterol enzymatic oxidation or auto-oxidation further exacerbate cell-damage by sustaining free-radical chain reactions (Palozza et al., 2008; Niki, 2018). Furthermore, increased oxysterols levels were detected in AD brains compared to controls (Hascalovici et al., 2009; Testa et al., 2016). Gamba et al. address this topic by discussing how cholesterol oxidation products, i.e., oxysterols, may favor brain insulin resistance development, thus contributing to disrupt glucose uptake resulting in increased accumulation and reduced clearance of both Aβ and phospho-Tau in AD brain.

Similarly, Chatterjee et al. provide experimental evidence that the impairment of the PI3K/Akt axis due to insulin resistance favors Tau phosphorylation through a mechanism involving GSK3β and reduced autophagy in Drosophila. These authors show that co-expression of Chico (homolog of the mammalian IRS) and Tau leads to GSK3β inactivation and reduces Tau hyperphosphorylation in Drosophila. Conversely, the co-expression of insulin-resistant Chico loss of function results in hyper-active GSK3β and Tau hyperphosphorylation, thus suggesting that IRS1 would play a pivotal role in controlling downstream kinases in AD.

The detrimental impact of metabolic disorders, e.g., T2DM, obesity and metabolic syndrome, on brain structure and function has been also addressed. A research paper authored by Kavanagh et al. provides evidence that T2DM in vervet monkeys produces alterations in brain metabolism that foster the amyloidogenic pathway similar to what is observed in pre-symptomatic AD. This study shows that during the progression from healthy to pre-diabetes to T2DM, the brain moves into a state of altered metabolism that is characterized by higher glucose and lower amino acids and acylcarnitines levels. Then, increased cerebral metabolism seems to drive Aβ production and accelerates Aβ aggregation, in T2D similar to AD. These results shed light on the mechanisms through which T2DM development could lead to AD-related pathology and cognitive decline.

Movassat et al. briefly examined the main mechanisms linking T2DM to AD and provide the first evidence that certain circulating AD biomarkers can be found in goto-kakizaki (GK) rats, a model of non-obesity induced diabetes, suggesting that GK rats may be a model to investigate common molecular

mechanisms of both disorders. Furthermore, Duarte et al. tested the hypothesis that caffeine exposure ameliorates T2DM-induced hippocampal alterations in GK rat brain. Caffeine is a non-selective antagonist of adenosine receptors (both A_1R and $A_{2A}R$), whose activity impact on the molecular processes regulating cognitive and learning functions. A_1R and $A_{2A}R$ were found to be altered in the brain of T2D animal models. Caffeine-mediated neuroprotective effects were likely promoted through the reduction of $A_{2A}Rs$ activity at the synaptic level as well as in glial cells. Indeed, caffeine long-term intake was associated with improved memory functions, reduced astrogliosis, and reduced hippocampal synaptic degeneration in GK rats. Although long-term intake of caffeine did not prevent T2D-induced metabolic alterations in the hippocampus, its neuroprotective effects may be of help to delay the progression of T2D-related neurodegeneration.

With regard to obesity, Lloret et al. discuss the increased risk to develop AD for overweight and obese individuals, describing the role of obesity-associated hyper-leptinemia in promoting brain insulin resistance and glutamate-induced excitotoxicity. Under physiological conditions, insulin secretion stimulates leptin synthesis and release by adipocytes thus favoring satiety, whereby, as in a vicious cycle, leptin reduces insulin secretion and enhances insulin sensitivity to promote glucose uptake and metabolism. Moreover, leptin was shown to have neuroprotective functions by favoring long-term potentiation (LTP) and boosting the activity of N-methyl-D-aspartate (NMDA) receptors at synaptic levels. Conversely, increased circulating leptin levels would lead to leptin resistance and consequently to insulin resistance found to be associated with LTP dysfunction, and NMDA excitotoxicity in obese individuals and AD subjects. For that reasons, obesity in middle-age could be considered as a risk factor to develop AD in the elderly.

Among the intracellular processes dependent on the rate of glucose uptake, that are altered under insulin resistance conditions, O-GlcNAcylation post-translational modifications emerged as a key process regulating protein functions (Moll et al., 2020; Zuliani et al., 2021). Ansari and Emerald discuss the reciprocal interaction between insulin signaling and O-GlcNAcylation process in the brain, highlighting that increased O-GlcNAcylation of active sites of proteins of insulin signaling may promote the development of brain insulin resistance. In turn, brain insulin resistance, by reducing glucose uptake, likely prevent the O-GlcNAcylation process, thus impairing the activity of many proteins. In particular, reduced O-GlcNAcylation of amyloid precursor protein (APP) and Tau may be responsible for increased Aβ production as well as Tau phosphorylation, both processes associated with the development of AD.

Another key aspect that links metabolic disorders and development of neurodegenerative diseases is the lipid dysmetabolism (Trostchansky, 2019; Falabella et al., 2021). Indeed, insulin signaling, beyond glucose metabolism, also regulates lipid metabolism while insulin resistance leads to dyslipidemia (Arnold et al., 2018). By discussing current evidence, Le Stunff et al. propose that an excess of toxic lipids generated in the liver can be a cause of neurodegeneration. In particular, dyslipidemia may lead to increased ceramide levels that, due to their hydrophobic nature, can cross the BBB, thus promoting an exaggerate production of pro-inflammatory cytokines within the brain fostering development of brain insulin resistance and cognitive decline.

The relevance for brain insulin resistance in development of Parkinson disease (PD) was also described. Fiory et al. highlight how peripheral alterations, such those occurring in T2DM, have detrimental effects on PD, by negatively affecting PD phenotype, accelerating its progression and worsening cognitive impairment. These authors provide an extensive analysis of the recent lines of evidence supporting this idea, by showing how insulin resistance both in peripheral tissues and in the brain worsens functions of dopaminergic neurons, favors alpha-synuclein aggregation, impairs mitochondrial functions and promotes a pro-inflammatory state, that are all features of PD. Moreover, they provide an update about the neuroprotective effects of antidiabetic drugs on PD onset and progression collected both in humans and animal models.

Among the strategies to overcome T2DM-associated metabolic dysfunctions and cognitive decline the role of glucagon-like peptide-1 (GLP1) is gaining much attention. Indeed, GLP1 and insulin pathway share several targets downstream from IRS1, including Akt and MAPK, thus meaning that activation of GLP1 cascade may be useful to overcome IRS1 inhibition and thus insulin resistance (Tramutola et al., 2017; Holscher, 2019). Grieco et al. address this aspect by emphasizing how the increased activation of GLP1 signaling pathway obtained through the administration of GLP1 receptor (GLP1R) agonists in experimental models of AD, PD and T2DM promotes neuroprotective effects. These latter include reduced Aβ and hyperphosphorylated Tau, reduced oxidative stress and inflammatory processes and improved functions of dopaminergic neurons. Overall, by reducing neurotoxic events, GLP1R agonists ameliorate synaptic plasticity thus exerting beneficial effects on cognitive functions.

Finally, Dierssen et al. provide a comprehensive analysis of the literature addressing the role of insulin signaling in DS. DS is the most frequent chromosomal abnormality responsible for intellectual disability, due to the presence of an extra complete or segment of chromosome 21 (Hsa21). Furthermore, DS individuals are at high risk to develop AD after the age of 40. Multiple genes and factors are responsible for the major DS phenotypes and as explained in the review, insulin signaling in the brain is thought to mediate brain dysfunction associated with intellectual disability and the development of AD in DS.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

Arnold, S. E., Arvanitakis, Z., Macauley-Rambach, S. L., Koenig, A. M., Wang, H. Y., Ahima, R. S., et al. (2018). Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nat. Rev. Neurol*. 14, 168–181. doi: 10.1038/nrneurol.2017.185

Barone, E., Di Domenico, F., Cassano, T., Arena, A., Tramutola, A., Lavecchia, M. A., et al. (2016). Impairment of biliverdin reductase-A promotes brain insulin resistance in Alzheimer disease: a new paradigm. *Free Radic. Biol. Med*. 91, 127–142. doi: 10.1016/j.freeradbiomed.2015.12.012

Barone, E., Mosser, S., and Fraering, P. C. (2014). Inactivation of brain Cofilin-1 by age, Alzheimer's disease and gamma-secretase. *Biochim. Biophys. Acta* 1842(12 Pt A), 2500–2509. doi: 10.1016/j.bbadis.2014.10.004

Barone, E., Tramutola, A., Triani, F., Calcagnini, S., Di Domenico, F., Ripoli, C., et al. (2019). Biliverdin reductase-a mediates the beneficial effects of intranasal insulin in Alzheimer disease. *Mol. Neurobiol*. 56, 2922–2943. doi: 10.1007/s12035-018-1231-5

Bomfim, T. R., Forny-Germano, L., Sathler, L. B., Brito-Moreira, J., Houzel, J. C., Decker, H., et al. (2012). An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Abeta oligomers. *J Clin Invest.* 122, 1339–1353. doi: 10.1172/JCI57256

Brás, I. C., König, A., and Outeiro, T. F. (2019). Glycation in Huntington's disease: a possible modifier and target for intervention. *J. Huntingtons Dis.* 8, 245–256. doi: 10.3233/JHD-190366

Butterfield, D. A., Di Domenico, F., and Barone, E. (2014). Elevated risk of type 2 diabetes for development of Alzheimer disease: a key role for oxidative stress in brain. *Biochim. Biophys. Acta* 1842, 1693–1706. doi: 10.1016/j.bbadis.2014.06.010

de la Monte, S. M. (2019). The full spectrum of Alzheimer's disease is rooted in metabolic derangements that drive type 3 diabetes. *Adv. Exp. Med. Biol*. 1128, 45–83. doi: 10.1007/978-981-13-3540-2_4

Dearden, L., Buller, S., Furigo, I. C., Fernandez-Twinn, D. S., and Ozanne, S. E. (2020). Maternal obesity causes fetal hypothalamic insulin resistance and disrupts development of hypothalamic feeding pathways. *Mol. Metab.* 42:101079. doi: 10.1016/j.molmet.2020.101079

Falabella, M., Vernon, H. J., Hanna, M. G., Claypool, S. M., and Pitceathly, R. D. S. (2021). Cardiolipin, mitochondria, and neurological disease. *Trends Endocrinol. Metab*. 32, 224–237. doi: 10.1016/j.tem.2021.01.006

Franklin, W., Krishnan, B., and Taglialatela, G. (2019). Chronic synaptic insulin resistance after traumatic brain injury abolishes insulin protection from amyloid beta and tau oligomer-induced synaptic dysfunction. *Sci. Rep*. 9:8228. doi: 10.1038/s41598-019-44635-z

Fusco, S., Spinelli, M., Cocco, S., Ripoli, C., Mastrodonato, A., Natale, F., et al. (2019). Maternal insulin resistance multigenerationally impairs synaptic plasticity and memory via gametic mechanisms. *Nat. Commun*. 10:4799. doi: 10.1038/s41467-019-12793-3

Hascalovici, J. R., Vaya, J., Khatib, S., Holcroft, C. A., Zukor, H., Song, W., et al. (2009). Brain sterol dysregulation in sporadic AD and MCI: relationship to heme oxygenase-1. *J. Neurochem.* 110, 1241–1253. doi: 10.1111/j.1471-4159.2009.06213.x

Holscher, C. (2019). Insulin signaling impairment in the brain as a risk factor in Alzheimer's disease. *Front. Aging Neurosci.* 11:88. doi: 10.3389/fnagi.2019.00088

Hong, C. T., Chen, K. Y., Wang, W., Chiu, J. Y., Wu, D., Chao, T. Y., et al. (2020). Insulin resistance promotes parkinson's disease through aberrant expression of alpha-synuclein, mitochondrial dysfunction, and deregulation of the polo-like kinase 2 signaling. *Cells* 9:740. doi: 10.3390/cells9030740

Kellar, D., and Craft, S. (2020). Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. *Lancet Neurol*. 19, 758–766. doi: 10.1016/S1474-4422(20)30231-3

Kleinridders, A., Cai, W., Cappellucci, L., Ghazarian, A., Collins, W. R., Vienberg, S. G., et al. (2015). Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. *Proc. Natl. Acad. Sci. U.S.A*. 112, 3463–3468. doi: 10.1073/pnas.1500877112

Lanzillotta, C., Tramutola, A., Di Giacomo, G., Marini, F., Butterfield, D. A., Di Domenico, F., et al. (2021). Insulin resistance, oxidative stress and mitochondrial defects in Ts65dn mice brain: a harmful synergistic path in down syndrome. *Free Radic. Biol. Med*. 165, 152–170. doi: 10.1016/j.freeradbiomed.2021.01.042

Lourenco, M. V., Clarke, J. R., Frozza, R. L., Bomfim, T. R., Forny-Germano, L., Batista, A. F., et al. (2013). TNF-alpha mediates PKR-dependent memory impairment and brain IRS-1 inhibition induced by Alzheimer's beta-amyloid oligomers in mice and monkeys. *Cell Metab*. 18, 831–843. doi: 10.1016/j.cmet.2013.11.002

Manco, M., Guerrera, S., Ravà, L., Ciofi Degli Atti, M., Di Vara, S., Valeri, G., et al. (2021). Cross-sectional investigation of insulin resistance in youths with autism spectrum disorder. Any role for reduced brain glucose metabolism? *Transl Psychiatry*. 11:229. doi: 10.1038/s41398-021-01345-3

Melo, H. M., Seixas da Silva, G. D. S., Sant'Ana, M. R., Teixeira, C. V. L., Clarke, J. R., et al. (2020). Palmitate is increased in the cerebrospinal fluid of humans with obesity and induces memory impairment in mice via pro-inflammatory TNF-alpha. *Cell Rep.* 30, 2180.e8–2194.e8. doi: 10.1016/j.celrep.2020.01.072

Moll, T., Shaw, P. J., and Cooper-Knock, J. (2020). Disrupted glycosylation of lipids and proteins is a cause of neurodegeneration. *Brain* 143, 1332–1340. doi: 10.1093/brain/awz358

Nakabeppu, Y. (2019). Molecular pathophysiology of insulin depletion, mitochondrial dysfunction, and oxidative stress in Alzheimer's disease brain. *Adv. Exp. Med. Biol*. 1128, 27–44. doi: 10.1007/978-981-13-3540-2_3

Niki, E. (2018). Oxidant-specific biomarkers of oxidative stress. Association with atherosclerosis and implication for antioxidant effects. *Free Radic. Biol. Med.* 120, 425–440. doi: 10.1016/j.freeradbiomed.2018.04.001

Palozza, P., Barone, E., Mancuso, C., and Picci, N. (2008). The protective role of carotenoids against 7-keto-cholesterol formation in solution. *Mol. Cell Biochem.* 309, 61–68. doi: 10.1007/s11010-007-9643-y

Rhea, E. M., Raber, J., and Banks, W. A. (2020). ApoE and cerebral insulin: trafficking, receptors, and resistance. *Neurobiol. Dis*. 137:104755. doi: 10.1016/j.nbd.2020.104755

Sharma, N., Tramutola, A., Lanzillotta, C., Arena, A., Blarzino, C., Cassano, T., et al. (2019). Loss of biliverdin reductase-A favors Tau hyper-phosphorylation in Alzheimer's disease. *Neurobiol. Dis*. 125, 176–189. doi: 10.1016/j.nbd.2019.02.003

Spinelli, M., Fusco, S., Mainardi, M., Scala, F., Natale, F., Lapenta, R., et al. (2017). Brain insulin resistance impairs hippocampal synaptic plasticity and memory by increasing GluA1 palmitoylation through FoxO3a. *Nat. Commun*. 8:2009. doi: 10.1038/s41467-017-02221-9

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., et al. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J. Clin. Invest*. 122, 1316–1338. doi: 10.1172/JCI59903

Testa, G., Staurenghi, E., Zerbinati, C., Gargiulo, S., Iuliano, L., Giaccone, G., et al. (2016). Changes in brain oxysterols at different stages of Alzheimer's disease: their involvement in neuroinflammation. *Redox. Biol*. 10, 24–33. doi: 10.1016/j.redox.2016.09.001

Tramutola, A., Arena, A., Cini, C., Butterfield, D. A., and Barone, E. (2017). Modulation of GLP-1 signaling as a novel therapeutic approach in the treatment of Alzheimer's disease pathology. *Expert Rev. Neurother.* 17, 59–75. doi: 10.1080/14737175.2017.1246183

Tramutola, A., Lanzillotta, C., Arena, A., Barone, E., Perluigi, M., and Di Domenico, F. (2016). Increased mammalian target of rapamycin signaling contributes to the accumulation of protein oxidative damage in a mouse model of Down's syndrome. *Neurodegener. Dis.* 16, 62–68. doi: 10.1159/000441419

Tramutola, A., Lanzillotta, C., Di Domenico, F., Head, E., Butterfield, D. A., Perluigi, M., et al. (2020). Brain insulin resistance triggers early onset Alzheimer disease in Down syndrome. *Neurobiol. Dis*. 137:104772. doi: 10.1016/j.nbd.2020.104772

Tramutola, A., Triplett, J. C., Di Domenico, F., Niedowicz, D. M., Murphy, M. P., Coccia, R., et al. (2015). Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD. *J. Neurochem.* 133, 739–749. doi: 10.1111/jnc.13037

Triani, F., Tramutola, A., Di Domenico, F., Sharma, N., Butterfield, D. A., Head, E., et al. (2018). Biliverdin reductase-A impairment links brain insulin resistance with increased Abeta production in an animal model of aging: Implications for Alzheimer disease. *Biochim. Biophys. Acta Mol. Basis Dis*. 1864, 3181–3194. doi: 10.1016/j.bbadis.2018.07.005

Trostchansky, A. (2019). Overview of lipid biomarkers in amyotrophic lateral sclerosis (ALS). *Adv. Exp. Med. Biol.* 1161, 233–241. doi: 10.1007/978-3-030-21735-8_18

Zuliani, I., Lanzillotta, C., Tramutola, A., Barone, E., Perluigi, M., Rinaldo, S., et al. (2021). High-fat diet leads to reduced protein O-GlcNAcylation and mitochondrial defects promoting the development of Alzheimer's disease signatures. *Int. J. Mol. Sci.* 22:3746. doi: 10.3390/ijms22073746

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 09 January 2019
doi: 10.3389/fnins.2018.01015

Impact of Caffeine Consumption on Type 2 Diabetes-Induced Spatial Memory Impairment and Neurochemical Alterations in the Hippocampus

João M. N. Duarte[1,2,3,4], Cecilia Skoug[3,4], Henrique B. Silva[1], Rui A. Carvalho[5], Rolf Gruetter[2,6,7] and Rodrigo A. Cunha[1,8]*

[1] CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [2] Laboratory for Functional and Metabolic Imaging, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [3] Department of Experimental Medical Science, Faculty of Medicine, Lund University, Lund, Sweden, [4] Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden, [5] Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal, [6] Department of Radiology, University of Lausanne, Lausanne, Switzerland, [7] Department of Radiology, University of Geneva, Geneva, Switzerland, [8] Faculty of Medicine, University of Coimbra, Coimbra, Portugal

OPEN ACCESS

Edited by:
Eugenio Barone, Sapienza University of Rome, Italy

Reviewed by:
Joana M. Gaspar, Universidade Federal de Santa Catarina, Brazil
Marinette Van Der Graaf, Radboud University Nijmegen Medical Centre, Netherlands

***Correspondence:**
João M. N. Duarte
joao.duarte@med.lu.se

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *02 November 2018*
Accepted: *17 December 2018*
Published: *09 January 2019*

Citation:
Duarte JMN, Skoug C, Silva HB, Carvalho RA, Gruetter R and Cunha RA (2019) Impact of Caffeine Consumption on Type 2 Diabetes-Induced Spatial Memory Impairment and Neurochemical Alterations in the Hippocampus. Front. Neurosci. 12:1015. doi: 10.3389/fnins.2018.01015

Diabetes affects the morphology and plasticity of the hippocampus, and leads to learning and memory deficits. Caffeine has been proposed to prevent memory impairment upon multiple chronic disorders with neurological involvement. We tested whether long-term caffeine consumption prevents type 2 diabetes (T2D)-induced spatial memory impairment and hippocampal alterations, including synaptic degeneration, astrogliosis, and metabolic modifications. Control Wistar rats and Goto-Kakizaki (GK) rats that develop T2D were treated with caffeine (1 g/L in drinking water) for 4 months. Spatial memory was evaluated in a Y-maze. Hippocampal metabolic profile and glucose homeostasis were investigated by ^{1}H magnetic resonance spectroscopy. The density of neuronal, synaptic, and glial-specific markers was evaluated by Western blot analysis. GK rats displayed reduced Y-maze spontaneous alternation and a lower amplitude of hippocampal long-term potentiation when compared to controls, suggesting impaired hippocampal-dependent spatial memory. Diabetes did not impact the relation of hippocampal to plasma glucose concentrations, but altered the neurochemical profile of the hippocampus, such as increased in levels of the osmolites taurine ($P < 0.001$) and *myo*-inositol ($P < 0.05$). The diabetic hippocampus showed decreased density of the presynaptic proteins synaptophysin ($P < 0.05$) and SNAP25 ($P < 0.05$), suggesting synaptic degeneration, and increased GFAP ($P < 0.001$) and vimentin ($P < 0.05$) immunoreactivities that are indicative of astrogliosis. The effects of caffeine intake on hippocampal metabolism added to those of T2D, namely reducing *myo*-inositol levels ($P < 0.001$) and further increasing taurine levels ($P < 0.05$). Caffeine prevented

T2D-induced alterations of GFAP, vimentin and SNAP25, and improved memory deficits. We conclude that caffeine consumption has beneficial effects counteracting alterations in the hippocampus of GK rats, leading to the improvement of T2D-associated memory impairment.

Keywords: insulin, adenosine, caffeine, neuroprotection, synaptic dysfunction, gliosis, glucose, metabolic profiling

INTRODUCTION

Metabolic syndrome and diabetes *mellitus* affect brain function and increase the risk of age-related cognitive impairment, vascular dementia, and Alzheimer's disease (Frisardi et al., 2010; Duarte, 2015; Moheet et al., 2015). Diabetes conditions are particularly associated with atrophy of the hippocampus (Convit et al., 2003; Gold et al., 2007). We and others have reported that experimental diabetic conditions cause synaptic degeneration (Duarte et al., 2006, 2009a, 2012), increase astrocytic reactivity and proliferation (Saravia et al., 2002; Baydas et al., 2003; Duarte et al., 2012), and change metabolism (Duarte et al., 2009a; Girault et al., 2018) in the hippocampus. As important as understanding the mechanisms of diabetes-induced hippocampal alterations leading to memory impairment, is the design of novel strategies to prevent such degeneration. The neuromodulation system operated by adenosine is altered in diabetes, with reduced density of adenosine A_1 receptors (A_1Rs) and increased density of adenosine A_{2A} receptors (A_{2A}Rs) in membranes from the hippocampus (Duarte et al., 2006, 2012). Caffeine is a widely consumed non-selective antagonist of adenosine receptors (Fredholm et al., 1999), and both caffeine and selective adenosine A_{2A}R antagonists affect performance in learning and memory tasks (Takahashi et al., 2008; Cunha, 2016) and afford neuroprotection upon chronic brain insults (Cunha, 2005). In addition, caffeine may reduce the risk of developing glucose-intolerance and diabetes severity (e.g., van Dam and Hu, 2005; Greenberg et al., 2006; Higdon and Frei, 2006). We previously reported that caffeine consumption ameliorates diabetes-induced hippocampal degeneration and prevents diabetes-associated memory deficits in insulin-deficient rats (Duarte et al., 2009a) and in a mouse model of obesity-associated type 2 diabetes (T2D) (Duarte et al., 2012). The different etiology of lean T2D prompted us to investigate the effect of long-term caffeine intake on alterations in the hippocampus of Goto-Kakizaki (GK) rats, an animal model of non-obese T2D that was produced by selective breeding of non-diabetic Wistar rats which displayed high plasma glucose levels in oral glucose tolerance tests (Girault et al., 2018, and references therein). In this study, we tested the hypothesis that caffeine exposure ameliorates T2D-induced alterations of hippocampal metabolism, degeneration of synapses and astrogliosis, as well as concomitant spatial memory impairment.

Abbreviations: aCSF, artificial cerebrospinal fluid; Ala, alanine; Asc, ascorbate; Asp, aspartate; BBB, blood-brain-barrier; βHB, β-hydroxibutyrate; CADO, 2-chloroadenosine; CMR_{glc}, cerebral metabolic rate of glucose consumption; Cr, creatine; CRLB, Cramér-Rao lower bound; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; ELISA, enzyme-linked immunosorbent assay; fEPSP, field excitatory postsynaptic potential; GABA, γ-aminobutyrate; GFAP, glial fibrillary acidic protein; GK, Goto-Kakizaki; Glc, glucose; Gln, glutamine; Glu, glutamate; GPC, glycerophosphocholine; GSH, glutathione; I/O, input/output; Ins, *myo*-inositol; Lac, lactate; LTP, long-term potentiation; MAP2, microtubule-associated protein type 2; MRS, magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartatylglutamate; NMR, nuclear magnetic resonance; PCho, phosphocholine; PCr, phosphocreatine; PE, phosphoethanolamine; PSD95, postsynaptic density protein of 95 kDa; SCH58261, 2-(2-furanyl)-7-(2-phenylethyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine; scyllo, *scyllo*-inositol; SNAP25, synaptosome-associated protein of 25 kDa; STZ, streptozotocin; Tau, taurine; VOI, volume of interest.

METHODS

Animals

Animals were handled according to Swiss and Portuguese guidelines for the use of experimental animals, and authorized by the respective local ethics committees (EXPANIM-SCAV and ORBEA). Male GK rats, which spontaneously develop insulin resistance, and control Wistar-Hannover-Galas rats were obtained from Taconic (Lille Skensved, Denmark), or from the colony kept at the animal house of the Faculty of Medicine of the University of Coimbra (for electrophysiology recordings). We used a total of 22 GK rats and 22 Wistar rats. All the animals were maintained with food and water *ad libitum*. When tested, caffeine was administered in the drinking water at 1 g/L from 2 to 6 months of age (4 month period). Thus, the present experimental design included four animal groups: control Wistar, caffeine-treated Wistar, diabetic GK, and caffeine-treated GK. Body weight and caffeine consumption were monitored throughout the treatment period. Glycaemia was measured monthly in a 2 μL blood sample collected by tail pricking, using a glucose oxidase-based glucometer (Ascencia Contour, Bayer, Switzerland). At 2 and 4 months of caffeine exposure, blood samples (100 μL) were taken from the tail vein under brief isoflurane anesthesia (2% in oxygen) for determination of serum caffeine and/or insulin concentrations.

Behavioral Tasks

Exploratory behavior and locomotor activity were evaluated in a square open-field arena of 34 × 34 cm with 30 cm high, which was divided in 4 squares of 17 × 17 cm. The animals were placed in the central area of the arena and allowed to explore it over 5 min in the dark. The number of crossings of the squares and the number of rearing movements with forepaws were recorded. Rearing with the forepaws pressed against the walls was not considered.

Spontaneous alternation was observed in a Y-maze constructed in black Plexiglas, with three arms measuring 35 cm long, 9 cm wide and 30 cm height, and converging to equal

angles, which was placed in a dim-illuminated room (12 lux) with large visual cues hanged on the walls. The animals were placed at the bottom of one arm of the Y-maze and allowed to explore freely all three arms for a single 8 min session in the dark. The measured spontaneous alternation behavior was used to assess hippocampal-dependent spatial memory (Lalonde, 2002). If the rat remembers the arm it has just explored, it will therefore enter one of the other arms of the maze. Complete spontaneous alternations were defined as successive entries into the three arms, and were expressed as fraction of the possible alternations in the respective test. In addition to the open field test, the number of entries in the arms of the maze also allowed to access locomotor activity and exploratory behavior of the tested rats.

Localized ^{1}H Magnetic Resonance Spectroscopy (MRS)

Rats were anesthetized with 2% isoflurane (Attane, Minrad, USA) in oxygen (PanGas, Ecublens, Switzerland), and then intubated and ventilated with a pressure-driven ventilator (MRI-1, CWE incorporated, PA, USA). Catheters were placed into the femoral artery for monitoring blood gases, glucose and blood pressure, and into the femoral vein for infusion of saline solutions containing α-chloralose (Acros Organics, Geel, Belgium) or D-glucose (Sigma-Aldrich, Switzerland). Rats were placed in a home-built holder that ensures a fixed and stable position of the skull for extended scanning times. Body temperature was maintained around 37.5°C with a warm water circulation system based on the feedback from a rectal temperature probe. Temperature, arterial blood pressure, heart rate, and respiratory rate were continuously monitored with an animal monitoring system (SA Instruments, NY, USA). Before inserting the animal in the bore of the magnet, anesthesia was switched to α-chloralose (intravenous bolus of 80 mg/kg, and continuous infusion of 25 mg/kg/h). D-glucose [20% (w/v) solution] was infused at a rate adjusted based on the concomitantly measured arterial plasma glucose concentrations to achieve stable targeted glycaemia levels. NMR measurements were performed after each glucose level had been stable for at least 15 min (Duarte and Gruetter, 2012). Arterial pH and pressures of O_2 and CO_2 were measured using a blood gas analyser (AVL Compact 3, Diamond Diagnostics, MA, USA). Concentration of glucose in arterial plasma samples was quantified by the glucose oxidase method, using a multi-assay analyser (GM7 Micro-Stat, Analox Instruments, UK).

All experiments were carried out as previously described (Duarte et al., 2009a) using a Varian INOVA spectrometer (Agilent Technologies, Palo Alto, CA, USA) interfaced to an actively-shielded 9.4 T magnet with a 31 cm horizontal bore (Magnex Scientific, Abingdon, UK), and a homebuilt 10 mm ^{1}H quadrature surface coil. The rat brain was positioned in the isocentre of the magnet and located with fast-spin-echo images with 5 s repetition time, effective echo time of 52 ms and echo train length of 8. Shimming was performed with FAST(EST)MAP (Gruetter and Tkáč, 2000), and ^{1}H NMR spectra were acquired from a volume of interest (VOI) of 18 μL placed in the left dorsal hippocampus using SPECIAL spectroscopy, with echo time of 2.8 ms and repetition time of 4 s (Mlynárik et al., 2006). Spectra were analyzed using LCModel (Stephen Provencher Inc., Oakville, Ontario, Canada), including a macromolecule spectrum in the database, as previously described (Mlynárik et al., 2006; Duarte et al., 2009a). The unsuppressed water signal measured from the same VOI was used as an internal reference (assuming the existence of 80% of water in the brain tissue) for the absolute quantification of the following metabolites: glucose (Glc), ascorbate (Asc), phosphoethanolamine (PE), creatine (Cr), phosphocreatine (PCr), *myo*-inositol (Ins), taurine (Tau), *N*-acetylaspartate (NAA), aspartate (Asp), glutamate (Glu), glutamine (Gln), γ-aminobutyrate (GABA), alanine (Ala), lactate (Lac), β-hydroxybutyrate (βHB), glycerophosphocholine (GPC), phosphocholine (PCho), glutathione (GSH), *N*-acetylaspartylglutamate (NAAG), *scyllo*-inositol (scyllo). The Cramér-Rao lower bound (CRLB) was provided by LCModel as a measure of the reliability of the apparent metabolite concentration quantification. CRLBs above 30% were systematically associated to *scyllo*-inositol, which was thus not used for further analyses. The remaining metabolites were quantified with CRLBs below 30%.

Determination of Glucose Transport Kinetics

The determination of hippocampal glucose by MRS *in vivo* as function of plasma glucose was used to estimate kinetic parameters of glucose transport across the blood-brain-barrier (BBB). Steady-state brain glucose transport kinetics was modeled with a four-state conformational model that accounts for reversibility and trans-acceleration of the glucose carrier (Duarte et al., 2009b). Hippocampal glucose at steady-state was fitted to the following equation

$$G_{hipp} = V_d \frac{\left(\frac{T_{max}}{CMR_{glc}} - 1\right) G_p - K_t}{\frac{T_{max}}{CMR_{glc}} + 1 + \frac{G_p}{K_{ii}}}$$

where G_{hipp} and G_p are the concentrations of glucose in the hippocampus (in μmol/g) and plasma (in mmol/L), respectively. CMR_{glc} is the cerebral metabolic rate of glucose. T_{max} denotes the apparent maximal transport rate across the BBB (μmol/g/min), K_t and K_{ii} denote the apparent Michaelis and iso-inhibition constants (in mmol/L), $V_d = 0.77$ mL/g is the volume of the physical distribution space of glucose in the hippocampus (see Duarte et al., 2009b for details).

Western Blot Analysis

Immediately after the MRS experiment, rats were decapitated, the brain was rapidly removed, and the hippocampus dissected. Whole membranes and synaptosomes (i.e., synaptic-enriched) membranes were prepared (Rebola et al., 2005; Cunha et al., 2006), and Western blot analysis of proteins in these hippocampal membrane preparations was performed using previously detailed methods (Duarte et al., 2007; Kaster et al., 2015). Western blot analysis of $A_{2A}R$ was carried out as detailed by Hurtado-Alvarado et al. (2016), using the avidin-biotin Vectastain Elite kit (Vector Laboratories, Burlingame, CA-USA)

for immunoreactivity amplification. The primary antibodies against the synaptic protein synaptosome-associated protein of 25 kDa (SNAP25; from Sigma, Sintra, Portugal), and against the glial fibrillary acidic protein (GFAP; from Sigma) were used at a dilution of 1:5,000. Antibodies against synaptophysin, α-tubulin and β-actin were purchased from Sigma and used at 1:10,000. Anti-postsynaptic density protein of 95 kDa (PSD95; from Chemicon) was used at 1:20,000; anti-vimentin (Sigma) and anti-microtubule-associated protein type 2 (MAP2; from Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:1,000. Antibodies against A_1R (Affinity Bioreagents, Golden, CO-USA) and $A_{2A}R$ (Abcam, Cambridge, UK) were used at a dilution of 1:600.

Electrophysiological Recordings

Electrophysiological recordings of synaptic transmission and plasticity were performed in superfused hippocampal slices, as previously described (Costenla et al., 2011; Kaster et al., 2015; Silva et al., 2018). Briefly, a rat was deeply anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane; no reaction to handling or tail pinch, while still breathing) before decapitation. The brain was rapidly removed and cooled in an artificial cerebrospinal fluid (aCSF) solution containing (in mmol/L): 124 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 10 glucose, 26 $NaHCO_3$, pH 7.4; 290–310 mOsm, gassed with 95% O_2, and 5% CO_2. Coronal hippocampal slices (400 μm thick) were prepared with a manual Vibratome 1,500 sectioning system (Vibratome, Germany), and allowed to recover for 1 h at room temperature in a Harvard Apparatus resting chamber filled with gassed aCSF. Individual dorsomedial hippocampal slices were transferred to a submerged recording chamber and continuously superfused at a rate of 4 mL/min with gassed aCSF kept at 30.5°C. A bipolar concentric stimulation electrode (SNE-100; Kopf, Germany) was placed over the Schaffer fibers delivering rectangular pulses (550 μA) of 0.1 ms duration applied with a Digitimer DS3 stimulator (Digitimer LTD, UK) once every 20 s. The evoked field excitatory postsynaptic potentials (fEPSPs) were recorded through an extracellular borosilicate microelectrode filled with 4 mol/L NaCl (2–5 MΩ resistance) placed in the *stratum radiatum* of the CA1 area, coupled to an ISO-80 amplifier (World Precision Instruments, Hitchin, UK). Averages of four consecutive responses acquired with a 1 kHz cut-off were digitalized and continuously monitored on a personal computer with the WINLTP 1.1 program (Anderson and Collingridge, 2001) to quantify the initial slope of the averaged fEPSPs, used to estimate the effect of drugs, added to the superfusion solution.

After obtaining a stable baseline, we first carried out an input/output curve to select a stimulus intensity triggering 40–50% of the maximal amplitude. We then tested the effects of 2-chloroadenosine (CADO, the closest and chemically stable analog of adenosine; from Tocris, Bristol, UK) and 1,3-dipropyl-8-cyclopentylxantine (DPCPX, a selective antagonist of adenosine A_1R; from Tocris) on basal synaptic transmission. Alternatively, we tested the effect of 2-(2-furanyl)-7-(2-phenylethyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine (SCH58261, a selective antagonist of adenosine $A_{2A}R$; from Sigma) on long-term potentiation (LTP). LTP was induced with a high-frequency train (100 Hz for 1 s) and was quantified as the percentage change between the fEPSP slopes 60 min after and 15 min before the train.

Statistics

Data was analyzed using ANOVA with two factors (diabetes and caffeine treatment). For the metabolic profile analysis, all metabolite concentrations were analyzed together with a multivariate ANOVA. Significant differences were considered for $P < 0.05$. Multiple comparisons after ANOVA were performed with Fisher's least significant difference (LSD) tests upon significant diabetes effect or diabetes-caffeine interaction. Two-tailed Student *t*-tests were used to compare caffeine intake and caffeine serum concentration between GK and Wistar rats, as well as the effects of CADO and DPCPX on synaptic transmission. Results are reported as mean ± SEM unless otherwise stated.

RESULTS

To test the role of caffeine consumption in the prevention of diabetes-induced hippocampal alterations, GK rats and age-matched controls were allowed to consume caffeine for 4 months, starting at 2 months of age. During the period when the rats had free access to 1 g/L caffeine solution, body weight, and pre-prandial glycaemia were monitored and insulin plasma levels were quantified 2 months after starting caffeine intake and at the end of the experiment. GK rats were smaller than controls independent of caffeine consumption, which had no significant effect on body weight (diabetes $P = 0.002$, caffeine $P = 0.275$, interaction $P = 0.794$; **Figure 1A**). T2D had a significant effect on fed glycaemia ($P < 0.001$), which was not impacted by caffeine treatment (caffeine $P = 0.779$, interaction $P = 0.935$; **Figure 1B**). Relative to controls, GK rats showed an increase in serum insulin concentration after 2 and 4 months of treatment ($P = 0.002$ and $P = 0.036$, respectively). At 4 months of treatment, caffeine prevented the diabetes-associated hyperinsulinemia (caffeine $P = 0.212$, diabetes $P = 0.240$, interaction $P = 0.050$; **Figure 1C**). Caffeine intake was slightly lower in Wistar than GK rats, but not significantly different ($P = 0.063$; **Figure 1D**). Serum levels of caffeine at the end of the treatment period were similar in diabetic and control rats ($P = 0.558$; **Figure 1E**).

Caffeine Consumption Prevents Spatial Memory Impairment in GK Rats

Hippocampal-dependent spatial memory was tested in a Y-maze 2 days before MRS *in vivo* at 6 months of age, i.e., after 4 months of caffeine exposure. Both diabetes and caffeine treatment affected spatial memory performance in the Y-maze (diabetes $P = 0.011$, caffeine $P = 0.033$, interaction $P = 0.549$). *Post-hoc* testing revealed that diabetes in GK rats caused a reduction of the spontaneous alternation in the Y-maze task when compared to controls (−19 ± 3%; $P < 0.001$; **Figure 2A**), which was ameliorated by 4 months of caffeine consumption. GK rats also showed a significant reduction in the number of entries in the Y-maze arms, independently of caffeine intake (diabetes

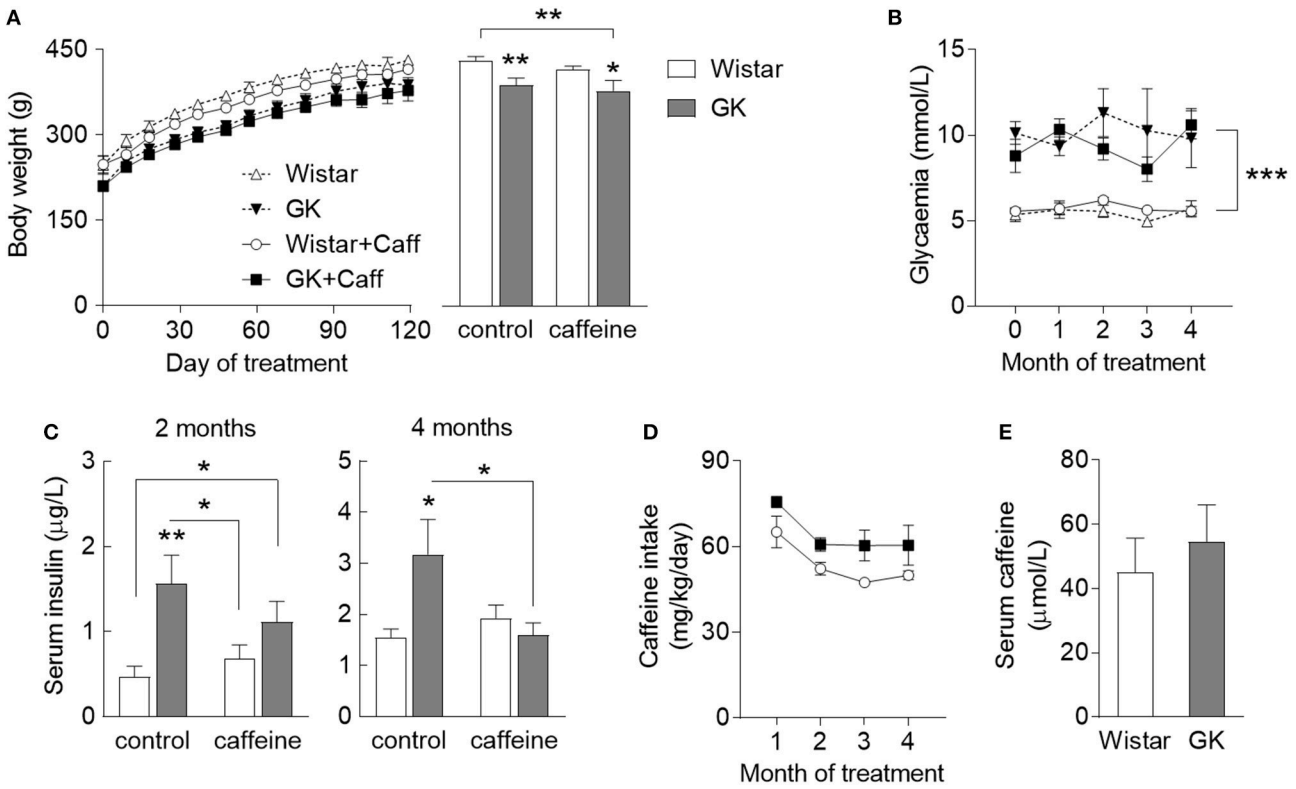

FIGURE 1 | Characteristics of diabetic GK rats (filed symbols/bars) and control Wistar rats (open symbols/bars), namely body weight **(A)** across the caffeine treatment period (line graph) and at the end of the study (bar graph), glycaemia **(B)**, concentration in the serum insulin determined after 2 and 4 months of treatment **(C)**, caffeine intake measured across the treatment period **(D)**, and concentration of serum caffeine after 4 months of caffeine exposure **(E)**. Caffeine (1 g/L) was provided through the drinking water for 4 months, starting at 2 months of age. Data are mean ± SEM of 8 rats per group. Symbols represent LSD test results after ANOVA with either significant diabetes effect or significant diabetes-caffeine interaction: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for GK vs. Wistar in the respective treatment group or as indicated.

$P = 0.003$, caffeine $P = 0.199$, interaction $P = 0.546$; **Figure 2B**). Nevertheless, diabetes was not associated with exploratory or locomotor impairment as gauged by similar exploration of the open-field arena (**Figures 2C,D**). Interestingly, caffeine impacted the number of rearing events in the open-field test (caffeine $P = 0.014$, diabetes $P = 0.974$, interaction $P = 0.974$), without impacting the number of crossing events between quadrants of the arena (caffeine $P = 0.908$, diabetes $P = 0.465$, interaction $P = 0.113$).

Diabetes-induced Metabolic Alterations

^{1}H spectra were acquired at 9.4 Tesla from the dorsal hippocampus of rats with half-height linewidths of 8–14 Hz and signal-to-noise ratios above 20, as reported by the LCModel. By inspecting these representative spectra (**Figure 3A**), one notices increased resonances of taurine in the hippocampus of GK rats relative to controls. This was in fact the most prominent metabolic alteration present in this model of T2D (see statistics below). Nineteen metabolites were quantified under normoglycaemia for the four experimental groups (**Figure 3B**). Analysis with a multivariate ANOVA to the whole metabolic profile indicated significant effects of diabetes ($P = 0.002$) and caffeine ($P = 0.028$) without diabetes-caffeine interaction ($P = 0.493$), which suggests cumulative effects of both factors. Furthermore, none of the levels of metabolites showed significant interaction between diabetes and caffeine in follow-up individual analyses.

Diabetes affected the concentration of taurine ($P < 0.001$), ascorbate ($P < 0.001$), creatine ($P = 0.002$), phosphocreatine ($P < 0.001$), glutamine ($P = 0.041$), *myo*-inositol ($P = 0.011$), lactate ($P = 0.005$), and glycerophosphorylcholine ($P < 0.001$). In *post-hoc* analyses comparing GK and Wistar rats in the absence of caffeine, GK rats only displayed significant increases in the levels of taurine (+22 ± 3%, $P < 0.001$), ascorbate (+20 ± 9%, $P = 0.038$), lactate (+34 ± 14%, $P = 0.035$), and phosphocreatine (+11 ± 4%, $P = 0.028$). Moreover, we observed a tendency for reduced creatine levels in GK rats (−7 ± 4%, $P = 0.080$ vs. Wistar), which resulted in a significant increase of phosphocreatine-to-creatine ratio (PCr/Cr; diabetes $P = 0.004$, caffeine $P = 0.150$, interaction $P = 0.378$). High PCr/Cr under normoglycaemia suggests that the hippocampus of GK rats is metabolically adapted to the diabetic condition in order to provide sufficient energy for basal oxidative metabolism.

On top of the effect of diabetes, caffeine consumption for 4 months had an effect on the concentration of creatine ($P = 0.027$), *myo*-inositol ($P < 0.001$), *N*-acetylaspartylglutamate

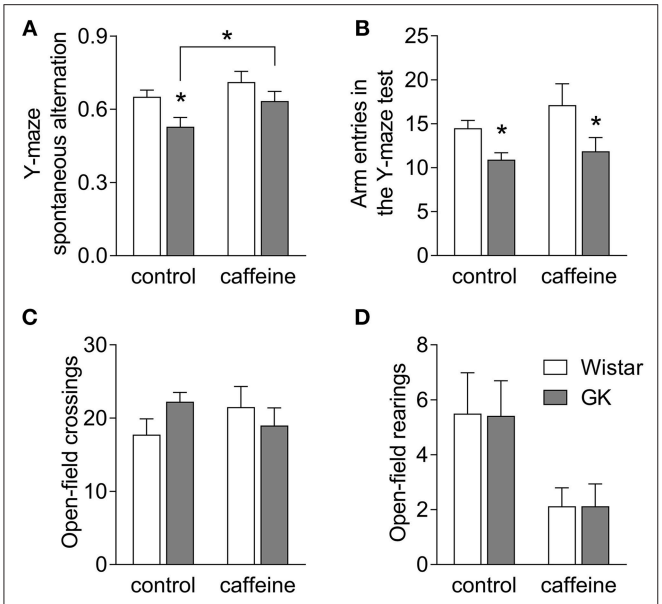

FIGURE 2 | Caffeine consumption improves spatial working memory deficits in diabetic GK rats. GK rats displayed reduced spontaneous alternation in the Y-maze, when compared to controls, but not if treated with caffeine for 4 months **(A)**. The number of entries in the Y-maze arms was lower in GK compared to control rats irrespective of caffeine consumption **(B)**. The number of crossings in the open-field arena was unaltered by diabetes or caffeine consumption **(C)**, but the number of rearing events was reduced upon chronic caffeine consumption **(D)**. Data are mean ± SEM of 8 rats per group. Symbols represent LSD test results after ANOVA with either significant diabetes effect or significant diabetes-caffeine interaction: $^*P < 0.05$ for GK vs. Wistar in the respective treatment group or as indicated.

($P = 0.036$) and taurine ($P = 0.023$). Notably, when compared to untreated controls, Wistar rats consuming caffeine exhibited reduced *myo*-inositol (−9 ± 2%, $P = 0.002$) and increased taurine (+15 ± 2%, $P < 0.001$) concentrations in the hippocampus. *Post-hoc* analyses within the caffeine-treated rats revealed higher levels of ascorbate (+35 ± 9%, $P = 0.002$), taurine (+14 ± 4%, $P = 0.004$), *myo*-inositol (7 ± 2%, $P = 0.006$), and phosphocreatine (7 ± 3%, $P = 0.045$) in GK than Wistar rats. Altogether, these results suggest that the caffeine-induced changes, namely in the osmolites *myo*-inositol and taurine, add to those induced by T2D.

Hippocampal Glucose Homeostasis

We have recently reported that diabetes impairs global glucose transport and consumption in the brain, without changes in the brain to plasma glucose levels (Girault et al., 2018). Since the hippocampus is particularly affected by T2D in experimental models, we measured hippocampal glucose concentration at several steady-state plasma glucose levels to test whether BBB transport of glucose in this region remains sufficient to feed metabolism. Physiology parameters measured during the periods of MRS were similar in all four experimental groups (**Table 1**). Glucose concentration in the hippocampus was similar for GK and control rats, and was dependent on plasma glucose levels (**Figure 4**). This indicates that the relation between glucose transport and consumption is not altered in the hippocampus of insulin-resistant GK rats, confirming previous observations in the whole brain (Girault et al., 2018). Kinetic parameters for hippocampal glucose transport estimated with the four-state conformational model were similar across the four experimental groups (**Table 2**). Indeed, neither T2D nor habitual caffeine consumption affected T_{max}/CMR_{glc}, suggesting that glucose transport at the BBB in our experimental conditions matches the glucose consumption needs in the hippocampus.

Synaptic Alterations in the Hippocampus of GK Rats

The putative degeneration of synapses was evaluated by quantifying the density of two presynaptic proteins in nerve terminal-enriched membrane preparations. As shown to occur in streptozotocin-induced diabetic rats (Duarte et al., 2006, 2009a) and NONcNZO10/Ltj diabetic mice (Duarte et al., 2012), the hippocampus of GK rats displayed synaptic degeneration, as suggested by reduced immunoreactivity of SNAP25 (−23 ± 5%, $P = 0.009$, $n = 8$, **Figure 5A**) and synaptophysin (−19 ± 3%, $P = 0.007$, $n = 5$, **Figure 5B**), when compared to control rats. Chronic caffeine consumption for 4 months did not significantly affect the immunoreactivity of these synaptic markers, whereas it prevented the T2D-induced reduction of SNAP25 (caffeine $P = 0.587$, diabetes $P = 0.176$, interaction $P = 0.0162$; **Figure 5A**) but not synaptophysin immunoreactivity (caffeine $P = 0.349$, diabetes $P = 0.001$, interaction $P = 0.681$; **Figure 5B**). To evaluate whether T2D also affected the post-synaptic compartment, we quantified the immunoreactivity of post-synaptic density-95 (PSD95), a prototypical postsynaptic marker. The immunoreactivity of PSD95 was not significantly altered in synaptic membranes of GK rats when compared to controls in the absence or presence of caffeine treatment (caffeine $P = 0.719$, diabetes $P = 0.053$, interaction $P = 0.692$; **Figure 5C**). Furthermore, total membranes from the hippocampus of GK and control rats also displayed similar MAP2 immunoreactivity (caffeine $P = 0.129$, diabetes $P = 0.154$, interaction $P = 0.915$; **Figure 5D**). Altogether these results suggest a main T2D-induced defect at presynaptic level.

Caffeine Consumption Prevents Diabetes-Induced Astrogliosis

Astrogliosis has been reported in several neurodegenerative diseases, including diabetes (e.g., Saravia et al., 2002; Baydas et al., 2003; Duarte et al., 2009a, 2012). This was now also found in the hippocampus of GK diabetic rats. Thus, when compared to controls, total hippocampal membranes prepared from GK rats exhibited increased immunoreactivity of GFAP (+20 ± 5%, $P < 0.001$, $n = 8$; **Figure 5E**) and vimentin (+65 ± 28%, $P = 0.010$, $n = 5$, **Figure 5F**). While caffeine intake was devoid of significant effects on the measured glial proteins in control Wistar rats, it prevented T2D-induced increase in the immunoreactivity of both astroglial-specific proteins GFAP (caffeine $P = 0.053$, diabetes $P = 0.026$, interaction $P = 0.003$, **Figure 5E**) and vimentin (caffeine $P = 0.280$, diabetes $P = 0.108$, interaction $P = 0.030$, **Figure 5F**).

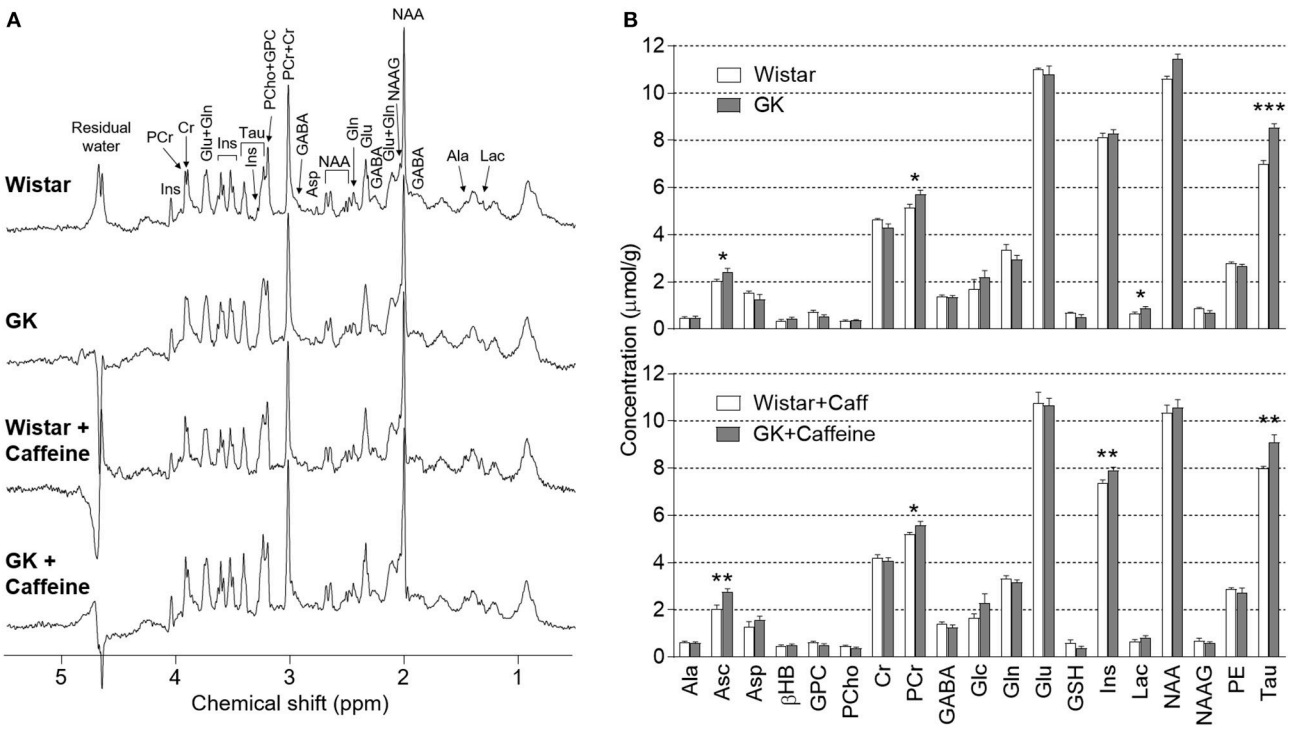

FIGURE 3 | Diabetes-induced metabolic alterations persisted upon caffeine treatment. **(A)** shows representative ^{1}H spectra acquired *in vivo* at 9.4 T from the dorsal hippocampus rats under normoglycaemia. An increase of taurine resonances at 3.25 and 3.42 ppm in the spectra from GK rats is clearly visible. The spectra were measured by the SPECIAL sequence with echo time of 2.8 ms, repetition time of 4 s, 640 scans and in a volume of 18 μL. For resolution enhancement, a shifted Gaussian function (gf = 0.12 and gsf = 0.05) was applied before Fourrier transformation. Zero-phase but not baseline was corrected. **(B)** shows mean metabolite concentrations in the hippocampus of Wistar (n = 6), GK (n = 8), caffeine-treated Wistar (n = 7) and caffeine-treated GK (n = 8) rats, at plasma glucose below 8 mmol/L. None of the metabolite concentrations showed caffeine-diabetes interaction. Results from *post-hoc* tests on metabolite levels after significant diabetes effect or caffeine-diabetes interaction are represented by *P < 0.05, **P < 0.01, and ***P < 0.001 for GK vs. Wistar in the respective treatment group.

Altered Density of Adenosine A_1 and A_{2A} Receptors in the Hippocampus

The density of adenosine receptors was evaluated by Western Blot in whole membranes and nerve terminal-enriched membranes prepared from the hippocampus. In synaptosomes, there was a significant interaction between effects of diabetes and caffeine on the density of A_1R (interaction $P < 0.001$, diabetes $P = 0.539$, caffeine $P = 0.002$, **Figure 6A**). When compared to controls, *post-hoc* analyses revealed a significant reduction of A_1R immunoreactivity in GK rats (-28 ± 7%, $P = 0.014$, $n = 8$), which was reversed upon caffeine consumption (+40 ± 10%, $P = 0.002$, $n = 8$). In contrast, diabetes caused a significant increase in levels of A_{2A}R in synaptic membranes, independently of caffeine consumption (diabetes $P < 0.001$, caffeine $P = 0.919$, interaction $P = 0.223$; **Figure 6A**). In the absence of caffeine, hippocampal synaptosomes from GK rats showed a 18±7% increase of A_{2A}R immunoreactivity ($P = 0.031$, $n = 3$). Within caffeine treated rats, there was a T2D-induced increase of A_{2A}R immunoreactivity of 32±6% ($P = 0.014$, $n = 3$). In total membranes, T2D was associated to a major reduction of A_1R levels (diabetes $P = 0.006$, caffeine $P = 0.210$, interaction $P = 0.181$), which was significantly different from controls only in the absence of caffeine treatment (−47 ± 8%, $P = 0.005$, $n = 6$; **Figure 6B**). In turn, the opposite effect was observed for A_{2A}R immunoreactivity, which increased in GK rats compared to controls in the absence (+71 ± 26%, $P = 0.046$, $n = 2$) but not in the presence of caffeine treatment (diabetes $P = 0.057$, caffeine $P = 0.205$, interaction $P = 0.232$). It should be noted however that the detection of changes on A_{2A}R density by Western blot suffered from technical challenges due to the known low immunoreactivity signal from the hippocampus of 6 month old rats (e.g., Rebola et al., 2003; Canas et al., 2009a). This is especially critical in total membranes from the rat hippocampus, in which the density of A_{2A}R is about half of that in synaptosomal membranes (e.g., Rebola et al., 2005; Duarte et al., 2006). Therefore, the present A_{2A}R density changes should be interpreted in a qualitative rather than quantitative manner.

Altered Efficiency of Adenosine Receptors Controlling Hippocampal Synaptic Transmission and Plasticity

The near superimposable input/output curves obtained in hippocampal slices from Wistar and GK rats ascertains that there were no changes in the density of excitatory inputs in Schaffer fibers CA1 pyramid synapses (**Figure 7A**), enabling a direct comparison of the efficiency of A_1R and A_{2A}R to control synaptic transmission and plasticity. Thus, we tested the ability of A_1R to control basal synaptic transmission

TABLE 1 | Mean physiologic parameters measured at 5 different intervals of steady-state plasma glucose concentration in MRS experiments.

Plasma glucose range (mM)		<8	8–14	14–20	20–26	>26
Body Temperature (°C)	Control	37.5 ± 0.2	37.4 ± 0.1	37.5 ± 0.1	37.2 ± 0.2	37.3 ± 0.1
	Caffeine	37.5 ± 0.2	37.2 ± 0.2	37.3 ± 0.1	37.1 ± 0.1	37.4 ± 0.1
	GK	37.0 ± 0.1	37.3 ± 0.2	37.2 ± 0.3	37.4 ± 0.2	37.0 ± 0.1
	GK + Caffeine	37.5 ± 0.1	37.3 ± 0.2	37.5 ± 0.2	37.6 ± 0.1	37.2 ± 0.1
Arterial pH	Control	7.34 ± 0.01	7.34 ± 0.01	7.34 ± 0.01	7.31 ± 0.02	7.33 ± 0.03
	Caffeine	7.42 ± 0.02	7.38 ± 0.01	7.35 ± 0.01	7.35 ± 0.01	7.33 ± 0.02
	GK	7.41 ± 0.02	7.40 ± 0.02	7.42 ± 0.02	7.40 ± 0.01	7.39 ± 0.01
	GK + Caffeine	7.44 ± 0.01	7.45 ± 0.01	7.39 ± 0.02	7.39 ± 0.02	7.38 ± 0.02
P_aCO_2 (mm Hg)	Control	44.7 ± 2.0	44.7 ± 1.6	46.8 ± 1.3	44.9 ± 1.9	42.1 ± 2.0
	Caffeine	39.9 ± 2.9	41.5 ± 2.7	45.7 ± 3.9	41.7 ± 2.7	41.4 ± 2.9
	GK	39.0 ± 3.4	38.6 ± 2.0	37.2 ± 2.7	40.3 ± 2.6	43.2 ± 2.3
	GK + Caffeine	35.7 ± 1.7	39.5 ± 4.7	38.9 ± 3.0	40.7 ± 3.0	40.1 ± 1.3

Data is mean ± SEM of 6 to 8 rats in each experimental group.

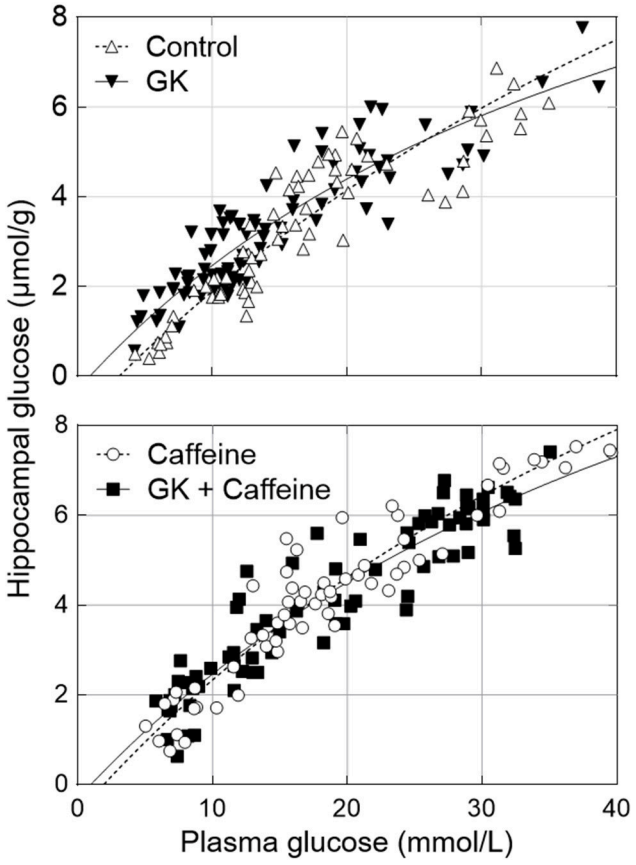

FIGURE 4 | Relationship between hippocampal and plasma glucose concentrations in control and GK rats treated or not with caffeine (1 g/L). Data corresponds to steady-state hippocampal glucose levels determined from ^{1}H NMR spectra measured during 40 min after plasma glucose was stable for at least 15 min. The kinetic parameters of glucose transport estimated from these datasets are presented in **Table 2** and all measurements from $n = 6$–8 animals per group were pooled together.

TABLE 2 | Apparent Michaelis-Menten constant (K_t), iso-inhibition constant (K_{ii}) and ratio of maximal transport rate (T_{max}) to cerebral glucose consumption rate (CMR_{glc}) for the glucose transport across the BBB, estimated with the 4-state conformational model from the relationship between hippocampal and plasma glucose concentrations in control, GK, caffeine-treated control, caffeine-treated GK rats (data in **Figure 4**).

	T_{max}/CMR_{glc}	K_t	K_{ii}
Control	2.3 (1.9–3.1)	4.2 (2.4–7.4)	22.9 (8.4–161.8)
GK	2.5 (2.0–4.7)	1.5 (0.0–8.2)	12.9 (3.3–55.0)
Caffeine-treated control	2.5 (1.9–4.3)	2.9 (0.1–10.4)	19.1 (5.0–201.9)
Caffeine-treated GK	2.4 (1.9–6.8)	1.4 (0.0–17.4)	16.2 (2.3–74.9)

Values are mean (95% confidence interval). Units of K_t and K_{ii} are mmol/L, T_{max}/CMR_{glc} is adimensional.

(Costenla et al., 2011) and found that the closest chemical analog of adenosine, 2-chloroadenosine (CADO), triggered a similar concentration-dependent inhibition of synaptic transmission (**Figure 7B**). In fact, the estimated EC_{50} of CADO to inhibit synaptic transmission was 0.56 µmol/L (95% confidence interval: 0.08–1.04 µmol/L, $n = 6$) in slices from Wistar rats, which was similar ($P = 0.833$) to the EC_{50} values obtained in slices from GK rats (0.61 µmol/L, 95% confidence interval: 0.23–1.00 µmol/L, $n = 6$). We then investigated if there were changes in the levels of endogenous adenosine tonically controlling basal excitatory transmission (Costenla et al., 2011). A supra-maximal but selective concentration (100 nmol/L) of the A_1R antagonist DPCPX (Sebastião et al., 2000) caused a greater disinhibition of hippocampal synaptic transmission in GK rats compared to Wistar rats ($P = 0.006$; $n = 6$; **Figures 7C,D**). This suggests a preserved efficiency of A_1R-mediated inhibition of synaptic transmission and higher levels of endogenous extracellular adenosine controlling synaptic transmission in GK rats.

We next compared synaptic plasticity in hippocampal slices from Wistar and GK rats to gauge the efficiency of $A_{2A}R$

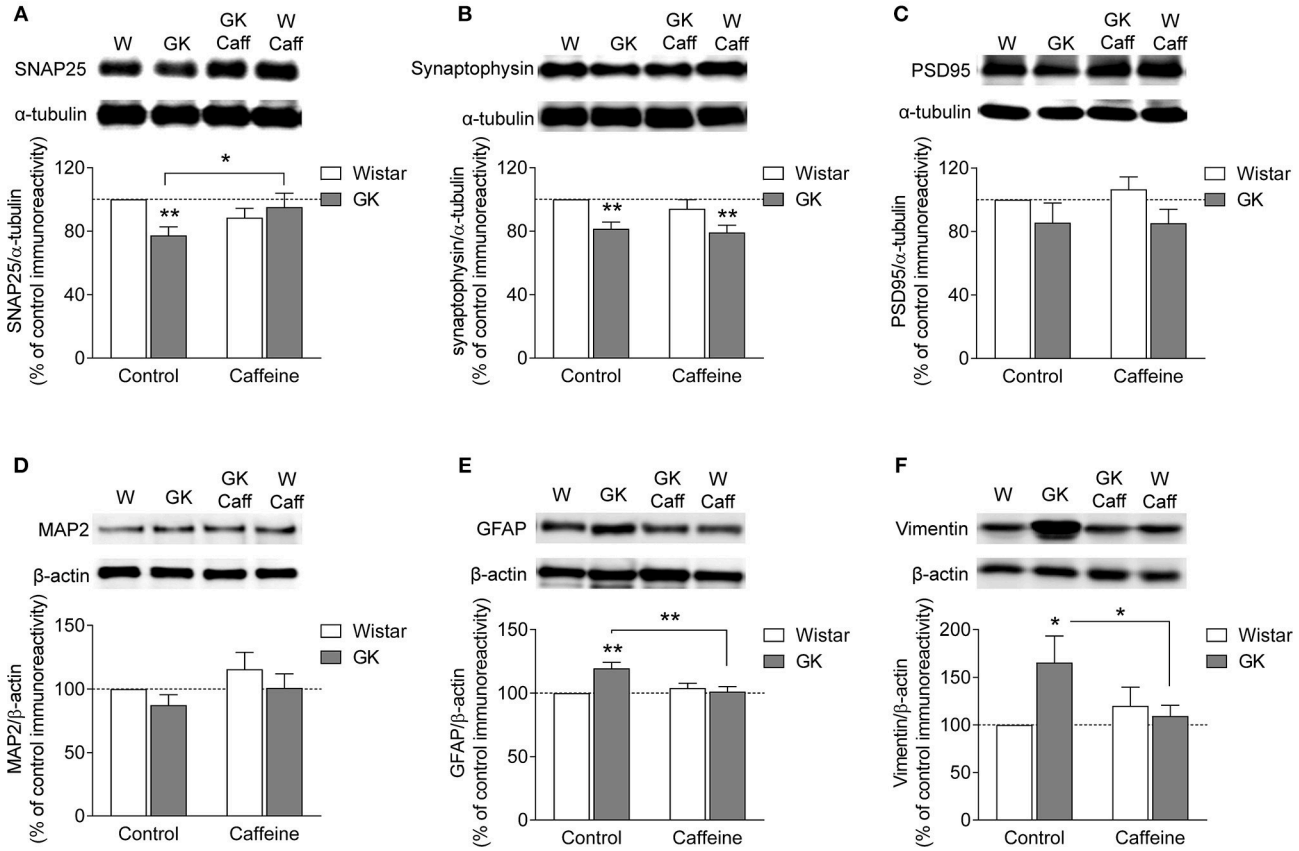

FIGURE 5 | Caffeine consumption attenuated diabetes-induced synaptic degeneration and prevented astrogliosis in the hippocampus of GK rats. Western blot analysis revealed that hippocampal synaptosomal membranes from diabetic GK rats displayed reduced immunoreactivity of both SNAP25 **(A)** and synaptophysin **(B)** but not PSD95 **(C)**, when compared to Wistar rats (W). In total hippocampal membranes, diabetes failed to alter MAP2 immunoreactivity relative to controls **(D)**. Caffeine (Caff) consumption prevented diabetes-induced reduction of SNAP25 but not synaptophysin. Western blot analysis revealed that both GFAP **(E)** and vimentin **(F)** immunoreactivity was increased in total hippocampal membranes from GK compared to control rats, an effect prevented by the consumption of caffeine. Samples were loaded in the SDS-PAGE gel at a protein concentration of 25 μg. Immunoreactivity of proteins of interest was normalized to either α-tubulin or β-actin, and expressed as percentage of control in the same Western blot experiment. In the graphs, open and filled bars represent Wistar and GK rats, respectively. Data are mean±SEM of $n = 5$–8 animals per experimental group. Symbols represent LSD test results after ANOVA with either significant diabetes effect or significant diabetes-caffeine interaction: $^{*}P < 0.05$, $^{**}P < 0.01$ for GK vs. Wistar in the respective treatment group or as indicated.

that selectively control hippocampal synaptic plasticity (Rebola et al., 2008; Costenla et al., 2011). The amplitude of long-term potentiation (LTP) was lower ($P = 0.0004$, **Figure 7E**) in GK rats (52 ± 4% over baseline, $n = 6$) than in Wistar rats (83 ± 2% over baseline, $n = 5$). The selective $A_{2A}R$ antagonist SCH58261, used in a supramaximal and selective concentration of 50 nmol/L (Lopes et al., 2004), caused a discrete inhibition of LTP amplitude in Wistar rats (70 ± 9% over baseline, $n = 5$) and recovered the depressed LTP amplitude in GK rats to near control values (74 ± 5% over baseline, $n = 6$). A two-way ANOVA on LTP amplitude results showed a significant effect of diabetes [$F_{(1,18)} = 5.0$; $P = 0.038$], no effect of applied drug [$F_{(1,18)} = 0.5$; $P = 0.496$], and a significant effect of their interaction [$F_{(1,18)} = 9.0$; $P = 0.008$]. *Post-hoc* analyses confirmed that LTP amplitude was decreased in GK compared to Wistar rats ($P = 0.002$), whereas SCH58261 recovered plasticity in GK rats ($P = 0.014$) but had a negligible effect in Wistar rats ($P = 0.136$).

DISCUSSION

The present study deepens our knowledge of the impact of T2D on cognitive function, which is not yet fully understood (Frisardi et al., 2010; Steculorum et al., 2014; Duarte, 2015). Diabetic rats displayed impaired hippocampal-dependent spatial memory, as suggested by reduced Y-maze spontaneous alternation. This diabetes-induced memory impairment was not accompanied by a modification of glucose transport to consumption ratio, that is, there was no alteration of hippocampal glucose concentration at a given glycaemia. Instead, when compared to controls, GK rats displayed alterations of the metabolic profile, synaptic dysfunction, and astrogliosis in the hippocampus. The causal relation between synaptic damage and astrogliosis and the memory impairment in GK rats is further emphasized by the observation that caffeine consumption had simultaneous beneficial effects on diabetes-induced spatial memory dysfunction, synaptic damage, and astrogliosis. We

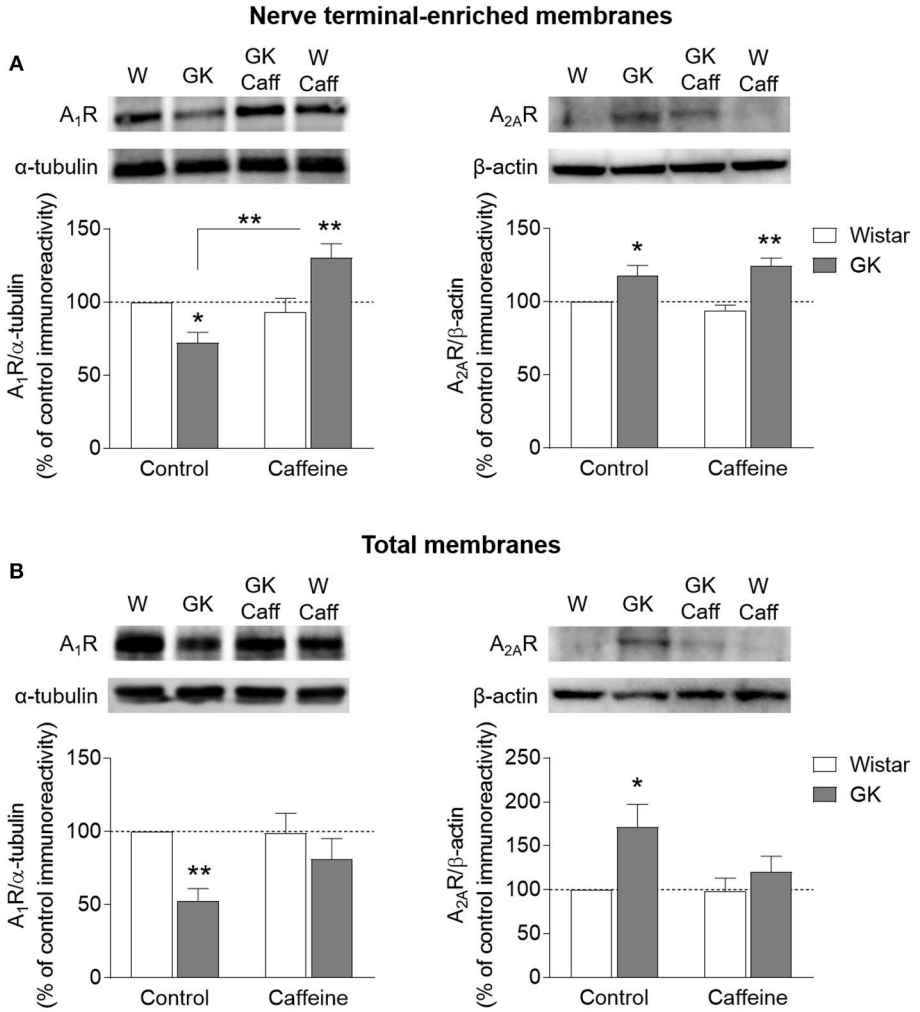

FIGURE 6 | Density A_1R and $A_{2A}R$ analyzed by Western blot in nerve terminal-enriched membranes **(A)** and total membranes **(B)** from the hippocampus of GK and Wistar (W) rats, either receiving 1 g/L caffeine (Caff) or tap water. Samples were loaded in SDS-PAGE gels at a protein concentration of 40 or 150 μg for A_1R ($n = 6$–8) and $A_{2A}R$ ($n = 2$–3), respectively. Immunoreactivity was normalized to either α-tubulin or β-actin, and calculated as percentage of control in the same Western blot experiment. Symbols represent LSD test results after ANOVA with either significant diabetes effect or significant diabetes-caffeine interaction: $^*P < 0.05$, $^{**}P < 0.01$ for GK vs. Wistar in the respective treatment group or as indicated.

further observed that both caffeine and T2D have an impact on metabolism in the hippocampus. However, caffeine consumption did not prevent diabetes-induced metabolic changes. It remains to be ascertained whether caffeine-associated metabolic changes are beneficial for the diabetic brain.

Brain function relies on glucose as main source of energy, and resting brain glucose uptake and consumption are largely independent from circulating insulin (Hasselbalch et al., 1999). Although diabetes leads to inadequate glucose transport in peripheral tissues, brain glucose utilization may eventually adapt to a new metabolic condition upon diabetes (Pelligrino et al., 1992). Some studies reported a lack of effect of type 1 diabetes on the transport of glucose into the brain (Kainulainen et al., 1993; Simpson et al., 1999). Likewise, previous studies in humans reported that poorly controlled diabetes did not affect glucose transport into the brain (Fanelli et al., 1998). We have previously found that insulin-dependent rats have hippocampus to plasma glucose concentrations similar to controls (Duarte et al., 2009a). In GK rats, we recently demonstrated that T2D is associated to reduced glucose transport and consumption rates in the whole brain, without modifying brain to plasma glucose relationship at steady-state. The present results confirmed this observation in the dorsal hippocampus, which controls learning and memory. Unaltered glucose transport to consumption ratio (T_{max}/CMR_{glc}) implies an increased glucose concentration in the hippocampus under sustained hyperglycaemia. The high glucose level in the hippocampus may trigger osmolarity alterations and thus induce metabolic adaptation. This is expected to translate into a modified neurochemical profile, as was observed in the hippocampus of GK rats compared to

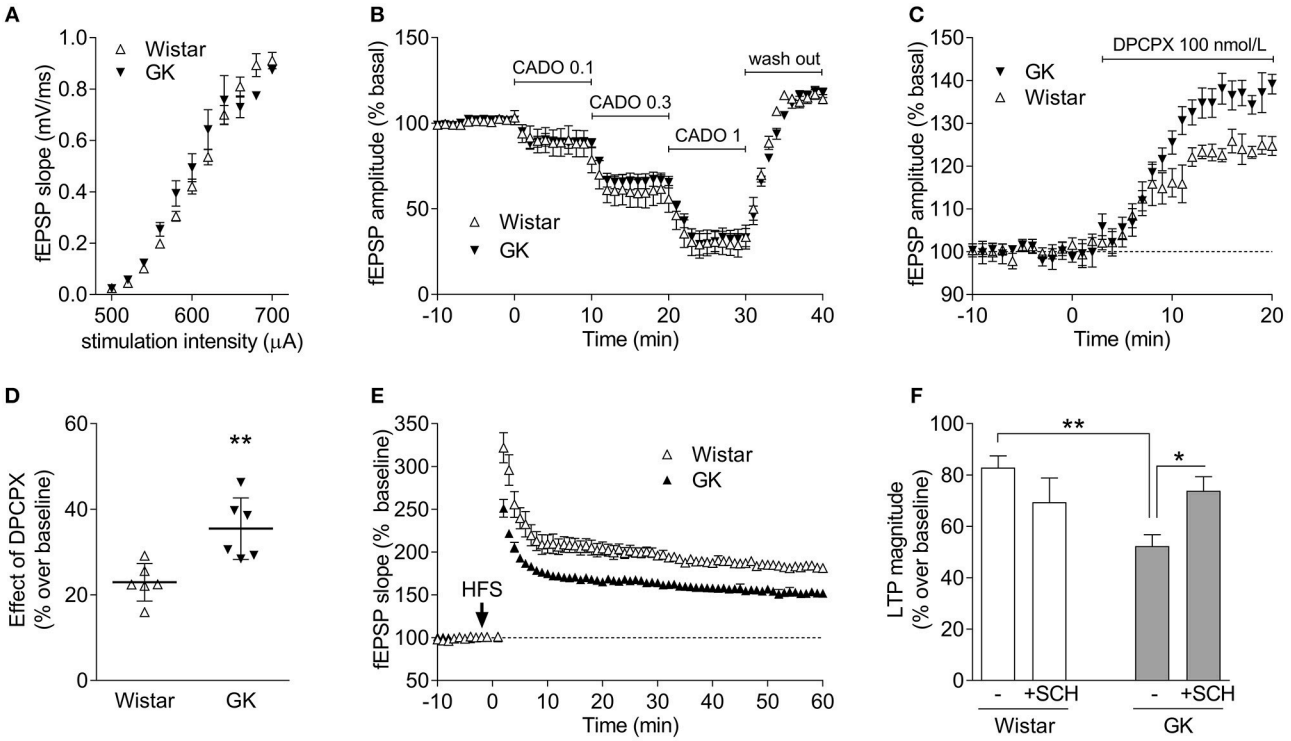

FIGURE 7 | GK rats display an unchanged A_1R-mediated inhibition of excitatory synaptic transmission in the hippocampus but an increased A_{2A}R-mediated control of synaptic plasticity. **(A)** Superimposed input-output curves in GK (filed symbols) and Wistar rats (open symbols), indicating a similar density of excitatory innervation in Schaffer fiber-CA1 pyramid synapses of hippocampal slices. **(B)** Superimposable concentration-dependent curves of inhibition of CA1 hippocampal field excitatory post-synaptic potentials (fEPSPs) by the closest chemical analog of adenosine, 2-chloroadenosine (CADO; applied at concentrations of 0.1, 0.3, and 1 μmol/L), showing that the A_1R-mediated inhibition of synaptic transmission is similar in GK and Wistar rats. The fEPSP slope before CADO application is normalized to 100%, and 0% corresponds to a complete inhibition of fEPSPs. **(C,D)** GK rats seem to have higher levels of endogenous extracellular adenosine tonically activating inhibitory A_1R, as revealed by the larger disinhibition of fEPSPs by the selective A_1R antagonist, DPCPX. **(E)** Averaged time course changes of fEPSP slope induced by a high-frequency stimulation train (HFS 100 Hz, 1 s, applied when indicated by the arrow) in hippocampal slices taken from Wistar (open symbols) or GK rats (filed symbols), showing that the amplitude of LTP is larger in Wistar than in GK rats. **(F)** The presence of the A_{2A}R antagonist SCH58261 (50 nM, +SHC), which was applied 30 min before LTP induction and remained in the bath up to the end of the experiment, has a discrete effect in Wistar rats (open bars) and efficiently restores LTP amplitude back to control levels in GK rats (gray bars). The electrophysiological data are mean ± SEM of 5–6 animals per experimental condition. $^*P < 0.05$, $^{**}P < 0.01$ using either an unpaired Student's *t* test in **(C)** or a two-way ANOVA followed by a LSD *post-hoc* test in **(F)**.

controls under normoglycaemia (**Figure 3**). The most prominent alteration was an increase in the hippocampal concentration of the osmolite taurine. Surprisingly, the concentration of *myo*-inositol (another osmolite) was not substantially altered in the hippocampus of GK rats in the absence of caffeine, in contrast to what was observed in streptozotocin-induced diabetic rats (Duarte et al., 2009a) or Zucker diabetic obese rats (van der Graaf et al., 2004). However, these models of diabetes are characterized by sustained hyperglycaemia ranging from 25 to 30 mM of plasma glucose (Wilkes et al., 2005; Duarte et al., 2009a), while GK rats are subjected to a rather mild hyperglycaemia state (below 15 mmol/L). This tentatively suggests that *myo*-inositol levels may only increase upon more extreme hyperosmolarity.

T2D was also associated with increased levels of ascorbate in the hippocampus, which is in line with stimulation of ascorbate production in the rat liver under mild hyperglycaemia (Küstermann et al., 1998). Ascorbate is involved in the regulation of brain glycolysis and pentose phosphate pathway, as well as astrocyte-neuron metabolic interactions (Cisternas et al., 2014), and changes of its concentration in the hippocampus may be related with T2D-induced adaptations of energy metabolism (Girault et al., 2018). The non-deleterious but rather adaptive nature of these diabetic-induced metabolic changes in hippocampal metabolic profile is supported by the observation that GK rats at euglycaemia displayed augmented phosphocreatine-to-creatine ratio, compared to controls.

As previously observed in the hippocampus of insulin-dependent diabetic rats, caffeine consumption lowered *myo*-inositol concentration, and increased hippocampal levels of taurine. Taurine is an amino acid that, although present at 1 μmol/g in the human brain, it reaches relatively large concentrations in the rodent brain (above 5 μmol/g in rats and above 8 μmol/g in mice; Duarte, 2016), playing a major role as osmolyte (Duarte et al., 2009a, and references therein). Indeed, caffeine is able to control osmotic swelling via adenosine

receptors (Wurm et al., 2008). In addition, taurine acts as an agonist at receptors of the GABAergic and glycinergic neurotransmitter systems (Albrecht and Schousboe, 2005), and caffeine controls taurine release from both neurons and glia via adenosine receptors (Hada et al., 1998). Taurine is transported into the mitochondrial matrix where it buffers pH to the optimal value for isocitrate dehydrogenase, which is a key enzyme of the tricarboxylic acid cycle regulating metabolism and oxidative phosphorylation, contributes to stabilize the pH gradient across the inner-membrane, and thus helps preserving mitochondrial function and preventing oxidative damage (Hansen et al., 2010). Therefore, this caffeine-associated increase of taurine levels in the diabetic hippocampus is likely related to neuroprotective functions.

Finally, it should be stressed that these adaptive metabolic modifications in the hippocampus of GK rats indeed seem to be caused by hyperglycaemia rather than by hyperinsulinemia since chronic consumption of caffeine prevented the later but not the former, and failed to prevent hippocampal metabolite alterations in GK rats, despite caffeine-induced metabolic changes.

The evaluation of hippocampal metabolite concentrations in Wistar and GK rats showed that this brain structure faces high glucose levels in diabetes at their fed glycaemia, which may lead to neurotoxicity and cellular damage. The present results indicate that T2D in GK rats caused neurodegeneration that does not affect the entire neuron, as suggested by unaltered MAP2 immunoreactivity, but instead occurs selectively at the presynaptic component of the nerve terminal, as previously proposed (Duarte et al., 2006, 2009a, 2012; Gaspar et al., 2010). In fact, GK rats displayed a reduced density of the presynaptic proteins SNAP25 and synaptophysin in the hippocampus, whereas the density of the postsynaptic protein PSD95 was not significantly altered relative to controls. The alteration of these presynaptic markers allowed sustaining synaptic transmission but was associated with an alteration of synaptic plasticity typified by a reduced amplitude of long-term potentiation in the hippocampal CA1 area of GK compared to Wistar rats. These synaptic modifications may eventually underlie the memory impairment observed in GK rats, as proposed to occur in Alzheimer's disease-associated neurodegeneration (Selkoe, 2002; Coleman et al., 2004). Together with synaptic dysfunction, we further found increased immunoreactivity of the glial-specific proteins GFAP and vimentin in the hippocampus of GK rats. This is in accordance with the occurrence of astrocytosis in the hippocampus, which was observed in other animal models of diabetes (e.g., Saravia et al., 2002; Baydas et al., 2003; Duarte et al., 2009a, 2012). Astrocytosis can be triggered by neuronal damage and contribute to further neuronal deterioration through the production of free radicals (e.g., Chao et al., 1996) and apoptotic factors (e.g., Crutcher et al., 1993; Fahnestock et al., 1996), leading to memory impairment (see Halassa and Haydon, 2010). In line with synaptic degeneration and astrocytosis, we have previously reported that GK rats show whole brain depression of neuronal oxidative metabolism and glutamate-glutamine cycle, and exacerbation of oxidative metabolism in astrocytes (Girault et al., 2018).

The relation between synaptotoxicity and astrogliosis with memory impairment in GK rats was further supported by the common ability of chronic caffeine consumption to simultaneously ameliorate or prevent these T2D-induced modifications. This is in agreement with the general neuroprotective action of chronic caffeine consumption against brain damage, which is largely mimicked by antagonists of adenosine A_{2A}Rs (Cunha, 2005; Chen et al., 2007). In particular, both caffeine and selective A_{2A}R antagonists are effective in improving memory performance upon noxious insults (Takahashi et al., 2008; Cunha, 2016), which was also observed in this study. Thus, neuroprotection and preservation of memory function by caffeine is likely associated with antagonism of A_{2A}Rs at the synaptic level as well as in glial cells (Cunha, 2016). Notably, the over-functioning of A_{2A}Rs is sufficient to impair memory performance (Li et al., 2015; Pagnussat et al., 2015). Accordingly, A_{2A}Rs were up-regulated in the hippocampus of GK rats, similarly to what was observed in other animal models of T1D (Duarte et al., 2009a) or T2D (Duarte et al., 2012) and in a variety of conditions associated with memory dysfunction, such as aging (Rebola et al., 2003; Canas et al., 2009a; Temido-Ferreira et al., 2018) or Alzheimer's disease (Canas et al., 2009b; Espinosa et al., 2013; Viana da Silva et al., 2016; Silva et al., 2018). In fact, the only established molecular targets for caffeine at non-toxic concentrations, which were achieved in the present study, are adenosine receptors, mainly A_1Rs and A_{2A}Rs (Fredholm et al., 1999). Hippocampal A_{2A}Rs are concentrated in synapses, where they selectively control synaptic plasticity processes (Rebola et al., 2008; Costenla et al., 2011; Temido-Ferreira et al., 2018) and play a prominent role in controlling the synaptic damage (Cunha et al., 2006; Silva et al., 2007, 2018; Canas et al., 2009b; Viana da Silva et al., 2016) that tightly correlates with memory impairment for instance in Alzheimer's disease (Selkoe, 2002; Coleman et al., 2004). Interestingly, we found that caffeine prevented the diabetes-induced loss of SNAP25 but not of synaptophysin. This is in agreement with previous observations suggesting that proteins of the SNARE complex are more robust indicators of synaptic dysfunction than proteins located in synaptic vesicles, such as synaptophysin (Reddy et al., 2005; Gao et al., 2006). We also observed that GK rats displayed a reduction of A_1R immunoreactivity. This was not associated with a modification of A_1R function controlling basal synaptic transmission in GK rats, in accordance with our observation that the modification of A_1R density mostly occurs in total membranes. Since extra-synaptic A_1Rs have recently been associated with modified information processing in cortical circuits (Florian et al., 2011; Serchov et al., 2015), future studies should focus on the possible role of A_1Rs on memory performance through a control of neuron-glia communication.

In whole hippocampal membranes we observed a diabetes-induced reduction in A_1R and increase of A_{2A}R levels, which was normalized upon caffeine treatment. Therefore, limiting excessive activation of A_{2A}Rs in extra-synaptic compartments, namely in glial cells, might also be a mechanism of neuroprotection by caffeine in T2D (Cunha, 2016). Indeed, apart from its synapto-protective action, caffeine had beneficial effects on T2D-induced astrogliosis, which emphasizes the

potential neuroprotective role of glial A_{2A}Rs (Daré et al., 2007), as reported in animal models of Alzheimer's (Matos et al., 2012) and Parkinson's disease (Yu et al., 2008), as well as exposure to LPS (Rebola et al., 2011) or glaucoma (Madeira et al., 2015).

It is important to stress that the present results do not exclude the possibility that the beneficial effects of chronic caffeine consumption might also involve the control of peripheral metabolism and circulating insulin concentration, such as via adenosine receptors in the pancreatic islet (e.g., Johansson et al., 2007; Töpfer et al., 2008; Salehi et al., 2009), or via regulation of peripheral metabolic rates and energy expenditure (van Dam and Hu, 2005; Greenberg et al., 2006; Higdon and Frei, 2006). It is of interest to note that GK rats chronically consuming caffeine displayed hyperglycemia but not hyperinsulinemia. The chronic caffeine treatment used in the present study was also previously found to improve peripheral insulin sensitivity and reduce circulating insulin concentration in aged rats (Guarino et al., 2013) and rats under diets rich in sugar or fat (Conde et al., 2012). In the brain, insulin and insulin-like growth factor 1 (IGF1) may be involved in regulating the presence of glucose carriers at the membrane of astrocytes (Fernandez et al., 2017), the expression of synaptic proteins and number of synapses (Chiu et al., 2008), the reactivity of astrocytes (e.g., Wilczak and De Keyser, 1997), and learning and memory processes (Zhao and Alkon, 2001). Notably, while insulin and insulin-sensitizing drugs have beneficial effect in dementia, it has also been proposed that persistent activation of insulin receptors could be the trigger for brain insulin resistance (e.g., Mullins et al., 2017). Therefore, further research is needed to understand the role of insulin in T2D-induced brain dysfunction.

In summary, long-term caffeine intake improved T2D-induced memory impairment, prevented astrogliosis, and ameliorated hippocampal synaptic degeneration in GK rats. Caffeine did not prevent T2D-associated metabolic modifications in the hippocampus. Nevertheless, it had an impact on metabolite concentrations in the hippocampus of both Wistar and GK rats.

Therefore, we conclude that the hippocampus is adaptable to different metabolic conditions, and that synaptic degeneration and astrogliosis rather than metabolic modifications contribute to diabetes-induced memory dysfunction. Finally, the present study also emphasizes the neuroprotective potential of chronic caffeine consumption as a prophylactic strategy to prevent memory impairment in T2D.

AUTHOR CONTRIBUTIONS

JD and RC designed the study. JD, CS, and HS performed experiments and analyzed data. JD wrote the manuscript. All authors contributed to the interpretation of the results and revised the manuscript.

ACKNOWLEDGMENTS

This work was supported by the Knut and Alice Wallenberg Foundation, Swedish Research Council (Strategic Research Area EXODIAB, grant 2009-1039), Swedish Foundation for Strategic Research (grant IRC15-0067), Swiss National Science Foundation (Ambizione grant 148250), Fundação para a Ciência e a Tecnologia (grants POCTI/SAU-NEU/56098/2004 and POCI-01-0145-FEDER-031274), Centro 2020 (projects CENTRO-01-0145-FEDER-000008:BrainHealth 2020 and CENTRO-01-0246-FEDER-000010), and Center d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL, and the Leenaards and Jeantet Foundations. We thank Professor Raquel Seiça for supplying brain tissue from some Wistar and GK rats.

REFERENCES

Albrecht, J., and Schousboe, A. (2005). Taurine interaction with neurotransmitter receptors in the CNS: an update. *Neurochem. Res.* 30, 1615–1621. doi: 10.1007/s11064-005-8986-6

Anderson, W. W., and Collingridge, G. L. (2001). The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. *J. Neurosci. Methods.* 108, 71–83. doi: 10.1016/S0165-0270(01)00374-0

Baydas, G., Nedzvetskii, V. S., Tuzcu, M., Yasar, A., and Kirichenko, S. V. (2003). Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E. *Eur. J. Pharmacol.* 462, 67–71. doi: 10.1016/S0014-2999(03)01294-9

Canas, P. M., Duarte, J. M. N., Rodrigues, R. J., Köfalvi, A., and Cunha, R. A. (2009a). Modification upon aging of the density of presynaptic modulation systems in the hippocampus. *Neurobiol. Aging* 30, 1877–1884. doi: 10.1016/j.neurobiolaging.2008.01.003

Canas, P. M., Porciúncula, L. O., Cunha, G. M., Silva, C. G., Machado, N. J., Oliveira, J. M., et al. (2009b). Adenosine A_{2A} receptor blockade prevents synaptotoxicity and memory dysfunction caused by β-amyloid peptides via p38 mitogen-activated protein kinase pathway. *J. Neurosci.* 29, 14741–14751. doi: 10.1523/JNEUROSCI.3728-09.2009

Chao, C. C., Hu, S., Sheng, W. S., Bu, D., Bukrinsky, M. I., and Peterson, P. K. (1996). Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. *Glia* 16, 276–284. doi: 10.1002/(SICI)1098-1136(199603)16:3<276::AID-GLIA10>3.0.CO;2-X

Chen, J. F., Sonsalla, P. K., Pedata, F., Melani, A., Domenici, M. R., Popoli, P., et al. (2007). Adenosine A_{2A} receptors and brain injury: broad spectrum of neuroprotection, multifaceted actions and "fine tuning" modulation. *Prog. Neurobiol.* 83, 310–331. doi: 10.1016/j.pneurobio.2007.09.002

Chiu, S. L., Chen, C. M., and Cline, H. T. (2008). Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function *in vivo*. *Neuron* 58, 708–719. doi: 10.1016/j.neuron.2008.04.014

Cisternas, P., Silva-Alvarez, C., Martínez, F., Fernandez, E., Ferrada, L., Oyarce, K., et al. (2014). The oxidized form of vitamin C, dehydroascorbic acid, regulates neuronal energy metabolism. *J. Neurochem.* 129, 663–671. doi: 10.1111/jnc.12663

Coleman, P., Federoff, H., and Kurlan, R. (2004). A focus on the synapse for neuroprotection in Alzheimer disease and other dementias. *Neurology* 63, 1155–1162. doi: 10.1212/01.WNL.0000140626.48118.0A

Conde, S. V., Nunes da Silva, T., Gonzalez, C., Mota Carmo, M., Monteiro, E. C., and Guarino, M. P. (2012). Chronic caffeine intake decreases circulating catecholamines and prevents diet-induced insulin resistance and hypertension in rats. *Br. J. Nutr.* 107, 86–95. doi: 10.1017/S0007114511002406

Convit, A., Wolf, O. T., Tarshish, C., and de Leon, M. J. (2003). Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2019–2022. doi: 10.1073/pnas.0336073100

Costenla, A. R., Diógenes, M. J., Canas, P. M., Rodrigues, R. J., Nogueira, C., Maroco, J., et al. (2011). Enhanced role of adenosine A_{2A} receptors in the

modulation of LTP in the rat hippocampus upon ageing. *Eur. J. Neurosci.* 34, 12–21. doi: 10.1111/j.1460-9568.2011.07719.x

Crutcher, K. A., Scott, S. A., Liang, S., Everson, W. V., and Weingartner, J. (1993). Detection of NGF-like activity in human brain tissue: increased levels in Alzheimer's disease. *J. Neurosci.* 13, 2540–2550. doi: 10.1523/JNEUROSCI.13-06-02540.1993

Cunha, G. M., Canas, P. M., Oliveira, C. R., and Cunha, R. A. (2006). Increased density and synapto-protective effect of adenosine A_{2A} receptors upon sub-chronic restraint stress. *Neuroscience* 141, 1775–1781. doi: 10.1016/j.neuroscience.2006.05.024

Cunha, R. A. (2005). Neuroprotection by adenosine in the brain: from A_1 receptor activation to A_{2A} receptor blockade. *Purinergic. Signal.* 1, 111–134. doi: 10.1007/s11302-005-0649-1

Cunha, R. A. (2016). How does adenosine control neuronal dysfunction and neurodegeneration? *J. Neurochem.* 139, 1019–1055. doi: 10.1111/jnc.13724

Daré, E., Schulte, G., Karovic, O., Hammarberg, C., and Fredholm, B. B. (2007). Modulation of glial cell functions by adenosine receptors. *Physiol. Behav.* 92, 15–20. doi: 10.1016/j.physbeh.2007.05.031

Duarte, J. M.(2015). Metabolic alterations associated to brain dysfunction in diabetes. *Aging Dis.* 6, 304–321. doi: 10.14336/AD.2014.1104

Duarte, J. M., Carvalho, R. A., Cunha, R. A., and Gruetter, R. (2009a). Caffeine consumption attenuates neurochemical modifications in the hippocampus of streptozotocin-induced diabetic rats. *J. Neurochem.* 111, 368–379. doi: 10.1111/j.1471-4159.2009.06349.x

Duarte, J. M., and Gruetter, R. (2012). Characterization of cerebral glucose dynamics *in vivo* with a four-state conformational model of transport at the blood-brain barrier. *J. Neurochem.* 121, 396–406. doi: 10.1111/j.1471-4159.2012.07688.x

Duarte, J. M., Morgenthaler, F. D., Lei, H., Poitry-Yamate, C., and Gruetter, R. (2009b). Steady-state brain glucose transport kinetics re-evaluated with a four-state conformational model. *Front. Neuroenergetics* 1:6. doi: 10.3389/neuro.14.006.2009

Duarte, J. M., Oses, J. P., Rodrigues, R. J., and Cunha, R. A. (2007). Modification of purinergic signaling in the hippocampus of streptozotocin-induced diabetic rats. *Neuroscience* 149, 382–391. doi: 10.1016/j.neuroscience.2007.08.005

Duarte, J. M. N. (2016). Metabolism in the diabetic brain: neurochemical profiling by ^{1}H magnetic resonance spectroscopy. *J. Diabetes. Metab. Disord.* 3:011. doi: 10.24966/DMD-201X/100011

Duarte, J. M. N., Agostinho, P. M., Carvalho, R. A., and Cunha, R. A. (2012). Caffeine consumption prevents diabetes-induced memory impairment and synaptotoxicity in the hippocampus of NONcNZO10/LtJ mice. *PLoS ONE* 7:e21899. doi: 10.1371/journal.pone.0021899

Duarte, J. M. N., Oliveira, C. R., Ambrósio, A. F., and Cunha, R. A. (2006). Modification of adenosine A_1 and A_{2A} receptor density in the hippocampus of streptozotocin-induced diabetic rats. *Neurochem. Int.* 48, 144–150. doi: 10.1016/j.neuint.2005.08.008

Espinosa, J., Rocha, A., Nunes, F., Costa, M. S., Schein, V., Kazlauckas, V., et al. (2013). Caffeine consumption prevents memory impairment, neuronal damage and adenosine A_{2A} receptors up-regulation in the hippocampus of a rat model of sporadic dementia. *J. Alzheimer. Dis.* 34, 509–518. doi: 10.3233/JAD-111982

Fahnestock, M., Scott, S. A., Jetté, N., Weingartner, J. A., and Crutcher, K. A. (1996). Nerve growth factor mRNA and protein levels measured in the same tissue from normal and Alzheimer's disease parietal cortex. *Mol. Brain Res.* 42, 175–178. doi: 10.1016/S0169-328X(96)00193-3

Fanelli, C. G., Dence, C. S., Markham, J., Videen, T. O., Paramore, D. S., Cryer, P. E., et al. (1998). Blood-to-brain glucose transport and cerebral glucose metabolism are not reduced in poorly controlled type 1 diabetes. *Diabetes* 47, 1444–1450. doi: 10.2337/diabetes.47.9.1444

Fernandez, A. M., Hernandez-Garzón, E., Perez-Domper, P., Perez-Alvarez, A., Mederos, S., Matsui, T., et al. (2017). Insulin regulates astrocytic glucose handling through cooperation with IGF-I. *Diabetes* 66, 64–74. doi: 10.2337/db16-0861

Florian, C., Vecsey, C. G., Halassa, M. M., Haydon, P. G., and Abel, T. (2011). Astrocyte-derived adenosine and A_1 receptor activity contribute to sleep loss-induced deficits in hippocampal synaptic plasticity and memory in mice. *J. Neurosci.* 31, 6956–6962. doi: 10.1523/JNEUROSCI.5761-10.2011

Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A., and Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol. Rev.* 51, 83–133.

Frisardi, V., Solfrizzi, V., Seripa, D., Capurso, C., Santamato, A., Sancarlo, D., et al. (2010). Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease. *Ageing Res. Rev.* 9, 399–417. doi: 10.1016/j.arr.2010.04.007

Gao, Y., Bezchlibnyk, Y. B., Sun, X., Wang, J. F., McEwen, B. S., and Young, L. T. (2006). Effects of restraint stress on the expression of proteins involved in synaptic vesicle exocytosis in the hippocampus. *Neuroscience* 141, 1139–1148. doi: 10.1016/j.neuroscience.2006.04.066

Gaspar, J. M., Baptista, F. I., Galvão, J., Castilho, A. F., Cunha, R. A., and Ambrósio, A. F. (2010). Diabetes differentially affects the content of exocytotic proteins in hippocampal and retinal nerve terminals. *Neuroscience* 169, 1589–1600. doi: 10.1016/j.neuroscience.2010.06.021

Girault, F. M., Sonnay, S., Gruetter, R., and Duarte, J. M. N. (2018). Alterations of brain energy metabolism in type 2 diabetic Goto-Kakizaki rats measured *in vivo* by ^{13}C magnetic resonance spectroscopy. *Neurotox. Res.* doi: 10.1007/s12640-017-9821-y. [Epub ahead of print].

Gold, S. M., Dziobek, I., Sweat, V., Tirsi, A., Rogers, K., Bruehl, H., et al. (2007). Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia* 50, 711–719. doi: 10.1007/s00125-007-0602-7

Greenberg, J. A., Boozer, C. N., and Geliebter, A. (2006). Coffee, diabetes, and weight control. *Am. J. Clin. Nutr.* 84, 682–693. doi: 10.1093/ajcn/84.4.682

Gruetter, R., and Tkáč, I. (2000). Field mapping without reference scan using asymmetric echo-planar techniques. *Magn. Reson. Med.* 43, 319–323. doi: 10.1002/(SICI)1522-2594(200002)43:2<319::AID-MRM22>3.0.CO;2-1

Guarino, M. P., Ribeiro, M. J., Sacramento, J. F., and Conde, S. V. (2013). Chronic caffeine intake reverses age-induced insulin resistance in the rat: effect on skeletal muscle Glut4 transporters and AMPK activity. *Age (Dordr)* 35, 1755–1765. doi: 10.1007/s11357-012-9475-x

Hada, J., Kaku, T., Morimoto, K., Hayashi, Y., and Nagai, K. (1998). Activation of adenosine A_2 receptors enhances high K^+-evoked taurine release from rat hippocampus: a microdialysis study. *Amino Acids* 15, 43–52. doi: 10.1007/BF01345279

Halassa, M. M., and Haydon, P. G. (2010). Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu. Rev. Physiol.* 72, 335–355. doi: 10.1146/annurev-physiol-021909-135843

Hansen, S. H., Andersen, M. L., Cornett, C., Gradinaru, R., and Grunnet, N. (2010). A role for taurine in mitochondrial function. *J. Biomed. Sci.* 17:S23. doi: 10.1186/1423-0127-17-S1-S23

Hasselbalch, S. G., Knudsen, G. M., Videbaek, C., Pinborg, L. H., Schmidt, J. F., Holm, S., et al. (1999). No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. *Diabetes* 48, 1915–1921. doi: 10.2337/diabetes.48.10.1915

Higdon, J. V., and Frei, B. (2006). Coffee and health: a review of recent human research. *Crit. Rev. Food Sci. Nutr.* 46, 101–123. doi: 10.1080/10408390500400009

Hurtado-Alvarado, G., Domínguez-Salazar, E., Velázquez-Moctezuma, J., and Gómez-González, B. (2016). A_{2A} Adenosine receptor antagonism reverts the blood-brain barrier dysfunction induced by sleep restriction. *PLoS ONE* 11:e0167236. doi: 10.1371/journal.pone.0167236

Johansson, S. M., Salehi, A., Sandström, M. E., Westerblad, H., Lundquist, I., Carlsson, P. O., et al. (2007). A_1 receptor deficiency causes increased insulin and glucagon secretion in mice. *Biochem. Pharmacol.* 74, 1628–1635. doi: 10.1016/j.bcp.2007.08.006

Kainulainen, H., Schürmann, A., Vilja, P., and Joost, H. G. (1993). *in vivo* glucose uptake and glucose transporter proteins GLUT1 and GLUT3 in brain tissue from streptozotocin-diabetic rats. *Acta Physiol. Scand.* 149, 221–225. doi: 10.1111/j.1748-1716.1993.tb09615.x

Kaster, M. P., Machado, N. J., Silva, H. B., Nunes, A., Ardais, A. P., Santana, M., et al. (2015). Caffeine acts through neuronal adenosine A_{2A} receptors to prevent mood and memory dysfunction triggered by chronic stress. *Proc. Natl. Acad. Sci. U.S.A.* 112, 7833–7838. doi: 10.1073/pnas.1423088112

Küstermann, E., Seelig, J., and Künnecke, B. (1998). Ascorbic acid, a vitamin, is observed by *in vivo* ^{13}C nuclear magnetic resonance spectroscopy of rat liver. *Am. J. Physiol.* 274(1 Pt 1), E65–E71.

Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. *Neurosci. Biobehav. Rev.* 26, 91–104. doi: 10.1016/S0149-7634(01)00041-0

Li, P., Rial, R., Canas, P. M., Yoo, J. H., Li, W., Zhou, X., et al. (2015). Optogenetic activation of intracellular adenosine A_{2A} receptor signaling in hippocampus

is sufficient to trigger CREB phosphorylation and impair memory. *Mol. Psychiatry* 20, 1339–1349. doi: 10.1038/mp.2014.182

Lopes, L. V., Halldner, L., Rebola, N., Johansson, B., Ledent, C., Chen, J. F., et al. (2004). Binding of the prototypical adenosine A_{2A} receptor agonist CGS 21680 to the cerebral cortex of adenosine A_1 and A_{2A} receptor knockout mice. *Br. J. Pharmacol*. 141, 1006–1014. doi: 10.1038/sj.bjp.0705692

Madeira, M. H., Elvas, F., Boia, R., Gonçalves, F. Q., Cunha, R. A., Ambrósio, A. F., et al. (2015). Adenosine A_{2A}R blockade prevents neuroinflammation-induced death of retinal ganglion cells caused by elevated pressure. *J. Neuroinflammation* 12:115. doi: 10.1186/s12974-015-0333-5

Matos, M., Augusto, E., Machado, N. J., dos Santos-Rodrigues, A., Cunha, R. A., and Agostinho, P. (2012). Astrocytic adenosine A_{2A} receptors control the amyloid-β peptide-induced decrease of glutamate uptake. *J. Alzheimers Dis.* 31, 555–567. doi: 10.3233/JAD-2012-120469

Mlynárik, V., Gambarota, G., Frenkel, H., and Gruetter, R. (2006). Localized short-echo-time proton MR spectroscopy with full signal-intensity acquisition. *Magn. Reson. Med*. 56, 965–970. doi: 10.1002/mrm.21043

Moheet, A., Mangia, S., and Seaquist, E. R. (2015). Impact of diabetes on cognitive function and brain structure. *Ann. N. Y. Acad. Sci*. 1353, 60–71 doi: 10.1111/nyas.12807

Mullins, R. J., Diehl, T. C., Chia, C. W., and Kapogiannis, D. (2017). Insulin resistance as a link between amyloid-beta and tau pathologies in Alzheimer's disease. *Front. Aging Neurosci.* 9:118. doi: 10.3389/fnagi.2017.00118

Pagnussat, N., Almeida, A. S., Marques, D. M., Nunes, F., Chenet, G. C., Botton, P. H., et al. (2015). Adenosine A_{2A} receptors are necessary and sufficient to trigger memory impairment in adult mice. *Br. J. Pharmacol*. 172, 3831–3845. doi: 10.1111/bph.13180

Pelligrino, D. A., LaManna, J. C., Duckrow, R. B., Bryan, R. M., and Harik, S. I. (1992). Hyperglycemia and blood-brain barrier glucose transport. *J. Cereb. Blood Flow Metab*. 12, 887–899. doi: 10.1038/jcbfm.1992.126

Rebola, N., Canas, P. M., Oliveira, C. R., and Cunha, R. A. (2005). Different synaptic and subsynaptic localization of adenosine A_{2A} receptors in the hippocampus and striatum of the rat. *Neuroscience* 132, 893–903. doi: 10.1016/j.neuroscience.2005.01.014

Rebola, N., Lujan, R., Cunha, R. A., and Mulle, C. (2008). Adenosine A_{2A} receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. *Neuron* 57, 121–134. doi: 10.1016/j.neuron.2007.11.023

Rebola, N., Sebastião, A. M., de Mendonça, A., Oliveira, C. R., Ribeiro, J. A., and Cunha, R. A. (2003). Enhanced adenosine A_{2A} receptor facilitation of synaptic transmission in the hippocampus of aged rats. *J. Neurophysiol.* 90, 1295–1303. doi: 10.1152/jn.00896.2002

Rebola, N., Simões, A. P., Canas, P. M., Tomé, A. R., Andrade, G. M., Barry, C. E., et al. (2011). Adenosine A_{2A} receptors control neuroinflammation and consequent hippocampal neuronal dysfunction. *J. Neurochem*. 117, 100–111. doi: 10.1111/j.1471-4159.2011.07178.x

Reddy, P. H., Mani, G., Park, B. S., Jacques, J., Murdoch, G., Whetsell, W., et al. (2005). Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. *J. Alzheimers Dis*. 7, 103–117. doi: 10.3233/JAD-2005-7203

Salehi, A., Parandeh, F., Fredholm, B. B., Grapengiesser, E., and Hellman, B. (2009). Absence of adenosine A_1 receptors unmasks pulses of insulin release and prolongs those of glucagon and somatostatin. *Life Sci*. 85, 470–476. doi: 10.1016/j.lfs.2009.08.001

Saravia, F. E., Revsin, Y., Gonzalez Deniselle, M. C., Gonzalez, S. L., Roig, P., Lima, A., et al. (2002). Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the non-obese diabetic (NOD) and streptozotocin-treated mice. *Brain Res*. 957, 345–353. doi: 10.1016/S0006-8993(02)03675-2

Sebastião, A. M., Cunha, R. A., de Mendonça, A., and Ribeiro, J. A. (2000). Modification of adenosine modulation of synaptic transmission in the hippocampus of aged rats. *Br. J. Pharmacol*. 131, 1629–1634. doi: 10.1038/sj.bjp.0703736

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science* 298, 789–791. doi: 10.1126/science.1074069

Serchov, T., Clement, H. W., Schwarz, M. K., Iasevoli, F., Tosh, D. K., Idzko, M., et al. (2015). Increased signaling via adenosine A_1 receptors, sleep deprivation, imipramine, and ketamine inhibit depressive-like behavior via induction of homer1a. *Neuron* 87, 549–562. doi: 10.1016/j.neuron.2015.07.010

Silva, A. C., Lemos, C., Gonçalves, F. Q., Pliássova, A. V., Machado, N. J., Silva, H. B., et al. (2018). Blockade of adenosine A_{2A} receptors recovers early deficits of memory and plasticity in the triple transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis*. 117, 72–81. doi: 10.1016/j.nbd.2018.05.024

Silva, C. G., Porciúncula, L. O., Canas, P. M., Oliveira, C. R., and Cunha, R. A. (2007). Blockade of adenosine A_{2A} receptors prevents staurosporine-induced apoptosis of rat hippocampal neurons. *Neurobiol. Dis*. 27, 182–189. doi: 10.1016/j.nbd.2007.04.018

Simpson, I. A., Appel, N. M., Hokari, M., Oki, J., Holman, G. D., Maher, F., et al. (1999). Blood-brain barrier glucose transporter: effects of hypo- and hyperglycemia revisited. *J. Neurochem*. 72, 238–247. doi: 10.1046/j.1471-4159.1999.0720238.x

Steculorum, S. M., Solas, M., and Brüning, J. C. (2014). The paradox of neuronal insulin action and resistance in the development of aging-associated diseases. *Alzheimers Dement*. 10(1 Suppl.), S3–S11. doi: 10.1016/j.jalz.2013.12.008

Takahashi, R. N., Pamplona, F. A., and Prediger, R. D. (2008). Adenosine receptor antagonists for cognitive dysfunction: a review of animal studies. *Front. Biosci.* 13, 2614–2632. doi: 10.2741/2870

Temido-Ferreira, M., Ferreira, D. G., Batalha, V. L., Marques-Morgado, I., Coelho, J. E., Pereira, P., et al. (2018). Age-related shift in LTD is dependent on neuronal adenosine A_{2A} receptors interplay with mGluR5 and NMDA receptors. *Mol. Psychiatry*. doi: 10.1038/s41380-018-0110-9. [Epub ahead of print].

Töpfer, M., Burbiel, C. E., Müller, C. E., Knittel, J., and Verspohl, E. J. (2008). Modulation of insulin release by adenosine A_1 receptor agonists and antagonists in INS-1 cells: the possible contribution of $^{86}Rb^+$ efflux and $^{45}Ca^{2+}$ uptake. *Cell Biochem. Funct*. 26, 833–843. doi: 10.1002/cbf.1514

van Dam, R. M., and Hu, F. B. (2005). Coffee consumption and risk of type 2 diabetes: a systematic review. *JAMA* 294, 97–104. doi: 10.1001/jama.294.1.97

van der Graaf, M., Janssen, S. W., van Asten, J. J., Hermus, A. R., Sweep, C. G., Pikkemaat, J. A., et al. (2004). Metabolic profile of the hippocampus of Zucker Diabetic Fatty rats assessed by *in vivo* ^{1}H magnetic resonance spectroscopy. *NMR Biomed*. 17, 405–410. doi: 10.1002/nbm.896

Viana da Silva, S., Haberl, M. G., Zhang, P., Bethge, P., Lemos, C., Gonçalves, N., et al. (2016). Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A_{2A} receptors. *Nature Comm*. 7:11915. doi: 10.1038/ncomms11915

Wilczak, N., and De Keyser, J. (1997). Insulin-like growth factor-I receptors in normal appearing white matter and chronic plaques in multiple sclerosis. *Brain Res*. 772, 243–246. doi: 10.1016/S0006-8993(97)00940-2

Wilkes, J. J., Nelson, E., Osborne, M., Demarest, K. T., and Olefsky, J. M. (2005). Topiramate is an insulin-sensitizing compound *in vivo* with direct effects on adipocytes in female ZDF rats. *Am. J. Physiol. Endocrinol. Metab*. 288, E617–E624. doi: 10.1152/ajpendo.00437.2004

Wurm, A., Iandiev, I., Hollborn, M., Wiedemann, P., Reichenbach, A., Zimmermann, H., et al. (2008). Purinergic receptor activation inhibits osmotic glial cell swelling in the diabetic rat retina. *Exp. Eye. Res*. 87, 385–393. doi: 10.1016/j.exer.2008.07.004

Yu, L., Shen, H. Y., Coelho, J. E., Araújo, I. M., Huang, Q. Y., Day, Y. J., et al. (2008). Adenosine A_{2A} receptor antagonists exert motor and neuroprotective effects by distinct cellular mechanisms. *Ann. Neurol*. 63, 338–346. doi: 10.1002/ana.21313

Zhao, W. Q., and Alkon, D. L. (2001). Role of insulin and insulin receptor in learning and memory. *Mol. Cell Endocrinol*. 177, 125–134. doi: 10.1016/S0303-7207(01)00455-5

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 09 May 2019
doi: 10.3389/fnins.2019.00473

The Role of Insulin Resistance and Protein O-GlcNAcylation in Neurodegeneration

Suraiya A. Ansari[1]* *and Bright Starling Emerald*[2]

[1] Department of Biochemistry, College of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates,
[2] Department of Anatomy, College of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates

Metabolic syndrome including obesity and type 2 diabetes is increasing at an alarming rate worldwide. Similarly, there has been an increase in the cases of neurodegenerative diseases such as Alzheimer's disease (AD) possibility due to increase in elderly population in the past few decades. Both, metabolic diseases and AD have one common feature that is insulin resistance. Recent studies suggest a link between the regulatory functions of insulin in the brain and AD. Hypoglycemia, a characteristic feature of AD may be a result of impaired insulin signaling in the affected regions of the brain. O-GlcNAcylation is a post-translational protein modification, the levels of which are dependent on the availability of glucose inside the cells. Hyperphosphorylation of Tau is a major molecular feature, which leads to its aggregation and neurotoxicity in AD. In addition, impaired processing of Amyloid precursor protein (APP) leading to toxic amyloid β (Aβ) aggregation is also implicated in the pathogenesis of AD. Both APP and Tau are also found to be O-GlcNAcylated. Reduced O-GlcNAcylation of APP and Tau due to hypoglycemia is found to be associated with their pathological features in AD brain. Recent studies have also identified perturbed O-GlcNAcylation/phosphorylation of several other proteins important for normal neuronal function, which may be contributing to the neuropathological development in AD. Herein, we discuss about the uptake and distribution of insulin inside the brain, brain insulin signaling and insulin resistance as well as its relation to neurodegenerative diseases with a special focus on protein O-GlcNAcylation and its potential role in the treatment of AD.

Keywords: insulin resistance, brain insulin uptake, Alzhemier's disease, O-GlcNAc cycling, neurodegeneration

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Fabio Di Domenico,
Sapienza University of Rome, Italy
Roland Brandt,
University of Osnabrück, Germany

***Correspondence:**
Suraiya A. Ansari
sansari@uaeu.ac.ae

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *26 February 2019*
Accepted: *26 April 2019*
Published: *09 May 2019*

Citation:
Ansari SA and Emerald BS (2019) The Role of Insulin Resistance and Protein O-GlcNAcylation in Neurodegeneration. Front. Neurosci. 13:473. doi: 10.3389/fnins.2019.00473

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder affecting millions of people worldwide. DM is classified into two major forms, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T2DM is mainly due to insulin resistance and is one of the key components of the metabolic syndrome that also contributes to obesity. T2DM is also the most common form of DM, accounting for more than 90% of all types of DM, and thus taking up most of the funds available for the management of patients with this condition. Unfortunately, the prevalence of T2DM has reached an epidemic state in the last few decades, accounting for more than 425 million diabetics

around the globe. It is projected that there will 629 million people with diabetes by the year 2045 (IDF Diabetes Atlas, 2017).

Central nervous system controls important physiological and metabolic processes such as feeding, acquisition of energy and expenditure, regulation of body weight and glucose homeostasis, (Schwartz and Porte, 2005; Prodi and Obici, 2006). Although a link between diabetes, glucose homeostasis and brain has been established by classical experiments almost a century ago using experiments in dogs, where impairment of the fourth ventricle resulted in marked glycosuria (Bernard, 1854), the brain was considered as an insulin insensitive organ for decades because passive diffusion into the brain is limited by the blood-brain barrier (BBB). Experiments using radioactive phosphorus and radiolabeled 131I-insulin in the early 60s have established that at least some parts of the brain are sensitive to insulin and insulin may have regulatory roles in the brain (Chowers et al., 1966). According to more recent studies, insulin in addition to its major regulatory roles in the peripheral tissues such as liver, muscle, and adipose tissue has emerged as a major regulatory molecule in the central nervous system. It has been suggested that hormones such as insulin circulate through the central nervous system and integrate the feeding behavior, autonomic outflow and metabolism so that a homeostasis is maintained between energy metabolism and storage of excess energy (Schwartz and Porte, 2005). Studies have established the presence of insulin like peptides (IGF1/2) and insulin receptors (IR) in the brain and shown to be involved in the regulation of different metabolic processes such as feeding and cognition (Schechter et al., 1990, 1994, 1996). The IRs of the brain were shown to be similar in structure to the ones present in peripheral tissues but there are differences in their distribution in different cell types which is more in the neurons compared to the glial cells (Schwartz et al., 1992; Wozniak et al., 1993). Furthermore, although insulin insensitive glucose transporters, Glut1 and Glut3 are mainly responsible for glucose uptake inside the brain, it is also shown that, in the neurons of the hypothalamus, glucose is transported via insulin sensitive Glut4 transporter (McEwen and Reagan, 2004; Ren et al., 2014).

Although, it is well accepted that T2DM is predominantly a genetic disease, the drastic increase in the worldwide prevalence of T2DM highlights the changes in regulatory mechanism that happens at multiple levels of metabolism. Insulin, the primary hormone which was isolated almost a century ago (Banting et al., 1922) is secreted by the β-cells of the islets, exerts its function through different peripheral tissues and prevents postprandial hyperglycemia, and maintains euglycaemic levels. Through its action in the skeletal muscle, it promotes the uptake of glucose from the blood stream and inside the liver, it prevents the production of glucose by preventing gluconeogenesis and glycogenolysis and promoting glycogen synthesis (DeFronzo and Ferrannini, 1991). Studies aimed at the estimation of insulin in both human and rodent brain have noticed a higher concentration of insulin in brain extract compared to that of the plasma (Havrankova et al., 1978). This abundance of insulin in brain suggests a possible important regulatory role for it in the brain. In this review, we discuss about insulin signaling in the brain and the role of insulin resistance in neurodegenerative diseases such as Alzheimer's disease (AD) with a particular focus on the association of protein O-GlcNAcylation with impaired glucose utilization possibly due to insulin resistance and the role of O-GlcNAcylation on the pathology of AD.

DISTRIBUTION OF INSULIN IN THE BRAIN

Insulin is a peptide hormone of 51-amino acid, which has a helical native structure and a molecular weight of about 6,000 Da (Jimenez et al., 2002). Its entry into the brain is limited by the BBB and the precise mechanisms of how and where insulin enters the brain remains unclear. Recent studies have suggested that the entry of insulin into the brain parenchyma can occur directly via the median eminence (ME), indirectly through the cerebrospinal fluid (CSF) or through brain interstitial fluid (ISF). The possibility that insulin may reach the brain through ME is based on the fact that some part of the brain such as circumventricular organs (CVOs) are without BBB and are with fenestrated capillaries which make the passage connection between the blood circulation and the underlying brain parenchyma (Siso et al., 2010). In fact, administration of radioactive insulin has been shown to reach the arcuate nucleus (ARH) of the hypothalamus through ME (Corp et al., 1986). Increase in the levels of insulin led to the activation of AKT signaling (insulin receptor activation) and c-Fos signaling (neuronal activation) in the ARH. This is important as defects in AKT and c-Fos signaling contributes to insulin resistance which leads to T2D and obesity (Olson et al., 1993; Koch et al., 2008; Williams et al., 2010; Clegg et al., 2011). Although the tight junctions between the tanycytes may restrict the movement of insulin and other hormones, they may reach other parts of the brain through ISF. This possibility is supported by the fact that the quantitation of ARH ISF showed a low level of insulin upon fasting which increased after feeding or peripheral administration of insulin (Gerozissis et al., 1997; Langlet et al., 2013) and this effect was very rapid suggestive of diffusion through ME. CSF, which is produced by the choroid plexus in the brain reaches Virchow-Robin space through the third and fourth ventricles and the microvasculature helps in the transfer of insulin and other hormones from blood circulation to CSF (Iliff et al., 2012). However, the significance of these modes of entry of insulin to the brain is still not well understood. Insulin binds to IR, which is a heterotetrameric receptor (2 α subunits and 2 β-subunits) (Boucher et al., 2014). In humans, the IR gene codes for two isoforms A and B and the uniformly expressed isoform A is expressed predominantly in the brain (Frasca et al., 1999). Although it shows variations, it is expressed in the hypothalamus, olfactory and limbic areas, neocortex, hippocampus, basal ganglia, cerebellum and choroid plexus (Hill et al., 1986). It was also shown that highest IR expression was seen in the ARH (Plum et al., 2005). Binding of insulin to IR results in its autophosphorylation and signals through the insulin receptor substrate proteins (IRS) which leads to the activation of P13K/AKT signaling pathway, an important

member of the complex network mediating insulin signaling (Rojas et al., 2003). In this pathway, P13K a lipid kinase that acts as a heterodimeric enzyme, phosphorylates the D-3 position of the inositol ring in phosphoinositides (Whitman et al., 1988; Carpenter et al., 1990; Stephens et al., 1991) and when insulin secretion is stimulated by the presence of glucose in the circulation, this signaling cascade that phosphorylates the serine-threonine kinase, AKT is activated. AKT phosphorylation then triggers the uptake and metabolism of glucose and coordinates neuromodulatory gene transcription (Kan et al., 1994; Hill et al., 1999). Insulin signaling is also negatively regulated by protein tyrosine phosphatases (PTPs), T cell protein tyrosine phosphatase (TCPTP) and suppressor of cytokine signaling 3 (SOCS3) (Wunderlich et al., 2013; Zhang et al., 2015). In addition, the other members of insulin family of peptides such as insulin like growth factor 1 and 2 (IGF1/2) also play important roles in the control of energy metabolism in the brain and neuronal plasticity (Fernandez and Torres-Aleman, 2012).

INSULIN SIGNALING IN THE BRAIN

The main target of insulin signaling in the brain is shown to be the hypothalamus (Heni et al., 2015). Functional analysis have shown several regions in the hypothalamus such as the ARH, the ventromedial nucleus (VMH), lateral hypothalamic area (LHA), dorsomedial nucleus of the hypothalamus (DMH) and paraventricular nucleus of the hypothalamus (PVH) respond to the changes in the level of insulin (Porter and Bokil, 1997; Qiu et al., 2014). Specifically, in the hypothalamus, the response to the insulin level had been shown to be with the two group of neurons, the POMC [α-melanocyte-stimulating hormone (MSH) precursor] which is anorectic in function and the agouti-related peptide (AgRP)/neuropeptide Y (NPY)-neuropeptide expressing neurons which are orexigenic (Varela and Horvath, 2012). While activation of AgRP neurons promotes feeding and weight gain, the activation of POMC neurons increase satiation, increase energy expenditure and attenuate weight gain (Xu and Xie, 2016). Insulin signaling in the brain has been shown to inhibit AgRP/NPy neurons while it activates POMC neurons (Varela and Horvath, 2012; Qiu et al., 2014; Roh et al., 2016). It was also suggested that from these neurons through the neuronal extensions insulin may be reaching the different regions of the hypothalamus such as the PVH, VMH, DMH, lateral hypothalamus (LH), amygdala, bed nucleus of stria terminalis, parabrachial nucleus and the dorsal vagal complex (Wang et al., 2015). Apart from these, insulin was also shown to act on the SF1 neurons of the VMH and in the dopaminergic neurons of the midbrain and in the higher cortical regions (Guthoff et al., 2010; Klockener et al., 2011; Konner et al., 2011).

THE EFFECTS OF SYSTEMIC INSULIN RESISTANCE IN THE BRAIN

Insulin resistance, the main contributor of T2D and obesity results from that fact that although there is high levels of insulin present in the circulation, the response to this is defective and brain is not an exception to this (Zhang et al., 2015). Interestingly, it has been shown that the effect of insulin in the brain is completely lost in the case of obesity and is restored with weight loss (Tschritter et al., 2006; Tuulari et al., 2013). Among the contribution of different factors in the development of insulin resistance, the intake of high fat diet has been suggested to reduce the sensitivity of hypothalamic insulin (Clegg et al., 2011). With the increase in the availability of nutrient rich food, this may be one reason which could contribute to the increase in obesity worldwide. It has been shown that saturated fatty acid such as palmitate or stearate can cross the BBB and can activate inflammatory signaling in the hypothalamus resulting in insulin resistance (Kleinridders et al., 2009). An increase in the levels of the negative regulators of insulin signaling in the hypothalamus such as TCPTP, PTP1B, and SOCS3 also have been shown and these factors also increase as a result of obesity induced inflammation (Loh et al., 2011; Zhang et al., 2015). Another factor which can contribute to insulin resistance is the decreased BBB permeability resulting in defective signaling (Kern et al., 2006; Hsu and Kanoski, 2014). Diet induced obesity also results from gliosis which makes both POMC and AgRP neurons insensitive to the peripheral insulin (Horvath et al., 2010; Dorfman and Thaler, 2015). So the main roles of insulin in the brain, regulation of body homeostasis and balancing of nutrient intake and energy expenditure are all altered in insulin resistance.

INSULIN RESISTANCE IN NEURODEGENERATION

An association of T2DM and neurodegenerative diseases such as AD has also been described (Holscher, 2011; Chen et al., 2014). AD is a neurodegenerative disease characterized by progressive dementia and loss of cognitive abilities. The incidence of both T2DM and AD increase with age and various epidemiological studies have shown an increased risk of dementia and neurodegeneration in people with T2DM (Mushtaq et al., 2014; Alam et al., 2016). Further, there are several common biological mechanisms associated with the pathogenesis of T2DM and AD such as insulin resistance and impaired glucose metabolism, Aβ plaque formation in the brain and increased oxidative stress (Zhao and Townsend, 2009; Mushtaq et al., 2014). Interestingly, the disruption of normal glucose metabolism in the affected region of AD brain is shown to promote Aβ aggregation that is associated with neurodegeneration observed in AD (Li et al., 2007; Zhao and Townsend, 2009).

Although vascular complications of diabetes may also be responsible for the development of neurodegenerative diseases in advanced age, a direct contribution of impaired insulin signaling pathway has been implicated repeatedly (Steen et al., 2005; Zhao and Townsend, 2009; Ma et al., 2015). Hyperglycemia or chronically elevated blood glucose level is associated with insulin resistance and development of T2DM. At the molecular level, the hallmarks of insulin resistance in major insulin target tissues such as liver, muscle and adipose tissue include

defects in the expression and phosphorylation of many effector molecules of insulin signaling pathway such as insulin receptor (IR), IRS1/2, PI3K and AKT (PKB), reduced expression of Glut4, and increased hepatic gluconeogenesis and lipogenesis (Mlinar et al., 2007; Samuel and Shulman, 2012).

As explained above, IR is expressed throughout the brain and in larger part, the uptake of glucose takes place with the help of glucose transporters, Glut1 or Glut3 in an insulin independent manner, the dependence on insulin for glucose uptake has been observed in specific areas of brain (Vannucci et al., 1998; Reno et al., 2017) through insulin dependent glucose transporter, Glut4. Therefore, a defect in brain insulin signaling may lead to reduced glucose uptake. Earlier works, which tried to determine the concentrations of insulin as well as IR in aging and AD brains, have shown by immunohistochemical staining in the neocortex of postmortem brains that the levels of insulin and connecting peptide (c-peptide) of insulin are reduced in both aging and AD brains. Furthermore, the levels of IR was decreased in the aging brain whereas it increased in AD brain compared to age matched controls (Frolich et al., 1998). Relatively a recent study, with real time PCR technique as well as immunostaining has shown reductions in the expression of IR, IGF1 and IGF2 polypeptides and their receptors in the AD brains. This correlated with increased levels of amyloid protein precursor (APP), glial fibrillary acidic protein, and the IBA1/AIF1 microglial mRNA transcripts (Rivera et al., 2005; Steen et al., 2005). As there is reduced levels of IR, along with decreased production of growth factors and their receptors (IGF1/2) and if neurons are dependent on the local production of these growth factors then a reduced production may lead to neuronal cell death which is what is seen in case of AD (Steen et al., 2005). Further on this, the role of inhibitory serine phosphorylation on IRS1 in the case of insulin resistance has been reported in most of the insulin target tissues including brain (Aguirre et al., 2002; Gual et al., 2005; Lerner-Marmarosh et al., 2005; Draznin, 2006; Barone et al., 2016). It is well established that Tyrosine phosphorylation of IRS1 during insulin signaling is responsible for signal activating function of IRS1 through its association with downstream effector, PI3K whereas ser307, S612, and S632 (ser312, S616, and/or S636 in human) of IRS1 is inhibitory to downstream insulin signal transduction (Aguirre et al., 2002; Draznin, 2006; Talbot et al., 2012). Consistent with this, increased ser307 phosphorylation is reported in insulin resistance of insulin target tissues such as skeletal muscle as well as in AD brain (Bandyopadhyay et al., 2005; Lerner-Marmarosh et al., 2005; Talbot et al., 2012; Barone et al., 2016). Interestingly, increased phosphorylation of inhibitory ser616 and ser636 of IRS1 were reported in AD brains even without peripheral insulin resistance (Talbot et al., 2012). Furthermore, brain insulin resistance is also suggested a risk factor for cognitive impairment (Ma et al., 2015). Although a clear molecular understanding behind insulin resistance and cognitive impairment is not known, various mechanisms such as defects in the neuronal plasticity and increased inflammation due to altered PI3K/AKT/GSK-3 signaling have been described based on animal models and epidemiological data (Calvo-Ochoa and Arias, 2015; Neergaard et al., 2017). Overall, these studies suggest a clear correlation between insulin receptor signaling and insulin resistance in the development of AD.

ROLE OF PROTEIN O-GlcNAcylation IN INSULIN SIGNALING

The role of glucose responsive protein O-GlcNAcylation has emerged as an important player in insulin signaling as it has been reported that many effector molecules of insulin signaling pathway are also O-GlcNAcylated, often in a reciprocal manner to phosphorylation on specific serine residues (Love and Hanover, 2005; Ma and Hart, 2013, 2014; Myslicki et al., 2014). O-GlcNAcylation is a post-translational protein modification on the serine/threonine amino acid of various proteins through the transfer of single sugar molecule, β-*N*-acetylglucosamine from the substrate UDP-GlcNAc. Protein O-GlcNAcylation was first discovered by Gerald Hart and colleagues in early 1980s as a dynamic protein mono-glycosylation present on nucleocytoplasmic proteins. Since then, thousands of proteins have been identified to be modified through this protein modification (Nandi et al., 2006; Hahne et al., 2013; Li et al., 2016). UDP-GlcNAc, the substrate needed for protein O-GlcNAcylation is produced through a metabolic pathway known as Hexosamine Biosynthesis Pathway (HBP) which is activated after the entry of glucose into the cells, where a small percentage of this glucose (2–5%) is shoveled into HBP resulting in the generation of UDP-GlcNAc through several steps (Love and Hanover, 2005; Hanover et al., 2010). The dynamic addition or removal of O-GlcNAc on serine/threonine amino acids of target proteins is achieved with the help of a pair of enzymes, O-GlcNAc-transferase (OGT) and O-GlcNAase (OGA). OGT attaches O-GlcNAc moiety provided by UDP-GlcNAc on to the target proteins whereas OGA removes it (Iyer and Hart, 2003; Hanover et al., 2010; Bond and Hanover, 2015; Eustice et al., 2017). Hyperglycemia is shown to increase the total levels of O-GlcNAc inside the cells as has been shown in several previous studies (Copeland et al., 2008). Interestingly, it has been reported that almost all of the major players of insulin signaling pathway such as IRS1, PI3K, PDK1, AKT, and FOXO1 are also O-GlcNAcylated often reciprocal to the phosphorylation sites on these proteins and thereby regulate insulin signaling through positive/negative feedback. Therefore, chronic elevation of O-GlcNAc could be considered a mechanism in the development of insulin resistance at least in part through O-GlcNAcylation of PI3K or AKT on stimulatory serine residues (Whelan et al., 2010; Ma and Hart, 2013). Toward this end, studies using animal models and cell culture experiments have shown that increasing O-GlcNAc levels due to either genetic or pharmacological inhibition of OGA activity leads to development of symptoms of insulin resistance and T2DM (Vosseller et al., 2002; Arias et al., 2004; Park et al., 2005; Keembiyehetty et al., 2015). However, Inhibition of O-GlcNAcase in 3T3-L1 adipocytes using a potent inhibitor on OGA did not induce insulin resistance (Macauley et al., 2010) suggesting a more complex relationship between phosphorylation and O-GlcNAcylation rather than just a yin-yang mechanism. Interestingly, inhibitory serine residues

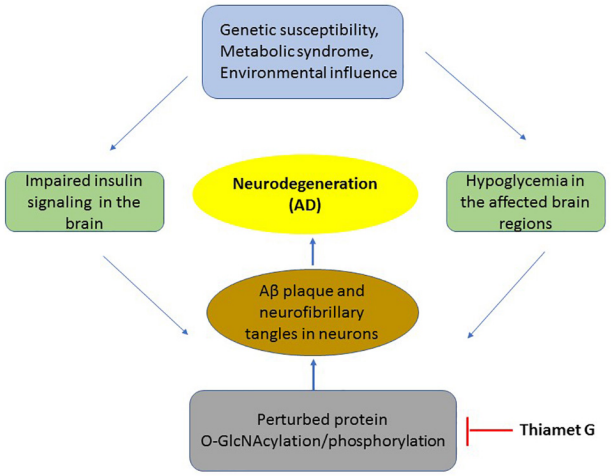

FIGURE 1 | The mechanism of insulin resistance and hypoglycaemia on pathology of neurons in AD. A flowchart of key events occurring in the development of AD which includes metabolic syndrome or disease susceptibility to neurodegeneration which results in impaired insulin signaling and hypoglycaemia leading to reduced O-GlcNAcylation of several proteins including APP and Tau. This results in the production of toxic Aβ aggregation and neurofibrillary tangles leading in neuronal death. Thiamet G by inhibition of OGA increases O-GlcNAcylation thus blocking downstream pathological events of AD.

of IRS1 can also be O-GlcNAcylated (Jahangir et al., 2014) leading to the possibility of hypoglycemia mediated reduced O-GlcNAcylation on inhibitory serine residues of IRS1 that may result in its increased phosphorylation and development of impaired insulin signaling in AD brain.

This suggests the possibility of a harmonious balance between O-GlcNAcylation and phosphorylation at the physiological levels is necessary for proper functioning of insulin pathway and an impairment in this homeostasis might be responsible for the development of pathology (Yang and Qian, 2017).

LINK BETWEEN BRAIN INSULIN RESISTANCE, O-GlcNAcylation AND NEURODEGENERATION

Hypoglycemia is one of the common features of many neurodegenerative diseases including AD (Hoyer, 2004). Several studies have identified insufficient glucose uptake and utilization in the affected regions of AD brain (Pedersen et al., 1999; Daulatzai, 2017). Insulin resistance in the brain is observed due to defects in insulin receptors signaling (Kuljis and Salkovic-Petrisic, 2011) and decreased levels of brain glucose transporters, Glut1 and Glut3 (Liu et al., 2008; Szablewski, 2017) which is also observed in AD brain. Furthermore, using postmortem AD brains, Talbot et al show that both IR and IGF1 responsiveness and downstream signaling through these receptors were significantly reduced in AD brains (Talbot et al., 2012). Therefore, although insulin has several functions in the brain, a defect in the effectors of insulin signaling pathway as observed in peripheral insulin resistance may also be responsible for brain insulin resistance (Candeias et al., 2012; Duarte et al., 2012; Chen and Zhong, 2013). Hypoglycemia in AD brain leads to decreased protein O-GlcNAcylation. Several studies have recently associated decreased O-GlcNAcylation to the pathogenesis of AD using both *in-vitro* and *in-vivo* experiments (Liu Y. et al., 2009; Gong et al., 2016; Pinho et al., 2018). The common theme that had emerged from these studies suggests that decreased O-GlcNAcylation of beta-amyloid precursor protein (APP) and Tau, two main culprits associated with neurodegeneration in Alzheimer's are associated with increased phosphorylation thus leading to classical Aβ plaque formation and Tau aggregation (Dias and Hart, 2007). The initial studies led by Robertson et al. (2004) and later by Liu F. et al. (2009) showed a reciprocal relationship between phosphorylation and O-GlcNAcylation on Tau protein suggesting that changes in Tau glycosylation may influence its phosphorylation state (Robertson et al., 2004). The levels of total O-GlcNAc were found to be reduced in AD brain, which negatively correlated with phosphorylation of Tau (Liu F. et al., 2009). These results suggested that impaired glucose metabolism leading to reduced O-GlcNAcylation of Tau results in its hyperphosphorylation [3–4 folds more phosphate than normal Tau (Liu et al., 2004)] and neurofibrillary degeneration in AD. Similarly, APP had been found be O-GlcNAcylated (Griffith et al., 1995) and that this plays an important role in its processing (Jacobsen and Iverfeldt, 2011; Chun et al., 2015). The accumulation of hydrophobic amyloid-beta (Aβ) peptide is a hallmark feature of AD. APP is processed through two proteolytic cleavage pathways termed as non-amyloidogenic pathway and amyloidogenic pathway where former is favored in normal brain whereas later pathway is found to be more active in AD brain leading to increased formation of pathogenic Aβ peptide. A study by Jacobsen et al showed that increasing the levels of total O-GlcNAc through PuGNAc to inhibit the function of OGA resulted in an increase in the level of O-GlcNAcylated APP, with increased secretion of sAPPα and decreased Aβ secretion (Jacobsen and Iverfeldt, 2011). Furthermore, Yuzwa et al., 2012 used a hemizygous JNPL3 tau mouse model (which express mutant human P301L tau whose expression is roughly equivalent to that of endogenous mouse tau and these animals undergo progressive neurodegeneration) and showed that increasing the levels of O-GlcNAc stabilized Tau aggregation and slowed down neurodegeneration. Later studies further confirmed the effect of OGA inhibition on preventing Tau aggregation and amelioration of pathological features in mouse model of tauopathy (Graham et al., 2014). Similarly, beneficial effect of OGA inhibition on the Aβ plaque formation and memory impairment has been observed in a mouse model of AD (Kim et al., 2013). Therefore, there is a significant link between hypoglycaemia and AD where protein O-GlcNAcylation plays an important role in the production of toxic APP and Tau aggregation due to a decrease in O-GlcNAcylation of these proteins (**Figure 1**). Recent studies have identified several other proteins which belong to important functional categories such as memory associated proteins, cytoskeleton and synaptic proteins with altered O-GlcNAc levels in the postmortem AD brain (Wang et al., 2017). Wang et al show that among the altered O-GlcNAcylation in AD brains, proteins

of particular interest which showed reduced O-GlcNAcylation in AD brain are ANK3 (ankyrin-3) and SYNPO (synaptopodin) which are involved in membrane integrity/axon polarity and synaptic plasticity, respectively, (Wang et al., 2017). In another recent study, using a triple transgenic mouse model of AD (3 × Tg-AD) and using an antibody specific for O-GlcNAc sugar to enrich all proteins O-GlcNAcylated in control and AD brains, Tramutola et al have identified several proteins which are important for neuronal function including structural proteins such as α tubulin, NF-L (neurofilament light chain) and energy metabolism such as Gapdh, Eno1, and Madh which are important enzymes of glycolysis and Kreb cycle (Tramutola et al., 2018).

Therefore, we cannot exclude the possibility of the role played by altered O-GlcNAcylation of these proteins as discussed above in the pathogenesis of AD and further studies are needed to functionally characterize the role of protein O-GlcNAcylation in relation to phosphorylation on these proteins and their relevance to AD.

CONCLUSION

It is clear that insulin, either through its uptake from systemic blood circulation or through local production plays significant roles in glucose homeostasis and energy metabolism in the brain. Insulin resistance, as observed in case of metabolic syndrome and T2DM or in neurodegenerative disease such as AD plays an important role in the pathogenesis of these diseases. Protein O-GlcNAcylation has emerged as an important mechanism in the pathogenesis of AD. Hypoglycemia, possibly as a result of brain insulin resistance leads to decreased O-GlcNAcylation of APP and Tau resulting in their hyperphosphorylation and production on toxic Aβ amyloid and Tau aggregates which are hallmark features of AD. Interestingly, increasing the levels of total O-GlcNAc through highly specific inhibitor of OGA enzyme, Thiamet G has shown promise in the treatment of AD through alleviation of major symptoms of AD through increased O-GlcNAcylation of APP and Tau and reduction in the production of toxic species of these proteins in preclinical studies (Yuzwa et al., 2012; Graham et al., 2014; Hastings et al., 2017). Recent studies have also identified several other proteins with defect in O-GlcNAcylation in AD brain involved in neuronal structure, energy metabolism and insulin signaling. Based on these results, although Thiamet G is considered a potential candidate to be used in the treatment of AD and is likely to be soon in clinics (**Figure 1**). However, other mechanisms of impaired insulin signaling cascade such as IRS1 and GSK-3 phosphorylation in the pathogenesis of AD as well as other cellular processes need further investigation for development of more effective therapeutic strategy for this devastating neurodegenerative disease.

AUTHOR CONTRIBUTIONS

SA and BE read and corrected the manuscript and approved its final content.

FUNDING

Research in the laboratory of SA is supported by research grants from United Arab Emirates University (UAEU) – Center based research grant # 31R170, Al Jalila Foundation grant # 21M116 and ADEK Award for Research Excellence (AARE) grant # 21M121. Research in the lab of BSE is supported by research grants from United Arab Emirates University, UPAR grants # 3IM188 and # 3IM307, Research Start-up grant # 3IM179 and Research Centre grant # 3IR034.

REFERENCES

Aguirre, V., Werner, E. D., Giraud, J., Lee, Y. H., Shoelson, S. E., and White, M. F. (2002). Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J. Biol. Chem.* 277, 1531–1537. doi: 10.1074/jbc.m101521200

Alam, F., Islam, M. A., Sasongko, T. H., and Gan, S. H. (2016). Type 2 diabetes mellitus and alzheimer's disease: bridging the pathophysiology and management. *Curr. Pharm. Des.* 22, 4430–4442. doi: 10.2174/1381612822666160527160236

Arias, E. B., Kim, J., and Cartee, G. D. (2004). Prolonged incubation in PUGNAc results in increased protein O-Linked glycosylation and insulin resistance in rat skeletal muscle. *Diabetes* 53, 921–930. doi: 10.2337/diabetes.53.4.921

Bandyopadhyay, G. K., Yu, J. G., Ofrecio, J., and Olefsky, J. M. (2005). Increased p85/55/50 expression and decreased phosphotidylinositol 3-kinase activity in insulin-resistant human skeletal muscle. *Diabetes* 54, 2351–2359. doi: 10.2337/diabetes.54.8.2351

Banting, F. G., Best, C. H., Collip, J. B., Campbell, W. R., and Fletcher, A. A. (1922). Pancreatic extracts in the treatment of diabetes mellitus. *Indian J. Med. Res.* 2007, 141–146.

Barone, E., Di Domenico, F., Cassano, T., Arena, A., Tramutola, A., Lavecchia, M. A., et al. (2016). Impairment of biliverdin reductase-A promotes brain insulin resistance in Alzheimer disease: a new paradigm. *Free Radic. Biol. Med.* 91, 127–142. doi: 10.1016/j.freeradbiomed.2015.12.012

Bernard C. (1854). *Leçons de Physiologie Expérimentale Appliquées á la Médecine*. Paris: Bailliére Et Fils.

Bond, M. R., and Hanover, J. A. (2015). A little sugar goes a long way: the cell biology of O-GlcNAc. *J. Cell Biol.* 208, 869–880. doi: 10.1083/jcb.201501101

Boucher, J., Kleinridders, A., and Kahn, C. R. (2014). Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb. Perspect. Biol.* 6:a009191. doi: 10.1101/cshperspect.a009191

Calvo-Ochoa, E., and Arias, C. (2015). Cellular and metabolic alterations in the hippocampus caused by insulin signalling dysfunction and its association with cognitive impairment during aging and Alzheimer's disease: studies in animal models. *Diabetes Metab. Res. Rev.* 31, 1–13. doi: 10.1002/dmrr.2531

Candeias, E., Duarte, A. I., Carvalho, C., Correia, S. C., Cardoso, S., Santos, R. X., et al. (2012). The impairment of insulin signaling in Alzheimer's disease. *IUBMB Life* 64, 951–957. doi: 10.1002/iub.1098

Carpenter, C. L., Duckworth, B. C., Auger, K. R., Cohen, B., Schaffhausen, B. S., and Cantley, L. C. (1990). Purification and characterization of phosphoinositide 3-kinase from rat liver. *J. Biol. Chem.* 265, 19704–19711.

Chen, Y., Deng, Y., Zhang, B., and Gong, C. X. (2014). Deregulation of brain insulin signaling in Alzheimer's disease. *Neurosci. Bull.* 30, 282–294. doi: 10.1007/s12264-013-1408-x

Chen, Z., and Zhong, C. (2013). Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. *Prog. Neurobiol.* 108, 21–43. doi: 10.1016/j.pneurobio.2013.06.004

Chowers, I., Lavy, S., and Halpern, L. (1966). Effect of insulin administered intracisternally on the glucose level of the blood and the cerebrospinal fluid in vagotomized dogs. *Exp. Neurol.* 14, 383–389. doi: 10.1016/0014-4886(66)90122-1

Chun, Y. S., Park, Y., Oh, H. G., Kim, T. W., Yang, H. O., Park, M. K., et al. (2015). O-GlcNAcylation promotes non-amyloidogenic processing of amyloid-beta protein precursor via inhibition of endocytosis from the plasma membrane. *J. Alzh. Dis.* 44, 261–275. doi: 10.3233/JAD-140096

Clegg, D. J., Gotoh, K., Kemp, C., Wortman, M. D., Benoit, S. C., Brown, L. M., et al. (2011). Consumption of a high-fat diet induces central insulin resistance independent of adiposity. *Physiol. Behav.* 103, 10–16. doi: 10.1016/j.physbeh.2011.01.010

Copeland, R. J., Bullen, J. W., and Hart, G. W. (2008). Cross-talk between GlcNAcylation and phosphorylation: roles in insulin resistance and glucose toxicity. *Am. J. Physiol. Endocrinol. Metab.* 295, E17–E28. doi: 10.1152/ajpendo.90281.2008

Corp, E. S., Woods, S. C., Porte, D. Jr., Dorsa, D. M., Figlewicz, D. P., and Baskin, D. G. (1986). Localization of 125I-insulin binding sites in the rat hypothalamus by quantitative autoradiography. *Neurosci. Lett.* 70, 17–22. doi: 10.1016/0304-3940(86)90430-1

Daulatzai, M. A. (2017). Cerebral hypoperfusion and glucose hypometabolism: Key pathophysiological modulators promote neurodegeneration, cognitive impairment, and Alzheimer's disease. *J. Neurosci. Res.* 95, 943–972. doi: 10.1002/jnr.23777

DeFronzo, R. A., and Ferrannini, E. (1991). Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14, 173–194. doi: 10.2337/diacare.14.3.173

Dias, W. B., and Hart, G. W. (2007). O-GlcNAc modification in diabetes and Alzheimer's disease. *Mol. Biosyst.* 3, 766–772.

Dorfman, M. D., and Thaler, J. P. (2015). Hypothalamic inflammation and gliosis in obesity. *Curr. Opin. Endocrinol. Diabetes Obes.* 22, 325–330. doi: 10.1097/MED.0000000000000182

Draznin, B. (2006). Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85alpha: the two sides of a coin. *Diabetes* 55, 2392–2397. doi: 10.2337/db06-0391

Duarte, A. I., Moreira, P. I., and Oliveira, C. R. (2012). Insulin in central nervous system: more than just a peripheral hormone. *J. Aging Res.* 2012:384017. doi: 10.1155/2012/384017

Eustice, M., Bond, M. R., and Hanover, J. A. (2017). O-GlcNAc cycling and the regulation of nucleocytoplasmic dynamics. *Biochem. Soc. Trans.* 45, 427–436. doi: 10.1042/BST20160171

Fernandez, A. M., and Torres-Aleman, I. (2012). The many faces of insulin-like peptide signalling in the brain. *Nat. Rev. Neurosci.* 13, 225–239. doi: 10.1038/nrn3209

Frasca, F., Pandini, G., Scalia, P., Sciacca, L., Mineo, R., Costantino, A., et al. (1999). Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol. Cell Biol.* 19, 3278–3288. doi: 10.1128/mcb.19.5.3278

Frolich, L., Blum-Degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., et al. (1998). Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J. Neural Transm.* 105, 423–438.

Gerozissis, K., Orosco, M., Rouch, C., and Nicolaidis, S. (1997). Insulin responses to a fat meal in hypothalamic microdialysates and plasma. *Physiol. Behav.* 62, 767–772. doi: 10.1016/s0031-9384(97)00195-9

Gong, C. X., Liu, F., and Iqbal, K. (2016). O-GlcNAcylation: A regulator of tau pathology and neurodegeneration. *Alzh. Dem.* 12, 1078–1089. doi: 10.1016/j.jalz.2016.02.011

Graham, D. L., Gray, A. J., Joyce, J. A., Yu, D., O'Moore, J., Carlson, G. A., et al. (2014). Increased O-GlcNAcylation reduces pathological tau without affecting its normal phosphorylation in a mouse model of tauopathy. *Neuropharmacology* 79, 307–313. doi: 10.1016/j.neuropharm.2013.11.025

Griffith, L. S., Mathes, M., and Schmitz, B. (1995). Beta-amyloid precursor protein is modified with O-linked N-acetylglucosamine. *J. Neurosci. Res.* 41, 270–278. doi: 10.1002/jnr.490410214

Gual, P., Le Marchand-Brustel, Y., and Tanti, J. F. (2005). Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 87, 99–109. doi: 10.1016/j.biochi.2004.10.019

Guthoff, M., Grichisch, Y., Canova, C., Tschritter, O., Veit, R., Hallschmid, M., et al. (2010). Insulin modulates food-related activity in the central nervous system. *J. Clin. Endocrinol. Metab.* 95, 748–755. doi: 10.1210/jc.2009-1677

Hahne, H., Sobotzki, N., Nyberg, T., Helm, D., Borodkin, V. S., van Aalten, D. M., et al. (2013). Proteome wide purification and identification of O-GlcNAc-modified proteins using click chemistry and mass spectrometry. *J. Proteome Res.* 12, 927–936. doi: 10.1021/pr300967y

Hanover, J. A., Krause, M. W., and Love, D. C. (2010). The hexosamine signaling pathway: O-GlcNAc cycling in feast or famine. *Biochim. Biophys. Acta* 1800, 80–95. doi: 10.1016/j.bbagen.2009.07.017

Hastings, N. B., Wang, X., Song, L., Butts, B. D., Grotz, D., Hargreaves, R., et al. (2017). Inhibition of O-GlcNAcase leads to elevation of O-GlcNAc tau and reduction of tauopathy and cerebrospinal fluid tau in rTg4510 mice. *Mol. Neurodegener.* 12:39. doi: 10.1186/s13024-017-0181-0

Havrankova, J., Schmechel, D., Roth, J., and Brownstein, M. (1978). Identification of insulin in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 75, 5737–5741. doi: 10.1073/pnas.75.11.5737

Heni, M., Kullmann, S., Preissl, H., Fritsche, A., and Haring, H. U. (2015). Impaired insulin action in the human brain: causes and metabolic consequences. *Nat. Rev. Endocrinol.* 11, 701–711. doi: 10.1038/nrendo.2015.173

Hill, J. M., Lesniak, M. A., Pert, C. B., and Roth, J. (1986). Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience* 17, 1127–1138. doi: 10.1016/0306-4522(86)90082-5

Hill, M. M., Clark, S. F., Tucker, D. F., Birnbaum, M. J., James, D. E., and Macaulay, S. L. (1999). A role for protein kinase Bbeta/Akt2 in insulin-stimulated GLUT4 translocation in adipocytes. *Mol. Cell Biol.* 19, 7771–7781. doi: 10.1128/mcb.19.11.7771

Holscher, C. (2011). Diabetes as a risk factor for Alzheimer's disease: insulin signalling impairment in the brain as an alternative model of Alzheimer's disease. *Biochem. Soc. Trans.* 39, 891–897. doi: 10.1042/BST0390891

Horvath, T. L., Sarman, B., Garcia-Caceres, C., Enriori, P. J., Sotonyi, P., Shanabrough, M., et al. (2010). Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14875–14880. doi: 10.1073/pnas.1004282107

Hoyer, S. (2004). Causes and consequences of disturbances of cerebral glucose metabolism in sporadic Alzheimer disease: therapeutic implications. *Adv. Exp. Med. Biol.* 541, 135–152. doi: 10.1007/978-1-4419-8969-7_8

Hsu, T. M., and Kanoski, S. E. (2014). Blood-brain barrier disruption: mechanistic links between Western diet consumption and dementia. *Front. Aging Neurosci.* 6:88. doi: 10.3389/fnagi.2014.00088

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. *Sci. Transl. Med.* 4:147ra111. doi: 10.1126/scitranslmed.3003748

Iyer, S. P., and Hart, G. W. (2003). Dynamic nuclear and cytoplasmic glycosylation: enzymes of O-GlcNAc cycling. *Biochemistry* 42, 2493–2499. doi: 10.1021/bi020685a

Jacobsen, K. T., and Iverfeldt, K. (2011). O-GlcNAcylation increases non-amyloidogenic processing of the amyloid-beta precursor protein (APP). *Biochem. Biophys. Res. Commun.* 404, 882–886. doi: 10.1016/j.bbrc.2010.12.080

Jahangir, Z., Ahmad, W., and Shabbiri, K. (2014). Alternate Phosphorylation/O-GlcNAc modification on human insulin IRSs: a road towards impaired insulin signaling in alzheimer and diabetes. *Adv. Bioinform.* 2014:324753. doi: 10.1155/2014/324753

Jimenez, J. L., Nettleton, E. J., Bouchard, M., Robinson, C. V., Dobson, C. M., and Saibil, H. R. (2002). The protofilament structure of insulin amyloid fibrils. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9196–9201. doi: 10.1073/pnas.142459399

Kan, O., Baldwin, S. A., and Whetton, A. D. (1994). Apoptosis is regulated by the rate of glucose transport in an interleukin 3 dependent cell line. *J. Exp. Med.* 180, 917–923. doi: 10.1084/jem.180.3.917

Keembiyehetty, C., Love, D. C., Harwood, K. R., Gavrilova, O., Comly, M. E., and Hanover, J. A. (2015). Conditional knock-out reveals a requirement for O-linked N-Acetylglucosaminase (O-GlcNAcase) in metabolic homeostasis. *J. Biol. Chem.* 290, 7097–7113. doi: 10.1074/jbc.M114.617779

Kern, W., Benedict, C., Schultes, B., Plohr, F., Moser, A., Born, J., et al. (2006). Low cerebrospinal fluid insulin levels in obese humans. *Diabetologia* 49, 2790–2792. doi: 10.1007/s00125-006-0409-y

Kim, C., Nam, D. W., Park, S. Y., Song, H., Hong, H. S., Boo, J. H., et al. (2013). O-linked beta-N-acetylglucosaminidase inhibitor attenuates beta-amyloid plaque and rescues memory impairment. *Neurobiol. Aging* 34, 275–285. doi: 10.1016/j.neurobiolaging.2012.03.001

Kleinridders, A., Schenten, D., Konner, A. C., Belgardt, B. F., Mauer, J., Okamura, T., et al. (2009). MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell Metab.* 10, 249–259. doi: 10.1016/j.cmet.2009.08.013

Klockener, T., Hess, S., Belgardt, B. F., Paeger, L., Verhagen, L. A., Husch, A., et al. (2011). High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. *Nat. Neurosci.* 14, 911–918. doi: 10.1038/nn.2847

Koch, L., Wunderlich, F. T., Seibler, J., Konner, A. C., Hampel, B., Irlenbusch, S., et al. (2008). Central insulin action regulates peripheral glucose and fat metabolism in mice. *J. Clin. Invest.* 118, 2132–2147. doi: 10.1172/JCI31073

Konner, A. C., Hess, S., Tovar, S., Mesaros, A., Sanchez-Lasheras, C., Evers, N., et al. (2011). Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. *Cell Metab.* 13, 720–728. doi: 10.1016/j.cmet.2011.03.021

Kuljis, R. O., and Salkovic-Petrisic, M. (2011). Dementia, diabetes, Alzheimer's disease, and insulin resistance in the brain: progress, dilemmas, new opportunities, and a hypothesis to tackle intersecting epidemics. *J. Alzh. Dis.* 25, 29–41. doi: 10.3233/JAD-2011-101392

Langlet, F., Levin, B. E., Luquet, S., Mazzone, M., Messina, A., Dunn-Meynell, A. A., et al. (2013). Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting. *Cell Metab.* 17, 607–617. doi: 10.1016/j.cmet.2013.03.004

Lerner-Marmarosh, N., Shen, J., Torno, M. D., Kravets, A., Hu, Z., and Maines, M. D. (2005). Human biliverdin reductase: a member of the insulin receptor substrate family with serine/threonine/tyrosine kinase activity. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7109–7114. doi: 10.1073/pnas.0502173102

Li, J., Wang, J., Wen, L., Zhu, H., Li, S., Huang, K., et al. (2016). An OGA-resistant probe allows specific visualization and accurate identification of O-GlcNAc-modified proteins in cells. *ACS Chem. Biol.* 11, 3002–3006. doi: 10.1021/acschembio.6b00678

Li, Z. G., Zhang, W., and Sima, A. A. (2007). Alzheimer-like changes in rat models of spontaneous diabetes. *Diabetes* 56, 1817–1824. doi: 10.2337/db07-0171

Liu, F., Iqbal, K., Grundke-Iqbal, I., Hart, G. W., and Gong, C. X. (2004). O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* 101, 10804–10809. doi: 10.1073/pnas.0400348101

Liu, F., Shi, J., Tanimukai, H., Gu, J., Grundke-Iqbal, I., Iqbal, K., et al. (2009). Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. *Brain* 132, 1820–1832. doi: 10.1093/brain/awp099

Liu, Y., Liu, F., Grundke-Iqbal, I., Iqbal, K., and Gong, C. X. (2009). Brain glucose transporters, O-GlcNAcylation and phosphorylation of tau in diabetes and Alzheimer's disease. *J. Neurochem.* 111, 242–249. doi: 10.1111/j.1471-4159.2009.06320.x

Liu, Y., Liu, F., Iqbal, K., Grundke-Iqbal, I., and Gong, C. X. (2008). Decreased glucose transporters correlate to abnormal hyperphosphorylation of tau in Alzheimer disease. *FEBS Lett.* 582, 359–364. doi: 10.1016/j.febslet.2007.12.035

Loh, K., Fukushima, A., Zhang, X., Galic, S., Briggs, D., Enriori, P. J., et al. (2011). Elevated hypothalamic TCPTP in obesity contributes to cellular leptin resistance. *Cell Metab.* 14, 684–699. doi: 10.1016/j.cmet.2011.09.011

Love, D. C., and Hanover, J. A. (2005). The hexosamine signaling pathway: deciphering the "O-GlcNAc code". *Sci. STKE* 2005:re13. doi: 10.1126/stke.3122005re13

Ma, J., and Hart, G. W. (2013). Protein O-GlcNAcylation in diabetes and diabetic complications. *Exp. Rev. Proteo.* 10, 365–380. doi: 10.1586/14789450.2013.820536

Ma, J., and Hart, G. W. (2014). O-GlcNAc profiling: from proteins to proteomes. *Clin. Proteo.* 11:8. doi: 10.1186/1559-0275-11-8

Ma, L., Wang, J., and Li, Y. (2015). Insulin resistance and cognitive dysfunction. *Clin. Chim. Acta* 444, 18–23. doi: 10.1016/j.cca.2015.01.027

Macauley, M. S., He, Y., Gloster, T. M., Stubbs, K. A., Davies, G. J., and Vocadlo, D. J. (2010). Inhibition of O-GlcNAcase using a potent and cell-permeable inhibitor does not induce insulin resistance in 3T3-L1 adipocytes. *Chem. Biol.* 17, 937–948. doi: 10.1016/j.chembiol.2010.07.006

McEwen, B. S., and Reagan, L. P. (2004). Glucose transporter expression in the central nervous system: relationship to synaptic function. *Eur. J. Pharmacol.* 490, 13–24. doi: 10.1016/j.ejphar.2004.02.041

Mlinar, B., Marc, J., Janez, A., and Pfeifer, M. (2007). Molecular mechanisms of insulin resistance and associated diseases. *Clin. Chim. Acta* 375, 20–35. doi: 10.1016/j.cca.2006.07.005

Mushtaq, G., Khan, J. A., and Kamal, M. A. (2014). Biological mechanisms linking Alzheimer's disease and type-2 diabetes mellitus. *CNS Neurol. Disord. Drug Targets* 13, 1192–1201. doi: 10.2174/1871527313666140917114537

Myslicki, J. P., Belke, D. D., and Shearer, J. (2014). Role of O-GlcNAcylation in nutritional sensing, insulin resistance and in mediating the benefits of exercise. *Appl. Physiol. Nutr Metab.* 39, 1205–1213. doi: 10.1139/apnm-2014-0122

Nandi, A., Sprung, R., Barma, D. K., Zhao, Y., Kim, S. C., and Falck, J. R. (2006). Global identification of O-GlcNAc-modified proteins. *Anal. Chem.* 78, 452–458. doi: 10.1021/ac051207j

Neergaard, J. S., Dragsbaek, K., Christiansen, C., Nielsen, H. B., Brix, S., Karsdal, M. A., et al. (2017). Metabolic syndrome, insulin resistance, and cognitive dysfunction: does your metabolic profile affect your brain? *Diabetes* 66, 1957–1963. doi: 10.2337/db16-1444

Olson, B. R., Freilino, M., Hoffman, G. E., Stricker, E. M., Sved, A. F., and Verbalis, J. G. (1993). c-Fos expression in rat brain and brainstem nuclei in response to treatments that alter food intake and gastric motility. *Mol. Cell Neurosci.* 4, 93–106. doi: 10.1006/mcne.1993.1011

Park, S. Y., Ryu, J., and Lee, W. (2005). O-GlcNAc modification on IRS-1 and Akt2 by PUGNAc inhibits their phosphorylation and induces insulin resistance in rat primary adipocytes. *Exp. Mol. Med.* 37, 220–229. doi: 10.1038/emm.2005.30

Pedersen, W. A., Culmsee, C., Ziegler, D., Herman, J. P., and Mattson, M. P. (1999). Aberrant stress response associated with severe hypoglycemia in a transgenic mouse model of Alzheimer's disease. *J. Mol. Neurosci.* 13, 159–165.

Pinho, T. S., Verde, D. M., Correia, S. C., Cardoso, S. M., and Moreira, P. I. (2018). O-GlcNAcylation and neuronal energy status: implications for Alzheimer's disease. *Ageing Res. Rev.* 46, 32–41. doi: 10.1016/j.arr.2018.05.003

Plum, L., Schubert, M., and Bruning, J. C. (2005). The role of insulin receptor signaling in the brain. *Trends Endocrinol. Metab* 16, 59–65. doi: 10.1016/j.tem.2005.01.008

Porter, J. P., and Bokil, H. S. (1997). Effect of intracerebroventricular and intravenous insulin on Fos-immunoreactivity in the rat brain. *Neurosci. Lett.* 224, 161–164. doi: 10.1016/s0304-3940(97)00162-6

Prodi, E., and Obici, S. (2006). Minireview: the brain as a molecular target for diabetic therapy. *Endocrinology* 147, 2664–2669. doi: 10.1210/en.2006-0143

Qiu, J., Zhang, C., Borgquist, A., Nestor, C. C., Smith, A. W., Bosch, M. A., et al. (2014). Insulin excites anorexigenic proopiomelanocortin neurons via activation of canonical transient receptor potential channels. *Cell Metab.* 19, 682–693. doi: 10.1016/j.cmet.2014.03.004

Ren, H., Yan, S., Zhang, B., Lu, T. Y., Arancio, O., and Accili, D. (2014). Glut4 expression defines an insulin-sensitive hypothalamic neuronal population. *Mol. Metab.* 3, 452–459. doi: 10.1016/j.molmet.2014.04.006

Reno, C. M., Puente, E. C., Sheng, Z., Daphna-Iken, D., Bree, A. J., Routh, V. H., et al. (2017). Brain GLUT4 knockout mice have impaired glucose tolerance, decreased insulin sensitivity, and impaired hypoglycemic counterregulation. *Diabetes* 66, 587–597. doi: 10.2337/db16-0917

Rivera, E. J., Goldin, A., Fulmer, N., Tavares, R., Wands, J. R., and de la Monte, S. M. (2005). Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. *J. Alzh. Dis.* 8, 247–268. doi: 10.3233/jad-2005-8304

Robertson, L. A., Moya, K. L., and Breen, K. C. (2004). The potential role of tau protein O-glycosylation in Alzheimer's disease. *J. Alzh. Dis.* 6, 489–495. doi: 10.3233/jad-2004-6505

Roh, E., Song, D. K., and Kim, M. S. (2016). Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. *Exp. Mol. Med.* 48:e216. doi: 10.1038/emm.2016.4

Rojas, F. A., Hirata, A. E., and Saad, M. J. (2003). Regulation of insulin receptor substrate-2 tyrosine phosphorylation in animal models of insulin resistance. *Endocrine* 21, 115–122. doi: 10.1385/endo:21:2:115

Samuel, V. T., and Shulman, G. I. (2012). Mechanisms for insulin resistance: common threads and missing links. *Cell* 148, 852–871. doi: 10.1016/j.cell.2012.02.017

Schechter, R., Beju, D., Gaffney, T., Schaefer, F., and Whetsell, L. (1996). Preproinsulin I and II mRNAs and insulin electron microscopic immunoreaction are present within the rat fetal nervous system. *Brain Res.* 736, 16–27. doi: 10.1016/s0006-8993(96)00664-6

Schechter, R., Sadiq, H. F., and Devaskar, S. U. (1990). Insulin and insulin mRNA are detected in neuronal cell cultures maintained in an insulin-free/serum-free medium. *J. Histochem. Cytochem.* 38, 829–836. doi: 10.1177/38.6.2186089

Schechter, R., Whitmire, J., Wheet, G. S., Beju, D., Jackson, K. W., Harlow, R., et al. (1994). Immunohistochemical and in situ hybridization study of an insulin-like substance in fetal neuron cell cultures. *Brain Res.* 636, 9–27. doi: 10.1016/0006-8993(94)90170-8

Schwartz, M. W., Figlewicz, D. P., Baskin, D. G., Woods, S. C., and Porte, D. (1992). Insulin in the brain: a hormonal regulator of energy balance. *Endocr. Rev.* 13, 387–414. doi: 10.1210/er.13.3.387

Schwartz, M. W., and Porte, D. Jr. (2005). Diabetes, obesity, and the brain. *Science* 307, 375–379.

Siso, S., Jeffrey, M., and Gonzalez, L. (2010). Sensory circumventricular organs in health and disease. *Acta Neuropathol.* 120, 689–705. doi: 10.1007/s00401-010-0743-5

Steen, E., Terry, B. M., Rivera, E. J., Cannon, J. L., Neely, T. R., Tavares, R., et al. (2005). Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *J. Alzheimers Dis.* 7, 63–80. doi: 10.3233/jad-2005-7107

Stephens, L. R., Hughes, K. T., and Irvine, R. F. (1991). Pathway of phosphatidylinositol(3,4,5)-trisphosphate synthesis in activated neutrophils. *Nature* 351, 33–39. doi: 10.1038/351033a0

Szablewski, L. (2017). Glucose Transporters in Brain: In Health and in Alzheimer's Disease. *J. Alzheimers Dis.* 55, 1307–1320. doi: 10.3233/JAD-160841

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., et al. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J. Clin. Invest.* 122, 1316–1338. doi: 10.1172/JCI59903

Tramutola, A., Sharma, N., Barone, E., Lanzillotta, C., Castellani, A., Iavarone, F., et al. (2018). Proteomic identification of altered protein O-GlcNAcylation in a triple transgenic mouse model of Alzheimer's disease. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 3309–3321. doi: 10.1016/j.bbadis.2018.07.017

Tschritter, O., Preissl, H., Hennige, A. M., Stumvoll, M., Porubska, K., Frost, R., et al. (2006). The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: a magnetoencephalographic study. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12103–12108. doi: 10.1073/pnas.0604404103

Tuulari, J. J., Karlsson, H. K., Hirvonen, J., Hannukainen, J. C., Bucci, M., Helmio, M., et al. (2013). Weight loss after bariatric surgery reverses insulin-induced increases in brain glucose metabolism of the morbidly obese. *Diabetes* 62, 2747–2751. doi: 10.2337/db12-1460

Vannucci, S. J., Koehler-Stec, E. M., Li, K., Reynolds, T. H., Clark, R., and Simpson, I. A. (1998). GLUT4 glucose transporter expression in rodent brain: effect of diabetes. *Brain Res.* 797, 1–11. doi: 10.1016/s0006-8993(98)00103-6

Varela, L., and Horvath, T. L. (2012). Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. *EMBO Rep.* 13, 1079–1086. doi: 10.1038/embor.2012.174

Vosseller, K., Wells, L., Lane, M. D., and Hart, G. W. (2002). Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. *Proc. Natl. Acad. Sci. U.S.A.* 99, 5313–5318. doi: 10.1073/pnas.072072399

Wang, D., He, X., Zhao, Z., Feng, Q., Lin, R., Sun, Y., et al. (2015). Whole-brain mapping of the direct inputs and axonal projections of POMC and AgRP neurons. *Front. Neuroanat.* 9:40. doi: 10.3389/fnana.2015.00040

Wang, S., Yang, F., Petyuk, V. A., Shukla, A. K., Monroe, M. E., Gritsenko, M. A., et al. (2017). Quantitative proteomics identifies altered O-GlcNAcylation of structural, synaptic and memory-associated proteins in Alzheimer's disease. *J. Pathol.* 243, 78–88. doi: 10.1002/path.4929

Whelan, S. A., Dias, W. B., Thiruneelakantapillai, L., Lane, M. D., and Hart, G. W. (2010). Regulation of insulin receptor substrate 1 (IRS-1)/AKT kinase-mediated insulin signaling by O-Linked beta-N-acetylglucosamine in 3T3-L1 adipocytes. *J. Biol. Chem.* 285, 5204–5211. doi: 10.1074/jbc.M109.077818

Whitman, M., Downes, C. P., Keeler, M., Keller, T., and Cantley, L. (1988). Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* 332, 644–646. doi: 10.1038/332644a0

Williams, K. W., Margatho, L. O., Lee, C. E., Choi, M., Lee, S., Scott, M. M., et al. (2010). Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *J. Neurosci.* 30, 2472–2479. doi: 10.1523/JNEUROSCI.3118-09.2010

Wozniak, M., Rydzewski, B., Baker, S. P., and Raizada, M. K. (1993). The cellular and physiological actions of insulin in the central nervous system. *Neurochem. Int.* 22, 1–10. doi: 10.1016/0197-0186(93)90062-a

Wunderlich, C. M., Hovelmeyer, N., and Wunderlich, F. T. (2013). Mechanisms of chronic JAK-STAT3-SOCS3 signaling in obesity. *Jakstat* 2:e23878. doi: 10.4161/jkst.23878

Xu, B., and Xie, X. (2016). Neurotrophic factor control of satiety and body weight. *Nat. Rev. Neurosci.* 17, 282–292. doi: 10.1038/nrn.2016.24

Yang, X., and Qian, K. (2017). Protein O-GlcNAcylation: emerging mechanisms and functions. *Nat. Rev. Mol. Cell Biol.* 18, 452–465. doi: 10.1038/nrm.2017.22

Yuzwa, S. A., Shan, X., Macauley, M. S., Clark, T., Skorobogatko, Y., Vosseller, K., et al. (2012). Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. *Nat. Chem. Biol.* 8, 393–399. doi: 10.1038/nchembio.797

Zhang, Z. Y., Dodd, G. T., and Tiganis, T. (2015). Protein tyrosine phosphatases in hypothalamic insulin and leptin signaling. *Trends Pharmacol. Sci.* 36, 661–674. doi: 10.1016/j.tips.2015.07.003

Zhao, W. Q., and Townsend, M. (2009). Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease. *Biochim. Biophys. Acta* 1792, 482–496. doi: 10.1016/j.bbadis.2008.10.014

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

MINI REVIEW
published: 22 May 2019
doi: 10.3389/fnins.2019.00508

Obesity as a Risk Factor for Alzheimer's Disease: Implication of Leptin and Glutamate

Ana Lloret[1], Paloma Monllor[1], Daniel Esteve[1], Ana Cervera-Ferri[2] and Maria-Angeles Lloret[3]*

[1] Department of Physiology, Faculty of Medicine, University of Valencia, Health Research Institute INCLIVA, Valencia, Spain, [2] Department of Human Anatomy and Embryology, Faculty of Medicine, University of Valencia, Valencia, Spain, [3] Department of Clinic Neurophysiology, University Clinic Hospital of Valencia, Valencia, Spain

Obesity is known to induce leptin and insulin resistance. Leptin is a peptide hormone synthesized in adipose tissue that mainly regulates food intake. It has been shown that insulin stimulates the production of leptin when adipocytes are exposed to glucose to encourage satiety; while leptin, via a negative feedback, decreases the insulin release and enhances tissue sensitivity to it, leading to glucose uptake for energy utilization or storage. Therefore, resistance to insulin is closely related to leptin resistance. Obesity in middle age has also been related to Alzheimer's disease (AD). In recent years, the relation between impaired leptin signaling pathway and the onset of AD has been studied. In all this context the role of the blood brain barrier (BBB) is crucial. Slow excitotoxicity happens in AD due to an excess of the neurotransmitter glutamate. Since leptin has been shown to regulate *N*-methyl-D-aspartate (NMDA) receptors, we want to review the link between these pathological pathways, and how they are affected by other AD triggering factors and its role in the onset of AD.

Keywords: leptin-resistance, dementia, overweight, excitotoxicity, LTP

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
João M. N. Duarte,
Lund University, Sweden
Salvatore Fusco,
Università Cattolica del Sacro Cuore, Italy

****Correspondence:***
Ana Lloret
ana.lloret@uv.es

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *01 March 2019*
Accepted: *02 May 2019*
Published: *22 May 2019*

Citation:
Lloret A, Monllor P, Esteve D, Cervera-Ferri A and Lloret M-A (2019) Obesity as a Risk Factor for Alzheimer's Disease: Implication of Leptin and Glutamate. Front. Neurosci. 13:508. doi: 10.3389/fnins.2019.00508

INTRODUCTION

In the last years obesity has changed from a mere aesthetic problem to become into a serious health problem worldwide. Nowadays it is considered by medical authorities as a genuine epidemic, consuming enormous technical, human, and economic resources. Obesity and also overweight affect near than 300 million people from child to elderly, and it is not related with the development level of the country (World Health Organization [WHO], 2003). Moreover, research has shown that obese children are more likely to be overweight or obese as adults (Sahoo et al., 2015). The growing incidence caused by a change in eating habits, by an increased consumption of fat and also by a substantial reduction in physical activity. This nutritional disorder implicates a number of conditions associated with excess weight, such as heart diseases, type 2 diabetes, high blood pressure, different types of cancer, and even neurodegeneration (Friedemann et al., 2012; Odegaard and Chawla, 2013; Hotamisligil, 2017). All these chronic pathologies associated with obesity, englobe the main causes of death and also monopolize 80% of healthcare expense (The World Health Organization [WHO], 2019).

The body mass index (BMI) is the most widely used method to classify a person in relation to his/her weight. In adults a BMI of 18.5 to 24.9 stands for a healthy, normal weight, while a value between 25 and 29.9 means is considered overweight. From values from 30 to 39.9 implies you are obese and from 40 to above means you are severely obese. A BMI lower than 18.5 is considered underweight and may indicate an eating disorder or malnutrition. However, BMI is not representative of overweight in the case of people with high percentage of muscle mass. In these population a high BMI would not indicate excess of fat. Perhaps a more accurate method to assess excess fat is waist circumference, which can be used as an additional measure in people who are overweight or moderately obese. Usually, men with a waist circumference of 94 cm (37 in) or more and women with a waist circumference of 80 cm (31.5 in) or more are in risk of obesity-related diseases.

In the last 15 years, obesity and dementia risk have been related (Whitmer et al., 2005). An increase in adipose tissue could promote a decrease in the blood flow to the brain, leading to vascular injury. In fact, obesity is related to changes in cerebral vascularization, because perivascular adipose tissue is not found around the cerebral arteries (Dorrance et al., 2014). A decrease in blood flow to the brain causes ischemia in vulnerable brain areas. The most sensitive areas, specifically vulnerable, are neurons located in the hippocampal regions CA1, CA3, and CA4, portions of the caudate nucleus, cerebellum, and layers III, V, VI of the neocortex (Payabvash et al., 2011). The hippocampal areas, due to its high baseline metabolic activity, are extremely susceptible to reduced oxygen and glucose intake and it is believed that it can be one of the causes of increased memory loss (Kivipelto et al., 2005). Chronic peripheral inflammation caused by the release of adipokines as leptin and other cytokines, may spread to the brain and the neuroinflammation is linked to a decrease in the brain white matter, leading to impair neuronal connections (Arnoldussen et al., 2014; Kiliaan et al., 2014). Moreover, neuroinflammation could be triggered by an imbalance in the gut microbiota due to the consumption of diets high in fats and sugars (Solas et al., 2017), which could provoke an alteration in the "gut-brain axis."

In this review, we are going to discuss the role of the cytokine leptin in brain function and specially in the memory decline associated with Alzheimer's disease (AD).

LEPTIN AND ITS ROLE IN THE BRAIN

Leptin was discovered in Zhang et al. (1994) by Friedman and co-workers using modern molecular biology tools such as positional cloning. After cloning the *ob* gene in mice and its homolog in humans, the gene product was purified and called leptin (Maffei et al., 1995). Leptin is a hormone mainly produced by adipose tissue which is released to the bloodstream and circulates throughout the body proportionally to the body fat mass (Friedman and Halaas, 1998). Moreover, leptin is expressed either in subcutaneous and visceral adipose tissue (Lieb et al., 2009), and also in placenta, skeletal muscle, ovaries, mammary epithelial cells, (Margetic et al., 2002), or even in the gastrointestinal tract with both endocrine and exocrine actions (Cammisotto et al., 2005). Leptin can be found into the bloodstream either associated to binding proteins or in a free, bioactive form (Sinha et al., 1996). Obese individuals show a higher proportion of the free circulating leptin form and in contrast, in lean subjects leptin circulates mainly bound to its soluble receptor (Sinha et al., 1996). This is in line with the fact that one of the functions attributed to leptin is to regulate food intake and energy expenditure. When adipose tissue decreases plasma leptin levels also decrease, and when adipose tissue increases leptin levels increase and suppresses appetite (Maffei et al., 1995). But we know today that the functions of leptin are many others: it is a growth factor, a permissive factor for puberty, controls metabolism and immune system and is also implicated in memory (Margetic et al., 2002; Kelesidis et al., 2010; McGregor and Harvey, 2018a). All these effects are mediated by binding to specific leptin receptors (LepR) expressed in the central nervous system (CNS) as well as in peripheral tissues.

The LepR has six different isoforms: five of them (LepRa, LepRc, LepRd, LepRf, and LepRb) show transmembrane domain, whereas LepRe only presents an extracellular domain and acts as a soluble receptor. LepRb is the long form of the receptor while the others isoforms are shorter (Chua et al., 1997; Tartaglia, 1997; Cui et al., 2017). LepRs are widely expressed all along the body, but focusing in the brain, both short and long isoforms are broadly expressed. LepR is found in the hypothalamus (specifically, in the arcuate, ventromedial, paraventricular, and ventral premammillary nuclei) but LepRs are also present in other areas primarily non-associated with energy balance such as the neocortex, hippocampus, thalamus, leptomeninges, choroid plexus (Mercer et al., 1996; Fei et al., 1997; De Matteis and Cinti, 1998), entorhinal cortex, amygdala, and rostral medulla (Savioz et al., 1997; Burguera et al., 2000).

LEPTIN AND OBESITY

To reach the CNS, leptin crosses the blood-brain barrier (BBB) through a saturable transport system (Banks et al., 1996). Brain microvessels express short leptin receptors which bind and internalize leptin (Karlsson et al., 1997; Bjørbaek et al., 1998). It has been proposed that leptin enters via cerebrospinal fluid (CSF) from plasma because the choroid plexus contains many leptin receptors (Schwartz et al., 1996; Golden et al., 1997). In the hypothalamus, a very specific type of cell, the tanycyte has a remarkable role conducting leptin. Tanycytes are ependymal cells located in the third ventricle and also in the floor of the fourth ventricle. They have cellular extensions that communicate deep into the hypothalamus, and thank to these cellular prolongations, the leptin is conducted to its target areas through transcytosis (Balland et al., 2014). When leptin binds to its receptor it activates several signaling cascades such as the Janus tyrosine kinase 2 (JAK2), the signal transducer activator of transcription 3 (STAT3), the phosphatidylinositol 3-kinase (PI3 kinase), and the AKT pathways (Flak and Myers, 2016) that culminates in the modification of neurons releasing three hormone-derived peptides: neuropeptide Y (NPY), pro-opiomelanocortin

(POMC), and agouti-related peptide (AgRP). If the amount of NPY and AgRP, are increased, it leads to increased food intake but the activation of the POMC triggers factors (mainly the α-melanocyte–stimulating hormone) that inhibit food intake (Varela and Horvath, 2012). The administration of leptin increases POMC mRNA expression, and inhibits NPY and AgRP mRNAs translation (Elias et al., 1999; Balland and Cowley, 2015).

Nevertheless, we have a hormone that is central in the regulation of food intake and glucose levels control, and of course, we are referring to insulin. An increase in the level of circulating insulin produced by its prandial release from endogenous stores is associated with the state of satiety. Given this fact, the relation of leptin and insulin is a point to discuss. As, Kahn and Flier (2000) propose, leptin has an insulin-sensitizing effect after both an acute or a chronic administration. This could happen because LepRs are present in pancreatic β-cells (Pallett et al., 1997; Amitani et al., 2013) and when leptin binds to them, it is able to inhibit insulin synthesis and release to the bloodstream. On the contrary, insulin stimulates leptin secretion from adipose tissue closing the feedback loop. In this line, Kulkarni et al. (1997) show that leptin administration lowers insulin secretion *in vivo* not only in mice but also in isolated human islets. Making tissues more sensitive to insulin, leptin causes glucose uptake for energy utilization, or storage.

Given that insulin resistance happens in obesity, the role of leptin in obesity etiology and pathophysiology is worth to discuss. In obese subjects, levels of leptin increase in plasma compared to lean subjects. This is, probably, a physiological response to reduce food intake, and also aims to use all the energy derived from the lipid metabolism (Myers et al., 2010). However, what actually happens is that obese subjects show resistance to leptin actions over time. In this line, leptin resistance is associated with both increased circulating levels of leptin and also with inability of exogenous leptin to decrease body fat or food intake (Myers, 2015). Actually, augmented circulating levels of leptin in obesity caused hypothalamic leptin resistance, reducing the anorexigenic and energy expenditure signals and aggravating obesity (Waterson and Horvath, 2015). The cause of leptin resistance is not well elucidated, but it seems to have its origin in a defect in the transport of leptin across the BBB, maybe in deficits involving intracellular signaling mechanisms downstream of leptin receptor or even in development alterations (Banks, 2004; Myers et al., 2008; Farr et al., 2015).

OBESITY AND RISK OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common form of dementia. It is characterized by two main lesions in the brain: senile plaques, predominantly formed by amyloid-beta (Aβ) peptide and neurofibrillary tangles, mainly compound by hyperphosphorylated tau protein (p-tau). Aβ is product of the processed of the amyloid precursor protein (APP) by β- and γ-secretase enzymes (Mattson, 2004; Patterson et al., 2008). The first symptom of the disease is episodic memory loss associated with hippocampal affectation. But some pathological modifications related to obesity, such as neuroinflammation, insulin resistance, or mitochondrial dysfunction, also occur in AD pathological progression (O'Brien et al., 2017).

The number of studies relating an increase in fat body mass and the risk of suffer AD has increased in the last years. However, the results are controversial and many times inconclusive. It seems that it is important to differentiate between mid-life and late-life overweight (Xu et al., 2011). Specifically, obesity in midlife and a weight loss in the preclinical phase characterizes dementia (Singh-Manoux et al., 2018). In fact, in a recent meta-analysis of 21 studies, the authors conclude that obesity below the age of 65 years (midlife obesity) correlates with the incident of dementia, but not the late-life obesity (over 65 years) (Pedditizi et al., 2016). Very recently, Kivimäki et al. (2018) analyzed 1,349,857 people from 39 different cohorts with BMI data assessed at baseline. The authors find that 20 years before dementia diagnosis, higher BMI is associated with increased dementia risk in mid-life. Moreover, they describe that this risk is reversed in late-life and a higher BMI could even be protective (Kivimäki et al., 2018).

Furthermore, a meta-analysis of 15 prospective studies including more than 72000 participants used BMI measures and the authors found that both underweight and obese are related to an increase risk of AD but only in mid-life; high BMI in late-life was not associated with any dementia (Anstey et al., 2011). Moreover, the authors conclude that underweight could be a useful marker for identifying mild cognitive impairment (MCI) subjects at increased risk to convert to AD (Joo et al., 2018). Another very large retrospective cohort study with two million people analyzed, concludes that underweight in both middle and old age increases the risk of dementia over two decades (Qizilbash et al., 2015), although this study is not focused specifically in AD. In spite of these publications, the hypothesis that being overweight in mid-life is linked to dementia in late-life seems to be widely accepted by scientific community. A recent analysis explains that the duration of the preclinical weight loss phase could be a negative confounding parameter and a plausible explanation of this paradox (Pegueroles et al., 2018).

THE ROLE OF LEPTIN IN AD

Since obesity and dementia were related, many studies tried to find a link between brain leptin activity and AD development. In this line, Bonda et al. (2014) show that leptin is increased in the CSF and also in the hippocampus of AD patients, but leptin receptor mRNA is decreased within degenerating neurons and this could suggest a novel neuronal leptin resistance in AD. On the other hand, Maioli et al. (2015) show no changes in leptin concentration in CSF, but LepR also diminishes in post-mortem brains of AD patients, confirming that leptin resistance occurs. LepR decreased expression related to age, is also shown in an animal model of AD (King et al., 2018).

Brain leptin resistance is proposed as part of the neurodegenerative process. Leptin has both neurotrophic and neuroprotective properties therefore, leptin signaling deficits may lead to susceptibility to AD-related neurotoxic conditions.

In fact, leptin is able to modify the levels of Aβ peptide by limiting its production in neurons via reducing β-secretase activity (Fewlass et al., 2004; Marwarha et al., 2010). Likewise, leptin protects hippocampal neurons in primary cell culture from Aβ derived insults such as oxidative stress (Martins et al., 2013). Moreover, leptin enhances the removal of Aβ by promoting its clearance and degradation and activating the insulin degrading enzyme (Patterson et al., 2008). Furthermore, in neurons treated with Aβ, leptin prevents glycogen synthase kinase 3β (GSK3β) activation (Greco et al., 2009; Marwarha et al., 2010; Martins et al., 2013). This is very significant for AD pathogenesis since GSK3β is a kinase of tau and is implicated in the formation of neurofibrillary tangles. Besides, development of leptin resistance is linked with higher tau pathology in transgenic mouse models of AD suggesting that a defect in LepR-mediated signaling cascade could increase p-tau levels (Platt et al., 2016).

An important target of leptin action is the hippocampus, where it has a role in synaptic plasticity process, in memory preservation, and has pro-cognitive effects (Harvey, 2007, 2013). All these effects seem to be mediated by modulating glutamate receptors: the ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA). These receptors are involved in long-term potentiation (LTP) and in long-term depression (LTD). Leptin enhances LTP and decreases LTD, increasing the efficacy of excitatory synaptic transmission (Shanley et al., 2001; Wayner et al., 2004; Moult and Harvey, 2011; McGregor and Harvey, 2018b; McGregor et al., 2018). Moreover, leptin resistance is determinant for hippocampal dysfunction (Mainardi et al., 2017). In AD models,

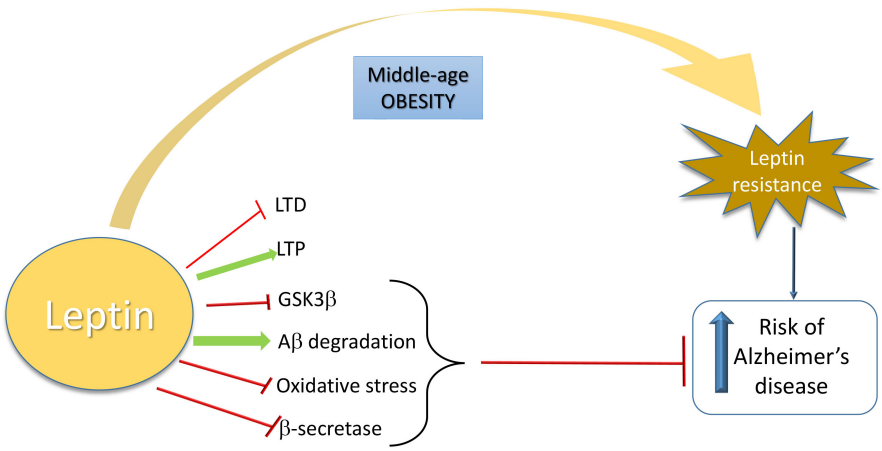

FIGURE 1 | Schematic overview of the relationship between leptin and increased risk of Alzheimer's Disease. Leptin is neuroprotective in AD by inhibiting LTD, GSK3β, oxidative stress, and β-secretase activity and by inducing LTP and Aβ degradation. When a leptin-resistance takes place due to middle-age obesity, AD risk is increased.

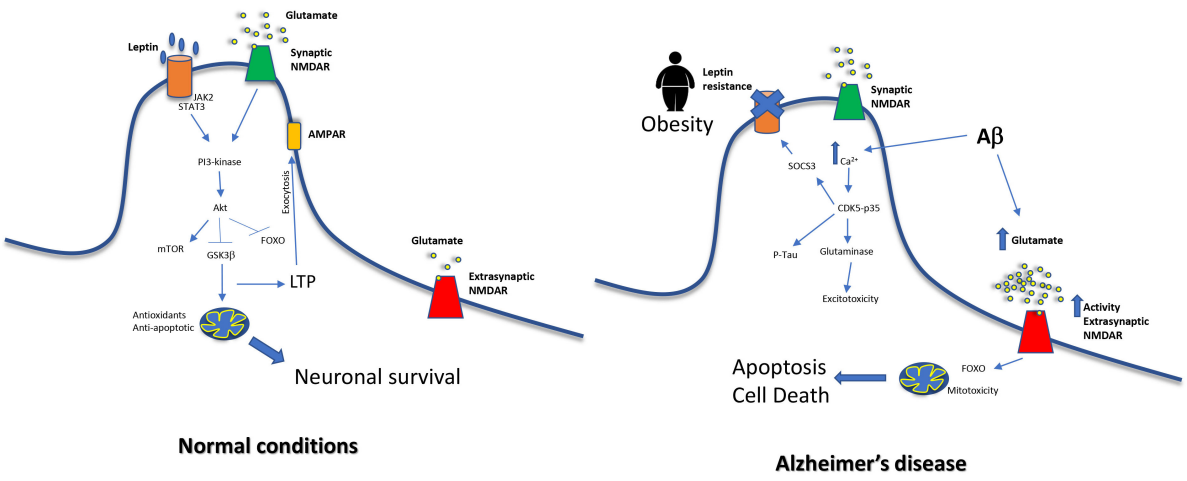

FIGURE 2 | Relationship between middle-age obesity, glutamate excitotoxicity and increased risk of Alzheimer's disease. In normal conditions, NMDA signaling and leptin receptor signal merge, exerting neuroprotective effects on cells. On the other hand, leptin resistance impairs this pathway. Moreover, Aβ causes an increase of glutamate levels and this leads to a dysfunction in extrasynaptic NMDAR, a decrease in LTP and mitochondrial alterations.

leptin prevents the anomalous effects of Aβ on hippocampal LTP and LTD, restoring normal hippocampal synaptic function (Doherty et al., 2013), and also increasing the synaptic density and rescuing memory deficits (Perez-Gonzalez et al., 2014).

Taken together, the studies described above indicate that brain leptin resistance could be central in AD pathophysiology, including the regulation of glutamatergic connections involved in hippocampal LTP and LTD. A schematic view is shown in **Figure 1**.

GLUTAMATE, OBESITY AND AD ARE LINKED VIA LEPTIN-RESISTANCE

Mild cognitive impairment and AD patients show an increase in plasma glutamate and glutamine (Miulli et al., 1993; Trushina et al., 2013). This increment is also reflected in brain, since some studies identify an increase in glutamate and glutamine levels in CSF from AD (Pomara et al., 1992; Jimenez-Jimenez et al., 1998; Kaiser et al., 2010; Madeira et al., 2018) and from MCI patients (D'Aniello et al., 2005). If this increase comes directly from the arise in the peripheral levels of glutamate and glutamine or if it is an indirect phenomenon is not yet known in AD. Curiously, glutamine levels increase in hippocampus from mice fed with a high-fat diet during 6 months (Lizarbe et al., 2019). Moreover, the BBB is disrupted in early phases of the disease and the consequences of this disruption in the amino acid transport are not yet studied in depth (Montagne et al., 2017). In any case, a slow excitotoxicity is shown in AD and this consist of an overexcitation of NMDA receptors by glutamate (Beal, 1992; Ong et al., 2013). Glutamate overexcite the NMDA receptors in a tonic manner and a good evidence of this, is that memantine, an uncompetitive NMDA receptor antagonist, is a well-established treatment of AD (Parsons et al., 2007). In fact, Aβ causes the increase of glutamate (Fuchsberger et al., 2016) and the intraneuronal Ca^{2+} levels (Kuchibhotla et al., 2008). A pathological signaling cascade is triggered, involving an increase of Cdk5-p35 levels, a decrease of Cdh1 and finally glutaminase increase, causing a positive feedback loop of excitotoxicity (Fuchsberger et al., 2016). Interestingly, Cdk5-p35 also modulates signaling induced by leptin (He et al., 2009). Cdk5-p35 causes SOCS3 activation, a negative feedback regulator which inhibits leptin-induced signal transduction and causes leptin resistance (He et al., 2009). So, the excess of glutamate levels can cause a cascade of events that also induce leptin-resistance.

Interestingly, in AD the aforementioned overactivation is produced in extrasynaptic NMDA receptors rather than in synaptic NMDA receptors (Zhang et al., 2016). Overstimulation of synaptic NMDA receptors is considered neuroprotective and in contrast, the overstimulation of extrasynaptic NMDA receptors induces tau hyperphosphorylation (Sun et al., 2016) and cell death (Hardingham and Bading, 2010). In fact, memantine blocks preferentially extrasynaptic over synaptic NMDA receptors (Xia et al., 2010) as part of its action as AD treatment. NR2-A is a subunit mainly present in synaptic NMDA receptors and it has shown that leptin mediates neuroprotection activating them (O'Malley et al., 2007), and this is critical for the induction of LTP and LTD (Muller et al., 2009). When leptin binds to its receptor, activates JAK2, which in turn promotes the activation of STAT3, and then, PI3K-Akt signaling pathways are induced. Activation of synaptic NR2A-containing NMDARs by glutamate also induces the PI3K-dependent pathway (Lee et al., 2002), so both common signals are highly potentiated. The signal cascade will induce AMPA exocytosis and LTP (Moult et al., 2010) but also neuronal survival by promoting expression of mitochondrial antioxidant enzymes and anti-apoptotic proteins such as Bcl-xl (Guo et al., 2008), and by inhibiting Foxo (Al-Mubarak et al., 2009) and GSK3β (Greco et al., 2009). In contrast, in AD extrasynaptic NMDA receptors are overstimulated and this leads to neuronal death. Extrasynaptic NMDAR induces the pro-apoptotic transcription factor Foxo (Dick and Bading, 2010) and also mitotoxicity. The consequences are mitochondrial calcium sustained increase, compromised ATP production and mitochondrial dysregulation finally inducing cell death (Bading, 2017). A global scheme is shown in **Figure 2**.

CONCLUSION

Leptin is a hormone secreted by adipose tissue that matters for the correct functioning of the brain, including the memory, and learning processes in the hippocampus. Leptin is neuroprotective and increase LTP, potentiating the activity of synaptic NMDA receptors of glutamate. We discussed how in AD both leptin resistance, LTP dysfunction, and also an increase in glutamate happen. For all this, obesity in middle-age could be considered as a risk factor to develop AD in the elderly.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by SAF2016-75508-R (Ministerio de Economía y Competitividad), CB16/10/00435 (CIBERFES-ISCIII), PROMETEOII2014/056 (Conselleria de Educación, Investigación, Cultura y Deporte).

REFERENCES

Al-Mubarak, B., Soriano, F. X., and Hardingham, G. E. (2009). Synaptic NMDAR activity suppresses FOXO1 expression via a cis-acting FOXO binding site: FOXO1 is a FOXO target gene. *Channels* 3, 233–238.

Amitani, M., Asakawa, A., Amitani, H., and Inui, A. (2013). The role of leptin in the control of insulin-glucose axis. *Front. Neurosci.* 7:51. doi: 10.3389/fnins.2013.00051

Anstey, K., Cherbuin, N., Budge, M., and Young, J. (2011). Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. *Obes. Rev.* 12, e426–e437. doi: 10.1111/j.1467-789X.2010.00825.x

Arnoldussen, I. A., Kiliaan, A. J., and Gustafson, D. R. (2014). Obesity and dementia: adipokines interact with the brain. *Eur. Neuropsychopharmacol.* 24, 1982–1999. doi: 10.1016/j.euroneuro.2014.03.002

Bading, H. (2017). Therapeutic targeting of the pathological triad of extrasynaptic NMDA receptor signaling in neurodegenerations. *J. Exp. Med.* 214, 569–578. doi: 10.1084/jem.20161673

Balland, E., and Cowley, M. A. (2015). New insights in leptin resistance mechanisms in mice. *Front. Neuroendocrinol.* 39, 59–65. doi: 10.1016/j.yfrne.2015.09.004

Balland, E., Dam, J., Langlet, F., Caron, E., Steculorum, S., Messina, A., et al. (2014). Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain. *Cell. Metab.* 19, 293–301. doi: 10.1016/j.cmet.2013.12.015

Banks, W. A. (2004). The many lives of leptin. *Peptides* 25, 331–338. doi: 10.1016/j.peptides.2004.02.014

Banks, W. A., Kastin, A. J., Huang, W., Jaspan, J. B., and Maness, L. M. (1996). Leptin enters the brain by a saturable system independent of insulin. *Peptides* 17, 305–311. doi: 10.1016/0196-9781(96)00025-3

Beal, M. F. (1992). Mechanisms of excitotoxicity in neurologic diseases. *FASEB J.* 6, 3338–3344. doi: 10.1096/fasebj.6.15.1464368

Bjørbaek, C., Elmquist, J. K., Michl, P., Ahima, R. S., van Bueren, A., McCall, A. L., et al. (1998). Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology* 139, 3485–3491. doi: 10.1210/en.139.8.3485

Bonda, D. J., Stone, J. G., Torres, S. L., Siedlak, S. L., Perry, G., Kryscio, R., et al. (2014). Dysregulation of leptin signaling in Alzheimer disease: evidence for neuronal leptin resistance. *J. Neurochem.* 128, 162–172. doi: 10.1111/jnc.12380

Burguera, B., Couce, M. E., Long, J., Lamsam, J., Laakso, K., Jensen, M. D., et al. (2000). The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. *Neuroendocrinology* 71, 187–195. doi: 10.1159/000054536

Cammisotto, P. G., Renaud, C., Gingras, D., Delvin, E., Levy, E., and Bendayan, M. (2005). Endocrine and exocrine secretion of leptin by the gastric mucosa. *J. Histochem. Cytochem.* 53, 851–860. doi: 10.1369/jhc.5a6620.2005

Chua, S. C., Koutras, I. K., Han, L., Liu, S., Kay, J., Young, S. J., et al. (1997). Fine structure of the murine leptin receptor gene: splice site suppression is required to form two alternatively spliced transcripts. *Genomics* 45, 264–270. doi: 10.1006/geno.1997.4962

Cui, H., López, M., and Rahmouni, K. (2017). The cellular and molecular bases of leptin and ghrelin resistance in obesity. *Nat. Rev. Endocrinol.* 13, 338–351. doi: 10.1038/nrendo.2016.222

D'Aniello, A., Fisher, G., Migliaccio, N., Cammisa, G., D'Aniello, E., and Spinelli, P. (2005). Amino acids and transaminases activity in ventricular CSF and in brain of normal and Alzheimer patients. *Neurosci. Lett.* 388, 49–53. doi: 10.1016/j.neulet.2005.06.030

De Matteis, R., and Cinti, S. (1998). Ultrastructural immunolocalization of leptin receptor in mouse brain. *Neuroendocrinology* 68, 412–419. doi: 10.1159/000054391

Dick, O., and Bading, H. (2010). Synaptic activity and nuclear calcium signaling protect hippocampal neurons from death signal-associated nuclear translocation of FoxO3a induced by extrasynaptic N-methyl-d-aspartate receptors. *J. Biol. Chem.* 285, 19354–19361. doi: 10.1074/jbc.M110.127654

Doherty, G. H., Beccano-Kelly, D., Du Yan, S., Gunn-Moore, F. J., and Harvey, J. (2013). Leptin prevents hippocampal synaptic disruption and neuronal cell death induced by amyloid β. *Neurobiol. Aging* 34, 226–237. doi: 10.1016/j.neurobiolaging.2012.08.003

Dorrance, A. M., Matin, N., and Pires, P. W. (2014). The effects of obesity on the cerebral vasculature. *Curr. Vasc. Pharmacol.* 12, 462–472. doi: 10.2174/1570161112666140423222411

Elias, C. F., Aschkenasi, C., Lee, C., Kelly, J., Ahima, R. S., Bjorbæk, C., et al. (1999). Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* 23, 775–786. doi: 10.1016/s0896-6273(01)80035-0

Farr, O. M., Gavrieli, A., and Mantzoros, C. S. (2015). Leptin applications in 2015: what have we learned about leptin and obesity? *Curr. Opin. Endocrinol. Diabetes. Obes.* 22, 353–359. doi: 10.1097/MED.0000000000000184

Fei, H., Okano, H. J., Li, C., Lee, G. H., Zhao, C., Darnell, R., et al. (1997). Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc. Natl. Acad. Sci. U.S.A.* 94, 7001–7005. doi: 10.1073/pnas.94.13.7001

Fewlass, D. C., Noboa, K., Pi-Sunyer, F. X., Johnston, J. M., Yan, S. D., and Tezapsidis, N. (2004). Obesity-related leptin regulates Alzheimer's Aβ. *FASEB J.* 18, 1870–1878. doi: 10.1096/fj.04-2572com

Flak, J. N., and Myers, M. G. Jr. (2016). Minireview: CNS mechanisms of leptin action. *Mol. Endocrinol.* 30, 3–12. doi: 10.1210/me.2015-1232

Friedemann, C., Heneghan, C., Mahtani, K., Thompson, M., Perera, R., and Ward, A. M. (2012). Cardiovascular disease risk in healthy children and its association with body mass index: systematic review and meta-analysis. *BMJ* 345:e4759. doi: 10.1136/bmj.e4759

Friedman, J. M., and Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature* 395, 763–770. doi: 10.1038/27376

Fuchsberger, T., Martínez-Bellver, S., Giraldo, E., Teruel-Martí, V., Lloret, A., and Viña, J. (2016). Aβ induces excitotoxicity mediated by APC/C-Cdh1 depletion that can be prevented by glutaminase inhibition promoting neuronal survival. *Sci. Rep.* 6:31158. doi: 10.1038/srep31158

Golden, P. L., Maccagnan, T. J., and Pardridge, W. M. (1997). Human blood-brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. *J. Clin. Invest.* 99, 14–18. doi: 10.1172/jci119125

Greco, S. J., Sarkar, S., Casadesus, G., Zhu, X., Smith, M. A., Ashford, J. W., et al. (2009). Leptin inhibits glycogen synthase kinase-3β to prevent tau phosphorylation in neuronal cells. *Neurosci. Lett.* 455, 191–194. doi: 10.1016/j.neulet.2009.03.066

Guo, Z., Jiang, H., Xu, X., Duan, W., and Mattson, M. P. (2008). Leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization. *J. Biol. Chem.* 283, 1754–1763. doi: 10.1074/jbc.m703753200

Hardingham, G. E., and Bading, H. (2010). Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* 11, 682–696. doi: 10.1038/nrn2911

Harvey, J. (2007). Leptin regulation of neuronal excitability and cognitive function. *Curr. Opin. Pharmacol.* 7, 643–647. doi: 10.1016/j.coph.2007.10.006

Harvey, J. (2013). Leptin regulation of neuronal morphology and hippocampal synaptic function. *Front. Syn. Neurosci.* 5:3. doi: 10.3389/fnsyn.2013.00003

He, Y., Kastin, A. J., Hsuchou, H., and Pan, W. (2009). The Cdk5/p35 kinases modulate leptin-induced STAT3 signaling. *J. Mol. Neurosci.* 39, 49–58. doi: 10.1007/s12031-008-9174-3

Hotamisligil, G. S. (2017). Foundations of immunometabolism and implications for metabolic health and disease. *Immunity* 47, 406–420. doi: 10.1016/j.immuni.2017.08.009

Jimenez-Jimenez, F. J., Molina, J. A., Gomez, P., Vargas, C., de Bustos, F., Benito-Leon, J., et al. (1998). Neurotransmitter amino acids in cerebrospinal fluid of patients with Alzheimer's disease. *J. Neural. Transm.* 105, 269–277. doi: 10.1007/s007020050056

Joo, S. H., Yun, S. H., Kang, D. W., Hahn, C. T., Lim, H. K., and Lee, C. U. (2018). Body mass index in mild cognitive impairment according to age, sex, cognitive intervention and hypertension and risk of progression to alzheimer's disease. *Front. Psychiatry* 9:142.

Kahn, B. B., and Flier, J. S. (2000). Obesity and insulin resistance. *J. Clin. Invest.* 106, 473–481.

Kaiser, E., Schoenknecht, P., Kassner, S., Hildebrandt, W., Kinscherf, R., and Schroeder, J. (2010). Cerebrospinal fluid concentrations of functionally important amino acids and metabolic compounds in patients with mild cognitive impairment and Alzheimer's disease. *Neurodegener. Dis.* 7, 251–259. doi: 10.1159/000287953

Karlsson, C., Lindell, K., Svensson, E., Bergh, C., Lind, P., Billig, H., et al. (1997). Expression of functional leptin receptors in the human ovary. *J. Clin. Endocrinol. Metab.* 82, 4144–4148. doi: 10.1210/jc.82.12.4144

Kelesidis, T., Kelesidis, I., Chou, S., and Mantzoros, C. S. (2010). Narrative review: the role of leptin in human physiology: emerging clinical applications. *Ann. Intern. Med.* 152, 93–100. doi: 10.7326/0003-4819-152-2-201001190-00008

Kiliaan, A. J., Arnoldussen, I. A., and Gustafson, D. R. (2014). Adipokines: a link between obesity and dementia? *Lancet Neurol.* 13, 913–923. doi: 10.1016/S1474-4422(14)70085-7

King, A., Brain, A., Hanson, K., Dittmann, J., Vickers, J., and Fernandez-Martos, C. (2018). Disruption of leptin signalling in a mouse model of Alzheimer's disease. *Metab. Brain Dis.* 33, 1097–1110. doi: 10.1007/s11011-018-0203-9

Kivimäki, M., Luukkonen, R., Batty, G. D., Ferrie, J. E., Pentti, J., Nyberg, S. T., et al. (2018). Body mass index and risk of dementia: analysis of individual-level data from 1.3 million individuals. *Alzheimers Dem.* 14, 601–609. doi: 10.1016/j.jalz.2017.09.016

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., et al. (2005). Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch. Neurol.* 62, 1556–1560.

Kuchibhotla, K. V., Goldman, S. T., Lattarulo, C. R., Wu, H. Y., Hyman, B. T., and Bacskai, B. J. (2008). Abeta plaques lead to aberrant regulation of calcium homeostasis in vivo resulting in structural and functional disruption of neuronal networks. *Neuron* 59, 214–225. doi: 10.1016/j.neuron.2008.06.008

Kulkarni, R. N., Wang, Z. L., Wang, R. M., Hurley, J. D., Smith, D. M., Ghatei, M. A., et al. (1997). Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. *J. Clin. Invest.* 100, 2729–2736. doi: 10.1172/jci119818

Lee, F. J. S., Xue, S., Pei, L., Vukusic, B., Chéry, N., Wang, Y., et al. (2002). Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. *Cell* 111, 219–230. doi: 10.1016/s0092-8674(02)00962-5

Lieb, W., Beiser, A. S., Vasan, R. S., Tan, Z. S., Au, R., Harris, T. B., et al. (2009). Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging. *JAMA* 302, 2565–2572. doi: 10.1001/jama.2009.1836

Lizarbe, B., Soares, A. F., Larsson, S., and Duarte, J. M. N. (2019). Neurochemical modifications in the hippocampus, cortex and hypothalamus of mice exposed to long-term high-fat diet. *Front. Neurosci.* 12:985. doi: 10.3389/fnins.2018.00985

Madeira, C., Vargas-Lopes, C., Brandão, C. O., Reis, T., Laks, J., Panizzutti, R., et al. (2018). Elevated glutamate and glutamine levels in the cerebrospinal fluid of patients with probable alzheimer's disease and depression. *Front. Psychiatry* 9:561. doi: 10.3389/fpsyt.2018.00561

Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., et al. (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight reduced subjects. *Nat. Med.* 1, 1155–1161. doi: 10.1038/nm1195-1155

Mainardi, M., Spinelli, M., Scala, F., Mattera, A., Fusco, S., D'Ascenzo, M., et al. (2017). Loss of leptin-induced modulation of hippocampal synaptic trasmission and signal transduction in high-fat diet-fed mice. *Front. Cell. Neurosci.* 11:225. doi: 10.3389/fncel.2017.00225

Maioli, S., Lodeiro, M., Merino-Serrais, P., Falahati, F., Khan, W., Puerta, E., et al. (2015). Alterations in brain leptin signalling in spite of unchanged CSF leptin levels in Alzheimer's disease. *Aging Cell* 14, 122–129. doi: 10.1111/acel.12281

Margetic, S., Gazzola, C., Pegg, G., and Hill, R. (2002). Leptin: a review of its peripheral actions and interactions. *Int. J. Obes.* 26:1407. doi: 10.1038/sj.ijo.0802142

Martins, I., Gomes, S., Costa, R., Otvos, L., Oliveira, C., Resende, R., et al. (2013). Leptin and ghrelin prevent hippocampal dysfunction induced by Aβ oligomers. *Neuroscience* 241, 41–51. doi: 10.1016/j.neuroscience.2013.02.062

Marwarha, G., Dasari, B., Prasanthi, J. R., Schommer, J., and Ghribi, O. (2010). Leptin reduces the accumulation of Aβ and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. *J. Alzheimers Dis.* 19, 1007–1019. doi: 10.3233/JAD-2010-1298

Mattson, M. P. (2004). Pathways towards and away from Alzheimer's disease. *Nature* 430, 631–639. doi: 10.1038/nature02621

McGregor, G., Clements, L., Farah, A., Irving, A. J., and Harvey, J. (2018). Age-dependent regulation of excitatory synaptic transmission at hippocampal temporoammonic-CA1 synapses by leptin. *Neurobiol. Aging* 69, 76–93. doi: 10.1016/j.neurobiolaging.2018.05.007

McGregor, G., and Harvey, J. (2018a). Food for thought: Leptin regulation of hippocampal function and its role in Alzheimer's disease. *Neuropharmacology* 136, 298–306. doi: 10.1016/j.neuropharm.2017.09.038

McGregor, G., and Harvey, J. (2018b). Regulation of hippocampal synaptic function by the metabolic hormone, leptin: Implications for health and neurodegenerative disease. *Front. Cell. Neurosci.* 12:340. doi: 10.3389/fncel.2018.00340

Mercer, J. G., Hoggard, N., Williams, L. M., Lawrence, C. B., Hannah, L. T., and Trayhurn, P. (1996). Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. *FEBS Lett.* 387, 113–116. doi: 10.1016/0014-5793(96)00473-5

Miulli, D. E., Norwell, D. Y., and Schwartz, F. N. (1993). Plasma concentrations of glutamate and its metabolites in patients with Alzheimer's disease. *J. Am. Osteopath. Assoc.* 93, 670–676.

Montagne, A., Zhao, Z., and Zlokovic, B. V. (2017). Alzheimer's disease: a matter of blood-brain barrier dysfunction? *J. Exp. Med.* 214, 3151–3169. doi: 10.1084/jem.20171406

Moult, P. R., Cross, A., Santos, S. D., Carvalho, A. L., Lindsay, Y., Connolly, C. N., et al. (2010). Leptin regulates AMPA receptor trafficking via PTEN inhibition. *J. Neurosci.* 30, 4088–4101. doi: 10.1523/JNEUROSCI.3614-09.2010

Moult, P. R., and Harvey, J. (2011). NMDA receptor subunit composition determines the polarity of leptin-induced synaptic plasticity. *Neuropharmacology* 61, 924–936. doi: 10.1016/j.neuropharm.2011.06.021

Muller, T., Albrecht, D., and Gebhardt, C. (2009). Both NR2A and NR2B subunits of the NMDA receptor are critical for long-term potentiation and long-term depression in the lateral amygdala of horizontal slices of adult mice. *Learn. Mem.* 16, 395–405. doi: 10.1101/lm.1398709

Myers, M. G. Jr. (2015). Leptin keeps working, even in obesity. *Cell Metab.* 21, 791–792. doi: 10.1016/j.cmet.2015.05.017

Myers, M. G. Jr., Leibel, R. L., Seeley, R. J., and Schwartz, M. W. (2010). Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol. Metab.* 21, 643–651. doi: 10.1016/j.tem.2010.08.002

Myers, M. G., Cowley, M. A., and Münzberg, H. (2008). Mechanisms of leptin action and leptin resistance. *Annu. Rev. Physiol.* 70, 537–556. doi: 10.1146/annurev.physiol.70.113006.100707

O'Brien, P. D., Hinder, L. M., Callaghan, B. C., and Feldman, E. L. (2017). Neurological consequences of obesity. *Lancet Neurol.* 16, 465–477.

Odegaard, J. I., and Chawla, A. (2013). Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. *Science* 339, 172–177. doi: 10.1126/science.1230721

O'Malley, D., MacDonald, N., Mizielinska, S., Connolly, C. N., Irving, A. J., and Harvey, J. (2007). Leptin promotes rapid dynamic changes in hippocampal dendritic morphology. *Mol. Cell. Neurosci.* 35, 559–572. doi: 10.1016/j.mcn.2007.05.001

Ong, W. Y., Tanaka, K., Dawe, G. S., Ittner, L. M., and Farooqui, A. A. (2013). Slow excitotoxicity in Alzheimer's disease. *J. Alzheimers Dis.* 35, 643–668. doi: 10.3233/JAD-121990

Pallett, A. L., Morton, N. M., Cawthorne, M. A., and Emilsson, V. (1997). Leptin inhibits insulin secretion and reduces insulin mRNA levels in rat isolated pancreatic islets. *Biochem. Biophys. Res. Commun.* 238, 267–270. doi: 10.1006/bbrc.1997.7274

Parsons, C. G., Stöffler, A., and Danysz, W. (2007). Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system-too little activation is bad, too much is even worse. *Neuropharmacology* 53, 699–723. doi: 10.1016/j.neuropharm.2007.07.013

Patterson, C., Feightner, J. W., Garcia, A., Hsiung, G. Y., MacKnight, C., and Sadovnick, A. D. (2008). Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of Alzheimer disease. *CMAJ* 178, 548–556. doi: 10.1503/cmaj.070796

Payabvash, S., Souza, L. C., Wang, Y., Schaefer, P. W., Furie, K. L., Halpern, E. F., et al. (2011). Regional ischemic vulnerability of the brain to hypoperfusion: the need for location specific computed tomography perfusion thresholds in acute stroke patients. *Stroke* 42, 1255–1260. doi: 10.1161/STROKEAHA.110.600940

Pedditizi, E., Peters, R., and Beckett, N. (2016). The risk of overweight/obesity in mid-life and late life for the development of dementia: a systematic review and meta-analysis of longitudinal studies. *Age Ageing* 45, 14–21. doi: 10.1093/ageing/afv151

Pegueroles, J., Jiménez, A., Vilaplana, E., Montal, V., Carmona-Iragui, M., Pané, A., et al. (2018). Obesity and Alzheimer's disease, does the obesity paradox

really exist? A magnetic resonance imaging study. *Oncotarget* 9, 34691–34698. doi: 10.18632/oncotarget.26162

Perez-Gonzalez, R., Alvira-Botero, M. X., Robayo, O., Antequera, D., Garzon, M., Martín-Moreno, A. M., et al. (2014). Leptin gene therapy attenuates neuronal damages evoked by amyloid-β and rescues memory deficits in APP/PS1 mice. *Gene Ther.* 21, 298–308. doi: 10.1038/gt.2013.85

Platt, T. L., Beckett, T. L., Kohler, K., Niedowicz, D. M., and Murphy, M. P. (2016). Obesity, diabetes, and leptin resistance promote tau pathology in a mouse model of disease. *Neuroscience* 315, 162–174. doi: 10.1016/j.neuroscience.2015.12.011

Pomara, N., Singh, R., Deptula, D., Chou, J. C., Schwartz, M. B., and LeWitt, P. A. (1992). Glutamate and other CSF amino acids in Alzheimer's disease. *Am. J. Psychiatry* 149, 251–254. doi: 10.1176/ajp.149.2.251

Qizilbash, N., Gregson, J., Johnson, M. E., Pearce, N., Douglas, I., Wing, K., et al. (2015). BMI and risk of dementia in two million people over two decades: a retrospective cohort study. *Lancet Diabetes Endocrinol.* 3, 431–436. doi: 10.1016/S2213-8587(15)00033-9

Sahoo, K., Sahoo, B., Choudhury, A. K., Sofi, N. Y., Kumar, R., and Bhadoria, A. S. (2015). Childhood obesity: causes and consequences. *J. Fam. Med. Prim. Care* 4:187.

Savioz, A., Charnay, Y., Huguenin, C., Graviou, C., Greggio, B., and Bouras, C. (1997). Expression of leptin receptor mRNA (long form splice variant) in the human cerebellum. *Neuroreport* 8, 3123–3126. doi: 10.1097/00001756-199709290-00023

Schwartz, M. W., Peskind, E., Raskind, M., Boyko, E. J., and Porte, D. Jr. (1996). Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat. Med.* 2, 589–593. doi: 10.1038/nm0596-589

Shanley, L. J., Irving, A. J., and Harvey, J. (2001). Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. *J. Neurosci.* 21:24RC186.

Singh-Manoux, A., Dugravot, A., Shipley, M., Brunner, E. J., Elbaz, A., Sabia, S., et al. (2018). Obesity trajectories and risk of dementia: 28 years of follow-up in the Whitehall II Study. *Alzheimers Dem.* 14, 178–186. doi: 10.1016/j.jalz.2017.06.2637

Sinha, M. K., Opentanova, I., Ohannesian, J. P., Kolaczynski, J. W., Heiman, M. L., Hale, J., et al. (1996). Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. *J. Clin. Invest.* 98, 1277–1282. doi: 10.1172/jci118913

Solas, M., Milagro, F. I., Ramirez, M. J., and Martínez, J. A. (2017). Inflammation and gut-brain axis link obesity to cognitive dysfunction: plausible pharmacological interventions. *Curr. Opin. Pharmacol.* 37, 87–92. doi: 10.1016/j.coph.2017.10.005

Sun, X. Y., Tuo, Q. Z., Liuyang, Z. Y., Xie, A. J., Feng, X. L., Yan, X., et al. (2016). Extrasynaptic NMDA receptor-induced tau overexpression mediates neuronal death through suppressing survival signaling ERK phosphorylation. *Cell Death Dis.* 7:e2449. doi: 10.1038/cddis.2016.329

Tartaglia, L. A. (1997). The leptin receptor. *J. Biol. Chem.* 272, 6093–6096.

The World Health Organization [WHO] (2019). *Obesity, High Blood Pressure, High Cholesterol, Alcohol And Tobacco: The World Health Organization's Response*. Geneva: WHO.

Trushina, E., Dutta, T., Persson, X. T., Mielke, M. M., and Petersen, R. C. (2013). Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. *PLoS One* 8:e63644. doi: 10.1371/journal.pone.0063644

Varela, L., and Horvath, T. L. (2012). Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. *EMBO Rep.* 13, 1079–1086. doi: 10.1038/embor.2012.174

Waterson, M. J., and Horvath, T. L. (2015). Neuronal regulation of energy homeostasis: beyond the hypothalamus and feeding. *Cell. Metab.* 22, 962–970. doi: 10.1016/j.cmet.2015.09.026

Wayner, M., Armstrong, D., Phelix, C., and Oomura, Y. (2004). Orexin-A (Hypocretin-1) and leptin enhance LTP in the dentate gyrus of rats in vivo. *Peptides* 25, 991–996. doi: 10.1016/j.peptides.2004.03.018

Whitmer, R. A., Gunderson, E. P., Barrett-Connor, E., Quesenberry, C. P. Jr., and Yaffe, K. (2005). Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. *BMJ* 330:1360. doi: 10.1136/bmj.38446.466238.e0

World Health Organization [WHO] (2003). *Controlling the Global Obesity Epidemic*. Geneva: WHO.

Xia, P., Chen, H. S., Zhang, D., and Lipton, S. A. (2010). Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *J. Neurosci.* 30, 11246–11250. doi: 10.1523/JNEUROSCI.2488-10.2010

Xu, W. L., Atti, A. R., Gatz, M., Pedersen, N. L., Johansson, B., and Fratiglioni, L. (2011). Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. *Neurology* 76, 1568–1574. doi: 10.1212/WNL.0b013e3182190d09

Zhang, Y., Li, P., Feng, J., and Wu, M. (2016). Dysfunction of NMDA receptors in Alzheimer's disease. *Neurol. Sci.* 37, 1039–1047. doi: 10.1007/s10072-016-2546-5

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425. doi: 10.1038/372425a0

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

MINI REVIEW
published: 31 May 2019
doi: 10.3389/fnins.2019.00556

A Crosstalk Between Brain Cholesterol Oxidation and Glucose Metabolism in Alzheimer's Disease

Paola Gamba†, Erica Staurenghi*†, Gabriella Testa, Serena Giannelli, Barbara Sottero and Gabriella Leonarduzzi*

Department of Clinical and Biological Sciences, University of Turin, San Luigi Hospital, Turin, Italy

In Alzheimer's disease (AD), both cholesterol and glucose dysmetabolism precede the onset of memory deficit and contribute to the disease's progression. It is indeed now believed that oxidized cholesterol in the form of oxysterols and altered glucose uptake are the main triggers in AD affecting production and clearance of Aβ, and tau phosphorylation. However, only a few studies highlight the relationship between them, suggesting the importance of further extensive studies on this topic. Recently, a molecular link was demonstrated between cholesterol oxidative metabolism and glucose uptake in the brain. In particular, 27-hydroxycholesterol, a key linker between hypercholesterolemia and the increased AD risk, is considered a biomarker for reduced glucose metabolism. In fact, its excess increases the activity of the renin-angiotensin system in the brain, thus reducing insulin-mediated glucose uptake, which has a major impact on brain functioning. Despite this important evidence regarding the role of 27-hydroxycholesterol in regulating glucose uptake by neurons, the involvement of other cholesterol oxidation products that have been clearly demonstrated to be key players in AD cannot be ruled out. This review highlights the current understanding of the potential role of cholesterol and glucose dysmetabolism in AD progression, and the bidirectional crosstalk between these two phenomena.

Keywords: Alzheimer's disease, cholesterol metabolism, oxysterols, glucose metabolism, insulin resistance, renin-angiotensin system

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Gérard Lizard,
Université de Bourgogne, France
Marzia Perluigi,
Sapienza University of Rome, Italy

***Correspondence:**
Paola Gamba
paola.gamba@unito.it

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *05 April 2019*
Accepted: *14 May 2019*
Published: *31 May 2019*

Citation:
Gamba P, Staurenghi E, Testa G, Giannelli S, Sottero B and Leonarduzzi G (2019) A Crosstalk Between Brain Cholesterol Oxidation and Glucose Metabolism in Alzheimer's Disease. Front. Neurosci. 13:556. doi: 10.3389/fnins.2019.00556

Abbreviations: α-EPOX, 5α,6α-epoxycholesterol; β-EPOX, 5β,6β-epoxycholesterol; 24-OHC, 24-hydroxycholesterol; 25-OHC, 25-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; 7α-OHC, 7α-hydroxycholesterol; 7β-OHC, 7β-hydroxycholesterol; 7Hoca, 7α-hydroxy-3-oxo-4-cholestenoic acid; 7-KC, 7-ketocholesterol; Aβ, amyloid β; ABC, ATP-binding cassette; ACE, angiotensin I-converting enzyme; Acetyl-CoA, acetyl coenzyme A; AD, Alzheimer's disease; Ang, angiotensin; AP, aminopeptidase; ApoE, apolipoprotein E; BBB, blood-brain barrier; BVR-A, biliverdin reductase A; CH25H, cholesterol 25-hydroxylase; CSF, cerebrospinal fluid; CYP27A1, cholesterol 27-hydroxylase; CYP46A1, cholesterol 24-hydroxylase; CYP7A1, cholesterol 7α-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; GLUT, glucose transporter; GPR91, G-protein-coupled receptor 91; GSK3β, glycogen synthase kinase 3β; HDL, high density lipoprotein; HFD, high fat diet; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HO-1, heme oxygenase 1; HSD3B7, 3β-hydroxy-C27-steroid dehydrogenase/isomerase; IR, insulin resistance; IRAP, insulin-regulated aminopeptidase; IRS, insulin receptor substrate; LDLR, low density lipoprotein receptor; LRP, LDL receptor-like protein; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; NFT, neurofibrillary tangle; PI3K, phosphoinositide 3-kinase; PPARγ, peroxisome proliferator-activated receptor γ; RAS, renin-angiotensin system; SREBP, sterol-regulatory element-binding protein.

INTRODUCTION

Several events in the brain contribute to AD development, including neuroinflammation, oxidative stress, Aβ toxicity, NFT formation, mitochondrial dysfunction, defective insulin signaling, decreased glucose utilization, and dysregulated cholesterol homeostasis. Deficiency in insulin signaling and IR, together with alteration in glucose and cholesterol metabolism, may lead to the occurrence of neuronal dysfunction and death and, consequently, to dementia. However, the molecular mechanisms involved in AD development are not completely clear, especially as regards the interaction between the different aspects of this pathology.

Cholesterol is particularly important in the brain since it is a major component of cell membranes, thus altered cholesterol metabolism may contribute to AD development (Gamba et al., 2015). Insulin is another important regulator of brain function. It affects neuronal synaptic function and plasticity, and glucose/cholesterol metabolism in the brain (Najem et al., 2014). Substantial glucose is required during memory processing especially in the hippocampus (McNay et al., 2001) and several neurodegenerative diseases are characterized by glucose hypometabolism (Teune et al., 2010). During AD progression, glucose dysmetabolism precedes the onset of memory deficit and it is speculated to predict the disease progression (Nordberg et al., 2010).

Both brain cholesterol and glucose dysmetabolism are recognized as important features of AD, affecting the production and clearance of Aβ and tau phosphorylation, and inducing neurodegeneration (Sato and Morishita, 2015). Recently, a connection between these two processes has been highlighted; however, a more integrated understanding of the interactions between cholesterol and glucose metabolism is required in order to develop new therapeutic strategies to counteract AD. This review provides a brief summary of the rationale on the bidirectional relationship between two main risk factors in AD pathogenesis, i.e., brain cholesterol and glucose dysmetabolism due to insulin signaling deficiency.

THE COMPLEX ROLE OF CHOLESTEROL IN THE BRAIN

Brain Cholesterol Metabolism

The brain is the most cholesterol-rich organ, since it contains a quarter of the whole body non-esterified cholesterol pool (Dietschy, 2009). Cholesterol, as the main lipid component of neuronal and glial membranes and key constituent of myelin, plays essential roles in plasma membrane compartmentalization, signaling, myelination, and formation and maintenance of synapses (Petrov et al., 2017; Hussain et al., 2019).

Plasma and brain cholesterol pools are separated by two barriers: (i) the BBB, that prevents lipoprotein-bound cholesterol uptake from the circulation; (ii) the blood-CSF barrier, through which plasma is ultrafiltered to form part of the CSF. In addition, CSF interfaces the brain interstitial fluid exchanging water, ions, and other molecules (Johanson et al., 2011). Consequently, brain cholesterol metabolism is independent from that of peripheral tissues, and neurons rely on *de novo*-synthesized cholesterol delivery from astrocytes.

As shown in **Figure 1**, cholesterol is synthesized from Acetyl-CoA through reactions catalyzed by over 20 enzymes, including HMG-CoA reductase. Newly synthesized cholesterol is loaded into lipoproteins similar to HDLs, containing the ApoE. Lipidation and secretion of ApoE are mediated by ABC transporters, such as ABCA1 and ABCG1. Then, lipoproteins are transported to neurons, where they are taken up by LDLRs and LRPs. Following receptor-mediated endocytosis, ApoE is recycled to the plasma membrane and cholesterol is used for cell membrane turnover and repair, myelin formation, synaptogenesis, and neurotransmitter release (Gamba et al., 2015; Petrov et al., 2016; Liao et al., 2017). In order to maintain brain cholesterol homeostasis, excess cholesterol is converted into oxysterols, important metabolites deriving from cholesterol enzymatic oxidation or auto-oxidation. Cholesterol is mainly converted into 24-OHC by CYP46A1, a cytochrome P-450 enzyme expressed by neurons. 24-OHC flows from the brain into the circulation across the BBB (∼99%) driven by the concentration gradient and, then, it is excreted by the liver in the form of bile acids (Björkhem et al., 2018; Dosch et al., 2019); less than 1% of 24-OHC flows into the CSF (Lütjohann et al., 1996). Brain cholesterol is also oxidized into 27-OHC by the enzyme CYP27A1, expressed by neurons and glial cells. In contrast to 24-OHC, most of the cerebral 27-OHC derives from the peripheral circulation since CYP27A1 is expressed in most of the organs and tissues (Marwarha and Ghribi, 2015). 27-OHC is indeed one of the major oxysterols in human circulation and its flux into the brain is likely driven by the concentration gradient, maintained by the high rate of its brain metabolism into 7Hoca by CYP7B1 and HSD3B7; subsequently, 7Hoca is eliminated in the systemic circulation and in the CSF (Meaney et al., 2007; Saeed et al., 2014; Björkhem et al., 2018). Both 24-OHC and 27-OHC can, in turn, regulate cholesterol synthesis and transport from glia to neurons by acting on the nuclear LXR, that regulates the expression and synthesis of ApoE and ABCA1/ABCG1 (Czuba et al., 2017). In addition to 24-OHC and 27-OHC, other oxysterols are present in the brain (Testa et al., 2016). Besides enzymatic oxidation, cholesterol auto-oxidation can be induced by different compounds, such as lipid peroxides, free radical species, and metal cations, resulting in the formation of various oxysterols. Among them, 7α-OHC, 7β-OHC, 7-KC, 25-OHC, α-EPOX, and β-EPOX are the most representative. Both 7α-OHC and 25-OHC can also derive from cholesterol enzymatic oxidation, respectively by CYP7A1 and CH25H (Leoni and Caccia, 2013). These oxysterols flow from the brain into the systemic circulation and *vice versa*, crossing the BBB (**Figure 1**).

The Involvement of Oxysterols in Alzheimer's Disease

There has been growing evidence about the involvement of altered cholesterol metabolism in AD (Wood et al., 2014; Zarrouk et al., 2014, 2018; Gamba et al., 2015; Testa et al., 2018a).

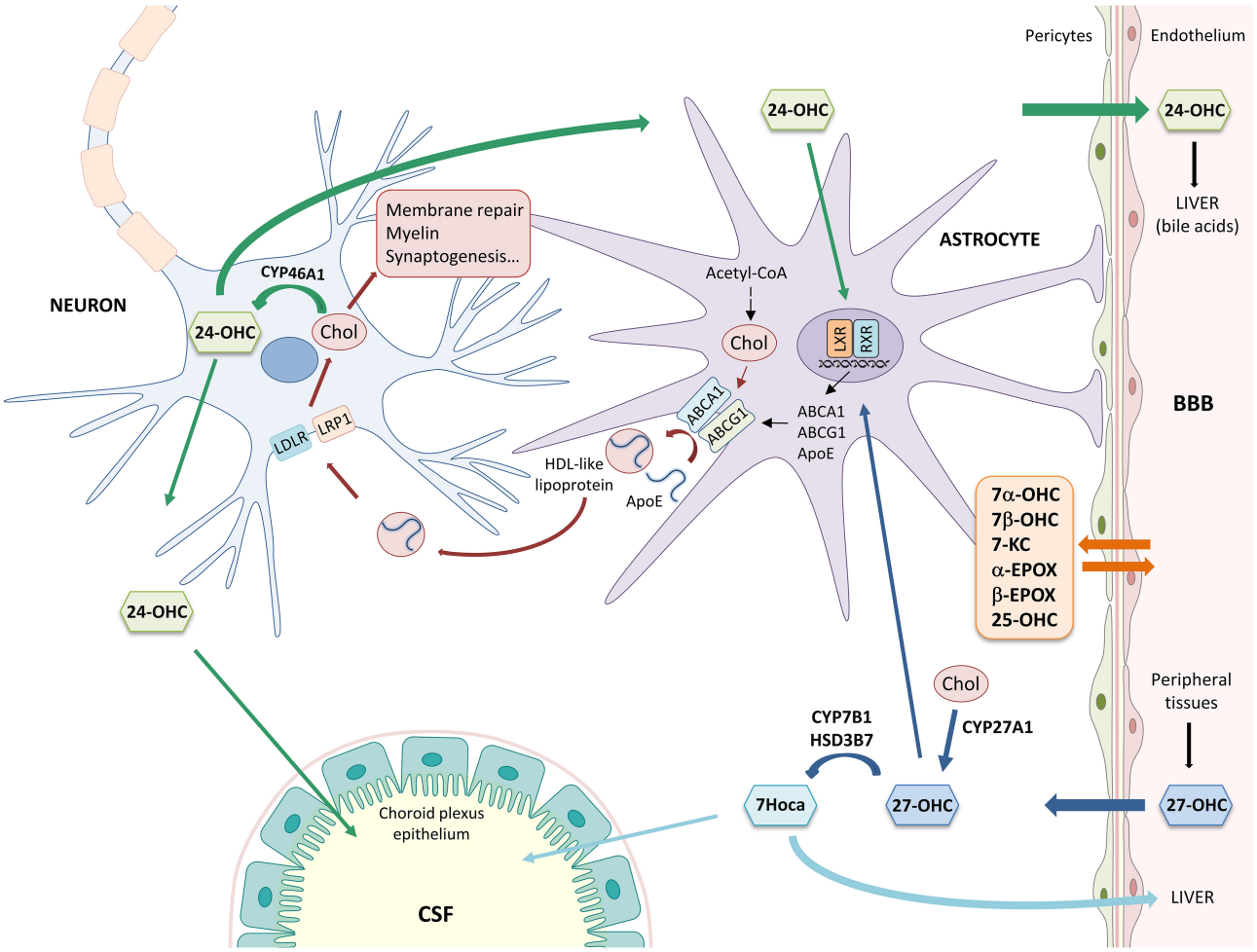

FIGURE 1 | Main mechanisms involved in brain cholesterol homeostasis. α-EPOX, 5α,6α-epoxycholesterol; β-EPOX, 5β,6β-epoxycholesterol; 24-OHC, 24-hydroxycholesterol; 25-OHC, 25-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; 7α-OHC, 7α-hydroxycholesterol; 7β-OHC, 7β-hydroxycholesterol; 7Hoca, 7α-hydroxy-3-oxo-4-cholestenoic acid; 7-KC, 7-ketocholesterol; ABC, ATP-binding cassette; Acetyl-CoA, acetyl coenzyme A; ApoE, apolipoprotein E; BBB, blood-brain barrier; Chol, cholesterol; CSF, cerebrospinal fluid; HDL, high density lipoprotein; HSD3B7, 3β-hydroxy-C27-steroid dehydrogenase/isomerase; LDLR, low density lipoprotein receptor; LRP1, LDL receptor-like protein 1; LXR, liver X receptor; and RXR, retinoid X receptor.

The AD brain, in particular the cortex and the hippocampus, is characterized by synaptic dysfunction, extracellular deposits of Aβ as senile plaques, and intracellular inclusions consisting of hyperphosphorylated tau protein as NFTs, all factors contributing to neuronal loss (Querfurth and LaFerla, 2010).

The presence of oxysterols in the brain could be one of the factors contributing to AD progression. It has been shown that some oxysterols (e.g., 27-OHC, 7β-OHC, and 7-KC) significantly increase in AD brains compared to healthy brains; in contrast, 24-OHC brain levels decrease likely due to neuronal loss (Hascalovici et al., 2009; Testa et al., 2016).

Concerning 27-OHC, its increased flux into the brain can be favored by hypercholesterolemia that induces oxidative stress, thus altering BBB permeability (Heverin et al., 2004; Dias et al., 2014). Moreover, under oxidative stress and inflammatory conditions, brain cholesterol metabolism into 27-OHC increases because the enzyme CYP27A1 is highly expressed by glial cells. Both these mechanisms cause the increase of 27-OHC/24-OHC brain ratio (Marwarha and Ghribi, 2015). 27-OHC has been observed to promote pro-inflammatory molecule release (Testa et al., 2014), to increase Aβ levels (Prasanthi et al., 2009; Gamba et al., 2014), in human neuroblastoma cell lines and both Aβ and hyperphosphorylated tau levels in rabbit organotypic hippocampal slices (Marwarha et al., 2010). Moreover, 27-OHC has been recently demonstrated to impact on lysosomal membrane permeabilization and pyroptosis in co-cultured SH-SY5Y and C6 cells (Chen et al., 2019). In addition, increased Aβ plaques were found in the hippocampus of 27-OHC-treated mice (Zhang et al., 2018), and 27-OHC has been shown to induce synaptic dysfunction and to impair neuron morphology (Merino-Serrais et al., 2019).

As regards 24-OHC, contrasting effects have been reported: on the one hand it promotes neuroinflammation, Aβ peptide

production, oxidative stress, and cell death in neuronal cell lines (Gamba et al., 2011, 2014; Yamanaka et al., 2011; Testa et al., 2014); on the other hand, it has been reported to play an important role in regulating brain cholesterol metabolism via LXR, and to exert beneficial effects such as preventing tau hyperphosphorylation in SK-N-BE cells, suppressing Aβ production in SH-SY5Y cells, and regulating synaptic function in rat hippocampal neurons and slices (Paul et al., 2013; Urano et al., 2013; Testa et al., 2018b). These opposite effects may depend on 24-OHC concentration, since low concentrations (1–10 μM) seem to induce adaptive responses and beneficial effects in neuronal cell lines as discussed by Testa et al. (2018a).

THE INTERPLAY BETWEEN CHOLESTEROL AND GLUCOSE METABOLISM IN THE BRAIN

The Role of Oxysterols in Brain Insulin Resistance

Insulin is an important regulator of brain cell function and metabolism: it affects neuronal synaptic function and plasticity and regulates both glucose and cholesterol metabolism. Like in peripheral tissues, insulin signaling in the brain is mediated by the binding of insulin to its receptor. Consequently, insulin receptor auto-phosphorylation leads to the phosphorylation of the IRS family, of which IRS1 is the best characterized. IRS1 activates two important signaling pathways: the PI3K/Akt pathway and the MAPK cascade (Akter et al., 2011). The activation of the insulin signaling cascade leads to the translocation of the insulin-sensitive GLUT4 to the plasma membrane to favor glucose uptake during memory-related cognitive functions (McEwen and Reagan, 2004).

However, the insulin-mediated glucose uptake in the brain is not as significant as in the periphery. Indeed, brain glucose uptake is also regulated by the cerebral RAS, which is essential for several brain functions, such as learning, memory, emotional responses, and processing of sensory information. A significant reduction of RAS activity has been reported in the AD brains (Mateos et al., 2008, 2011a,b). The downstream peptide Ang IV binds to its receptor, known as IRAP, which is localized in specialized vesicles containing GLUT4 within hippocampal neurons, as well as throughout other brain regions. This binding inhibits IRAP activity, thus preventing the cleavage of memory-enhancing peptides, and activates GLUT4 favoring glucose uptake, thus preserving cognitive functions (Wright and Harding, 2008).

Recently, a molecular link was demonstrated among cholesterol metabolism, brain glucose uptake, and the brain RAS, all of which are affected in neurodegenerative diseases. Besides being a link between hypercholesterolemia and the increased AD risk, 27-OHC is considered a biomarker for the reduced brain glucose metabolism in AD since it is able to increase brain RAS activity, thus impairing neuronal glucose uptake (**Figure 2**). In particular, 27-OHC is involved in the reduction of glucose uptake in the brain by modulating the activity of IRAP and GLUT4. To do this, 27-OHC increases the expression of two main factors involved in the cerebral RAS: AP-A, which transforms Ang II into Ang III, and AP-N, which degrades Ang IV (Ismail et al., 2017). Since Ang III and Ang IV have opposite effects (Ang III inhibits GLUT4 and activates IRAP and, *vice versa*, Ang IV activates GLUT4 and inhibits IRAP), it can be assumed that 27-OHC excess in the brain, as in the case of AD, may reduce brain glucose uptake which has a major impact on brain functioning (**Figure 2**). In this connection, *in vivo* experiments demonstrated that intracerebroventricular injection of 10 μM 27-OHC in WT mice significantly reduces the levels of GLUT4 and increases the levels of AP-A, AP-N and IRAP in the hippocampus. Moreover, a decrease in GLUT4 levels and an enhancement in IRAP levels were observed in cortical and hippocampal primary neurons treated with 1 μM 27-OHC (Ismail et al., 2017). The activation of IRAP by 27-OHC causes the cleavage of neuropeptides and thus contributes to memory deterioration (Lew et al., 2003). These results are supported by the fact that *CYP27A1* overexpressing mice show decreased glucose metabolism and memory deficit (Ismail et al., 2017). *In vitro* experiments also demonstrated that treatments of rat primary neurons, astrocytes, and human neuroblastoma cells with 1–10 μM 27-OHC stimulate the production of angiotensinogen, the precursor of Ang I. Moreover, in AD the activity of ACE correlates with 27-OHC levels both in plasma and CSF (Mateos et al., 2011a), although ACE levels have been shown to be reduced in the CSF (Miners et al., 2009).

Several effects exerted by 27-OHC on brain RAS have been observed to be mediated by LXRβ, since 27-OHC is a good LXR ligand (Ismail et al., 2017). However, besides 27-OHC, other oxysterols have been identified as endogenous ligands for LXR, including 24S-OHC (Nagy et al., 2012). In fact, both 24S-OHC and 27-OHC regulate the brain RAS in primary neurons and astrocytes through a LXR-dependent mechanism, by upregulating angiotensinogen, ACE and Ang II type 1 receptors, all involved in neuronal plasticity, learning, and memory (Mateos et al., 2011b). In addition to LXRβ, also LXRα regulates glucose uptake since the LXRα binding site has been found in the *GLUT4* promoter (Dalen et al., 2003).

In addition, the expression of *GLUT4*, together with the expression of other genes involved in glucose metabolism control, may be regulated by PPARγ (Komers and Vrána, 1998). This nuclear receptor is also involved in the increase of LRP1, a member of the LDL receptor family involved in cholesterol metabolism but also in AD pathogenesis (Shinohara et al., 2017). LRP1 participates in Aβ uptake and metabolism, and in amyloid precursor protein trafficking (Xue-Shan et al., 2016). Moreover, LRP1 is strongly associated to IR because it is involved in the insulin receptor trafficking and intracellular signaling, as well as in glucose uptake in several tissues, but mainly in the brain (Actis Dato and Chiabrando, 2018). In this regard, neuronal LRP1 deficiency leads to a reduced insulin receptor localization in the plasma membrane, an impaired insulin signaling, and decreased glucose uptake due to the lack of GLUT3 and GLUT4 (Liu C.C. et al., 2015).

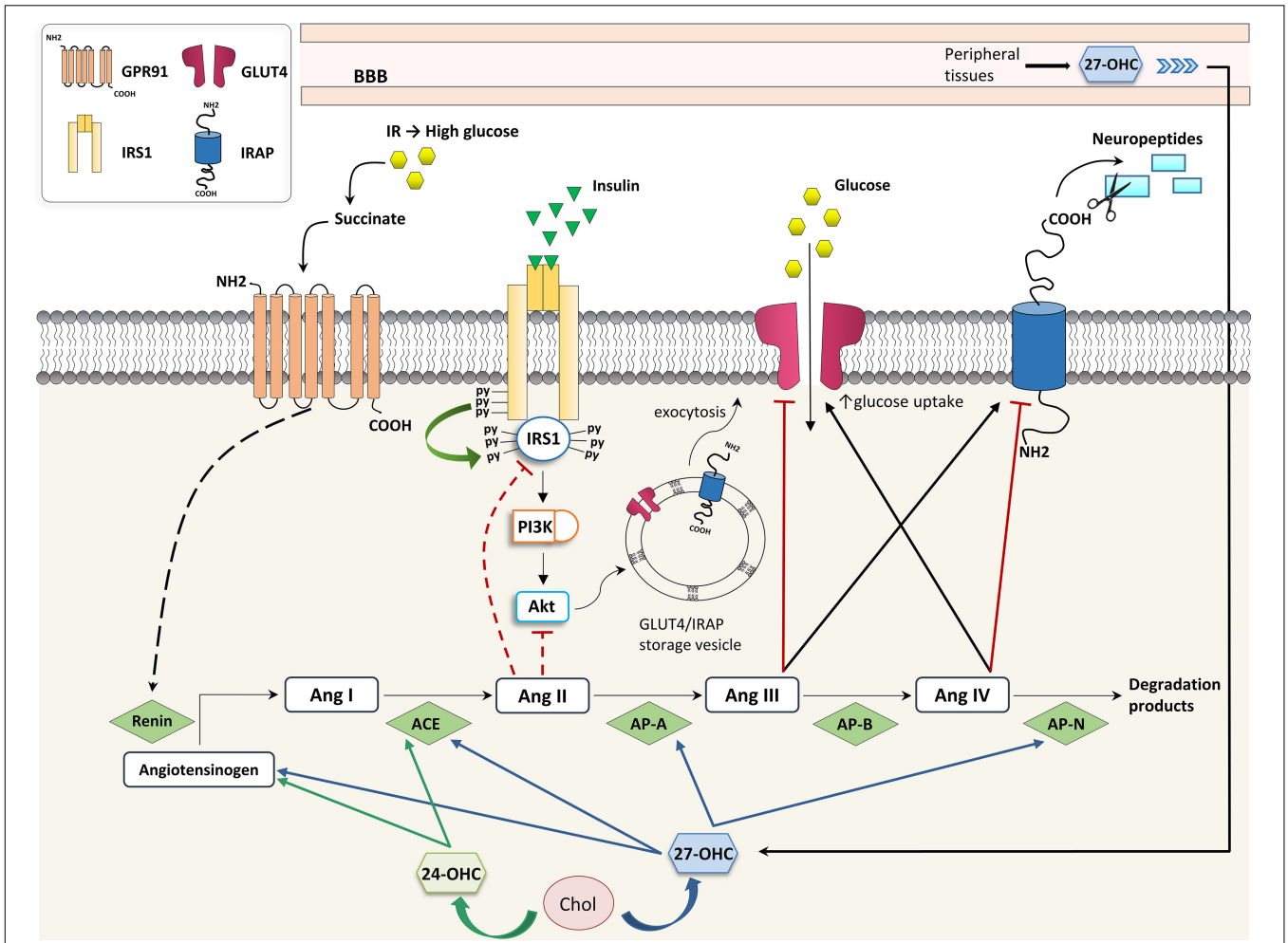

FIGURE 2 | Effects of oxysterols on RAS- and insulin-dependent glucose uptake. 24-OHC, 24-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; ACE, angiotensin I-converting enzyme; Ang, angiotensin; AP, aminopeptidase; BBB, blood-brain barrier; Chol, cholesterol; GLUT, glucose transporter; GPR91, G-protein-coupled receptor 91; IR, insulin resistance; IRAP, insulin-regulated aminopeptidase; IRS, insulin receptor substrate; and PI3K, phosphoinositide 3-kinase.

Both the nuclear receptors PPARγ and LXRs are also implicated in the regulation of lipid metabolism. In this context, *CYP27A1* gene expression, regulated by PPARγ and LXR signaling, results in increased levels of 27-OHC, which in turn up-regulates PPARγ and LXR-dependent processes (Szanto et al., 2004; An et al., 2017). Moreover, an oxysterol mixture compatible with that detectable in human hypercholesterolemic plasma, but not unoxidized cholesterol, has been shown to upregulate PPARγ (Leonarduzzi et al., 2010). In addition, oxidized derivatives of fatty acids, such as 9- and 13-hydroxyoctadecadienoic acid, both oxidized LDL components, activate PPARγ in macrophages (Nagy et al., 2012). Furthermore, macrophage-specific *PPARγ* knockout mice easily develop diet-induced obesity, glucose intolerance and IR (Hevener et al., 2007; Odegaard et al., 2007).

Brain IR is defined as the inadequate response to insulin by target cells and it has been considered a key feature in AD development since it is highly related to tau pathology. IR is, indeed, associated with higher tau levels in the CSF (Starks et al., 2015), and CSF tau predicts changes in brain glucose metabolism (Dowling et al., 2015). It has been observed that in the AD brain there are lower levels of insulin and of insulin receptors, resulting in reduced PI3K/Akt signaling (Schubert et al., 2003, 2004) and GSK3β activation, responsible for NFT formation (Doble and Woodgett, 2003).

A direct crosstalk between high glucose levels induced by IR and RAS has been highlighted in kidneys. In particular, hyperglycemia induced by IR modulates RAS by leading to renin release through the binding of succinate to its receptor GPR91 (Peti-Peterdi et al., 2008); *vice versa*, RAS contributes to IR because Ang II impairs insulin signaling through IRS1 or PI3K/Akt inhibition, as shown in **Figure 2** (Andreozzi et al., 2004). At present there is no evidence that this regulatory network exists also in the brain, but it has been demonstrated that 27-OHC and 24-OHC interfere in the brain's insulin-dependent glucose uptake through RAS.

The role of the HO-1/BVR-A system in the occurrence of IR in the brain, in particular in AD, is gaining attention (Barone and Butterfield, 2015).

The enzyme HO-1 is markedly overexpressed in cortical and hippocampal neurons and astroglia, and colocalizes with senile plaques and NFTs (Schipper et al., 1995). The upregulation of HO-1, in particular by the astrocytic compartment, may confer cytoprotection by enhancing the break-down of prooxidant heme to the radical scavenging biliverdin and bilirubin. However, under certain conditions, heme-derived iron and CO may exacerbate intracellular oxidative stress by provoking free radical generation within mitochondria and other subcellular organelles. The interplay between brain HO-1 and cholesterol homeostasis may have important implications in the pathogenesis of AD. In this connection, it has been demonstrated that HO-1 levels increase in the AD brain in parallel with the increased levels of oxysterols; indeed, HO-1 overexpression suppresses total cholesterol levels by favoring LXR-mediated cholesterol efflux, and enhances oxysterol formation (Vaya and Schipper, 2007; Hascalovici et al., 2014).

Brain IR may be due to increased phosphorylation of IRS1 on specific residues. In this connection, BVR-A is the kinase that phosphorylates and inhibits IRS1, consequently inhibiting the insulin signaling. For this reason, BVR-A is considered a novel mediator of IR. Interestingly, oxidative stress affects BVR-A function resulting in the impairment of the insulin signaling in AD subjects (Barone et al., 2011, 2016).

The Impact of High Fat Diet-Induced Hypercholesterolemia on Brain Insulin Resistance

As one of the most cholesterol-rich organs, brain cholesterol homeostasis is tightly regulated; however, there is growing evidence that the brain lipid profile may be modified by HFD-induced hypercholesterolemia (Czuba et al., 2017).

In this connection, in AD and aging animal models it has been observed that HFD induces cognitive decline (Pancani et al., 2013; Knight et al., 2014). Long-term exposure to HFD results in the increase of plasma cholesterol and, most importantly, disturbs brain cholesterol homeostasis leading to Aβ accumulation, hyperphosphorylation of tau, and neuronal death (Vance, 2006). Moreover, the HFD triggers astrocytic activation in the murine hippocampi and increases the expression of proteins involved in cholesterol transport across brain cell membranes, such as ApoE, thus HFD has a great impact on brain cholesterol homeostasis (Chen et al., 2016). Reactive astrocytes release various inflammatory mediators, that can promote senile plaque and NFT formation that, in turn, contribute to the redox imbalance and inflammation. Cholesterol fed rabbits exhibit high levels of both reactive oxygen species and antioxidant enzyme HO-1 in the brain, and the increment of HO-1 correlates well with oxysterol levels (Hascalovici et al., 2014). It has also been shown that the brain levels of 27-OHC, transported from the systemic circulation, increased in high cholesterol fed rabbits, thus leading to neurodegeneration in the hippocampus (Brooks et al., 2017).

Besides the increased risk of AD induced by HFD because of brain cholesterol dysmetabolism (Stapleton et al., 2008), it has also been demonstrated that HFD induces hepatic IR and impairment of synaptic plasticity (Liu Z. et al., 2015). Additionally, *in vivo* studies demonstrated that HFD-induced peripheral IR and *apoEε4* gene variant synergistically impair cerebral insulin signaling (Zhao et al., 2017). The influence of HFD on the development of brain IR has been demonstrated by the presence, in the hippocampi of HFD fed mice, of elevated levels of phospho-IRS1 (Ser616) (Arnold et al., 2014), phospho-Akt (Ser473), and phospho-GSK3β (Ser9) (Spinelli et al., 2017). Both short-term diet, with very high fat content, and long-term diet, with moderate fat, interfere with the insulin signaling pathways and induce IR in the brain (Arnold et al., 2014).

Furthermore, few studies highlight the importance of serum cholesterol in brain glucose uptake. Higher midlife serum total cholesterol levels are associated, in humans, with lower metabolic glucose rate in brain areas affected by AD, such as precuneus, parietotemporal, and prefrontal regions, but also in frontal regions that are commonly affected by normal aging (Reiman et al., 2010). Moreover, high levels of blood cholesterol enhance RAS activity in the brain: high cholesterol fed mice show increased levels of the precursor angiotensinogen and of ACE (Mateos et al., 2011b). Moreover, HFD fed mice exhibit increased IRAP catalytic activity in the brain (Ismail et al., 2017).

Insulin Resistance Regulates Cholesterol Metabolism in the Brain

The crosstalk between cholesterol dysmetabolism and IR is bidirectional: not only hypercholesterolemia and altered cholesterol homeostasis affect IR, but also IR may, conversely, affect cholesterol metabolism; in fact, insulin can activate the transcription factors SREBPs involved in cholesterol biosynthesis (Suzuki et al., 2010). In addition, insulin increases cholesterol biosynthesis in SH-SY5Y and N2a cells, by upregulating 24-dehydrocholesterol reductase, and HMG-CoA reductase through SREBP2, whereas Aβ-induced IR leads to dysregulation of cholesterol homeostasis (Najem et al., 2016). Moreover, insulin-deficient diabetes leads to a reduced cholesterol synthesis in the brain due to lower expression of SREBP2 and of its downstream genes in the hypothalamus and in other brain regions, resulting in altered synaptic formation, and function (Suzuki et al., 2010, 2013). Conversely, cholesterol depletion in GT1-7 hypothalamic neuron-derived cells contributes to IR, alters autophagy, and enhances apoptosis induced by cytotoxic stress (Fukui et al., 2015).

CONCLUSION

Disruption of cholesterol and glucose metabolism are key players in AD onset and progression, however, the crosstalk between these two phenomena is not yet clear. Despite the important evidence regarding the role of certain oxysterols in regulating glucose uptake by neurons, it would be crucial to deepen their role in modulating the insulin signaling pathway in the brain in order to develop new strategies aimed at preventing or delaying AD development.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by the San Paolo Foundation (Turin) and the University of Turin (Italy).

REFERENCES

Actis Dato, V., and Chiabrando, G. A. (2018). The role of low-density lipoprotein receptor-related protein 1 in lipid metabolism, glucose homeostasis and inflammation. *Int. J. Mol. Sci.* 19:E1780. doi: 10.3390/ijms19061780

Akter, K., Lanza, E. A., Martin, S. A., Myronyuk, N., Rua, M., and Raffa, R. B. (2011). Diabetes mellitus and Alzheimer's disease: shared pathology and treatment? *Br. J. Clin. Pharmacol.* 71, 365–376. doi: 10.1111/j.1365-2125.2010.03830.x

An, Y., Zhang, D. D., Yu, H. L., Ma, W. W., Lu, Y. H., Liu, Q. R., et al. (2017). 27-Hydroxycholesterol regulates cholesterol synthesis and transport in C6 glioma cells. *Neurotoxicology* 59, 88–97. doi: 10.1016/j.neuro.2017.02.001

Andreozzi, F., Laratta, E., Sciacqua, A., Perticone, F., and Sesti, G. (2004). Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser616 in human umbilical vein endothelial cells. *Circ. Res.* 94, 1211–1218. doi: 10.1161/01.RES.0000126501.34994.96

Arnold, S. E., Lucki, I., Brookshire, B. R., Carlson, G. C., Browne, C. A., Kazi, H., et al. (2014). High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. *Neurobiol. Dis.* 67, 79–87. doi: 10.1016/j.nbd.2014.03.011

Barone, E., and Butterfield, D. A. (2015). Insulin resistance in Alzheimer disease: is heme oxygenase-1 an Achille's heel? *Neurobiol. Dis.* 84, 69–77. doi: 10.1016/j.nbd.2015.02.013

Barone, E., Di Domenico, F., Cenini, G., Sultana, R., Cini, C., Preziosi, P., et al. (2011). Biliverdin reductase–a protein levels and activity in the brains of subjects with Alzheimer disease and mild cognitive impairment. *Biochim. Biophys. Acta* 1812, 480–487. doi: 10.1016/j.bbadis.2011.01.005

Barone, E., Di Domenico, F., Cassano, T., Arena, A., Tramutola, A., Lavecchia, M. A., et al. (2016). Impairment of biliverdin reductase-A promotes brain insulin resistance in Alzheimer disease: a new paradigm. *Free Radic. Biol. Med.* 91, 127–142. doi: 10.1016/j.freeradbiomed.2015.12.012

Björkhem, I., Leoni, V., and Svenningsson, P. (2018). On the fluxes of side-chain oxidized oxysterols across blood-brain and blood-CSF barriers and origin of these steroids in CSF. *J. Steroid Biochem. Mol. Biol.* 188, 86–89. doi: 10.1016/j.jsbmb.2018.12.009

Brooks, S. W., Dykes, A. C., and Schreurs, B. G. (2017). A high-cholesterol diet increases 27-hydroxycholesterol and modifies estrogen receptor expression and neurodegeneration in rabbit hippocampus. *J. Alzheimers Dis.* 56, 185–196. doi: 10.3233/JAD-160725

Chen, Y. L., Wang, L. M., Chen, Y., Gao, J. Y., Marshall, C., Cai, Z. Y., et al. (2016). Changes in astrocyte functional markers and β-amyloid metabolism-related proteins in the early stages of hypercholesterolemia. *Neuroscience* 316, 178–191. doi: 10.1016/j.neuroscience.2015.12.039

Chen, S., Zhou, C., Yu, H., Tao, L., An, Y., Zhang, X., et al. (2019). 27-hydroxycholesterol contributes to lysosomal membrane permeabilization-mediated pyroptosis in co-cultured SH-SY5Y cells and C6 cells. *Front. Mol. Neurosci.* 12:14. doi: 10.3389/fnmol.2019.00014

Czuba, E., Steliga, A., Lietzau, G., and Kowiański, P. (2017). Cholesterol as a modifying agent of the neurovascular unit structure and function under physiological and pathological conditions. *Metab. Brain Dis.* 32, 935–948. doi: 10.1007/s11011-017-0015-3

Dalen, K. T., Ulven, S. M., Bamberg, K., Gustafsson, J. A., and Nebb, H. I. (2003). Expression of the insulin-responsive glucose transporter GLUT4 in adipocytes is dependent on liver X receptor alpha. *J. Biol. Chem.* 278, 48283–48291. doi: 10.1074/jbc.M302287200

Dias, I. H., Polidori, M. C., and Griffiths, H. R. (2014). Hypercholesterolaemia-induced oxidative stress at the blood-brain barrier. *Biochem. Soc. Trans.* 42, 1001–1005. doi: 10.1042/BST20140164

Dietschy, J. M. (2009). Central nervous system: cholesterol turnover, brain development and neurodegeneration. *Biol. Chem.* 390, 287–293. doi: 10.1515/BC.2009.035

Doble, B. W., and Woodgett, J. R. (2003). GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell Sci.* 116, 1175–1186. doi: 10.1242/jcs.00384

Dosch, A. R., Imagawa, D. K., and Jutric, Z. (2019). Bile metabolism and lithogenesis: an update. *Surg. Clin. North Am.* 99, 215–229. doi: 10.1016/j.suc.2018.12.003

Dowling, N. M., Johnson, S. C., Gleason, C. E., Jagust, W. J., and Alzheimer's Disease Neuroimaging Initiative (2015). The mediational effects of FDG hypometabolism on the association between cerebrospinal fluid biomarkers and neurocognitive function. *Neuroimage* 105, 357–368. doi: 10.1016/j.neuroimage.2014.10.050

Fukui, K., Ferris, H. A., and Kahn, C. R. (2015). Effect of cholesterol reduction on receptor signaling in neurons. *J. Biol. Chem.* 290, 26383–26392. doi: 10.1074/jbc.M115.664367

Gamba, P., Leonarduzzi, G., Tamagno, E., Guglielmotto, M., Testa, G., Sottero, B., et al. (2011). Interaction between 24-hydroxycholesterol, oxidative stress, and amyloid-β in amplifying neuronal damage in Alzheimer's disease: three partners in crime. *Aging Cell* 10, 403–417. doi: 10.1111/j.1474-9726.2011.00681.x

Gamba, P., Guglielmotto, M., Testa, G., Monteleone, D., Zerbinati, C., Gargiulo, S., et al. (2014). Up-regulation of β-amyloidogenesis in neuron-like human cells by both 24- and 27-hydroxycholesterol: protective effect of N-acetyl-cysteine. *Aging Cell* 13, 561–572. doi: 10.1111/acel.12206

Gamba, P., Testa, G., Gargiulo, S., Staurenghi, E., Poli, G., and Leonarduzzi, G. (2015). Oxidized cholesterol as the driving force behind the development of Alzheimer's disease. *Front. Aging Neurosci.* 7:119. doi: 10.3389/fnagi.2015.00119

Hascalovici, J. R., Vaya, J., Khatib, S., Holcroft, C. A., Zukor, H., Song, W., et al. (2009). Brain sterol dysregulation in sporadic AD and MCI: relationship to heme oxygenase-1. *J. Neurochem.* 110, 1241–1253. doi: 10.1111/j.1471-4159.2009.06213.x

Hascalovici, J. R., Song, W., Liberman, A., Vaya, J., Khatib, S., Holcroft, C., et al. (2014). Neural HO-1/sterol interactions in vivo: implications for Alzheimer's disease. *Neuroscience* 280, 40–49. doi: 10.1016/j.neuroscience.2014.09.001

Hevener, A. L., Olefsky, J. M., Reichart, D., Nguyen, M. T., Bandyopadyhay, G., Leung, H. Y., et al. (2007). Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. *J. Clin. Invest.* 117, 1658–1669. doi: 10.1172/JCI31561

Heverin, M., Bogdanovic, N., Lütjohann, D., Bayer, T., Pikuleva, I., Bretillon, L., et al. (2004). Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease. *J. Lipid Res.* 45, 186–193. doi: 10.1194/jlr.M300320-JLR200

Hussain, G., Wang, J., Rasul, A., Anwar, H., Imran, A., Qasim, M., et al. (2019). Role of cholesterol and sphingolipids in brain development and neurological diseases. *Lipids Health Dis.* 18:26. doi: 10.1186/s12944-019-0965-z

Ismail, M. A., Mateos, L., Maioli, S., Merino-Serrais, P., Ali, Z., Lodeiro, M., et al. (2017). 27-Hydroxycholesterol impairs neuronal glucose uptake through an IRAP/GLUT4 system dysregulation. *J. Exp. Med.* 214, 699–717. doi: 10.1084/jem.20160534

Johanson, C. E., Stopa, E. G., and McMillan, P. N. (2011). The blood-cerebrospinal fluid barrier: structure and functional significance. *Methods Mol. Biol.* 686, 101–131. doi: 10.1007/978-1-60761-938-3_4

Knight, E. M., Martins, I. V., Gümüsgöz, S., Allan, S. M., and Lawrence, C. B. (2014). High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. *Neurobiol. Aging* 35, 1821–1832. doi: 10.1016/j.neurobiolaging.2014.02.010

Komers, R., and Vrána, A. (1998). Thiazolidinediones–tools for the research of metabolic syndrome X. *Physiol. Res.* 47, 215–225.

Leonarduzzi, G., Gargiulo, S., Gamba, P., Perrelli, M. G., Castellano, I., Sapino, A., et al. (2010). Molecular signaling operated by a diet-compatible mixture of oxysterols in up-regulating CD36 receptor in CD68 positive cells. *Mol. Nutr. Food Res.* 54, S31–S41. doi: 10.1002/mnfr.200900493

Leoni, V., and Caccia, C. (2013). Potential diagnostic applications of side chain oxysterols analysis in plasma and cerebrospinal fluid. *Biochem. Pharmacol.* 86, 26–36. doi: 10.1016/j.bcp.2013.03.015

Lew, R. A., Mustafa, T., Ye, S., McDowall, S. G., Chai, S. Y., and Albiston, A. L. (2003). Angiotensin AT4 ligands are potent, competitive inhibitors of insulin regulated aminopeptidase (IRAP). *J. Neurochem.* 86, 344–350. doi: 10.1046/j.1471-4159.2003.01852.x

Liao, F., Yoon, H., and Kim, J. (2017). Apolipoprotein E metabolism and functions in brain and its role in Alzheimer's disease. *Curr. Opin. Lipidol.* 28, 60–67. doi: 10.1097/MOL.0000000000000383

Liu, C. C., Hu, J., Tsai, C. W., Yue, M., Melrose, H. L., Kanekiyo, T., et al. (2015). Neuronal LRP1 regulates glucose metabolism and insulin signaling in the brain. *J. Neurosci.* 35, 5851–5859. doi: 10.1523/JNEUROSCI.5180-14.2015

Liu, Z., Patil, I. Y., Jiang, T., Sancheti, H., Walsh, J. P., Stiles, B. L., et al. (2015). High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. *PLoS One* 10:e0128274. doi: 10.1371/journal.pone.0128274

Lütjohann, D., Breuer, O., Ahlborg, G., Nennesmo, I., Sidén, A., Diczfalusy, U., et al. (1996). Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9799–9804. doi: 10.1073/pnas.93.18.9799

Marwarha, G., and Ghribi, O. (2015). Does the oxysterol 27-hydroxycholesterol underlie Alzheimer's disease-Parkinson's disease overlap? *Exp. Gerontol.* 68, 13–18. doi: 10.1016/j.exger.2014.09.013

Marwarha, G., Dasari, B., Prasanthi, J. R., Schommer, J., and Ghribi, O. (2010). Leptin reduces the accumulation of Abeta and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. *J. Alzheimers Dis.* 19, 1007–1019. doi: 10.3233/JAD-2010-1298

Mateos, L., Akterin, S., Gil-Bea, F. J., Spulber, S., Rahman, A., Björkhem, I., et al. (2008). Activity-regulated cytoskeleton-associated protein in rodent brain is down-regulated by high fat diet in vivo and by 27-hydroxycholesterol in vitro. *Brain Pathol.* 19, 69–80. doi: 10.1111/j.1750-3639.2008.00174.x

Mateos, L., Ismail, M. A., Gil-Bea, F. J., Leoni, V., Winblad, B., Björkhem, I., et al. (2011a). Upregulation of brain renin angiotensin system by 27-hydroxycholesterol in Alzheimer's disease. *J. Alzheimers Dis.* 24, 669–679. doi: 10.3233/JAD-2011-101512

Mateos, L., Ismail, M. A., Gil-Bea, F. J., Schüle, R., Schöls, L., Heverin, M., et al. (2011b). Side chain-oxidized oxysterols regulate the brain renin-angiotensin system through a liver X receptor-dependent mechanism. *J. Biol. Chem.* 286, 25574–25585. doi: 10.1074/jbc.M111.236877

McEwen, B. S., and Reagan, L. P. (2004). Glucose transporter expression in the central nervous system: relationship to synaptic function. *Eur. J. Pharmacol.* 490, 13–24. doi: 10.1016/j.ejphar.2004.02.041

McNay, E. C., McCarty, R. C., and Gold, P. E. (2001). Fluctuations in brain glucose concentration during behavioral testing: dissociations between brain areas and between brain and blood. *Neurobiol. Learn. Mem.* 75, 325–337. doi: 10.1006/nlme.2000.3976

Meaney, S., Heverin, M., Panzenboeck, U., Ekström, L., Axelsson, M., Andersson, U., et al. (2007). Novel route for elimination of brain oxysterols across the blood-brain barrier: conversion into 7alpha-hydroxy-3-oxo-4-cholestenoic acid. *J. Lipid Res.* 48, 944–951. doi: 10.1194/jlr.M600529-JLR200

Merino-Serrais, P., Loera-Valencia, R., Rodriguez-Rodriguez, P., Parrado-Fernandez, C., Ismail, M. A., Maioli, S., et al. (2019). 27-Hydroxycholesterol induces aberrant morphology and synaptic dysfunction in hippocampal neurons. *Cereb. Cortex* 29, 429–446. doi: 10.1093/cercor/bhy274

Miners, S., Ashby, E., Baig, S., Harrison, R., Tayler, H., Speedy, E., et al. (2009). Angiotensin-converting enzyme levels and activity in Alzheimer's disease: differences in brain and CSF ACE and association with ACE1 genotypes. *Am. J. Transl. Res.* 1, 163–177.

Nagy, L., Szanto, A., Szatmari, I., and Széles, L. (2012). Nuclear hormone receptors enable macrophages and dendritic cells to sense their lipid environment and shape their immune response. *Physiol. Rev.* 92, 739–789. doi: 10.1152/physrev.00004.2011

Najem, D., Bamji-Mirza, M., Chang, N., Liu, Q. Y., and Zhang, W. (2014). Insulin resistance, neuroinflammation, and Alzheimer's disease. *Rev. Neurosci.* 25, 509–525. doi: 10.1515/revneuro-2013-0050

Najem, D., Bamji-Mirza, M., Yang, Z., and Zhang, W. (2016). Aβ-induced insulin resistance and the effects of insulin on the cholesterol synthesis pathway and Aβ secretion in neural cells. *Neurosci. Bull.* 32, 227–238. doi: 10.1007/s12264-016-0034-9

Nordberg, A., Rinne, J. O., Kadir, A., and Långström, B. (2010). The use of PET in Alzheimer disease. *Nat. Rev. Neurol.* 6, 78–87. doi: 10.1038/nrneurol.2009.217

Odegaard, J. I., Ricardo-Gonzalez, R. R., Goforth, M. H., Morel, C. R., Subramanian, V., Mukundan, L., et al. (2007). Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* 447, 1116–1120. doi: 10.1038/nature05894

Pancani, T., Anderson, K. L., Brewer, L. D., Kadish, I., DeMoll, C., Landfield, P. W., et al. (2013). Effect of high-fat diet on metabolic indices, cognition, and neuronal physiology in aging F344 rats. *Neurobiol. Aging* 34, 1977–1987. doi: 10.1016/j.neurobiolaging.2013.02.019

Paul, S. M., Doherty, J. J., Robichaud, A. J., Belfort, G. M., Chow, B. Y., Hammond, R. S., et al. (2013). The major brain cholesterol metabolite 24(S)-hydroxycholesterol is a potent allosteric modulator of N-methyl-D-aspartate receptors. *J. Neurosci.* 33, 17290–17300. doi: 10.1523/JNEUROSCI.2619-13.2013

Peti-Peterdi, J., Kang, J. J., and Toma, I. (2008). Activation of the renal renin-angiotensin system in diabetes–new concepts. *Nephrol. Dial. Transplant.* 23, 3047–3049. doi: 10.1093/ndt/gfn377

Petrov, A. M., Kasimov, M. R., and Zefirov, A. L. (2016). Brain cholesterol metabolism and its defects: linkage to neurodegenerative diseases and synaptic dysfunction. *Acta Naturae* 8, 58–73.

Petrov, A. M., Kasimov, M. R., and Zefirov, A. L. (2017). Cholesterol in the pathogenesis of Alzheimer's, Parkinson's diseases and autism: link to synaptic dysfunction. *Acta Naturae* 9, 26–37.

Prasanthi, J. R., Huls, A., Thomasson, S., Thompson, A., Schommer, E., and Ghribi, O. (2009). Differential effects of 24-hydroxycholesterol and 27-hydroxycholesterol on beta-amyloid precursor protein levels and processing in human neuroblastoma SH-SY5Y cells. *Mol. Neurodegener.* 4:1. doi: 10.1186/1750-1326-4-1

Querfurth, H. W., and LaFerla, F. M. (2010). Alzheimer's disease. *N. Engl. J. Med.* 362, 329–344. doi: 10.1056/NEJMra0909142

Reiman, E. M., Chen, K., Langbaum, J. B., Lee, W., Reschke, C., Bandy, D., et al. (2010). Higher serum total cholesterol levels in late middle age are associated with glucose hypometabolism in brain regions affected by Alzheimer's disease and normal aging. *Neuroimage* 49, 169–176. doi: 10.1016/j.neuroimage.2009.07.025

Saeed, A., Floris, F., Andersson, U., Pikuleva, I., Lövgren-Sandblom, A., Bjerke, M., et al. (2014). 7α-hydroxy-3-oxo-4-cholestenoic acid in cerebrospinal fluid reflects the integrity of the blood-brain barrier. *J. Lipid Res.* 55, 313–318. doi: 10.1194/jlr.P044982

Sato, N., and Morishita, R. (2015). The roles of lipid and glucose metabolism in modulation of β-amyloid, tau, and neurodegeneration in the pathogenesis of Alzheimer disease. *Front. Aging Neurosci.* 7:199. doi: 10.3389/fnagi.2015.00199

Schipper, H. M., Cissém, S., and Stopam, E. G. (1995). Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann. Neurol.* 37, 758–768. doi: 10.1002/ana.410370609

Schubert, M., Brazil, D. P., Burks, D. J., Kushner, J. A., Ye, J., Flint, C. L., et al. (2003). Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. *J. Neurosci.* 23, 7084–7092. doi: 10.1523/JNEUROSCI.23-18-07084.2003

Schubert, M., Gautam, D., Surjo, D., Ueki, K., Baudler, S., Schubert, D., et al. (2004). Role for neuronal insulin resistance in neurodegenerative diseases. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3100–3105. doi: 10.1073/pnas.0308724101

Shinohara, M., Tachibana, M., Kanekiyo, T., and Bu, G. (2017). Role of LRP1 in the pathogenesis of Alzheimer's disease: evidence from clinical and preclinical studies. *J. Lipid Res.* 58, 1267–1281. doi: 10.1194/jlr.R075796

Spinelli, M., Fusco, S., Mainardi, M., Scala, F., Natale, F., Lapenta, R., et al. (2017). Brain insulin resistance impairs hippocampal synaptic plasticity and memory

by increasing GluA1 palmitoylation through FoxO3a. *Nat. Commun.* 8:2009. doi: 10.1038/s41467-017-02221-9

Stapleton, P. A., James, M. E., Goodwill, A. G., and Frisbee, J. C. (2008). Obesity and vascular dysfunction. *Pathophysiology* 15, 79–89. doi: 10.1016/j.pathophys.2008.04.007

Starks, E. J., Patrick, O., Grady, J., Hoscheidt, S. M., Racine, A. M., Carlsson, C. M., et al. (2015). Insulin resistance is associated with higher cerebrospinal fluid tau levels in asymptomatic APOEε4 carriers. *J. Alzheimers Dis.* 46, 525–533. doi: 10.3233/JAD-150072

Suzuki, R., Lee, K., Jing, E., Biddinger, S. B., McDonald, J. G., Montine, T. J., et al. (2010). Diabetes and insulin in regulation of brain cholesterol metabolism. *Cell Metab.* 12, 567–579. doi: 10.1016/j.cmet.2010.11.006

Suzuki, R., Ferris, H. A., Chee, M. J., Maratos-Flier, E., and Kahn, C. R. (2013). Reduction of the cholesterol sensor SCAP in the brains of mice causes impaired synaptic transmission and altered cognitive function. *PLoS Biol.* 11:e1001532. doi: 10.1371/journal.pbio.1001532

Szanto, A., Benko, S., Szatmari, I., Balint, B. L., Furtos, I., Rühl, R., et al. (2004). Transcriptional regulation of human CYP27 integrates retinoid, peroxisome proliferator-activated receptor, and liver X receptor signaling in macrophages. *Mol. Cell. Biol.* 24, 8154–8166. doi: 10.1128/MCB.24.18.8154-8166.2004

Testa, G., Gamba, P., Badilli, U., Gargiulo, S., Maina, M., Guina, T., et al. (2014). Loading into nanoparticles improves quercetin's efficacy in preventing neuroinflammation induced by oxysterols. *PLoS One* 9:e96795. doi: 10.1371/journal.pone.0096795

Testa, G., Staurenghi, E., Zerbinati, C., Gargiulo, S., Iuliano, L., Giaccone, G., et al. (2016). Changes in brain oxysterols at different stages of Alzheimer's disease: their involvement in neuroinflammation. *Redox Biol.* 10, 24–33. doi: 10.1016/j.redox.2016.09.001

Testa, G., Rossin, D., Poli, G., Biasi, F., and Leonarduzzi, G. (2018a). Implication of oxysterols in chronic inflammatory human diseases. *Biochimie* 153, 220–231. doi: 10.1016/j.biochi.2018.06.006

Testa, G., Staurenghi, E., Giannelli, S., Gargiulo, S., Guglielmotto, M., Tabaton, M., et al. (2018b). A silver lining for 24-hydroxycholesterol in Alzheimer's disease: the involvement of the neuroprotective enzyme sirtuin 1. *Redox Biol.* 17, 423–431. doi: 10.1016/j.redox.2018.05.009

Teune, L. K., Bartels, A. L., de Jong, B. M., Willemsen, A. T., Eshuis, S. A., de Vries, J. J., et al. (2010). Typical cerebral metabolic patterns in neurodegenerative brain diseases. *Mov. Disord.* 25, 2395–2404. doi: 10.1002/mds.23291

Urano, Y., Ochiai, S., and Noguchi, N. (2013). Suppression of amyloid-β production by 24S-hydroxycholesterol via inhibition of intracellular amyloid precursor protein trafficking. *FASEB J.* 27, 4305–4315. doi: 10.1096/fj.13-231456

Vance, J. E. (2006). Lipid imbalance in the neurological disorder. *Niemann-Pick C disease. FEBS Lett.* 580, 5518–5524. doi: 10.1016/j.febslet.2006.06.008

Vaya, J., and Schipper, H. M. (2007). Oxysterols, cholesterol homeostasis, and Alzheimer disease. *J. Neurochem.* 102, 1727–1737. doi: 10.1111/j.1471-4159.2007.04689.x

Wood, W. G., Li, L., Müller, W. E., and Eckert, G. P. (2014). Cholesterol as a causative factor in Alzheimer's disease: a debatable hypothesis. *J. Neurochem.* 129, 559–572. doi: 10.1111/jnc.12637

Wright, J. W., and Harding, J. W. (2008). The angiotensin AT4 receptor subtype as a target for the treatment of memory dysfunction associated with Alzheimer's disease. *J. Renin Angiotensin Aldosterone Syst.* 9, 226–237. doi: 10.1177/1470320308099084

Yamanaka, K., Saito, Y., Yamamori, T., Urano, Y., and Noguchi, N. (2011). 24(S)-hydroxycholesterol induces neuronal cell death through necroptosis, a form of programmed necrosis. *J. Biol. Chem.* 286, 24666–24673. doi: 10.1074/jbc.M111.236273

Xue-Shan, Z., Juan, P., Qi, W., Zhong, R., Li-Hong, P., Zhi-Han, T., et al. (2016). Imbalanced cholesterol metabolism in Alzheimer's disease. *Clin. Chim. Acta* 456, 107–114. doi: 10.1016/j.cca.2016.02.024

Zarrouk, A., Vejux, A., Mackrill, J., O'Callaghan, Y., Hammami, M., O'Brien, N., et al. (2014). Involvement of oxysterols in age-related diseases and ageing processes. *Ageing Res. Rev.* 18, 148–162. doi: 10.1016/j.arr.2014.09.006

Zarrouk, A., Debbabi, M., Bezine, M., Karym, E. M., Badreddine, A., Rouaud, O., et al. (2018). Lipid biomarkers in Alzheimer's disease. *Curr. Alzheimer Res.* 15, 303–312. doi: 10.2174/1567205014666170505101426

Zhang, X., Xi, Y., Yu, H., An, Y., Wang, Y., Tao, L., et al. (2018). 27-hydroxycholesterol promotes Aβ accumulation via altering Aβ metabolism in mild cognitive impairment patients and APP/PS1 mice. *Brain Pathol.* [Epub ahead of print],

Zhao, N., Liu, C. C., Van Ingelgom, A. J., Martens, Y. A., Linares, C., Knight, J. A., et al. (2017). Apolipoprotein E4 impairs neuronal insulin signaling by trapping insulin receptor in the endosomes. *Neuron* 96:115-129.e5. doi: 10.1016/j.neuron.2017.09.003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 04 June 2019
doi: 10.3389/fnins.2019.00521

Role of the Blood-Brain Barrier in Central Nervous System Insulin Resistance

Elizabeth M. Rhea[1,2]* *and William A. Banks*[1,2]

[1] Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA, United States, [2] Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA, United States

The blood-brain barrier (BBB) mediates the communication between the periphery and the central nervous system (CNS). Recently, CNS insulin resistance has been elucidated to play a role in neurodegenerative disease. This has stimulated a wealth of information on the molecular impact of insulin in the brain, particularly in the improvement of cognition. Since the BBB regulates the transport of insulin into the brain and thus, helps to regulate CNS levels, alterations in the BBB response to insulin could impact CNS insulin resistance. In this review, we summarize the effect of insulin on some of the cell types that make up the BBB, including endothelial cells, neurons, astrocytes, and pericytes. We broadly discuss how these changes in specific cell types could ultimately impact the BBB. We also summarize how insulin can regulate levels of the pathological hallmarks of Alzheimer's disease, including amyloid beta (Aβ) and tau within each cell type. Finally, we suggest interventional approaches to overcome detrimental effects on the BBB in regards to changes in insulin transport.

Keywords: insulin, transport, blood-brain barrier, neurovascular unit, insulin resistance

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Lawrence Reagan,
University of South Carolina, United States
Ana Lloret,
University of Valencia, Spain

***Correspondence:**
Elizabeth M. Rhea
meredime@uw.edu

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *28 February 2019*
Accepted: *06 May 2019*
Published: *04 June 2019*

Citation:
Rhea EM and Banks WA (2019) Role of the Blood-Brain Barrier in Central Nervous System Insulin Resistance. Front. Neurosci. 13:521. doi: 10.3389/fnins.2019.00521

INTRODUCTION TO THE BLOOD-BRAIN BARRIER

The blood-brain barrier (BBB) is a component of the neurovascular unit (NVU) and acts as the blood-brain interface, mediating communication between the central nervous system (CNS) and the periphery. The BBB separates the circulation from the brain, allowing for protection from and transport regulation of serum factors and neurotoxins. The BBB is not just a physical barrier (due to the presence of specialized tight junctions and other changes that prevent unregulated leakage) but also acts more selectively as a transport interface (with specific transporters present on luminal and abluminal membranes), a secretory body, and a metabolic barrier (containing and releasing certain enzymes locally) (Abbott et al., 2006).

CELLS FORMING THE BLOOD-BRAIN BARRIER

We introduce the cells and structures that make up the BBB here so we can discuss insulin receptor signaling in each of them later. For more detailed reviews of these BBB components, we refer the reader to the following excellent reviews (Broadwell, 1989; Begley, 2004; Neuwelt et al., 2008; Banks, 2016; Sweeney et al., 2019). It is important to keep in mind that the BBB is not the same in all regions throughout the brain. Therefore, observations in one brain region or subregion might not

persist in another. The same will hold true for insulin signaling and insulin transport. The diameter of blood vessels and composition of the BBB can change depending on the requirements of the region and the type of blood vessel (i.e., arterioles to capillaries to venules). We do not discuss other components of the NVU including the extracellular matrix, vascular smooth muscle cells, and other glia cells due to limited available information on insulin signaling. However, with growing interest in CNS insulin signaling and the impact both centrally and peripherally, we expect further research endeavors exploring these other components of the NVU.

Brain Endothelial Cells

The first line of defense against circulating factors for the brain is a monolayer of brain endothelial cells which are connected to one another by tight junctions and adherens junctions (Tietz and Engelhardt, 2015). These endothelial cells are different from peripheral endothelial cells in that they express tight junction proteins, creating a stronger barrier, and have decreased pinocytosis, restricting vesicle-mediated transcellular transport and transporters (Reese and Karnovsky, 1967). They make up the largest surface area at the blood-CNS interface. With this large surface area, they can readily transport proteins and molecules into and out of the brain most efficiently. Endothelial cells are polarized, exhibiting a luminal and abluminal side, with different transporters and cellular machinery expressed at each side. While this specific cell type is most often modeled as the BBB *in vitro,* there are other cell types present that are a part of the NVU or that affect BBB functions, including neurons, astrocytes, and pericytes.

Neurons

Neurons remain close to the capillaries and connect with astrocytic endfeet in near proximity to the BBB. Neurons are rarely more than 8–20 μm from a brain capillary (Schlageter et al., 1999). It is estimated that nearly each neuron has its own capillary (Zlokovic, 2005). The close proximity to the endothelial cells, allows neurons to respond to the ever changing local milieu, especially in regards to ion balance. Neurons play a role in regulating blood flow, microvascular permeability, interact with the extracellular matrix, and can release factors to stimulate angiogenesis (Zlokovic, 2008). Following a vascular insult, signals from neurons and astrocytes can recruit microglia which secrete proinflammatory cytokines (Man et al., 2007). Neurons help tighten brain endothelial cells in culture by aiding in tight junction protein synthesis and localization (Savettieri et al., 2000). These data support a synergistic role for the regulation of other cell types by neurons and highlight how these cells communicate with one another. Indeed, the neuronal circuitry is linked to the blood vessels by water channels present in astrocytes.

Astrocytes

Astrocytes are the most abundant cells in the brain, providing an environment to help regulate all aspects of neuronal function (survival, development, metabolism, neurotransmission). They act as metabolic sensors in the brain responding to changes in the local environment (Garcia-Caceres et al., 2016). At the BBB, astrocytes help provide maintenance and repair support through release of several effector molecules (Wosik et al., 2007; Alvarez et al., 2011; Bell et al., 2012). The astrocytic endfeet ensheath the vascular tube and help to regulate ion and water regulation (Abbott et al., 2006). Aquaporin-4 is an astroglial water channel that regulates perivascular fluid and solute movement through the glymphatic system, a unique exchange between perivascular cerebrospinal fluid (CSF) and interstitial fluid present in the CNS (Iliff et al., 2012; Nedergaard, 2013; Mestre et al., 2018). Using this system, the brain can regulate fluid flow throughout the CNS and aid in clearance of toxins. In addition, the connection between neurons and blood vessels allows astrocytes to relay signals regarding blood flow (Hamilton and Attwell, 2010) as well as controlling brain water content (Zlokovic, 2008). Of the approximately 11 distinct phenotypes of astrocytes, 8 are involved in interactions with blood vessels (Reichenbach and Wolburg, 2005; Abbott et al., 2006). Astrocytes and endothelial cells have a symbiotic relation. Astrocytes secrete a range of chemical factors, including various growth factors that induce aspects of the BBB phenotype in endothelial cells *in vitro* and likely *in vivo* while endothelial cells aid in astrocytic differentiation (Mi et al., 2001; Lee et al., 2003; Abbott et al., 2006). Astrocytic end feet are polarized and guided to cerebral vessel walls by pericytes (Armulik et al., 2010).

Pericytes

Pericytes sit on the abluminal surface of the endothelial cell and are embedded in the vascular basement membrane and are physically connected to brain endothelial cells by way of gap junctions and peg and socket arrangements (Miller and Sims, 1986). Pericytes help to maintain and stabilize the monolayer of brain endothelial cells by regulating angiogenesis and depositing extracellular matrix. Pericytes are essential for development of tight junctions, including in the development of barrier functions in utero (Daneman et al., 2010; Hayden et al., 2013). In addition, there is cross talk from the brain endothelial cell to the pericyte on pericyte proliferation and migration. CNS pericytes also have distinct properties from their peripheral counterparts. The endothelial:pericyte ratio is much greater in the CNS, estimated to be about 4:1 in mice (Bonkowski et al., 2011), compared to other tissues which have just one pericyte per 100 endothelial cells (Shepro and Morel, 1993). Pericytes can regulate blood flow in response to neural activity (Armulik et al., 2010; Daneman and Prat, 2015) suggesting an important role in mediating vascular tone and highlighting the neural communication necessary for this particular function.

These cell types (brain endothelial cells, neurons, astrocytes, and pericytes) communicate with one another to not only help form the BBB but also to regulate its structure and function. As touched on above, these cells can communicate with secretory factors in addition to changes in fluid movement and water channels. Interruptions in signaling within one cell type could have detrimental effects in all cell types. For example, pericyte loss has been shown to occur in some animal models of

peripheral insulin resistance (Price et al., 2012; Salameh et al., 2016; Warmke et al., 2016) and are one of the first cell types of the BBB to degenerate in Alzheimer's disease (Sengillo et al., 2013). Loss of pericytes can lead to BBB breakdown, causing a dysfunction in the transport regulation of blood-to-brain and brain-to-blood factors. Pericyte loss accelerates development of Alzheimer's disease pathology including amyloid beta (Aβ) deposition, tau pathology, and neuronal loss (Sagare et al., 2013). In the next section, we will describe the role of the insulin receptor in each of these cell types and speculate how insulin resistance in one cell type might adversely affect some of the other BBB cell types.

INSULIN SIGNALING WITHIN CELLS OF THE BLOOD-BRAIN BARRIER

There is not a cell type in the CNS that we are aware of that does not express the insulin receptor. In mice, the expression of the insulin receptor gene is most abundant in endothelial cells, about two times greater than astrocytes, with neurons falling in close behind in terms of RNA expression levels[1] (Zhang et al., 2014). This same expression pattern was not observed in samples from human tissue (Zhang et al., 2016). Instead, expression of the insulin receptor is more evenly distributed between the cell types. Insulin interacts with receptors on neurons and glial cells (Unger et al., 1989), endothelial cells (Konishi et al., 2017; Rhea et al., 2018), and pericytes (Sweeney et al., 2016) to elicit various physiological effects, some of which are highlighted in **Figure 1**. The insulin receptor exists in two isoforms, an A and B form, due to differences in splicing of the α subunit, resulting in different binding affinities to insulin and insulin-like growth factor (Belfiore et al., 2017). However, until recently, the ability to detect these two isoforms by immunological methods *in vivo* in different cell types has been a challenge. With the advances in single cell RNA sequencing (Ofengeim et al., 2017) and a novel *in situ* RT-PCR/FISH assay (Spencer et al., 2018), we expect a growth in the knowledge of expression pattern of these isoforms and alterations in human disease within specific cell types and regional variations. The insulin receptor can also form heterodimers with the IGF-1 receptor and can have varying post-translational modifications leading to further diversity of insulin action (Wozniak et al., 1993; Chiu and Cline, 2010).

Figure 1 summarizes the role of the insulin receptor on each BBB cell type discussed in this review. Various groups have utilized Cre-loxP-mediated recombination (Gu et al., 1994) to generate cell specific knock-out models of the insulin receptor to investigate the impact of disrupted insulin signaling. We have included a table listing the studies generating and utilizing some of these CNS insulin receptor knock-out models (**Table 1**). This section describes the importance of the insulin receptor in components of the BBB from research generated primarily in these knock-out mice with a focus not only on organization of the BBB but also on Alzheimer's disease pathology and cognition.

Brain Endothelial Cell

Mice lacking the insulin receptor on vascular endothelial cells (VENIRKO) were first generated in Vicent et al. (2003). The original study introducing these mice investigated the role of the endothelial cell in regulating vascular tone and peripheral insulin resistance. Cerebrovascular microvessels and endothelial cells from the lungs were isolated and cultured to measure the effectiveness of the knock-out (which resulted in 95–97% decrease in mRNA levels). Abnormal architecture of capillary integrity could not be detected in brain (Vicent et al., 2003).

[1]http://www.brainrnaseq.org/

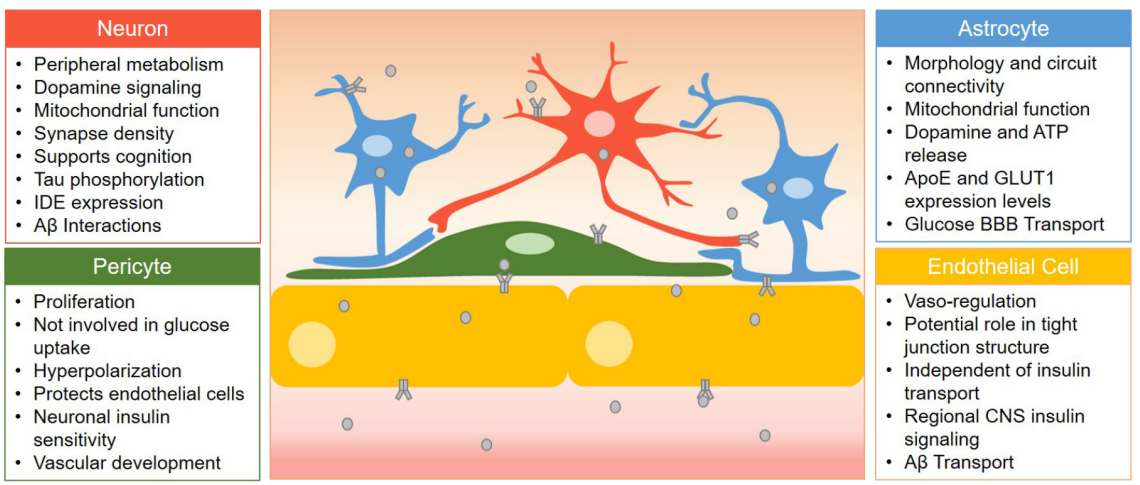

FIGURE 1 | Summary of the role of insulin receptor signaling in various cells of the BBB. Cell types are listed here with the role of insulin receptor signaling listed in bullet points. Insulin (gray circles) must first cross barrier cells in order to activate insulin receptors (gray receptor) in the CNS located on neurons, astrocytes, and pericytes.

TABLE 1 | Summary of the generation of CNS insulin receptor knock-out models relating to the BBB.

Cell type	Model	Cre driver	References
Endothelial Cell	VENIRKO	*Tie2*	Vicent et al., 2003; Kondo et al., 2004
	EndoIRKO	*Cdh5*	Konishi et al., 2017; Rhea et al., 2018
Neuron	NIRKO	*Nestin*	Bruning et al., 2000; Schubert et al., 2004; Kleinridders et al., 2015
	nIR −/−	*Synapsin*	Freude et al., 2009; Stohr et al., 2013
Astrocyte	GIRKO	*GFAP*	Garcia-Caceres et al., 2016; Cai et al., 2018
Pericyte	PIR −/−	*PDGFRβ2*	Warmke et al., 2017

These references highlight the critical work investigating the role of the insulin receptor on BBB function in addition to memory impairments in CNS insulin resistance.

Using a euglycemic-hyperinsulinemic clamp, the authors found endothelial cell insulin receptors do not play a role in the access of insulin to peripheral metabolically active tissues but did not investigate brain. Loss of the endothelial insulin receptor resulted in decreased levels of eNOS, an important regulator of vascular tone which could affect exposure to various circulatory factors. Indeed, the cerebrovascular response to insulin appears to be biphasic with vasoconstriction at low doses and vasodilation at higher doses (Katakam et al., 2009). Vascular integrity of the BBB was later investigated in these mice (Kondo et al., 2004). There was no disruption to Evans blue in the hemispheres or cerebellum in VENIRKO mice compared to littermate controls. Levels of ZO-1, a tight junction protein, were unchanged in addition to levels of astrocytes, as measured by GFAP staining. However, in another model of insulin receptor loss in endothelial cells (EndoIRKO), permeability of the BBB to a 3 kDa dextran was increased in the olfactory bulb and median eminence compared to controls and ZO-1 was decreased by 40% in the hypothalamus (Konishi et al., 2017). These differences between the two models could be due to regional regulation of tight junction proteins by the insulin receptor. Using the EndoIRKO model, it was demonstrated insulin receptors on brain endothelial cells control the kinetics of insulin signaling in certain regions of the brain, such as the hippocampus and hypothalamus, but not the olfactory bulb (Konishi et al., 2017). Decreased binding of insulin was observed in the frontal cortex endothelium of EndoIRKO mice. The results from these two models suggests the insulin receptor in endothelial cells has a regional effect in relaying insulin signaling to other cell types in addition to maintaining the BBB structure by regulating tight junction protein expression.

In addition to the genetically modified mouse models, some groups have utilized a selective inhibitor of the insulin receptor, S961, *in vitro* to investigate the role of the brain endothelial cell insulin receptor. This inhibitor has high affinity and selectivity, especially over the IGF-1 receptor (Schaffer et al., 2008). Insulin binding was decreased with S961 treatment (Hersom et al., 2018) and insulin-induced phosphorylation of Akt was decreased with S961 treatment (Gray et al., 2017; Hersom et al., 2018). It was also shown that while the downstream insulin receptor signaling mediator PI3K was inhibited, insulin uptake was not altered (Gray et al., 2017). On the other hand, high-fat diet decreased insulin uptake, yet insulin receptor signaling was unaltered (Gray et al., 2017). These data suggest a disconnect between insulin receptor signaling and insulin transport across the endothelial cell which we will discuss later.

Impaired brain clearance of Aβ across the BBB is thought to be part of the contribution of Alzheimer's disease pathogenesis. Insulin can regulate Aβ trafficking at the BBB in both the luminal-to-abluminal and abluminal-to-luminal directions (Vandal et al., 2014; Swaminathan et al., 2018). Systemic insulin increased Aβ40 plasma clearance and transport from blood-to-brain but decreased Aβ42 (Swaminathan et al., 2018). On the other hand, brain Aβ40 BBB clearance is decreased while Aβ42, the more toxic and amyloidogenic form, is increased after insulin treatment. If insulin is administered intracerebroventricularly, Aβ40 clearance is inhibited (Shiiki et al., 2004). Therefore, it is important to consider the effects of insulin at the BBB when administered luminally versus abluminally. The impact of insulin on Aβ transport at the BBB has also been investigated in obese mice in the triple-transgenic model of Alzheimer's disease (3xTg-AD) (Vandal et al., 2014). An acute intravenous injection of insulin (5 min before sacrifice) restored cortical soluble Aβ40 and Aβ42 back to the level of 3xTg-Ad mice fed a control diet. There was a concomitant increase in plasma Aβ following insulin injection. In two separate human studies, it was found plasma Aβ42 levels increased following insulin administration (Kulstad et al., 2006; Karczewska-Kupczewska et al., 2013). These studies suggest BBB clearance is likely the process by which insulin decreases brain Aβ levels. Based on the density of the cerebravasculature throughout the brain, it is possible that Aβ can be excreted rapidly out of the brain with the widespread network of capillaries, venules and veins.

Neurons

Insulin signaling in the CNS is important for promoting neuronal survival and regulating key processes involved in learning and memory (synapse density, plasticity, and connectivity). Insulin in the brain is more closely linked to its ancestral roles by acting more as a growth factor rather than acting as a metabolite to regulate glucose uptake as occurs in the periphery (Banks et al., 2012). Peripheral injection of 1 mU insulin increased cerebral insulin signaling within 5 min, which was localized to the plasma membrane of a subset of neurons (Freude et al., 2005).

Mice lacking the insulin receptor specifically in the brain (NIRKO) were generated in Bruning et al. (2000). NIRKO mice have a 95% reduction in total insulin receptor expression in brain with no detectable change in peripheral tissues such as skeletal

muscle, heart, and liver. The loss of the insulin receptor leads to impaired peripheral metabolism as the mice aged. NIRKO mice exhibit decreased dopamine signaling and impairments in mitochondrial function (Kleinridders et al., 2015). In *Xenopus* tadpoles, loss of the insulin receptor specifically in tectal neurons reduces synapse density, decreases activation, and alters morphology (Chiu et al., 2008).

Young and aged NIRKO mice do not have memory impairments compared to age-matched controls (Schubert et al., 2004). In addition, the insulin receptor was not required for neuronal survival *in vivo* (Bruning et al., 2000). While this data is not in line with other reports on the mechanism of insulin in the CNS to promote neuronal survival and play a role in memory, it is likely compensation has occurred due to complete loss of the insulin receptor throughout the brain for the entire life of the animal, as previously suggested (Grillo et al., 2015). If the insulin receptor is downregulated specifically in the hippocampus of adult rats using a lentivirus, long-term memory is impaired (Grillo et al., 2015). It is important to note that there were no metabolic or endocrine changes with specific knock-down in the hippocampus despite prior work by this group showing metabolic changes when targeted to the hypothalamus (Grillo et al., 2007). These differences when the insulin receptor is targeted in specific regions highlight the need to further investigate the role of the insulin receptor in a regional context.

Loss of the insulin receptor increases phosphorylation of tau, suggesting CNS insulin resistance could lead to an increase in this pathological mediator of Alzheimer's disease. In addition, the insulin stimulated phosphorylation of tau was at a site demonstrated to form tangles (Kimura et al., 1996). This effect of insulin on tau phosphorylation seems to be time dependent based on studies *in vitro* (Lesort et al., 1999; Lesort and Johnson, 2000). Tau phosphorylation was completely abolished in NIRKO mice (Schubert et al., 2004). These data show that insulin receptor signaling can impact tau phosphorylation given the right time and environment.

While numerous groups have shown insulin can affect tau phosphorylation, there are fewer studies suggesting tau can regulate insulin signaling. Insulin accumulates intraneuronally together with hyperphosphorylated tau in Alzheimer's disease (Rodriguez-Rodriguez et al., 2017). Tau pathology triggers insulin accumulation and oligomerization. Inhibition of tau phosphorylation using okadaic acid decreased insulin receptor expression levels in neurons, and this was dependent on the presence of extracellular insulin. In addition, neurons with increased tau hyperphosphorylation have enhanced insulin uptake (Rodriguez-Rodriguez et al., 2017).

Primary neuronal cultures with dysfunctional insulin receptor (transfected with kinase dead insulin receptor) have increased levels of Aβ oligomers and exacerbated aggregation (Zhao et al., 2009). It is thought that stimulation of the insulin receptor in neurons activates insulin degrading enzyme (IDE), reducing the risk of Aβ buildup. Indeed, activation of insulin receptor signaling enhances IDE expression in neurons (Zhao et al., 2004). Insulin can protect cultured rat hippocampal neurons from Aβ-induced toxicity (Takadera et al., 1993). In addition, Aβ competes with insulin in binding the insulin receptor, decreasing the phosphorylation and thus, activity of the insulin receptor (Xie et al., 2002). Because these studies were completed with purified components (i.e., insulin receptor isolated from plasma membranes, insulin, and Aβ), it was only later shown by Rensink et al. (2004) *in vitro* that insulin interacted with Aβ directly to limit membrane binding and fibril formation (see pericyte section). In addition, Aβ oligomers have been shown to lead to the removal of insulin receptors from the membranes of neuronal processes (Zhao et al., 2008; De Felice et al., 2009). Neuronal specific knockout of the insulin receptor using a *synapsin-1* Cre driver reduced Aβ (1–40 and 1–42) accumulation in the Tg2576 Alzheimer's disease mouse model (Stohr et al., 2013). IDE preferentially degrades insulin over Aβ (Qiu et al., 1998) suggesting levels of insulin can regulate levels of Aβ. Lastly, gene expression of amyloid processing genes, such as APP and PSEN1/2, have varying degrees of correlation with expression levels of the insulin receptor and IRS1 within the brain and are considered "co-expressed" genes that share similar spatial expression patterns (Diehl et al., 2017). These results greatly support the interaction of insulin, the insulin receptor, and Aβ within neurons linking changes in CNS insulin with the development of AD pathology. However, it is still unclear whether disrupted insulin receptor signaling leads to alterations in Aβ clearance and degradation or whether Aβ accumulation leads to disruptions in insulin receptor signaling.

Astrocytes

Primary human astrocytes express the insulin receptor and downstream signaling mediators and are responsive to insulin by altering glycogen synthesis and cell proliferation (Heni et al., 2011). Astrocytes can respond to insulin concentrations as low as 1 nM, concentrations that are commonly exceeded in the blood of healthy humans following feeding. These data suggest that even if CSF insulin levels are low, astrocytes might be exposed to comparable blood levels due to their close contact with blood vessels. Specifically in astrocytes, insulin receptor gene expression increases with age in the mouse (Clarke et al., 2018). By 9.5 months in mice, levels have reached their highest in the cortex and striatum. Interestingly, it is not until the age of 2 years that mouse hippocampal astrocytes reach their peak in insulin receptor gene expression. Astrocytes are one type of brain cell that have the ability to proliferate in adults. Astrocyte cell numbers increase after addition of insulin to the culture medium but high glucose inhibits astrocyte proliferation (Li et al., 2018). Astrocytes predominantly express the insulin receptor-B isoform (Garwood et al., 2015).

While the loss of insulin receptor signaling in neurons has been studied for decades, the loss in astrocytes has only recently begun to be investigated. Astrocyte insulin receptor knock-out mice (GIRKO) have been generated recently (Garcia-Caceres et al., 2016; Cai et al., 2018). Insulin receptor levels in astrocytes were decreased by about 50–70% (Garcia-Caceres et al., 2016; Cai et al., 2018). Postnatal loss of the insulin receptor in astrocytes affects morphology, circuit connectivity, and mitochondrial function (Garcia-Caceres et al., 2016). Insulin signaling in astrocytes also plays a role in potentiating release of dopamine and ATP (Cai et al., 2018). In an insulin-deficient

mouse model of diabetes, astrocytes retract at the BBB (Salameh et al., 2016). This ultimately leads to a disruption of the BBB, both structurally and via permeability to sucrose. BBB permeability in models lacking the astrocytic insulin receptor has not been investigated. The loss of the insulin receptor reduces activation of neurons by glucose, which ultimately alters glucose transport across the BBB (Garcia-Caceres et al., 2016). GIRKO mice have decreased expression of GLUT1 and shifts fuel preference of astrocytes from glucose to lipids. GLUT1 is more abundantly expressed in astrocytes compared to brain endothelial cells (Simpson et al., 1999). Decreased GLUT1 due to loss of astrocyte insulin receptor likely decreases glucose transport across the BBB. Alterations in GLUT1 expression at the BBB is associated with Alzheimer's disease (Winkler et al., 2015). Astrocytes are a major source of apolipoprotein E (apoE) in the brain (Kim et al., 2009). The secretion of apoE4 from astrocytes led to impaired barrier function *in vitro* (Nishitsuji et al., 2011) which has been implicated in Alzheimer's disease. Loss of the insulin receptor increased apoE expression by approximately 40% in astrocytes (Cai et al., 2018). This data suggests insulin can regulate multiple aspects of astrocyte function, which can ultimately affect neuronal plasticity and activity in the brain.

In humans, systemically administered insulin can increase CSF Aβ42 levels (Watson and Craft, 2003). It is possible the aquaporin-4 water channels present in astrocytes can aid in the insulin-dependent BBB clearance of CNS Aβ levels either through BBB clearance or by increasing CSF turnover to shuttle Aβ out through the glymphatic system (Vandal et al., 2015). Loss of the insulin receptor present in astrocytes or impairment in insulin response could weaken this clearance.

Pericytes

Human pericytes express the insulin receptor (James and Cotlier, 1983) yet the alpha subunit is undetectable in cultured human brain pericytes (Rensink et al., 2004). Insulin does not stimulate glucose uptake in cultured human brain pericytes (Rensink et al., 2004) or retinal capillary pericytes (Mandarino et al., 1994). It has also been shown that cell proliferation is enhanced more in pericytes due to insulin exposure compared to endothelial cells (King et al., 1983). Most studies investigating the role of the insulin receptor in pericytes have been done on cells isolated from bovine retinal capillaries (Escudero et al., 2017). Insulin can induce hyperpolarization of pericytes through calcium sensitive potassium channels (Berweck et al., 1993). Pericyte insulin signaling reduces endothelial cell death (Kobayashi and Puro, 2007). Pericyte-derived media, but not astrocyte-derived media, was able to increase the insulin stimulated phosphorylation of Akt and insulin receptor in a hypothalamic neuronal cell line, suggesting pericytes can increase insulin sensitivity in these neurons (Takahashi et al., 2015).

A mouse model with a pericyte specific knockout of the insulin receptor was used to investigate the role of insulin signaling in retinal angiogenesis (Warmke et al., 2017). Early on (postnatal day 5), retinas are hypervascularized in the knockout mice, which did not persist into adulthood. Alternatively, while pericyte coverage was similar between controls and knock-outs at this age, pericyte levels were reduced by 20% in the adult retinal vasculature. Changes in insulin signaling, pericyte function, or BBB changes were not reported in this abstract. In addition, PDGFRβ2, a marker commonly used for pericyte specificity, is expressed in other mural cells including vascular smooth muscle cells which would delete the insulin receptor in these cells as well.

Insulin can protect primary human brain pericytes from the toxic nature of Aβ (Rensink et al., 2004). Insulin inhibits Aβ fibril formation, binding of Aβ to the cell surface, and potentially interacts with Aβ itself (Rensink et al., 2004). Therefore, the protective effects of insulin in Alzheimer's disease could be due to pericyte protection from Aβ toxicity.

Even though the loss of the insulin receptor in specific cell types of the NVU has not been exhaustively investigated within the last two decades, there is still much to learn from these various models. For example, it is largely unknown what combinatorial effects might occur due to the loss of the insulin receptor in multiple cell types. Second, it is largely unknown how the loss of the insulin receptor in one cell type impacts another cell type. The use of the novel *ex vivo* technique utilizing BBB organoids (Bergmann et al., 2018) in addition to *in vitro* co-culture experiments could help answer some of these questions about basic interactions. Third, the regional effect of the insulin receptor has hardly been studied. More studies utilizing targeted knock-down of the insulin receptor should be performed in order to better understand the specific role of the insulin receptor in regions dedicated to different processes. Another way to get at this question would be to utilize optogenetics to inhibit the insulin receptor in certain sub-populations of cell types to determine the downstream impact. Fourth, as a recent study suggests that 25% of the neurons present in the adult human frontal cortex does not express the insulin receptor (Spencer et al., 2018), it will be important to also understand why insulin receptor signaling might not be necessary in this rather large subset of cells. Lastly, something that has not really been touched on here but is important to consider is the location of the insulin receptor within the cell types. It has been shown in cultured hippocampal neurons that the insulin receptor is present primarily in the postsynaptic density (Abbott et al., 1999), suggesting a role for insulin receptor signaling in mediating communication between neurons. However, the localization of the insulin receptor in other cell types in other regions has largely been uninvestigated. The molecular impact of the insulin receptor present within these cell types of the BBB is gaining great interest, likely due to the link in CNS insulin resistance and Alzheimer's disease.

INSULIN TRANSPORT AT THE BBB

Investigators have been examining the transport of insulin into the brain since 1954 when it was observed that minimal amounts of radioactively labeled insulin appeared in brain tissue following intravenous or subcutaneous injection (Haugaard et al., 1954). It was later more definitively shown that serum insulin appeared in CSF in dogs following insulin infusion (Margolis and Altszuler, 1967). Intravenously administered insulin is detected in brain within 1 min (Banks and Kastin, 1998; Banks et al., 1999).

Transport of insulin across the BBB has been validated many times using various techniques including perfusions (Schwartz et al., 1991), species-specific immunoassays (Banks et al., 1997c), and state-of-the art kinetic analyses (Banks et al., 1997a). The transporter for insulin at the BBB is not static but rather a dynamic protein regulated by the current physiological state of the body. In fact, during a time in which the brain is developing the greatest, the neonatal period, insulin transport across the BBB and binding to the brain endothelial cells is increased compared to weanling and adult periods (Frank et al., 1985). CSF and brain insulin levels are also significantly greater in the neonatal period. Insulin binding to brain capillaries is highest in the newborn rabbits compared to adults suggesting the presence of higher levels of insulin binding sites (Frank et al., 1985). These discrepancies between neonates and adults is likely due to the mitogenic nature of insulin action in the CNS rather than the metabolic role. Other physiological regulators of insulin transport that relate to insulin resistance are discussed in the next section.

We also know the rate of transport of insulin BBB transport varies between brain regions based on requirement. Insulin transport is not flow dependent like glucose. For some time now, it has been thought the insulin receptor present on the brain endothelial cell mediates this transport. This concept has some validity to it as regions in which insulin receptor expression is greatest, such as the olfactory bulb (Schulingkamp et al., 2000; Ghasemi et al., 2013), transport is greatest (Banks et al., 1999; Rhea et al., 2018). However, what is not necessarily taken into consideration is the amount of insulin receptor present on neurons, astrocytes, pericytes, and other CNS cell types in these brain regions versus the levels present on brain endothelial cells. With the increasing use of single-cell RNA sequencing, we are beginning to learn more about the expression pattern of the insulin receptor in different CNS cell types (Zhang et al., 2014) and will further be able to identify regional differences between the cell types. In addition, variable cerebral blood circulation, diverse capillary density in the brain, or other factors, such as expression levels of insulin transport protein mediators could also drive the regional transport differences. Studies using *in vitro* transport models (Gray et al., 2017), capillary binding assays (Frank et al., 1985; King and Johnson, 1985), and *in vivo* static measurements of transport (Meijer et al., 2016) suggest the insulin receptor is responsible for physically transporting insulin across the brain endothelial cell and into the brain. However, we have recently shown using dynamic, pharmacokinetic *in vivo* techniques in a mouse model lacking the insulin receptor in brain endothelial cells and use of pharmacological inhibition of the insulin receptor, insulin transport across the BBB is unchanged (Rhea et al., 2018). It was also confirmed by a separate group using primary brain endothelial cells and capillaries from bovine, rat, and mouse that inhibition of the insulin receptor did not affect transport (Hersom et al., 2018). Regional expression of the insulin transporter could be responsible for the regional transport differences. The rationale that the insulin transporter is separate from the insulin receptor is not far-fetched. An important signaling peptide such as insulin should have a protein that it can bind to and elicit an internal signaling cascade in addition to having another protein that can transport this signaling peptide to other areas necessary for signaling (**Figure 2**). In addition, these separate proteins should be able to be regulated differently, depending on the physiological necessity at any given time. For example, insulin receptor signaling in the brain endothelial cell relays a signal to neurons (Konishi et al., 2017) which we know has multiple effects on growth and survival. In addition, insulin signaling in endothelial cells is a vasoregulator (Vicent et al., 2003). Since endothelial cells create a barrier to other cells within organs, it can be speculated these cells would also contain a transporter to independently get insulin to other cell types in order to act physiologically there. Insulin transport is not related to amino acid transport, the *p*-glycoprotein system, a slow calcium channel, alpha-adrenergic action, or growth hormone, somatostatin, glucagon, or leptin transport (Frank et al., 1985; Banks et al., 1997a; Banks, 2001; Yu et al., 2006). Excess IGF-1 can inhibit the transport of insulin across the BBB, suggesting competitive transport (Yu et al., 2006). However, this study used about a 220-fold excess amount of IGF-1 so the physiological relevance on insulin transport is still unclear. Based on the lack of competitive inhibition with most substrates tested and the data showing changes in insulin transport under various physiological conditions, it is likely that the insulin transporter is rather specific for insulin but is regulated in an allosteric manner.

CONDITIONS WITH ALTERED INSULIN BBB TRANSPORT

While the molecular mediators regulating insulin transport across the BBB are not exactly clear, there are specific conditions and factors that are known to alter the transport rate, total amount, and level of endothelial binding of insulin at the BBB (Banks, 2004). However, since the year of that review (2004), the impact of other factors on BBB insulin transport have been investigated. Estrogen does not impact the transport of insulin into the CSF (May et al., 2016a), while the gastrointestinal hormone cholecystokinin (CCK) increases transport (May et al., 2016b). Other conditions and factors including starvation (Urayama and Banks, 2008), triglycerides (Urayama and Banks, 2008), and nitric oxide (Banks et al., 2008) have also been investigated and will be discussed below with the conditions related to insulin resistance (**Figure 3**).

Obesity and Diabetes

Nearly 2 months of high-fat diet feeding in dogs led to an approximate 60% decrease in insulin BBB transport that was inversely proportional to body weight (Kaiyala et al., 2000). Levels of brain insulin were also decreased in obese Zucker rats compared to lean counterparts (Baskin et al., 1985). In addition to overall decreases in brain insulin levels in obese models, it has been shown the transport rate of insulin across the BBB is about half that in obese mice compared to lean mice (Urayama and Banks, 2008). This study also showed starvation (72 h) of obese mice can lead to an increase in transport. However, these changes in transport were eliminated when the contribution of serum factors was removed. This suggests the effect of obesity on insulin BBB transport is mediated by

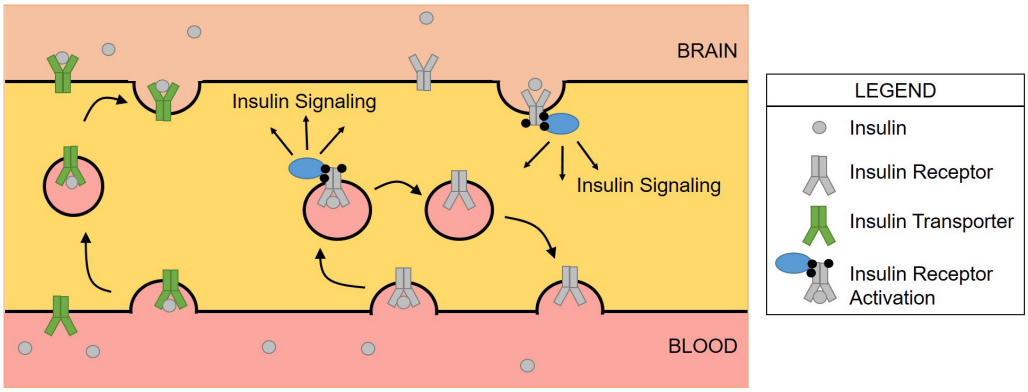

FIGURE 2 | Schematic of insulin transport versus insulin receptor signaling at the BBB. Insulin binds the insulin receptor where the complex is internalized via endocytosis. Insulin activates the receptor, triggering a signaling cascade before insulin then dissociates from the receptor. The receptor is then recycled to the membrane for subsequent signaling. The insulin transporter is responsible for delivering insulin from the blood to the brain, navigating across the brain endothelial cell. This has been demonstrated in only the luminal to abluminal direction suggesting a modification of the insulin transporter that prevents binding on the abluminal surface.

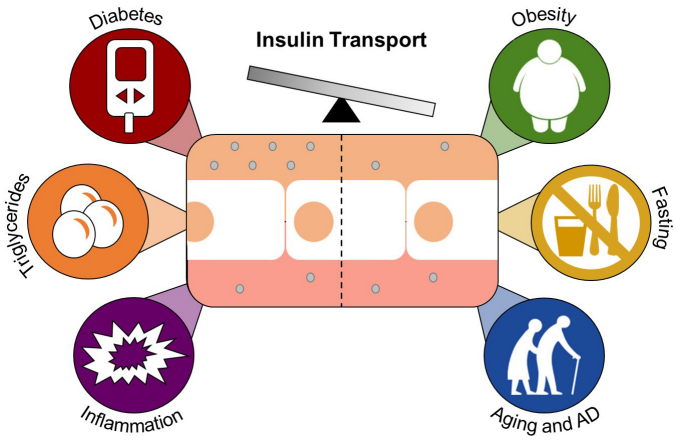

FIGURE 3 | Conditions and factors that regulate insulin transport across the BBB. Brain endothelial cells (center white) separate the blood from the brain and make up the BBB. Insulin (gray circle) is transported across the BBB by a carrier-mediated system. Diabetes, triglycerides, and inflammation are known enhancers of insulin transport. Obesity, fasting, and aging/Alzheimer's disease attenuate this transport.

changes in circulating factors, one of which is the triglyceride triolein. Triglycerides increase insulin BBB transport, can cross the BBB, and can induce CNS insulin receptor resistance (Urayama and Banks, 2008; Banks et al., 2018). These data show how a peripheral condition such as obesity can have detrimental effects on CNS insulin signaling.

Contrary to obesity, diabetes mellitus increases BBB insulin transport and endothelial insulin binding, correlating with the onset of diabetes (Banks et al., 1997b). This occurred independent of changes in leptin transport (which share similarities with insulin). Also opposite of obesity, the enhanced transport of insulin across the BBB in the streptozotocin-model of diabetes was not due to changes in serum factors. Instead, there is likely molecular reorganization of the insulin transport system at the BBB due to diabetes. A steady increase in serum glucose following a glucose bolus does not increase the uptake of insulin by the brain but does increase insulin binding to the endothelium (Banks et al., 1997b). These data suggest glucose does not necessarily affect insulin BBB transport but rather can affect interaction with the brain endothelial cell insulin receptor.

Diabetes can also alter the overall structure and function of the BBB. In streptozotocin-treated mice, insulin increases cerebral microvessel expression of tight junction proteins including occludin, claudin-5, and ZO-1 (Sun et al., 2015). Again, this data supports the role of insulin in regulating tight junction protein expression, similar to the data observed in the EndoIRKO mice (Konishi et al., 2017). Expression of the transport protein lipoprotein receptor-related protein 1 (LRP1) is downregulated in mouse brain capillaries (Hong et al., 2009) and CSF LRP1 is increased in type 1 diabetic patients (Ouwens et al., 2014), suggesting insulin may regulate CNS LRP1. Indeed, insulin treatment in an immortalized human brain endothelial cell line increased plasma membrane LRP1 expression but not total cell expression, suggesting a role for insulin in LRP1 translocation

(Swaminathan et al., 2018). LRP1 is an important functional regulator within the brain endothelial cell and interacts with multiple substrates, including Aβ, aiding in clearance of this peptide from the CNS. This is one hypothesis as to why people with diabetes are at a higher risk for developing Alzheimer's disease. LRP1 is also downregulated due to inflammation (Jaeger et al., 2009). Diabetes and obesity can increase inflammation and serum inflammatory mediators, which can have an adverse effect on the BBB.

Inflammation has been shown to have multiple detrimental effects on BBB structure and function. This topic has been extensively reviewed elsewhere (Erickson and Banks, 2018; Van Dyken and Lacoste, 2018). Inflammation due to lipopolysaccharide (LPS) administration enhances insulin BBB transport by up to 2–3 fold (Xaio et al., 2001). Nitric oxide is a signaling molecule in inflammation, released by nitric oxide synthase. Nitric oxide isoenzymes can regulate insulin transport across the BBB under LPS inflammation (Banks et al., 2008). Transcytosis and paracellular transport is increased due to inflammation. Inflammation leads to decreased expression levels of the tight junction proteins claudin-5, ZO-1, and occludin. *P*-glycoprotein, LRP1, and amino acid transporters are also downregulated (Jaeger et al., 2009; Erickson et al., 2012; Ransohoff et al., 2015) while TNF-alpha and Aβ transporters are increased (Jaeger et al., 2009; Varatharaj and Galea, 2017). Proteins responsible for improving transport of immune cells are also increased (Van Dyken and Lacoste, 2018). It is difficult to tease apart the contribution of inflammation, glucose, and changes in hormone levels on BBB permeability in diabetes and obesity. In addition, it is still unclear what the molecular mediators are either at the brain endothelial cell or other cell types of the BBB that alter insulin BBB transport in these various conditions.

Aging and Alzheimer's Disease

Due to the data supporting a role for CNS insulin resistance in Alzheimer's disease, efforts have been made to investigate the transport of insulin in Alzheimer's disease compared to 'healthy' aging. While there was no difference in the rate of insulin BBB transport in an aged non-transgenic Alzheimer's disease mouse model (SAMP8) compared to young ones, the level of reversible binding at the endothelium was increased regionally (Banks et al., 2000). While this has not been shown molecularly, the level of binding could be considered as a marker for the amount of insulin receptor present. However, in a different mouse model of Alzheimer's disease (APP/PS1), insulin BBB transport rate is significantly increased in specific brain regions including the hippocampus compared to a wild-type mouse (Poduslo et al., 2001). This could be due to the Aβ interactions with insulin as noted above, as the APP/PS1 mice have higher levels of Aβ that are more fibrillating (and hence more toxic) than the SAMP8. A comparative study utilizing different mouse models of Alzheimer's disease investigating the transport rate of insulin across the BBB will help us determine what factors might control the rate of transport due to various Alzheimer's disease pathologies.

There is no disruption of the BBB to serum albumin either in aged SAMP8 mice (Banks et al., 2000) or in APP/PS1 mice that have substantial Aβ levels in the plasma and brain (Poduslo et al., 2001). Instead, there was increased binding of serum albumin, suggestive of a thickened basement membrane. Whether or not BBB breakdown occurs in patients with Alzheimer's disease is still controversial. A lack of BBB permeability to imaging tracers has been shown in a limited number of subjects (Friedland et al., 1983; Schlageter et al., 1987; Caserta et al., 1998) while others have suggested BBB breakdown precedes pathological hallmarks of Alzheimer's disease (Nation et al., 2019). The discrepancies reported in the literature could be due to multiple factors including technique used, level of cognitive impairment, or sensitivity of detection.

Patients with Alzheimer's disease have increased plasma insulin levels, decreased CSF insulin levels, and thus a reduced CSF-to-plasma insulin ratio (Craft et al., 1998). This suggests in Alzheimer's disease in humans that insulin BBB transport might be impaired. It has also been shown brain insulin levels and insulin receptor levels and signaling are decreased in Alzheimer's disease (Frolich et al., 1998; Talbot et al., 2012). The insulin immunoreactivity was localized primarily to pyramidal neurons and not glial and endothelial cells. There were also decreases in the insulin receptor expression due to age in the frontal and parietal cortex while expression of the IGF-1 receptor was unchanged suggesting a specific susceptibility.

THERAPIES TO INCREASE BBB INSULIN TRANSPORT AND CNS INSULIN LEVELS

In this review, we have summarized the detrimental effects of altered insulin signaling within specific cell types at the BBB. However, many of these detrimental effects are due to decreased exposure to insulin and hence insulin BBB transport. Therefore, if CNS insulin can be increased, some of these detrimental effects could be overcome. Alternative routes to increase CNS insulin has recently been reviewed by our group in detail (Rhea et al., 2019). We have highlighted here a couple of therapies that are the most translational to increase CNS insulin levels.

Intranasal

Intranasal insulin can improve memory in young, healthy adults (Benedict et al., 2004), people with mild cognitive impairment and Alzheimer's disease (Reger et al., 2008a,b), and in mouse models of Alzheimer's disease (Salameh et al., 2015; Mao et al., 2016). When insulin is delivered intranasally, it reaches most brain regions in both young and aged wild-type and SAMP8 mice (Salameh et al., 2015; Rhea et al., 2017). This therapy has been shown to improve parts of insulin receptor signaling in the forebrain (Chen et al., 2014) and hippocampus (Mao et al., 2016). It is possible insulin acts independent of the insulin receptor to improve memory. One potential mechanism could be via the interaction of insulin and Aβ. Intranasal insulin is able to reduce Aβ plaque deposits by altering the processing of the APP peptide (Mao et al., 2016). Even though insulin signaling in the CNS cannot be studied in living humans, brain slices taken

post-mortem from patients with Alzheimer's disease respond to *ex vivo* insulin stimulation (in terms of insulin receptor signaling phosphorylation), but not at the level of age-matched controls (Talbot et al., 2012). This suggests that while insulin signaling is impaired in Alzheimer's disease, the brain still has the ability to respond to insulin. Therefore, delivering insulin to the site of action as occurs with intranasal delivery will help to overcome CNS insulin resistance.

Weight Loss

Since obesity is commonly linked with insulin resistance, weight loss should restore insulin sensitivity. As mentioned earlier, in animal models of obesity, insulin transport across the BBB is impaired (Schwartz et al., 1991; Urayama and Banks, 2008). In an animal study investigating the response of BBB insulin transport to weight loss, CSF insulin levels increase following diet reversal and weight loss in male Long-Evans rats (Begg et al., 2013). The rats were on a high-fat diet for 22 weeks before a group of them were switched to a low-fat diet for 8 weeks. Therefore, diet-induced CNS insulin resistance can be reversed if switched to a low-fat diet. Weight loss is associated with a decrease in triglyceride levels. Triglycerides increase insulin BBB transport (Urayama and Banks, 2008) but can cross the BBB and impair CNS insulin signaling (Banks et al., 2018). It is unclear whether diet reversal can improve CNS insulin resistance in humans. One could measure CSF and serum insulin levels following weight loss to assess potential changes in transport. Indeed, weight loss is associated with improved cognition (Veronese et al., 2017). Triglycerides and other lipids including cholesterol and omega-3 fatty acids have an impact on memory and cognition (Morley and Banks, 2010). Studies investigating changes in CNS insulin sensitivity following dietary, behavior changes or surgery, such as bariatric surgery or liposuction, would help elucidate the true impact of weight loss on insulin BBB transport.

CONCLUSION

Here, we have summarized for the condition of insulin resistance the interplay of the BBB and various types of other brain cells that form the NVU. This interplay has emerged to be particularly important in Alzheimer's disease and provides mechanisms for specific interactions with Aβ and tau and links with metabolic disease. The BBB with its transport system for insulin is critical for defining the interactions between peripherally secreted insulin with its receptors located within the CNS. Thus, the BBB is also critical in considering therapeutic options. Overall, the barrier functions in combination with the communication functions of the BBB result in an operational blood-brain interface vital to understanding insulin resistance.

AUTHOR CONTRIBUTIONS

ER established the review topic. ER and WB wrote and edited the manuscript.

FUNDING

This work was supported in part by the VA Puget Sound Health Care System, the University of Washington Diabetes Research Center New Investigator Award (ER), and NIH funding (RO1AG046619 to WB).

REFERENCES

Abbott, M. A., Wells, D. G., and Fallon, J. R. (1999). The insulin receptor tyrosine kinase substrate p58/53 and the insulin receptor are components of CNS synapses. *J. Neurosci.* 19, 7300–7308. doi: 10.1523/jneurosci.19-17-07300.1999

Abbott, N. J., Ronnback, L., and Hansson, E. (2006). Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* 7, 41–53. doi: 10.1038/nrn1824

Alvarez, J. I., Dodelet-Devillers, A., Kebir, H., Ifergan, I., Fabre, P. J., Terouz, S., et al. (2011). The Hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. *Science* 334, 1727–1731. doi: 10.1126/science.1206936

Armulik, A., Genove, G., Mae, M., Nisancioglu, M. H., Wallgard, E., Niaudet, C., et al. (2010). Pericytes regulate the blood-brain barrier. *Nature* 468, 557–561.

Banks, W. A. (2001). Enhanced leptin transport across the blood-brain barrier by alpha 1-adrenergic agents. *Brain Res.* 899, 209–217. doi: 10.1016/s0006-8993(01)02242-9

Banks, W. A. (2004). The source of cerebral insulin. *Eur. J. Pharmacol.* 490, 5–12. doi: 10.1016/j.ejphar.2004.02.040

Banks, W. A. (2016). From blood-brain barrier to blood-brain interface: new opportunities for CNS drug delivery. *Nat. Rev. Drug Dis.* 15, 275–292. doi: 10.1038/nrd.2015.21

Banks, W. A., Dohgu, S., Lynch, J. L., Fleegal-DeMotta, M. A., Erickson, M. A., Nakaoke, R., et al. (2008). Nitric oxide isoenzymes regulate lipopolysaccharide-enhanced insulin transport across the blood-brain barrier. *Endocrinology* 149, 1514–1523. doi: 10.1210/en.2007-1091

Banks, W. A., Farr, S. A., and Morley, J. E. (2000). Permeability of the blood-brain barrier to albumin and insulin in the young and aged SAMP8 mouse. *J. Gerontol. A Biol. Sci. Med. Sci.* 55, B601–B606.

Banks, W. A., Farr, S. A., Salameh, T. S., Niehoff, M. L., Rhea, E. M., Morley, J. E., et al. (2018). Triglycerides cross the blood-brain barrier and induce central leptin and insulin receptor resistance. *Int. J. Obes.* 42, 391–397. doi: 10.1038/ijo.2017.231

Banks, W. A., Jaspan, J. B., Huang, W., and Kastin, A. J. (1997a). Transport of insulin across the blood-brain barrier: saturability at euglycemic doses of insulin. *Peptides* 18, 1423–1429. doi: 10.1016/s0196-9781(97)00231-3

Banks, W. A., Jaspan, J. B., and Kastin, A. J. (1997b). Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. *Peptides* 18, 1577–1584. doi: 10.1016/s0196-9781(97)00238-6

Banks, W. A., Jaspan, J. B., and Kastin, A. J. (1997c). Selective, physiological transport of insulin across the blood-brain barrier: novel demonstration by species-specific radioimmunoassays. *Peptides* 18, 1257–1262. doi: 10.1016/s0196-9781(97)00198-8

Banks, W. A., and Kastin, A. J. (1998). Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin. *Peptides* 19, 883–889. doi: 10.1016/s0196-9781(98)00018-7

Banks, W. A., Kastin, A. J., and Pan, W. (1999). Uptake and degradation of blood-borne insulin by the olfactory bulb. *Peptides* 20, 373–378. doi: 10.1016/s0196-9781(99)00045-5

Banks, W. A., Owen, J. B., and Erickson, M. A. (2012). Insulin in the brain: there and back again. *Pharmacol. Therap.* 136, 82–93. doi: 10.1016/j.pharmthera.2012.07.006

Baskin, D. G., Stein, L. J., Ikeda, H., Woods, S. C., Figlewicz, D. P., Porte, D. Jr., et al. (1985). Genetically obese Zucker rats have abnormally low brain insulin content. *Life Sci.* 36, 627–633. doi: 10.1016/0024-3205(85)90166-3

Begg, D. P., Mul, J. D., Liu, M., Reedy, B. M., D'Alessio, D. A., Seeley, R. J., et al. (2013). Reversal of diet-induced obesity increases insulin transport into cerebrospinal fluid and restores sensitivity to the anorexic action of central insulin in male rats. *Endocrinology* 154, 1047–1054. doi: 10.1210/en.2012-1929

Begley, D. J. (2004). Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. *Pharmacol. Therap.* 104, 29–45. doi: 10.1016/j.pharmthera.2004.08.001

Belfiore, A., Malaguarnera, R., Vella, V., Lawrence, M. C., Sciacca, L., Frasca, F., et al. (2017). Insulin receptor isoforms in physiology and disease: an updated view. *Endocr. Rev.* 38, 379–431. doi: 10.1210/er.2017-00073

Bell, R. D., Winkler, E. A., Singh, I., Sagare, A. P., Deane, R., Wu, Z., et al. (2012). Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. *Nature* 485, 512–516. doi: 10.1038/nature11087

Benedict, C., Hallschmid, M., Hatke, A., Schultes, B., Fehm, H. L., Born, J., et al. (2004). Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* 29, 1326–1334. doi: 10.1016/j.psyneuen.2004.04.003

Bergmann, S., Lawler, S. E., Qu, Y., Fadzen, C. M., Wolfe, J. M., Regan, M. S., et al. (2018). Blood-brain-barrier organoids for investigating the permeability of CNS therapeutics. *Nat. Protoc.* 13, 2827–2843. doi: 10.1038/s41596-018-0066-x

Berweck, S., Thieme, H., Lepple-Wienhues, A., Helbig, H., and Wiederholt, M. (1993). Insulin-induced hyperpolarization in retinal capillary pericytes. *Invest. Ophthalmol. Vis. Sci.* 34, 3402–3407.

Bonkowski, D., Katyshev, V., Balabanov, R. D., Borisov, A., and Dore-Duffy, P. (2011). The CNS microvascular pericyte: pericyte-astrocyte crosstalk in the regulation of tissue survival. *Fluids Barriers CNS* 8:8. doi: 10.1186/2045-8118-8-8

Broadwell, R. D. (1989). Transcytosis of macromolecules through the blood-brain barrier: a cell biological perspective and critical appraisal. *Acta Neuropathol.* 79, 117–128. doi: 10.1007/bf00294368

Bruning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., et al. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science* 289, 2122–2125. doi: 10.1126/science.289.5487.2122

Cai, W., Xue, C., Sakaguchi, M., Konishi, M., Shirazian, A., Ferris, H. A., et al. (2018). Insulin regulates astrocyte gliotransmission and modulates behavior. *J. Clin. Invest.* 128, 2914–2926. doi: 10.1172/JCI99366

Caserta, M. T., Caccioppo, D., Lapin, G. D., Ragin, A., and Groothuis, D. R. (1998). Blood-brain barrier integrity in Alzheimer's disease patients and elderly control subjects. *J. Neuropsychiatry Clin. Neurosci.* 10, 78–84. doi: 10.1176/jnp.10.1.78

Chen, Y., Zhao, Y., Dai, C. L., Liang, Z., Run, X., Iqbal, K., et al. (2014). Intranasal insulin restores insulin signaling, increases synaptic proteins, and reduces Abeta level and microglia activation in the brains of 3xTg-AD mice. *Exp. Neurol.* 261, 610–619. doi: 10.1016/j.expneurol.2014.06.004

Chiu, S. L., Chen, C. M., and Cline, H. T. (2008). Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo. *Neuron* 58, 708–719. doi: 10.1016/j.neuron.2008.04.014

Chiu, S. L., and Cline, H. T. (2010). Insulin receptor signaling in the development of neuronal structure and function. *Neural Dev* 5:7. doi: 10.1186/1749-8104-5-7

Clarke, L. E., Liddelow, S. A., Chakraborty, C., Munch, A. E., Heiman, M., and Barres, B. A. (2018). Normal aging induces A1-like astrocyte reactivity. *Proc. Natl. Acad. Sci. U.S.A.* 115, E1896–E1905. doi: 10.1073/pnas.1800165115

Craft, S., Peskind, E., Schwartz, M. W., Schellenberg, G. D., and Raskind, M. P. (1998). Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology* 50, 164–168. doi: 10.1212/wnl.50.1.164

Daneman, R., and Prat, A. (2015). The blood-brain barrier. *Cold Spring Harb. Perspect. Biol.* 7, a020412. doi: 10.1101/cshperspect.a020412

Daneman, R., Zhou, L., Kebede, A. A., and Barres, B. A. (2010). Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 468, 562–566. doi: 10.1038/nature09513

De Felice, F. G., Vieira, M. N., Bomfim, T. R., Decker, H., Velasco, P. T., Lambert, M. P., et al. (2009). Protection of synapses against Alzheimer's-linked toxins: insulin signaling prevents the pathogenic binding of Abeta oligomers. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1971–1976. doi: 10.1073/pnas.0809158106

Diehl, T., Mullins, R., and Kapogiannis, D. (2017). Insulin resistance in Alzheimer's disease. *Transl. Res.* 183, 26–40. doi: 10.1016/j.trsl.2016.12.005

Erickson, M. A., and Banks, W. A. (2018). Neuroimmune axes of the blood-brain barriers and blood-brain interfaces: bases for physiological regulation. disease states, and pharmacological interventions. *Pharmacol. Rev.* 70, 278–314. doi: 10.1124/pr.117.014647

Erickson, M. A., Hansen, K., and Banks, W. A. (2012). Inflammation-induced dysfunction of the low-density lipoprotein receptor-related protein-1 at the blood-brain barrier: protection by the antioxidant N-acetylcysteine. *Brain Behav. Immun.* 26, 1085–1094. doi: 10.1016/j.bbi.2012.07.003

Escudero, C. A., Herlitz, K., Troncoso, F., Guevara, K., Acurio, J., Aguayo, C., et al. (2017). Pro-angiogenic role of insulin: from physiology to pathology. *Front. Physiol.* 8:204. doi: 10.3389/fphys.2017.00204

Frank, H. J., Jankovic-Vokes, T., Pardridge, W. M., and Morris, W. L. (1985). Enhanced insulin binding to blood-brain barrier in vivo and to brain microvessels in vitro in newborn rabbits. *Diabetes Metab. Res. Rev* 34, 728–733. doi: 10.2337/diab.34.8.728

Freude, S., Plum, L., Schnitker, J., Leeser, U., Udelhoven, M., Krone, W., et al. (2005). Peripheral hyperinsulinemia promotes tau phosphorylation in vivo. *Diab. Metab. Res. Rev.* 54, 3343–3348. doi: 10.2337/diabetes.54.12.3343

Freude, S., Schilbach, K., and Schubert, M. (2009). The role of IGF-1 receptor and insulin receptor signaling for the pathogenesis of Alzheimer's disease: from model organisms to human disease. *Curr. Alzheimer Res.* 6, 213–223. doi: 10.2174/156720509788486527

Friedland, R. P., Budinger, T. F., Ganz, E., Yano, Y., Mathis, C. A., Koss, B., et al. (1983). Regional cerebral metabolic alterations in dementia of the Alzheimer type: positron emission tomography with [18F]fluorodeoxyglucose. *J. Comput. Assist. Tomogr.* 7, 590–598. doi: 10.1097/00004728-198308000-00003

Frolich, L., Blum-Degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., et al. (1998). Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J. Neural Transm.* 105, 423–438.

Garcia-Caceres, C., Quarta, C., Varela, L., Gao, Y., Gruber, T., Legutko, B., et al. (2016). Astrocytic insulin signaling couples brain glucose uptake with nutrient availability. *Cell* 166, 867–880. doi: 10.1016/j.cell.2016.07.028

Garwood, C. J., Ratcliffe, L. E., Morgan, S. V., Simpson, J. E., Owens, H., Vazquez-Villasenor, I., et al. (2015). Insulin and IGF1 signalling pathways in human astrocytes in vitro and in vivo; characterisation, subcellular localisation and modulation of the receptors. *Mol. Brain* 8:51. doi: 10.1186/s13041-015-0138-6

Ghasemi, R., Haeri, A., Dargahi, L., Mohamed, Z., and Ahmadiani, A. (2013). Insulin in the brain: sources, localization and functions. *Mol. Neurobiol.* 47, 145–171. doi: 10.1007/s12035-012-8339-9

Gray, S. M., Aylor, K. W., and Barrett, E. J. (2017). Unravelling the regulation of insulin transport across the brain endothelial cell. *Diabetologia* 60, 1512–1521. doi: 10.1007/s00125-017-4285-4

Grillo, C. A., Piroli, G. G., Lawrence, R. C., Wrighten, S. A., Green, A. J., Wilson, S. P., et al. (2015). hippocampal insulin resistance impairs spatial learning and synaptic plasticity. *Diabetes Metab. Res. Rev.* 64, 3927–3936. doi: 10.2337/db15-0596

Grillo, C. A., Tamashiro, K. L., Piroli, G. G., Melhorn, S., Gass, J. T., Newsom, R. J., et al. (2007). Lentivirus-mediated downregulation of hypothalamic insulin receptor expression. *Physiology & behavior* 92, 691–701. doi: 10.1016/j.physbeh.2007.05.043

Gu, H., Marth, J. D., Orban, P. C., Mossmann, H., and Rajewsky, K. (1994). Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. *Science* 265, 103–106. doi: 10.1126/science.8016642

Hamilton, N. B., and Attwell, D. (2010). Do astrocytes really exocytose neurotransmitters? *Nat. Rev. Neurosci.* 11, 227–238. doi: 10.1038/nrn2803

Haugaard, N., Vaughan, M., Haugaard, E. S., and Stadie, W. C. (1954). Studies of radioactive injected labeled insulin. *J. Biol. Chem.* 208, 549–563.

Hayden, M. R., Banks, W. A., Shah, G. N., Gu, Z., and Sowers, J. R. (2013). Cardiorenal metabolic syndrome and diabetic cognopathy. *Cardiorenal. Med.* 3, 265–282. doi: 10.1159/000357113

Heni, M., Hennige, A. M., Peter, A., Siegel-Axel, D., Ordelheide, A. M., Krebs, N., et al. (2011). Insulin promotes glycogen storage and cell proliferation in primary human astrocytes. *PLoS One* 6:e21594. doi: 10.1371/journal.pone.0021594

Hersom, M., Helms, H. C., Schmalz, C., Pedersen, T. A., Buckley, S. T., and Brodin, B. (2018). The insulin receptor is expressed and functional in cultured blood-brain barrier endothelial cells, but does not mediate insulin entry from blood-to-brain. *Am. J. Physiol. Endocrinol. Metab.* 315, E531–E542. doi: 10.1152/ajpendo.00350.2016

Hong, H., Liu, L. P., Liao, J. M., Wang, T. S., Ye, F. Y., Wu, J., et al. (2009). Downregulation of LRP1 [correction of LPR1] at the blood-brain barrier in streptozotocin-induced diabetic mice. *Neuropharmacology* 56, 1054–1059. doi: 10.1016/j.neuropharm.2009.03.001

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. *Sci. Transl. Med.* 4:147ra111. doi: 10.1126/scitranslmed.3003748

Jaeger, L. B., Dohgu, S., Sultana, R., Lynch, J. L., Owen, J. B., Erickson, M. A., et al. (2009). Lipopolysaccharide alters the blood-brain barrier transport of amyloid beta protein: a mechanism for inflammation in the progression of Alzheimer's disease. *Brain Behav. Immun.* 23, 507–517. doi: 10.1016/j.bbi.2009.01.017

James, C. R., and Cotlier, E. (1983). Fate of insulin in the retina: an autoradiographic study. *Br. J. Ophthalmol.* 67, 80–88. doi: 10.1136/bjo.67.2.80

Kaiyala, K. J., Prigeon, R. L., Kahn, S. E., Woods, S. C., and Schwartz, M. W. (2000). Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes Metab. Res. Rev.* 49, 1525–1533. doi: 10.2337/diabetes.49.9.1525

Karczewska-Kupczewska, M., Lelental, N., Adamska, A., Nikolajuk, A., Kowalska, I., Gorska, M., et al. (2013). The influence of insulin infusion on the metabolism of amyloid beta peptides in plasma. *Alzheimers. Dement.* 9, 400–405. doi: 10.1016/j.jalz.2012.01.013

Katakam, P. V., Domoki, F., Lenti, L., Gaspar, T., Institoris, A., Snipes, J. A., et al. (2009). Cerebrovascular responses to insulin in rats. *J. Cereb Blood Flow Metab.* 29, 1955–1967. doi: 10.1038/jcbfm.2009.177

Kim, J., Basak, J. M., and Holtzman, D. M. (2009). The role of apolipoprotein E in Alzheimer's disease. *Neuron* 63, 287–303. doi: 10.1016/j.neuron.2009.06.026

Kimura, T., Ono, T., Takamatsu, J., Yamamoto, H., Ikegami, K., Kondo, A., et al. (1996). Sequential changes of tau-site-specific phosphorylation during development of paired helical filaments. *Dementia* 7, 177–181. doi: 10.1159/000106875

King, G. L., Buzney, S. M., Kahn, C. R., Hetu, N., Buchwald, S., Macdonald, S. G., et al. (1983). Differential responsiveness to insulin of endothelial and support cells from micro- and macrovessels. *J. Clin. Invest.* 71, 974–979. doi: 10.1172/jci110852

King, G. L., and Johnson, S. M. (1985). Receptor-mediated transport of insulin across endothelial cells. *Science* 227, 1583–1586. doi: 10.1126/science.3883490

Kleinridders, A., Cai, W., Cappellucci, L., Ghazarian, A., Collins, W. R., Vienberg, S. G., et al. (2015). Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3463–3468. doi: 10.1073/pnas.1500877112

Kobayashi, T., and Puro, D. G. (2007). Loss of insulin-mediated vasoprotection: early effect of diabetes on pericyte-containing microvessels of the retina. *Invest. Ophthalmol. Vis. Sci.* 48, 2350–2355.

Kondo, T., Hafezi-Moghadam, A., Thomas, K., Wagner, D. D., and Kahn, C. R. (2004). Mice lacking insulin or insulin-like growth factor 1 receptors in vascular endothelial cells maintain normal blood-brain barrier. *Biochem. Biophys. Res. Commun.* 317, 315–320. doi: 10.1016/j.bbrc.2004.03.043

Konishi, M., Sakaguchi, M., Lockhart, S. M., Cai, W., Li, M. E., Homan, E. P., et al. (2017). Endothelial insulin receptors differentially control insulin signaling kinetics in peripheral tissues and brain of mice. *Proc. Natl. Acad. Sci. U.S.A.* 114, E8478–E8487. doi: 10.1073/pnas.1710625114

Kulstad, J. J., Green, P. S., Cook, D. G., Watson, G. S., Reger, M. A., Baker, L. D., et al. (2006). Differential modulation of plasma beta-amyloid by insulin in patients with Alzheimer disease. *Neurology* 66, 1506–1510. doi: 10.1212/01.wnl.0000216274.58185.09

Lee, S. W., Kim, W. J., Choi, Y. K., Song, H. S., Son, M. J., Gelman, I. H., et al. (2003). SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. *Nat. Med.* 9, 900–906. doi: 10.1038/nm889

Lesort, M., and Johnson, G. V. (2000). Insulin-like growth factor-1 and insulin mediate transient site-selective increases in tau phosphorylation in primary cortical neurons. *Neuroscience* 99, 305–316. doi: 10.1016/s0306-4522(00)00200-1

Lesort, M., Jope, R. S., and Johnson, G. V. (1999). Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase. *J. Neurochem.* 72, 576–584. doi: 10.1046/j.1471-4159.1999.0720576.x

Li, W., Roy Choudhury, G., Winters, A., Prah, J., Lin, W., Liu, R., et al. (2018). Hyperglycemia Alters Astrocyte Metabolism and Inhibits Astrocyte Proliferation. *Aging Dis.* 9, 674–684. doi: 10.14336/AD.2017.1208

Man, S., Ubogu, E. E., and Ransohoff, R. M. (2007). Inflammatory cell migration into the central nervous system: a few new twists on an old tale. *Brain Pathol.* 17, 243–250. doi: 10.1111/j.1750-3639.2007.00067.x

Mandarino, L. J., Finlayson, J., and Hassell, J. R. (1994). High glucose downregulates glucose transport activity in retinal capillary pericytes but not endothelial cells. *Invest. Ophthalmol. Vis. Sci.* 35, 964–972.

Mao, Y. F., Guo, Z., Zheng, T., Jiang, Y., Yan, Y., Yin, X., et al. (2016). Intranasal insulin alleviates cognitive deficits and amyloid pathology in young adult APPswe/PS1dE9 mice. *Aging Cell* 15, 893–902. doi: 10.1111/acel.12498

Margolis, R. U., and Altszuler, N. (1967). Insulin in the cerebrospinal fluid. *Nature* 215, 1375–1376.

May, A. A., Bedel, N. D., Shen, L., Woods, S. C., and Liu, M. (2016a). Estrogen and insulin transport through the blood-brain barrier. *Physiol. Behav.* 163, 312–321. doi: 10.1016/j.physbeh.2016.05.019

May, A. A., Liu, M., Woods, S. C., and Begg, D. P. (2016b). CCK increases the transport of insulin into the brain. *Physiol. Behav.* 165, 392–397. doi: 10.1016/j.physbeh.2016.08.025

Meijer, R. I., Gray, S. M., Aylor, K. W., and Barrett, E. J. (2016). Pathways for insulin access to the brain: the role of the microvascular endothelial cell. *Am. J. Physiol. Heart Circ. Physiol.* 311, H1132–H1138. doi: 10.1152/ajpheart.00081.2016

Mestre, H., Hablitz, L. M., Xavier, A. L., Feng, W., Zou, W., Pu, T., et al. (2018). Aquaporin-4-dependent glymphatic solute transport in the rodent brain. *Elife* 7:e40070. doi: 10.7554/eLife.40070

Mi, H., Haeberle, H., and Barres, B. A. (2001). Induction of astrocyte differentiation by endothelial cells. *J. Neurosci.* 21, 1538–1547. doi: 10.1523/jneurosci.21-05-01538.2001

Miller, F. N., and Sims, D. E. (1986). Contractile elements in the regulation of macromolecular permeability. *Fed. Proc.* 45, 84–88.

Morley, J. E., and Banks, W. A. (2010). Lipids and cognition. *J. Alzheimers Dis.* 20, 737–747. doi: 10.3233/jad-2010-091576

Nation, D. A., Sweeney, M. D., Montagne, A., Sagare, A. P., D'Orazio, L. M., Pachicano, M., et al. (2019). Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat. Med.* 25, 270–276. doi: 10.1038/s41591-018-0297-y

Nedergaard, M. (2013). Neuroscience. garbage truck of the brain. *Science* 340, 1529–1530. doi: 10.1126/science.1240514

Neuwelt, E., Abbott, N. J., Abrey, L., Banks, W. A., Blakley, B., Davis, T., et al. (2008). Strategies to advance translational research into brain barriers. *Lancet. Neurol.* 7, 84–96. doi: 10.1016/s1474-4422(07)70326-5

Nishitsuji, K., Hosono, T., Nakamura, T., Bu, G., and Michikawa, M. (2011). Apolipoprotein E regulates the integrity of tight junctions in an isoform-dependent manner in an in vitro blood-brain barrier model. *J. Biol. Chem.* 286, 17536–17542. doi: 10.1074/jbc.M111.225532

Ofengeim, D., Giagtzoglou, N., Huh, D., Zou, C., and Yuan, J. (2017). Single-Cell RNA sequencing: unraveling the brain one cell at a time. *Trends Mol. Med.* 23, 563–576. doi: 10.1016/j.molmed.2017.04.006

Ouwens, D. M., van Duinkerken, E., Schoonenboom, S. N., Herzfeld de Wiza, D., Klein, M., van Golen, L., et al. (2014). Cerebrospinal fluid levels of Alzheimer's disease biomarkers in middle-aged patients with type 1 diabetes. *Diabetologia* 57, 2208–2214. doi: 10.1007/s00125-014-3333-6

Poduslo, J. F., Curran, G. L., Wengenack, T. M., Malester, B., and Duff, K. (2001). Permeability of proteins at the blood-brain barrier in the normal adult mouse and double transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* 8, 555–567. doi: 10.1006/nbdi.2001.0402

Price, T. O., Eranki, V., Banks, W. A., Ercal, N., and Shah, G. N. (2012). Topiramate treatment protects blood-brain barrier pericytes from hyperglycemia-induced oxidative damage in diabetic mice. *Endocrinology* 153, 362–372. doi: 10.1210/en.2011-1638

Qiu, W. Q., Walsh, D. M., Ye, Z., Vekrellis, K., Zhang, J., Podlisny, M. B., et al. (1998). Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J. Biol. Chem.* 273, 32730–32738. doi: 10.1074/jbc.273.49.32730

Ransohoff, R. M., Schafer, D., Vincent, A., Blachere, N. E., and Bar-Or, A. (2015). Neuroinflammation: ways in which the immune system affects the brain. *Neurotherapeutics* 12, 896–909. doi: 10.1007/s13311-015-0385-3

Reese, T. S., and Karnovsky, M. J. (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J. Cell Biol.* 34, 207–217. doi: 10.1083/jcb.34.1.207

Reger, M. A., Watson, G. S., Green, P. S., Baker, L. D., Cholerton, B., Fishel, M. A., et al. (2008a). Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. *J. Alzheimers Dis.* 13, 323–331. doi: 10.3233/jad-2008-13309

Reger, M. A., Watson, G. S., Green, P. S., Wilkinson, C. W., Baker, L. D., Cholerton, B., et al. (2008b). Intranasal insulin improves cognition and modulates beta-amyloid in early AD. *Neurology* 70, 440–448. doi: 10.1212/01.wnl.0000265401.62434.36

Reichenbach, A., and Wolburg, H. (2005). Astrocytes and ependymal glia. *Neuroglia* 2, 19–35. doi: 10.1093/acprof:oso/9780195152227.003.0002

Rensink, A. A., Otte-Holler, I., de Boer, R., Bosch, R. R., ten Donkelaar, H. J., de Waal, R. M., et al. (2004). Insulin inhibits amyloid beta-induced cell death in cultured human brain pericytes. *Neurobiol. Aging* 25, 93–103. doi: 10.1016/s0197-4580(03)00039-3

Rhea, E. M., Humann, S. R., Nirkhe, S., Farr, S. A., Morley, J. E., and Banks, W. A. (2017). intranasal insulin transport is preserved in aged SAMP8 mice and is altered by albumin and insulin receptor inhibition. *J. Alzheimers Dis.* 57, 241–252. doi: 10.3233/JAD-161095

Rhea, E. M., Rask-Madsen, C., and Banks, W. A. (2018). Insulin transport across the blood-brain barrier can occur independently of the insulin receptor. *J. Physiol.* 596, 4753–4765. doi: 10.1113/JP276149

Rhea, E. M., Salameh, T. S., and Banks, W. A. (2019). Routes for the delivery of insulin to the central nervous system: a comparative review. *Exp. Neurol.* 313, 10–15. doi: 10.1016/j.expneurol.2018.11.007

Rodriguez-Rodriguez, P., Sandebring-Matton, A., Merino-Serrais, P., Parrado-Fernandez, C., Rabano, A., Winblad, B., et al. (2017). Tau hyperphosphorylation induces oligomeric insulin accumulation and insulin resistance in neurons. *Brain* 140, 3269–3285. doi: 10.1093/brain/awx256

Sagare, A. P., Bell, R. D., Zhao, Z., Ma, Q., Winkler, E. A., Ramanathan, A., et al. (2013). Pericyte loss influences Alzheimer-like neurodegeneration in mice. *Nat. Commun.* 4:2932. doi: 10.1038/ncomms3932

Salameh, T. S., Bullock, K. M., Hujoel, I. A., Niehoff, M. L., Wolden-Hanson, T., Kim, J., et al. (2015). Central nervous system delivery of intranasal insulin: mechanisms of uptake and effects on cognition. *J. Alzheimers Dis.* 47, 715–728. doi: 10.3233/JAD-150307

Salameh, T. S., Shah, G. N., Price, T. O., Hayden, M. R., and Banks, W. A. (2016). Blood-Brain barrier disruption and neurovascular unit dysfunction in diabetic mice: protection with the mitochondrial carbonic anhydrase inhibitor topiramate. *J. Pharmacol. Exp. Ther.* 359, 452–459. doi: 10.1124/jpet.116.237057

Savettieri, G., Di Liegro, I., Catania, C., Licata, L., Pitarresi, G. L., D'Agostino, S., et al. (2000). Neurons and ECM regulate occludin localization in brain endothelial cells. *Neuroreport* 11, 1081–1084. doi: 10.1097/00001756-200004070-00035

Schaffer, L., Brand, C. L., Hansen, B. F., Ribel, U., Shaw, A. C., Slaaby, R., et al. (2008). A novel high-affinity peptide antagonist to the insulin receptor. *Biochem. Biophys. Res. Commun.* 376, 380–383. doi: 10.1016/j.bbrc.2008.08.151

Schlageter, K. E., Molnar, P., Lapin, G. D., and Groothuis, D. R. (1999). Microvessel organization and structure in experimental brain tumors: microvessel populations with distinctive structural and functional properties. *Microvasc. Res.* 58, 312–328. doi: 10.1006/mvre.1999.2188

Schlageter, N. L., Carson, R. E., and Rapoport, S. I. (1987). Examination of blood-brain barrier permeability in dementia of the Alzheimer type with [68Ga]EDTA and positron emission tomography. *J. Cereb. Blood Flow Metab.* 7, 1–8. doi: 10.1038/jcbfm.1987.1

Schubert, M., Gautam, D., Surjo, D., Ueki, K., Baudler, S., Schubert, D., et al. (2004). Role for neuronal insulin resistance in neurodegenerative diseases. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3100–3105.

Schulingkamp, R. J., Pagano, T. C., Hung, D., and Raffa, R. B. (2000). Insulin receptors and insulin action in the brain: review and clinical implications. *Neurosci. Biobehav. Rev.* 24, 855–872. doi: 10.1016/s0149-7634(00)00040-3

Schwartz, M. W., Bergman, R. N., Kahn, S. E., Taborsky, G. J Jr., Fisher, L. D., Sipols, A. J., et al. (1991). Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. Quantitative aspects and implications for transport. *J. Clin. Invest.* 88, 1272–1281. doi: 10.1172/jci115431

Sengillo, J. D., Winkler, E. A., Walker, C. T., Sullivan, J. S., Johnson, M., and Zlokovic, B. V. (2013). Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol.* 23, 303–310. doi: 10.1111/bpa.12004

Shepro, D., and Morel, N. M. (1993). Pericyte physiology. *FASEB J.* 7, 1031–1038. doi: 10.1096/fasebj.7.11.8370472

Shiiki, T., Ohtsuki, S., Kurihara, A., Naganuma, H., Nishimura, K., Tachikawa, M., et al. (2004). Brain insulin impairs amyloid-beta(1-40) clearance from the brain. *J. Neurosci.* 24, 9632–9637. doi: 10.1523/jneurosci.2236-04.2004

Simpson, I. A., Appel, N. M., Hokari, M., Oki, J., Holman, G. D., Maher, F., et al. (1999). Blood-brain barrier glucose transporter: effects of hypo- and hyperglycemia revisited. *J. Neurochem.* 72, 238–247. doi: 10.1046/j.1471-4159.1999.0720238.x

Spencer, B., Rank, L., Metcalf, J., and Desplats, P. (2018). Identification of insulin receptor splice variant B in neurons by in situ detection in human brain samples. *Sci. Rep.* 8:4070. doi: 10.1038/s41598-018-22434-2

Stohr, O., Schilbach, K., Moll, L., Hettich, M. M., Freude, S., Wunderlich, F. T., et al. (2013). Insulin receptor signaling mediates APP processing and beta-amyloid accumulation without altering survival in a transgenic mouse model of Alzheimer's disease. *Age* 35, 83–101. doi: 10.1007/s11357-011-9333-2

Sun, Y. N., Liu, L. B., Xue, Y. X., and Wang, P. (2015). Effects of insulin combined with idebenone on blood-brain barrier permeability in diabetic rats. *J. Neurosci. Res.* 93, 666–677. doi: 10.1002/jnr.23511

Swaminathan, S. K., Ahlschwede, K. M., Sarma, V., Curran, G. L., Omtri, R. S., Decklever, T., et al. (2018). Insulin differentially affects the distribution kinetics of amyloid beta 40 and 42 in plasma and brain. *J. Cereb. Blood Flow Metab.* 38, 904–918. doi: 10.1177/0271678X17709709

Sweeney, M. D., Ayyadurai, S., and Zlokovic, B. V. (2016). Pericytes of the neurovascular unit: key functions and signaling pathways. *Nat. Neurosci.* 19, 771–783. doi: 10.1038/nn.4288

Sweeney, M. D., Zhao, Z., Montagne, A., Nelson, A. R., and Zlokovic, B. V. (2019). Blood-Brain barrier: from physiology to disease and back. *Physiol. Rev.* 99, 21–78. doi: 10.1152/physrev.00050.2017

Takadera, T., Sakura, N., Mohri, T., and Hashimoto, T. (1993). Toxic effect of a beta-amyloid peptide (beta 22-35) on the hippocampal neuron and its prevention. *Neurosci. Lett.* 161, 41–44. doi: 10.1016/0304-3940(93)90135-8

Takahashi, H., Takata, F., Matsumoto, J., Machida, T., Yamauchi, A., Dohgu, S., et al. (2015). Brain pericyte-derived soluble factors enhance insulin sensitivity in GT1-7 hypothalamic neurons. *Biochem. Biophys. Res. Commun.* 457, 532–537. doi: 10.1016/j.bbrc.2015.01.016

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., et al. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance. IRS-1 dysregulation, and cognitive decline. *J. Clin. Invest.* 122, 1316–1338. doi: 10.1172/JCI59903

Tietz, S., and Engelhardt, B. (2015). Brain barriers: crosstalk between complex tight junctions and adherens junctions. *J. Cell Biol.* 209, 493–506. doi: 10.1083/jcb.201412147

Unger, J., McNeill, T. H., Moxley, R. T. III, White, M., Moss, A., and Livingston, J. N. (1989). Distribution of insulin receptor-like immunoreactivity in the rat forebrain. *Neuroscience* 31, 143–157. doi: 10.1016/0306-4522(89)90036-5

Urayama, A., and Banks, W. A. (2008). Starvation and triglycerides reverse the obesity-induced impairment of insulin transport at the blood-brain barrier. *Endocrinology* 149, 3592–3597. doi: 10.1210/en.2008-0008

Van Dyken, P., and Lacoste, B. (2018). Impact of metabolic syndrome on neuroinflammation and the blood-brain barrier. *Front. Neurosci.* 12:930. doi: 10.3389/fnins.2018.00930

Vandal, M., Bourassa, P., and Calon, F. (2015). Can insulin signaling pathways be targeted to transport Abeta out of the brain? *Front. Aging Neurosci.* 7:114. doi: 10.3389/fnagi.2015.00114

Vandal, M., White, P. J., Tremblay, C., St-Amour, I., Chevrier, G., Emond, V., et al. (2014). Insulin reverses the high-fat diet-induced increase in brain Abeta and improves memory in an animal model of Alzheimer disease. *Diab. Metab. Res. Rev.* 63, 4291–4301. doi: 10.2337/db14-0375

Varatharaj, A., and Galea, I. (2017). The blood-brain barrier in systemic inflammation. *Brain Behav. Immun.* 60, 1–12. doi: 10.1016/j.bbi.2016.03.010

Veronese, N., Facchini, S., Stubbs, B., Luchini, C., Solmi, M., Manzato, E., et al. (2017). Weight loss is associated with improvements in cognitive function among overweight and obese people: a systematic review and meta-analysis. *Neurosci. Biobehav. Rev.* 72, 87–94. doi: 10.1016/j.neubiorev.2016.11.017

Vicent, D., Ilany, J., Kondo, T., Naruse, K., Fisher, S. J., Kisanuki, Y. Y., et al. (2003). The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J. Clin. Invest.* 111, 1373–1380. doi: 10.1172/jci15211

Warmke, N., Griffin, K. J., and Cubbon, R. M. (2016). Pericytes in diabetes-associated vascular disease. *J. Diab. Comp.* 30, 1643–1650. doi: 10.1016/j.jdiacomp.2016.08.005

Warmke, N., Griffin, K. J., Slater, C. I., Walker, A. M. N., Yuldasheva, N. Y., and Cubbon, R. M. (2017). 186 Reduced pericyte insulin signalling causes abnormal developmental angiogenesis. *Heart* 103, A127–A128.

Watson, G. S., and Craft, S. (2003). The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs* 17, 27–45. doi: 10.2165/00023210-200317010-00003

Winkler, E. A., Nishida, Y., Sagare, A. P., Rege, S. V., Bell, R. D., Perlmutter, D., et al. (2015). GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nat. Neurosci.* 18, 521–530. doi: 10.1038/nn.3966

Wosik, K., Cayrol, R., Dodelet-Devillers, A., Berthelet, F., Bernard, M., Moumdjian, R., et al. (2007). Angiotensin II controls occludin function and is required for blood brain barrier maintenance: relevance to multiple sclerosis. *J. Neurosci.* 27, 9032–9042. doi: 10.1523/jneurosci.2088-07.2007

Wozniak, M., Rydzewski, B., Baker, S. P., and Raizada, M. K. (1993). The cellular and physiological actions of insulin in the central nervous system. *Neurochem. Int.* 22, 1–10. doi: 10.1016/0197-0186(93)90062-a

Xaio, H., Banks, W. A., Niehoff, M. L., and Morley, J. E. (2001). Effect of LPS on the permeability of the blood-brain barrier to insulin. *Brain Res.* 896, 36–42. doi: 10.1016/s0006-8993(00)03247-9

Xie, L., Helmerhorst, E., Taddei, K., Plewright, B., Van Bronswijk, W., and Martins, R. (2002). Alzheimer's beta-amyloid peptides compete for insulin binding to the insulin receptor. *J. Neurosci.* 22:RC221.

Yu, Y., Kastin, A. J., and Pan, W. (2006). Reciprocal interactions of insulin and insulin-like growth factor I in receptor-mediated transport across the blood-brain barrier. *Endocrinology* 147, 2611–2615. doi: 10.1210/en.2006-0020

Zhang, Y., Chen, K., Sloan, S. A., Bennett, M. L., Scholze, A. R., O'Keeffe, S., et al. (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* 34, 11929–11947. doi: 10.1523/JNEUROSCI.1860-14.2014

Zhang, Y., Sloan, S. A., Clarke, L. E., Caneda, C., Plaza, C. A., Blumenthal, P. D., et al. (2016). Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron* 89, 37–53. doi: 10.1016/j.neuron.2015.11.013

Zhao, L., Teter, B., Morihara, T., Lim, G. P., Ambegaokar, S. S., Ubeda, O. J., et al. (2004). Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: implications for Alzheimer's disease intervention. *J. Neurosci.* 24, 11120–11126. doi: 10.1523/jneurosci.2860-04.2004

Zhao, W. Q., De Felice, F. G., Fernandez, S., Chen, H., Lambert, M. P., Quon, M. J., et al. (2008). Amyloid beta oligomers induce impairment of neuronal insulin receptors. *FASEB J.* 22, 246–260. doi: 10.1096/fj.06-7703com

Zhao, W. Q., Lacor, P. N., Chen, H., Lambert, M. P., Quon, M. J., Krafft, G. A., et al. (2009). Insulin receptor dysfunction impairs cellular clearance of neurotoxic oligomeric a{beta}. *J. Biol. Chem.* 284, 18742–18753. doi: 10.1074/jbc.M109.011015

Zlokovic, B. V. (2005). Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* 28, 202–208. doi: 10.1016/j.tins.2005.02.001

Zlokovic, B. V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57, 178–201. doi: 10.1016/j.neuron.2008.01.003

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

MINI REVIEW
published: 18 June 2019
doi: 10.3389/fnins.2019.00629

Altered Insulin Signaling in Alzheimer's Disease Brain – Special Emphasis on PI3K-Akt Pathway

Sami Gabbouj[1], Simo Ryhänen[1], Mikael Marttinen[1], Rebekka Wittrahm[1], Mari Takalo[1], Susanna Kemppainen[1], Henna Martiskainen[1], Heikki Tanila[2], Annakaisa Haapasalo[2], Mikko Hiltunen[1] and Teemu Natunen[1]**

[1] Institute of Biomedicine, University of Eastern Finland, Kuopio, Finland, [2] A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

Alzheimer's disease (AD) and type 2 diabetes (T2D) are both diseases with increasing prevalence in aging populations. T2D, characterized by insulin resistance and defective insulin signaling, is a common co-morbidity and a risk factor for AD, increasing the risk approximately two to fourfold. Insulin exerts a wide variety of effects as a growth factor as well as by regulating glucose, fatty acid, and protein metabolism. Certain lifestyle factors, physical inactivity and typical Western diet (TWD) containing high fat and high sugar are strongly associated with insulin resistance and T2D. The PI3K-Akt signaling pathway is a major mediator of effects of insulin and plays a crucial role in T2D pathogenesis. Decreased levels of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) subunits as well as blunted Akt kinase phosphorylation have been observed in the AD brain, characterized by amyloid-β and tau pathologies. Furthermore, AD mouse models fed with TWD have shown to display altered levels of PI3K subunits. How impaired insulin-PI3K-Akt signaling in peripheral tissues or in the central nervous system (CNS) affects the development or progression of AD is currently poorly understood. Interestingly, enhancement of PI3K-Akt signaling in the CNS by intranasal insulin (IN) treatment has been shown to improve memory *in vivo* in mice and in human trials. Insulin is known to augment neuronal growth and synapse formation through the PI3K-Akt signaling pathway. However, PI3K-Akt pathway mediates signaling related to different functions also in other cell types, like microglia and astrocytes. In this review, we will discuss the most prominent molecular mechanisms related to the PI3K-Akt pathway in AD and how T2D and altered insulin signaling may affect the pathogenesis of AD.

Keywords: Alzheimer's disease, type 2 diabetes, insulin, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), Akt (Protein kinase B, PKB), glucose metabolism, neuroinflammation, autophagy

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Roland Brandt,
University of Osnabrück, Germany
Paola Gamba,
University of Turin, Italy

***Correspondence:**
Mikko Hiltunen
mikko.hiltunen@uef.fi
Teemu Natunen
teemu.natunen@uef.fi

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *27 February 2019*
Accepted: *31 May 2019*
Published: *18 June 2019*

Citation:
Gabbouj S, Ryhänen S, Marttinen M, Wittrahm R, Takalo M, Kemppainen S, Martiskainen H, Tanila H, Haapasalo A, Hiltunen M and Natunen T (2019) Altered Insulin Signaling in Alzheimer's Disease Brain – Special Emphasis on PI3K-Akt Pathway. Front. Neurosci. 13:629. doi: 10.3389/fnins.2019.00629

INTRODUCTION

Both Alzheimer's disease (AD) and type 2 diabetes (T2D) are diseases reaching epidemic proportions. The main neuropathological findings in AD, the most common form of dementia, are β-amyloid plaques, composed of extracellular aggregates of the β-amyloid (Aβ) peptide, and intracellular neurofibrillary tangles (NFT) formed of hyperphosphorylated tau protein (Hardy and Selkoe, 2002). However, there are several other pathological features related to AD, including loss of

synapses and neurons, inflammatory activation of microglia and astrocytes as well as impairment in glucose metabolism and insulin-phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3)-Akt signaling in the brain (Mosconi et al., 2008; Serrano-Pozo et al., 2011; Talbot et al., 2012).

Type 2 diabetes is a complex, age- and lifestyle-related chronic disease. It is characterized by increased glucose and insulin levels in the blood, insulin resistance, metabolic abnormalities, and chronic inflammation (Sjöholm and Nyström, 2006; Ashcroft and Rorsman, 2012). T2D is one of the most important co-morbidities of AD, increasing the risk of AD two to fourfold (Craft, 2007; Sims-Robinson et al., 2010). Lifestyle factors, such as physical inactivity and excess calories gained from the typical Western diet (TWD), play a central role in T2D (Khazrai et al., 2014). Several *in vivo* studies in AD mouse models have shown that TWD induces T2D phenotype and exacerbates memory impairments, which are linked to altered PI3K-Akt signaling in the brain (Kang et al., 2017; Kothari et al., 2017; Salas et al., 2018). Insulin is a crucial factor controlling blood glucose levels and it facilitates cellular glucose uptake in peripheral tissues by activating the PI3K-Akt pathway (Kim and Feldman, 2012). In the brain, insulin does not have a major role in glucose metabolism. However, insulin and the PI3K-Akt signaling pathway play a significant role in neuronal health as well as synapse formation and maintenance (Van Der Heide et al., 2005; Chiu et al., 2008; Lee et al., 2011). Apart from neurons, PI3K-Akt signaling pathway also plays a central role in other cell types in the brain and TWD has been shown to affect, e.g., the function of microglia (Spagnuolo et al., 2015; Spencer et al., 2019).

Imaging studies have shown that T2D is often associated with changes in the brain that are typically detected in patients with AD and related dementias, including decreased hippocampal volume, reduced glucose metabolism, and changes in cerebral blood flow (Baker et al., 2011; Moran et al., 2013; Willette et al., 2015). Furthermore, alterations in the insulin signaling pathway as well as decreased levels of insulin and insulin receptors (IR) have been observed in the AD brain (Steen et al., 2005; Talbot et al., 2012). Similarly, to T2D, other abnormalities such as metabolic stress and inflammation are also characteristic in AD (Moloney et al., 2010; Talbot et al., 2012). However, the relationship between T2D and the main neuropathological finding in AD, cerebral Aβ accumulation, remains unclear. While most of the studies have reported no association, two studies have found a significant correlation between peripheral insulin resistance and brain Aβ levels as measured by Pittsburgh compound B-positron emission tomography (PiB-PET) (Willette et al., 2015; Ekblad et al., 2018). The impaired insulin-PI3K-Akt signaling observed in the AD brain has led to clinical trials studying whether the enhancement of this pathway using intranasal insulin (IN) treatment is beneficial. Intranasally administered insulin reaches the brain via the olfactory and trigeminal nerves (Lochhead et al., 2019). Further, doses up to 40 IU in elderly subjects seem not to induce systemic hypoglycemia (Craft et al., 2012). The majority of results from studies in cognitively healthy humans as well as in AD patients are encouraging, suggesting that IN enhances memory and cognition (reviewed by Chapman et al., 2017). However, the effects appear to depend on the dose and the dose regime (acute vs. repeated), cognitive test used, and the *APOE* genotype. Interestingly, in a preclinical APP/PS1 AD mouse model, IN treatment led to the specific activation of the Akt2 isoform (Gabbouj et al., 2019). This suggests that Akt kinases may have isoform-specific roles in insulin signaling in the brain. Furthermore, the same study revealed differential effects of IN on the expression profile of homeostatic microglia and the markers of autophagy in the hippocampus of WT and APP/PS1 mice.

Despite the strong epidemiological association between AD and T2D, the underlying molecular mechanisms are still not fully understood. It is probable that T2D affects the development and progression of AD and related disorders via several mechanisms, some of which may be directly or indirectly linked to the insulin-PI3K-Akt signaling pathway. The aim in this review article is to summarize and discuss the effects of altered insulin-PI3K-Akt signaling and T2D on the pathogenesis of AD.

GLUCOSE METABOLISM AND INSULIN RESISTANCE IN THE BRAIN

Common findings in T2D are hyperglycemia and insulin resistance, meaning that peripheral tissues do not respond normally to insulin in order to take up glucose from the blood. Insulin resistance can be clinically assessed using, e.g., the homeostatic model assessment for insulin resistance (HOMA-IR) from fasting insulin and glucose levels (Matthews et al., 1985). However, the definition of insulin resistance in the brain is not as clear. While glucose uptake in peripheral tissues is heavily dependent on insulin, glucose uptake in the brain is mainly independent of insulin (Kim and Feldman, 2012). The term "brain insulin resistance" has been used to provide an underlying reason for the glucose hypometabolism observed in the AD brain. However, since insulin does not play a major role in brain glucose metabolism, insulin resistance in the brain is considered as an impairment in the insulin signaling pathway.

Glucose uptake in peripheral tissues is based on the insulin-dependent glucose transporter 4 (GLUT4) (Huang and Czech, 2007). Insulin activates the PI3K-Akt pathway and the activated Akt kinase subsequently phosphorylates Akt substrate 160 kDa (AS160), which recruits GLUT4 to the plasma membrane, allowing glucose to efficiently enter the cell (**Figure 1**). In the brain, endothelial cells and astrocytes, which are part of the blood-brain barrier (BBB), express mainly GLUT1, while the most common glucose transporter in neurons is GLUT3. Both GLUT1 and GLUT3 are insulin-independent. However, it has been shown that insulin-dependent GLUT4 is expressed to some extent in several brain regions, such as hippocampus, cerebellum, and olfactory bulb (Vannucci et al., 1998).

Recently, a rare P50T variant in *AKT2* gene was shown to increase the risk of T2D in humans as well as the fasting levels of insulin on average ∼15% as compared to matched controls (Manning et al., 2017). Interestingly, the carriers of this variant showed decreased glucose uptake in the peripheral tissues, such as skeletal muscle, whereas glucose uptake in the brain was elevated ∼20% upon hyperinsulinemic–euglycemic clamp

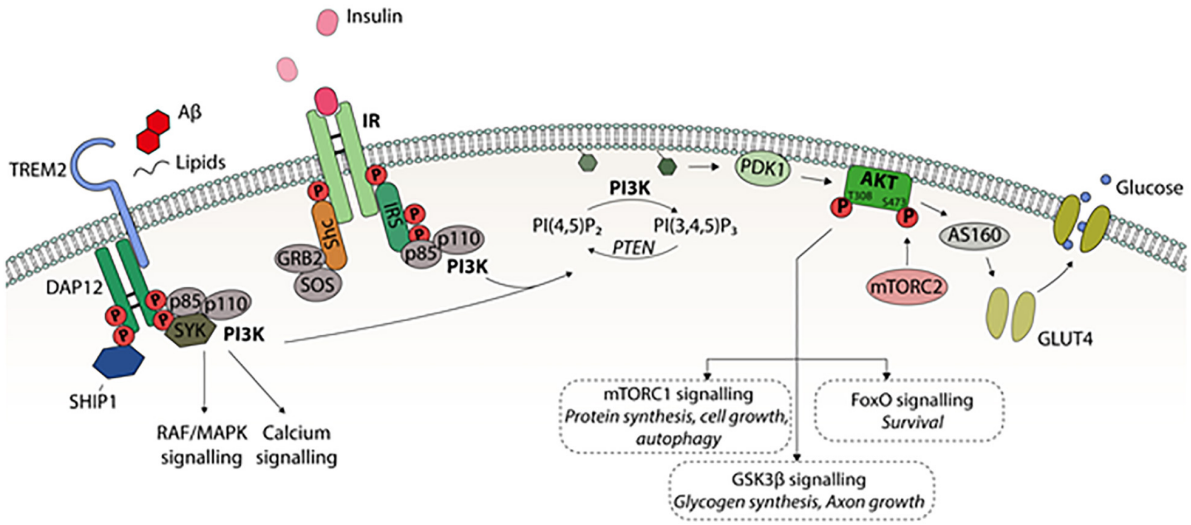

FIGURE 1 | A schematic presentation of PI3K-Akt intracellular signaling. Different extracellular stimuli, e.g., growth factors mediate their effects by receptors belonging to receptor tyrosine kinase (RTK) family locating in the cell membrane. Binding of insulin to IR leads to activating tyrosine phosphorylation of insulin receptor substrate (IRS). This activates phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) consisting of regulatory p85 and catalytic p110 subunits. PI3K converts phosphatidylinositol (3,4)-bisphosphate (PIP_2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), which recruits Akt-kinase to cell membrane. PIP_3 activates phosphoinositide-dependent protein kinase 1 (PDK1) to phosphorylate threonine 308 site in Akt1. For the full activation of Akt, serine 473 phosphorylation by mammalian target for rapamycin complex 2 (mTORC2) is needed. PI3K-Akt pathway regulates several cellular functions via downstream factors; Akt substrate 160 kDa (AS160) controls insulin dependently on glucose transporter 4 (GLUT4) translocation to the cell membrane and glucose uptake into the cell, mTORC1 regulates autophagy, protein synthesis, and cell growth, glycogen synthase kinase 3 (GSK3) affects glycogen synthesis, axon growth, and tau phosphorylation, and forkhead box (FOX) transcription factors regulate many functions, such as cell survival. In microglia, cell surface receptor TREM2 signaling regulates the phagocytosis, motility, autophagy, survival, and proliferation. TREM2 is activated by several ligands including phospholipids, lipoproteins, and oligomeric Aβ leading to interaction with activating adaptor protein DAP12. This in turn, leads to activation of PI3K-Akt pathway.

(Latva-Rasku et al., 2018). Given these observations, further studies are needed to uncover whether this genetic variation affects cognitive performance at the higher age or the risk of dementia.

INSULIN-PI3K-AKT SIGNALING IN THE BRAIN

Insulin, a hormone produced in the pancreas, has a wide variety of functions. Of these, the best known is the regulation of glucose uptake into peripheral tissues, such as skeletal muscle, liver, and adipose tissue (Dimitriadis et al., 2011). Insulin is able to penetrate the BBB via a saturable transport system (Banks et al., 2012), meaning that increased serum insulin levels elevate those of brain insulin only to a certain level.

Insulin transport varies between different brain regions. The olfactory bulb is reported to have the highest rate of insulin transport, probably due to having the highest concentration of IRs (Hill et al., 1986; Gupta et al., 1992). In general, IRs are more abundantly expressed in neurons as compared to other cell types in the brain (Frölich et al., 1998). IRs have been observed in all compartments of neurons, particularly in dendrites and presynaptic terminals, emphasizing their role in dendritic growth and synapse formation (Abbott et al., 1999; Lee et al., 2011).

The binding of insulin to the IR can activate two distinct branches of insulin signaling: the Ras-mitogen-activated protein kinase (MAPK) and PI3K-Akt pathways (**Figure 1**; Kim and Feldman, 2012). The IR and the insulin-like growth factor 1 (IGF1) receptor (IGF1R) are the major activators of PI3K. IR and IGF1R belong to the receptor tyrosine kinase (RTK) family, which includes receptors for many growth factors, such as vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) (Lemmon and Schlessinger, 2010). Furthermore, the different RTKs are able to activate the same intracellular PI3K-Akt pathway as insulin. The binding of insulin causes a conformational change in the IR, inducing autophosphorylation followed by the recruitment of intracellular insulin receptor substrate (IRS) proteins, which are subsequently tyrosine-phosphorylated. While the phosphorylation of specific tyrosine residues activates IRS, there are serine phosphorylation sites that inactivate IRSs by causing their dissociation from the IR and decreasing tyrosine phosphorylation (Copps and White, 2012). Tyrosine-phosphorylated IRS activates PI3Ks, a kinase family involved in several intracellular signaling transduction processes.

PI3K is a heteromeric protein consisting of a p110 catalytic subunit and a p85 regulatory subunit (**Figure 1**). The SH2 domain of the PI3K p85 subunit binds to the phosphotyrosines in the cytosolic domain of the plasma membrane-resident RTKs. P110 converts phosphatidylinositol (3,4)-bisphosphate (PIP_2) into phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), leading to the activation of numerous downstream kinases, such as Akt (Boura-Halfon and Zick, 2009).

Akt is a family of serine/threonine kinases consisting of three isoforms: Akt1, Akt2, and Akt3. These isoforms exhibit different expression patterns depending on the brain region and cell type. For example, Akt1 and Akt3 are distributed throughout the somatic layers of the hippocampus, while Akt2 is mostly expressed in astrocytes but not in neurons of the hippocampus (Levenga et al., 2017). Once activated, Akt regulates cell survival, proliferation, cytoskeletal organization, cell metabolism, vesicle trafficking, and glucose transport (Noguchi and Suizu, 2012). Akt is activated upon its interaction with the pleckstrin homology (PH)-domain of PIP_3 allowing phosphoinositide-dependent protein kinase 1 (PDK1) to phosphorylate threonine 308/309/305 of Akt1/2/3, respectively, at the plasma membrane. Full activation of Akt also requires the phosphorylation of serine 473/474/472 of Akt1/2/3, respectively. The kinase responsible for the serine phosphorylation of Akt is mammalian target for rapamycin complex 2 (mTORC2), although the exact mechanism of this mTORC2-mediated activation is still unclear (Noguchi and Suizu, 2012). PI3K/Akt regulates downstream factors, such as glycogen synthase kinase 3 (GSK3), mTORC1, and forkhead box (FOX) transcription factors, affecting a plethora of cellular functions in peripheral tissues and in the brain (**Figure 1**; Kim and Feldman, 2012).

ALTERATIONS OF PI3K-AKT SIGNALING IN THE AD BRAIN

In the AD brain, alterations in the PI3K-Akt pathway primarily manifest as decreased phosphorylation or total levels of the components in the insulin-PI3K-Akt signaling cascade (Steen et al., 2005; Liu et al., 2011). Previous studies have found that Aβ oligomers inhibit the PI3K-Akt pathway, which leads to neuronal death. Post-mortem analysis of different AD brain regions has revealed reduced levels of insulin, IR, IGF1, and IGF1R (Steen et al., 2005; Liu et al., 2011). In addition, the analysis of post-mortem AD brain samples showed decreased levels of PI3K subunits (both p85 and p110) and reduced phosphorylation of Akt and GSK3β (Steen et al., 2005; Moloney et al., 2010). Interestingly, these changes were associated with several important pathological hallmarks of AD, such as the NFT pathology as well as microglial and astroglial markers (Rivera et al., 2005). Progression of NFT pathology in AD brain from one brain region to another during the disease course exhibits a certain chronological pattern, which is defined by Braak staging and correlates relatively well with clinical dementia symptoms (Braak et al., 2006). GSK3β is one of the most important tau-phosphorylating kinases (Wilson et al., 2013). PI3K-Akt signaling regulates GSK3β by phosphorylating the serine 9 residue, which inhibits GSK3β activity. In cultured neurons, insulin and IGF1 have been shown to decrease tau phosphorylation through Akt-mediated GSK3β inhibition (Hong and Lee, 1997). Talbot et al. (2012) subjected hippocampal tissue from normal post-mortem brains and from AD brains to *ex vivo* insulin stimulation with physiological doses. The normal tissue responded strongly to insulin as measured by the enhanced phosphorylation of IRS-1, Akt, GSK3α, and GSK3β. In contrast, the AD hippocampal tissue demonstrated drastically reduced insulin-mediated downstream activation (Talbot et al., 2012). Interestingly, two separate studies showed abnormal basal phosphorylation levels of proteins in the insulin-IRS-1-Akt pathway in post-mortem AD brains. Furthermore, these changes correlated positively with Aβ and tau lesions and negatively with memory and global cognition scores. Intriguingly, hippocampal insulin resistance contributed to the presence of Aβ and tau lesions independently of cognitive impairment (Bomfim et al., 2012; Talbot et al., 2012).

Disturbances in autophagy play a significant role in many neurodegenerative diseases, including AD, which is characterized by the accumulation of toxic intracellular protein aggregates (Son et al., 2012). mTOR, a key regulator of autophagy induction, is a central protein in two complexes, mTORC1 and mTORC2, which are both important downstream factors in the PI3K-Akt signaling pathway. As mentioned above, mTORC2 activates Akt by phosphorylating it at serine 473, while Akt activates mTORC1. In turn, active mTORC1 inhibits the induction of autophagy and promotes protein synthesis in neurons (Stoica et al., 2011), making mTORC1 a crucial factor regulating the balance between autophagy and protein synthesis. Different stimuli, such as insulin, IGFs, growth factors, and amino acids activate the PI3K-Akt-mTORC1 pathway and inhibit autophagy, while e.g., starvation inactivates this pathway, leading to increased autophagy. Constitutive autophagy is considered highly efficient in healthy neurons. Neurons in the AD brain display large amounts of autophagosomal vesicles (Boland et al., 2008) but it is not currently clear whether this results from decreased activation mTORC1, leading to increased autophagosome formation, or from defects in the later steps of autophagy, such as impairment in the clearance of autophagosomes through lysosomes (Boland et al., 2008).

Neuroinflammation and PI3K-Akt Signaling in AD Brain

Chronic, low-grade inflammation is one of the main features observed in both T2D (Calle and Fernandez, 2012) and AD as well as other neurodegenerative diseases (Heneka et al., 2015). Elevated levels of inflammatory markers, such as tumor necrosis factor alpha (TNFα) and other cytokines, have been observed in brain and in blood samples of AD patients (Perry et al., 2010; Swardfager et al., 2010) and in peripheral tissues of subjects with T2D (Sjöholm and Nyström, 2006). TNFα plays a crucial role in peripheral insulin resistance. It activates c-Jun kinase (JNK), which leads to inhibitory serine phosphorylation of IRS and blockade of insulin signaling (Gregor and Hotamisligil, 2011). Aβ oligomers have also been shown to activate JNK, leading to inhibitory phosphorylation of IRS in hippocampal neurons of Aβ plaque producing transgenic mice (Bomfim et al., 2012). Importantly, this finding was also confirmed in post-mortem AD brains (Bomfim et al., 2012).

Microglia, the resident immune cells in the CNS, are responsible for neuroinflammation. High insulin levels promote inflammatory responses in the brain, based on increased TNFα, interleukin 1β and 6 (IL1β and IL6) levels observed in the CSF of healthy individuals after an acute dose of insulin

(Craft, 2005; Fishel et al., 2005). In addition to the secretion of these pro-inflammatory cytokines, the same study showed that hyperinsulinemia increased Aβ levels in the plasma, suggesting that hyperinsulinemia can exacerbate neuroinflammation and provoke AD pathogenesis (Fishel et al., 2005). This may be explained by the competition of insulin and Aβ for degradation by the same enzyme, insulin degrading enzyme (Zhao, 2004).

There are two extremes in the spectrum of the classical activation status of microglia: M1 and M2. M2 represents the anti-inflammatory phenotype, characterized by the secretion of anti-inflammatory cytokines, such as IL10, while M1 is pro-inflammatory (Tang and Le, 2016). Microglia adopt the M1 phenotype when Toll-like receptor 4 (TLR4) is activated by ligands, e.g., lipopolysaccharide. This leads to activation and secretion of high levels of pro-inflammatory cytokines, such as TNFα, IL1β, IL6, and nitric oxide (NO). Activation of TLR4 triggers the PI3K-Akt-mTORC1 pathway, which in turn regulates nuclear factor-kappa B (NFκB), which controls transcription, cytokine production and cell survival in immune cells (Fang et al., 2017).

Secretion of inflammatory mediators TNFα, IL1α and complement component 1q (C1q), by activated microglia leads to inflammatory responses also in astrocytes and to transition from A2 to the neurotoxic A1 phenotype in astrocytes (Liddelow et al., 2017). Reactive A1 astrocytes lose their ability to support neuronal outgrowth and synaptogenesis leading to death of neurons. Interestingly, transition to A1 phenotype can be rescued by upregulating PI3K-Akt pathway (Xu et al., 2018).

Importantly, a recent study utilizing a single cell RNA sequencing technology revealed a novel disease-associated microglia (DAM) phenotype in amyloid plaque producing transgenic mice (Keren-Shaul et al., 2017). DAMs are a subset of microglia occurring also in other neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), and they co-exist with Aβ plaques in AD (Keren-Shaul et al., 2017). DAM development is a two-step process where Trem2-PI3K-Akt pathway plays a central role. Stage 1 DAM transition is Trem2-independent, and factors driving this step are currently unknown.

At stage 1, there is a significant downregulation of homeostatic microglia genes, including *Cx3cr1, P2ry12,* and simultaneously increased expression of Trem2 regulators/adaptors Dap12 (Tyrobp) and Apoe. Interestingly, the levels of *Pik3r1* (p85) were decreased in microglia upon the transition from homeostatic to DAM phenotype (Keren-Shaul et al., 2017). Proceeding to stage 2 is Trem2-dependent and it is characterized by the elevated expression of certain genes such as *Trem2, Lpl, Cst7,* and *Clec7a*, which are involved in lysosomal, phagocytic and lipid metabolism

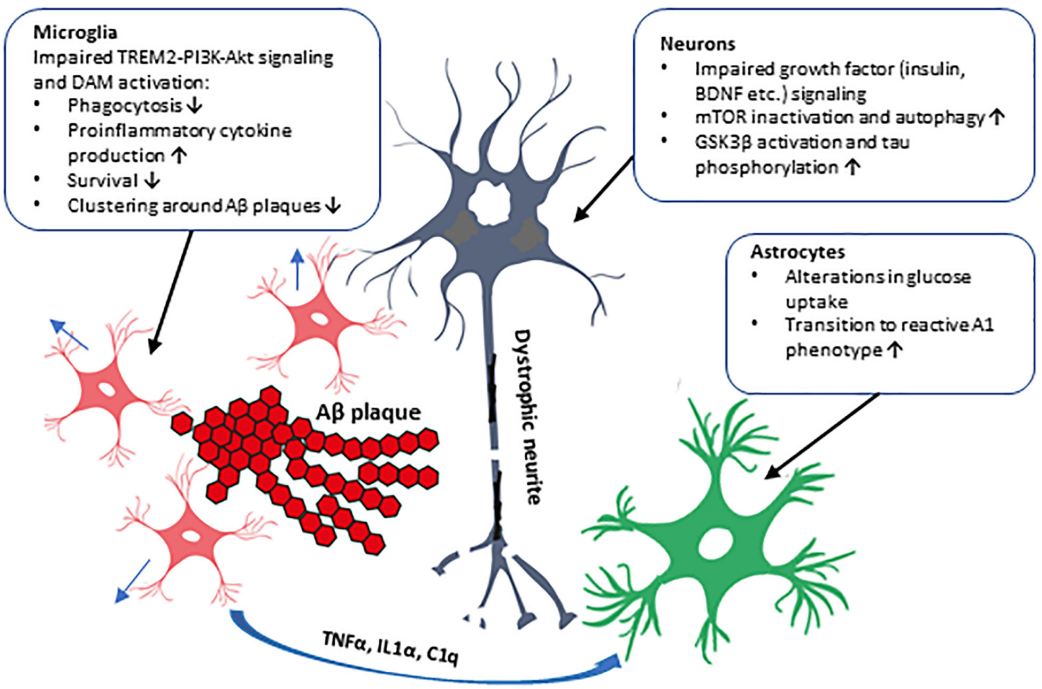

FIGURE 2 | Schematic illustration of the cell type-specific effects of impaired PI3K-Akt signaling in AD brain. The impaired PI3K-Akt signaling might be due to decreased PI3K subunit levels observed in the brain of AD patients or due to T2D, which alters insulin levels and affects PI3K-Akt pathway in the brain, augmenting AD pathogenesis. These alterations could have distinct effects in different cell types in the brain, as shown in the figure. Activation of PI3K-Akt signaling pathway by several extracellular stimuli, such as growth factors (insulin, BDNF, etc.), affects proliferation, metabolism, and survival in various cell types. In neurons, GSK3β activity and tau phosphorylation and mTOR activity affecting autophagy are influenced. Microglial transit to DAM phenotype, phagocytosis, and cytokine production, and formation of microglia clusters around Aβ plaques are affected. Inflammatory cytokines secreted by microglia activate astrocytes, which transit to the reactive A1 phenotype unable to support neuronal outgrowth and synaptogenesis. Furthermore, genetic variation in *AKT2* gene alters glucose uptake, probably in astrocytes, potentially affecting brain energy metabolism.

pathways (Keren-Shaul et al., 2017; Deczkowska et al., 2018). In addition to enhanced phagocytic activity, production of proinflammatory cytokines is suppressed in microglia with the DAM phenotype (Ma et al., 2015).

TREM2 activation leads to DAP12 phosphorylation via Src family kinases, initiating the downstream signaling cascades including PI3K, PKC, and ERK. The PI3K-Akt signaling pathway has been shown to be downstream of Trem2-mediated signaling in microglia (**Figure 1**), since siRNA-mediated knockdown of Trem2 in microglia leads to decreased serine 473 phosphorylation of Akt and consequently decreased phosphorylation of GSK3β at serine 9 (Zheng et al., 2017). Additionally, it was recently shown that defective Trem2 signaling in microglia of Aβ plaque producing transgenic mice resulted in impaired Akt-mTORC1 signaling with simultaneous activation of AMP activated protein kinase. This led to the accumulation of autophagosomes, metabolic impairment, and further, decreased ability of microglia to form clusters around Aβ-plaques and increased formation of dystrophic neurites (Ulland et al., 2017). Thus, TREM2-DAP12 signaling utilizes at least partially the same intracellular PI3K-Akt signaling as insulin to induce its downstream effects.

CONCLUDING REMARKS

The insulin-PI3K-Akt signaling pathway plays an important role in a variety of physiological functions in the brain, such as metabolism, synapse formation, and cell growth and survival. Results from epidemiological, clinical, and animal model-based studies have already established a strong association between T2D and AD, and alteration in PI3K-Akt signaling is the common denominator in these diseases. PI3K subunit (p85 and p110) levels are decreased in AD brain which might have versatile effects in different cell types (**Figure 2**). Insulin regulates cell growth, apoptosis, autophagy, and protein synthesis in the brain, but plays a minor role there in the control of glucose uptake. An interesting, recent finding revealed that P50T genetic variation in *AKT2* gene, which leads to insulin resistance and hyperinsulinemia in the periphery, increased the glucose uptake in the brain as assessed by [^{18}F]-FDG PET imaging (Latva-Rasku et al., 2018). This suggests that specific genetic alterations may exert differential, and also perhaps cell type-specific, functional outcomes in terms of glucose uptake and metabolism. Consistent with this idea, it was recently demonstrated that the signal in [^{18}F]-FDG PET imaging does not only represent neuronal glucose uptake but is also strongly affected by the activation of astrocytes (Zimmer et al., 2017). These findings emphasize the role of different cell types and diverse molecular mechanisms underlying the glucose uptake in the brain, but further studies are needed to determine how these novel observations linked to brain glucose uptake and metabolism may mechanistically affect cellular processes relevant for AD pathogenesis. Furthermore, recent findings related to neuroinflammation and TREM2-signaling further underscore the seminal role of the PI3K-Akt pathway in microglia in the context of AD-related pathogenesis. The levels of PI3K subunits p85 and p110 have been shown to be decreased in AD brain (Moloney et al., 2010), which is particularly interesting given that the microglial expression of p85 decreases upon the transition from homeostatic to DAM phenotype (Keren-Shaul et al., 2017). Also, the deficiency of TREM2 in microglia has been shown to impair Akt-mTOR signaling and hence affect autophagy and energy metabolism as well as decrease the ability of microglia to form clusters around Aβ-plaques leading to increased formation of dystrophic neurites (Ulland et al., 2017). These results raise the question whether T2D or life-style factors could affect TREM2-PI3K-Akt signaling and thus the function and activity of microglia.

IN has been shown to activate PI3K-Akt signaling in the brain and to have beneficial effects in individuals with cognitive impairment (Mao et al., 2016; Chapman et al., 2017). IN was also shown to differentially alter the expression of homeostatic microglia markers in AD mice as compared to wild-type mice, suggesting that IN affects the function and activity of microglia depending on the disease status (Gabbouj et al., 2019). Collectively, these genetic and functional findings reinforce the idea that PI3K-Akt signaling cascade in glial cells encompasses a central role in different cellular processes affecting AD pathogenesis beyond its conventional functions in glucose uptake and metabolism. Thus, unraveling the mechanisms in the PI3K-Akt signaling pathway related to altered glial cell function in AD may eventually provide much-needed novel therapeutic targets and treatment strategies for neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

SG, AH, MM, MH, and TN designed and outlined the structure and contents of the review. All authors contributed to the literature review, discussion, and writing of the manuscript.

FUNDING

This work was supported by the Academy of Finland (Grant Nos. 288659, 307866, and 315459), Sigrid Jusélius Foundation, Yrjö Jahnsson Foundation (Grant No. 20187070), the Strategic Neuroscience Funding of the University of Eastern Finland, and the Neurocenter Finland – AlzTrans pilot project.

REFERENCES

Abbott, M. A., Wells, D. G., and Fallon, J. R. (1999). The insulin receptor tyrosine kinase substrate p58/53 and the insulin receptor are components of CNS synapses. *J. Neurosci.* 19, 7300–7308. doi: 10.1523/jneurosci.4306-08.2009

Ashcroft, F. M., and Rorsman, P. (2012). Diabetes mellitus and the β cell: the last ten years. *Cell* 148, 1160–1171. doi: 10.1016/j.cell.2012.02.010

Baker, L. D., Cross, D. J., Minoshima, S., Belongia, D., Watson, G. S., and Craft, S. (2011). Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch. Neurol.* 68, 51–57. doi: 10.1001/archneurol.2010.225

Banks, W. A., Owen, J. B., and Erickson, M. A. (2012). Insulin in the brain: there and back again. *Pharmacol. Ther.* 136, 82–93. doi: 10.1016/j.pharmthera.2012.07.006

Boland, B., Kumar, A., Lee, S., Platt, F. M., Wegiel, J., Yu, W. H., et al. (2008). Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in alzheimer's disease. *J. Neurosci.* 28, 6926–6937. doi: 10.1523/JNEUROSCI.0800-08.2008

Bomfim, T. R., Forny-Germano, L., Sathler, L. B., Brito-Moreira, J., Houzel, J. C., Decker, H., et al. (2012). An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease-associated Aβ oligomers. *J. Clin. Invest.* 122, 1339–1353. doi: 10.1172/JCI57256

Boura-Halfon, S., and Zick, Y. (2009). Phosphorylation of IRS proteins, insulin action, and insulin resistance. *AJP Endocrinol. Metab.* 296, E581–E591. doi: 10.1152/ajpendo.90437.2008

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., and Tredici, K. (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 112, 389–404. doi: 10.1007/s00401-006-0127-z

Calle, M. C., and Fernandez, M. L. (2012). Inflammation and type 2 diabetes. *Diabetes Metab.* 38, 183–191. doi: 10.1016/j.diabet.2011.11.006

Chapman, C. D., Schiöth, H. B., Grillo, C. A., and Benedict, C. (2017). Intranasal insulin in Alzheimer's disease: food for thought. *Neuropharmacology* 136, 196–201. doi: 10.1016/J.NEUROPHARM.2017.11.037

Chiu, S.-L., Chen, C.-M., and Cline, H. T. (2008). Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo. *Neuron* 58, 708–719. doi: 10.1016/j.neuron.2008.04.014

Copps, K. D., and White, M. F. (2012). Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* 55, 2565–2582. doi: 10.1007/s00125-012-2644-2648

Craft, S. (2005). Insulin resistance syndrome and Alzheimer's disease: age- and obesity-related effects on memory, amyloid, and inflammation. *Neurobiol. Aging* 26, 65–69. doi: 10.1016/j.neurobiolaging.2005.08.021

Craft, S. (2007). Insulin resistance and Alzheimer's disease pathogenesis: potential mechanisms and implications for treatment. *Curr. Alzheimer Res.* 4, 147–152. doi: 10.2174/156720507780362137

Craft, S., Baker, L. D., Montine, T. J., Minoshima, S., Watson, G. S., Claxton, A., et al. (2012). Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment: a pilot clinical trial. *Arch. Neurol.* 69, 29–38. doi: 10.1001/archneurol.2011.233

Deczkowska, A., Keren-Shaul, H., Weiner, A., Colonna, M., Schwartz, M., and Amit, I. (2018). Disease-associated microglia: a universal immune sensor of neurodegeneration. *Cell* 173, 1073–1081. doi: 10.1016/j.cell.2018.05.003

Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., and Raptis, S. A. (2011). Insulin effects in muscle and adipose tissue. *Diabetes Res. Clin. Pract.* 93, S52–S59. doi: 10.1016/S0168-8227(11)70014-70016

Ekblad, L. L., Johansson, J., Helin, S., Viitanen, M., Laine, H., Puukka, P., et al. (2018). Midlife insulin resistance. APOE genotype, and late-life brain amyloid accumulation. *Neurology* 90, e1150–e1157. doi: 10.1212/WNL.0000000000005214

Fang, W., Bi, D., Zheng, R., Cai, N., Xu, H., Zhou, R., et al. (2017). Identification and activation of TLR4-mediated signalling pathways by alginate-derived guluronate oligosaccharide in RAW264.7 macrophages. *Sci. Rep.* 7:1663. doi: 10.1038/s41598-017-01868-0

Fishel, M. A., Watson, G. S., Montine, T. J., Wang, Q., Green, P. S., Kulstad, J. J., et al. (2005). Hyperinsulinemia provokes synchronous increases in central inflammation and β-amyloid in normal adults. *Arch. Neurol.* 62, 1539–1544. doi: 10.1001/archneur.62.10.noc50112

Frölich, L., Blum-Degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., et al. (1998). Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J. Neural. Transm.* 105, 423–438. doi: 10.1007/s007020050068

Gabbouj, S., Natunen, T., Koivisto, H., Jokivarsi, K., Takalo, M., Marttinen, M., et al. (2019). Intranasal insulin activates Akt2 signaling pathway in the hippocampus of wild-type but not in APP/PS1 Alzheimer model mice. *Neurobiol. Aging* 75, 98–108. doi: 10.1016/j.neurobiolaging.2018.11.008

Gregor, M. F., and Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annu. Rev. Immunol.* 29, 415–445. doi: 10.1146/annurev-immunol-031210-101322

Gupta, G., Azam, M., and Baquer, N. Z. (1992). Modulation of rat brain insulin receptor kinase activity in diabetes. *Neurochem. Int.* 20, 487–492. doi: 10.1016/0197-0186(92)90027-O

Hardy, J., and Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353–356. doi: 10.1126/science.1072994

Heneka, M. T., Golenbock, D. T., and Latz, E. (2015). Innate immunity in Alzheimer's disease. *Nat. Immunol.* 16, 229–236. doi: 10.1038/ni.3102

Hill, J. M., Lesniak, M. A., Pert, C. B., and Roth, J. (1986). Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience* 17, 1127–1138. doi: 10.1016/0306-4522(86)90082-90085

Hong, M., and Lee, V. M. Y. (1997). Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. *J. Biol. Chem.* 272, 19547–19553. doi: 10.1074/jbc.272.31.19547

Huang, S., and Czech, M. P. (2007). The GLUT4 glucose transporter. *Cell. Metab.* 5, 237–252. doi: 10.1016/j.cmet.2007.03.006

Kang, S., Kim, C. H., Jung, H., Kim, E., Song, H. T., and Lee, J. E. (2017). Agmatine ameliorates type 2 diabetes induced-Alzheimer's disease-like alterations in high-fat diet-fed mice via reactivation of blunted insulin signalling. *Neuropharmacology* 113, 467–479. doi: 10.1016/j.neuropharm.2016.10.029

Keren-Shaul, H., Spinrad, A., Weiner, A., Matcovitch-Natan, O., Dvir-Szternfeld, R., Ulland, T. K., et al. (2017). A unique microglia type associated with restricting development of alzheimer's disease. *Cell* 169, 1276.e–1290.e. doi: 10.1016/j.cell.2017.05.018

Khazrai, Y. M., Defeudis, G., and Pozzilli, P. (2014). Effect of diet on type 2 diabetes mellitus: a review. *Diabetes Metab. Res. Rev.* 30, 24–33. doi: 10.1002/dmrr.2515

Kim, B., and Feldman, E. L. (2012). Insulin resistance in the nervous system. *Trends Endocrinol. Metab.* 23, 133–141. doi: 10.1016/j.tem.2011.12.004

Kothari, V., Luo, Y., Tornabene, T., O'Neill, A. M., Greene, M. W., Geetha, T., et al. (2017). High fat diet induces brain insulin resistance and cognitive impairment in mice. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863, 499–508. doi: 10.1016/j.bbadis.2016.10.006

Latva-Rasku, A., Honka, M. J., Stancáková, A., Koistinen, H. A., Kuusisto, J., Guan, L., et al. (2018). A partial loss-of-function variant in AKT2 is associated with reduced insulin-mediated glucose uptake in multiple insulin-sensitive tissues: a genotype-based callback positron emission tomography study. *Diabetes* 67, 334–342. doi: 10.2337/db17-1142

Lee, C.-C., Huang, C.-C., and Hsu, K.-S. (2011). Insulin promotes dendritic spine and synapse formation by the PI3K/Akt/mTOR and Rac1 signaling pathways. *Neuropharmacology* 61, 867–879. doi: 10.1016/J.NEUROPHARM.2011.06.003

Lemmon, M. A., and Schlessinger, J. (2010). Cell signaling by receptor tyrosine kinases. *Cell* 141, 1117–1134. doi: 10.1016/j.cell.2010.06.011

Levenga, J., Wong, H., Milstead, R. A., Keller, B. N., Laplante, L. E., and Hoeffer, C. A. (2017). AKT isoforms have distinct hippocampal expression and roles in synaptic plasticity. *Elife* 6:e30640. doi: 10.7554/eLife.30640

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 541, 481–487. doi: 10.1038/nature21029

Liu, Y., Liu, F., Grundke-Iqbal, I., Iqbal, K., and Gong, C.-X. (2011). Deficient brain insulin signalling pathway in Alzheimer's disease and diabetes. *J. Pathol.* 225, 54–62. doi: 10.1002/path.2912

Lochhead, J. J., Kellohen, K. L., Ronaldson, P. T., and Davis, T. P. (2019). Distribution of insulin in trigeminal nerve and brain after intranasal administration. *Sci. Rep.* 9:2621. doi: 10.1038/s41598-019-39191-39195

Ma, J., Jiang, T., Tan, L., and Yu, J. T. (2015). TYROBP in Alzheimer's Disease. *Mol. Neurobiol.* 51, 820–826. doi: 10.1007/s12035-014-8811-8819

Manning, A., Highland, H. M., Gasser, J., Sim, X., Tukiainen, T., Fontanillas, P., et al. (2017). A low-frequency inactivating AKT2 variant enriched in the finnish population is associated with fasting insulin levels and type 2 diabetes risk. *Diabetes* 66, 2019–2032. doi: 10.2337/db16-1329

Mao, Y.-F., Guo, Z., Zheng, T., Jiang, Y., Yan, Y., Yin, X., et al. (2016). Intranasal insulin alleviates cognitive deficits and amyloid pathology in young adult APPswe/PS1dE9 mice. *Aging Cell* 15, 893–902. doi: 10.1111/acel.12498

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28, 412–419. doi: 10.1007/BF00280883

Moloney, A. M., Griffin, R. J., Timmons, S., O'Connor, R., Ravid, R., and O'Neill, C. (2010). Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiol. Aging* 31, 224–243. doi: 10.1016/j.neurobiolaging.2008.04.002

Moran, C., Phan, T. G., Chen, J., Blizzard, L., Beare, R., Venn, A., et al. (2013). Brain atrophy in type 2 diabetes: regional distribution and influence on cognition. *Diabetes Care* 36, 4036–4042. doi: 10.2337/dc13-0143

Mosconi, L., De Santi, S., Li, J., Tsui, W. H., Li, Y., Boppana, M., et al. (2008). Hippocampal hypometabolism predicts cognitive decline from normal aging. *Neurobiol. Aging* 29, 676–692. doi: 10.1016/j.neurobiolaging.2006.12.008

Noguchi, M., and Suizu, F. (2012). "Regulation of AKT by phosphorylation of distinct threonine and serine residues," in *Advances in Medicine and Biology*, ed. L. V. Berhardt (New York, NY: Nova Science Publishers), 139–162.

Perry, V. H., Nicoll, J. A. R., and Holmes, C. (2010). Microglia in neurodegenerative disease. *Nat. Rev. Neurol.* 6, 193–201. doi: 10.1038/nrneurol.2010.17

Rivera, E. J., Goldin, A., Fulmer, N., Tavares, R., Wands, J. R., and de la Monte, S. M. (2005). Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. *J. Alzheimer's Dis.* 8, 247–268. doi: 10.3233/JAD-2005-8304

Salas, I. H., Weerasekera, A., Ahmed, T., Callaerts-Vegh, Z., Himmelreich, U., D'Hooge, R., et al. (2018). High fat diet treatment impairs hippocampal long-term potentiation without alterations of the core neuropathological features of Alzheimer disease. *Neurobiol. Dis.* 113, 82–96. doi: 10.1016/j.nbd.2018.02.001

Serrano-Pozo, A., Frosch, M. P., Masliah, E., and Hyman, B. T. (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* 1:a006189. doi: 10.1101/cshperspect.a006189

Sims-Robinson, C., Kim, B., Rosko, A., and Feldman, E. L. (2010). How does diabetes accelerate Alzheimer disease pathology? *Nat. Rev. Neurol.* 6, 551–559. doi: 10.1038/nrneurol.2010.130

Sjöholm, Å, and Nyström, T. (2006). Inflammation and the etiology of type 2 diabetes. *Diabetes Metab. Res. Rev.* 22, 4–10. doi: 10.1002/dmrr.568

Son, J. H., Shim, J. H., Kim, K. H., Ha, J. Y., and Han, J. Y. (2012). Neuronal autophagy and neurodegenerative diseases. *Exp. Mol. Med.* 44, 89–98. doi: 10.1111/ejed.12265

Spagnuolo, M. S., Mollica, M. P., Maresca, B., Cavaliere, G., Cefaliello, C., Trinchese, G., et al. (2015). high fat diet and inflammation – modulation of haptoglobin level in rat brain. *Front Cell Neurosci.* 9:479. doi: 10.3389/fncel.2015.00479

Spencer, S. J., Basri, B., Sominsky, L., Soch, A., Ayala, M. T., Reineck, P., et al. (2019). High-fat diet worsens the impact of aging on microglial function and morphology in a region-specific manner. *Neurobiol. Aging* 74, 121–134. doi: 10.1016/j.neurobiolaging.2018.10.018

Steen, E., Terry, B. M., Rivera, E. J., Cannon, J. L., Neely, T. R., Tavares, R., et al. (2005). Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease - Is this type 3 diabetes? *J. Alzheimer's Dis.* 7, 63–80. doi: 10.3233/JAD-2005-7107

Stoica, L., Zhu, P. J., Huang, W., Zhou, H., Kozma, S. C., and Costa-Mattioli, M. (2011). Selective pharmacogenetic inhibition of mammalian target of rapamycin complex I (mTORC1) blocks long-term synaptic plasticity and memory storage. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3791–3796. doi: 10.1073/pnas.1014715108

Swardfager, W., Lanctt, K., Rothenburg, L., Wong, A., Cappell, J., and Herrmann, N. (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biol. Psychiatry* 68, 930–941. doi: 10.1016/j.biopsych.2010.06.012

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., et al. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J. Clin. Invest.* 122, 1316–1338. doi: 10.1172/JCI59903

Tang, Y., and Le, W. (2016). Differential roles of M1 and M2 microglia in neurodegenerative diseases. *Mol. Neurobiol.* 53, 1181–1194. doi: 10.1007/s12035-014-9070-5

Ulland, T. K., Song, W. M., Huang, S. C. C., Ulrich, J. D., Sergushichev, A., Beatty, W. L., et al. (2017). TREM2 maintains microglial metabolic fitness in alzheimer's disease. *Cell* 170, 649.e–663.e. doi: 10.1016/j.cell.2017.07.023

Van Der Heide, L. P., Kamal, A., Artola, A., Gispen, W. H., and Ramakers, G. M. J. (2005). Insulin modulates hippocampal activity-dependent synaptic plasticity in a N-methyl-D-aspartate receptor and phosphatidyl-inositol-3-kinase-dependent manner. *J. Neurochem.* 94, 1158–1166. doi: 10.1111/j.1471-4159.2005.03269.x

Vannucci, S. J., Koehler-Stec, E. M., Li, K., Reynolds, T. H., Clark, R., and Simpson, I. A. (1998). GLUT4 glucose transporter expression in rodent brain: effect of diabetes. *Brain Res.* 797, 1–11. doi: 10.1016/S0006-8993(98)00103-106

Willette, A. A., Johnson, S. C., Birdsill, A. C., Sager, M. A., Christian, B., Baker, L. D., et al. (2015). Insulin resistance predicts brain amyloid deposition in late middle-aged adults. *Alzheimer's Dem.* 11, 504–510. doi: 10.1016/j.jalz.2014.03.011

Wilson, M., Zou, S., Mattson, M. P., Martin, L., Latypova, X., Wilson, C. M., et al. (2013). Tau protein kinases: involvement in alzheimer's disease. *Ageing Res. Rev.* 12, 289–309. doi: 10.1016/j.arr.2012.06.003

Xu, X., Zhang, A., Zhu, Y., He, W., Di, W., Fang, Y., et al. (2018). MFG-E8 reverses microglial-induced neurotoxic astrocyte (A1) via NF-κB and PI3K-Akt pathways. *J. Cell Physiol.* 234, 904–914. doi: 10.1002/jcp.26918

Zhao, L. (2004). Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: implications for alzheimer's disease intervention. *J. Neurosci.* 24, 11120–11126. doi: 10.1523/jneurosci.2860-04.2004

Zheng, H., Jia, L., Liu, C.-C., Rong, Z., Zhong, L., Yang, L., et al. (2017). TREM2 promotes microglial survival by activating Wnt/β-catenin pathway. *J. Neurosci.* 37, 1772–1784. doi: 10.1523/JNEUROSCI.2459-16.2017

Zimmer, E. R., Parent, M. J., Souza, D. G., Leuzy, A., Lecrux, C., Kim, H. I., et al. (2017). [18F]FDG PET signal is driven by astroglial glutamate transport. *Nat. Neurosci.* 20, 393–395. doi: 10.1038/nn.4492

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

HYPOTHESIS AND THEORY
published: 26 June 2019
doi: 10.3389/fneur.2019.00649

Hypothesis and Theory: Circulating Alzheimer's-Related Biomarkers in Type 2 Diabetes. Insight From the Goto-Kakizaki Rat

Jamileh Movassat[1], *Etienne Delangre*[1], *Junjun Liu*[1], *YuChen Gu*[2] *and Nathalie Janel*[2]*

[1] *Univ Paris Diderot-Sorbonne Paris Cité, Laboratoire de Biologie et Pathologie du Pancréas Endocrine, Unité de Biologie Fonctionnelle et Adaptative (BFA), UMR 8251 CNRS, Paris, France,* [2] *Univ Paris Diderot-Sorbonne Paris Cité, Laboratoire Processus Dégénératifs, Stress et Vieillissement, Unité de Biologie Fonctionnelle et Adaptative (BFA), UMR 8251 CNRS, Paris, France*

Epidemiological data suggest an increased risk of developing Alzheimer's disease (AD) in individuals with type 2 diabetes (T2D). AD is anatomically associated with an early progressive accumulation of Aβ leading to a gradual Tau hyperphosphorylation, which constitute the main characteristics of damaged brain in AD. Apart from these processes, mounting evidence suggests that specific features of diabetes, namely impaired glucose metabolism and insulin signaling in the brain, play a key role in AD. Moreover, several studies report a potential role of Aβ and Tau in peripheral tissues such as pancreatic β cells. Thus, it appears that several biological pathways associated with diabetes overlap with AD. The link between peripheral insulin resistance and brain insulin resistance with concomitant cognitive impairment may also potentially be mediated by a liver/pancreatic/brain axis, through the excessive trafficking of neurotoxic molecules across the blood-brain barrier. Insulin resistance incites inflammation and pro-inflammatory cytokine activation modulates the homocysteine cycle in T2D patients. Elevated plasma homocysteine level is a risk factor for AD pathology and is also closely associated with metabolic syndrome. We previously demonstrated a strong association between homocysteine metabolism and insulin via cystathionine beta synthase (CBS) activity, the enzyme implicated in the first step of the trans-sulfuration pathway, in Goto-Kakizaki (GK) rats, a spontaneous model of T2D, with close similarities with human T2D. CBS activity is also correlated with DYRK1A, a serine/threonine kinase regulating brain-derived neurotrophic factor (BDNF) levels, and Tau phosphorylation, which are implicated in a wide range of disease such as T2D and AD. We hypothesized that DYRK1A, BDNF, and Tau, could be among molecular factors linking T2D to AD. In this focused review, we briefly examine the main mechanisms linking AD to T2D and provide the first evidence that certain circulating AD biomarkers are found in diabetic GK rats. We propose that the spontaneous model of T2D in GK rat could be a suitable model to investigate molecular mechanisms linking T2D to AD.

Keywords: diabetes, Alzheimer's disease, GK rat, BDNF, Dyrk1A, plasma biomarkers, tau

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Anne-Dominique Lajoix,
Université de Montpellier, France
Alberto Granzotto,
Centro Scienze Dell'Invecchiamento e Medicina Traslazionale (CeSI-MeT), Italy

***Correspondence:**
Nathalie Janel
janel@univ-paris-diderot.fr

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology

Received: *06 March 2019*
Accepted: *03 June 2019*
Published: *26 June 2019*

Citation:
Movassat J, Delangre E, Liu J, Gu Y and Janel N (2019) Hypothesis and Theory: Circulating Alzheimer's-Related Biomarkers in Type 2 Diabetes. Insight From the Goto-Kakizaki Rat. Front. Neurol. 10:649. doi: 10.3389/fneur.2019.00649

INTRODUCTION

Type 2 diabetes (T2D) and Alzheimer disease (AD) are both age-related, degenerative diseases, with increasing prevalence. Epidemiological data show strong association between AD and T2D (1). Although AD patients are not routinely evaluated for T2D or hyperinsulinemia (2), it is estimated that T2D nearly doubles the risk of dementia (3), cognitive dysfunction and AD (4). Despite the large body of epidemiological evidence linking AD to T2D, the precise molecular mechanisms underlying this association are yet unknown. Clinically, AD is a progressive neurodegenerative disease that begins with a subtle decline in the ability to encode new memories, and follows by more profound cognitive and behavioral/personality deterioration (5, 6). AD is anatomically associated with an early progressive accumulation of β-amyloid peptides (Aβ), leading to a gradual Tau hyperphosphorylation, which constitute the main characteristics of damaged brain in AD (7, 8). Apart from these processes, mounting evidence suggests that specific features of diabetes, namely impaired glucose metabolism and insulin signaling, play a key role in the brain during AD. This concept is supported by human postmortem studies showing that brain insulin resistance is consistently present in AD brains and worsens with disease progression (1, 9). The discovery of brain-specific insulin signaling deficiencies in the early stages of AD pathogenesis has led to the designation of AD as "type 3 diabetes" (10). Thus, AD lies on an intricate crosstalk between age-related metabolic, vascular, and hormonal changes that goes beyond its traditional central nervous system boundaries (6, 11).

THE CONNECTION BETWEEN T2D AND AD

There are several hypotheses in support of mechanistic links between AD and T2D. Numerous reviews have detailed the main and consolidated mechanisms linking these two conditions [review in (12–16)]. Some of the most documented mechanisms include defective insulin signaling and inflammation.

Importantly, T2D and AD might have a bi-directional relationship, showing both causative and consequential implications in their mutual development (**Figure 1**). Indeed, studies have shown that AD patients have an increased risk of developing T2D (17, 18). Studies in animal models of AD revealed increased susceptibility to develop metabolic disorders (19, 20). Several biological pathways associated with diabetes overlap with AD (21, 22). Similar to AD, pathological changes in insulin production and action occur years before patients receive a diagnosis of T2D (5, 6, 23). T2D is characterized by the association of peripheral insulin resistance and pancreatic β cell failure (24). The main organs involved in T2D development include the endocrine pancreas, liver, skeletal muscle, and adipose tissue, but also brain and small intestine. Emerging data suggest that insulin resistance (diabetic milieu) can either contribute to or serve as co-factor in its pathogenesis (1, 10). In the brain, insulin regulates peripheral Aβ and tau metabolism which influences the Aβ release in the brain by regulating amyloid precursor protein (APP) metabolism to modulate the balance between Aβ anabolism and catabolism (10). Lack of insulin or its action may link T2D to AD by modification of Aβ production and degradation.

Beyond its role in glucose metabolism in the body, insulin plays an important neurotrophic role in the nervous system [review in (12, 13)]. Numerous studies have demonstrated the implication of insulin and its signaling in neuronal survival and synaptic function and plasticity (25, 26). *In vitro*, insulin has been shown to promote neurite outgrowth in a population of dorsal root ganglions neurons (27).

Areas of the central nervous system such as the hippocampus, which are important for memory, have high expression of insulin receptor (28, 29). Therefore, impaired insulin levels or signaling in the brain can lead to neuronal and synaptic loss, and thus contribute to the development of AD and other neurodegenerative diseases.

Insulin can also have indirect effects, by inducing the expression of other neurotrophic factors. It has been shown that insulin treatment increases the expression of the brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) receptors in the hippocampus of young rats (30).

Some of the effects of insulin might also be mediated through its binding to insulin-like growth factor (IGF) receptors. IGF1 is a growth factor exerting trophic effects on neuronal regeneration. It stimulates protein synthesis in neurons, glia, oligodendrocytes, and Schwann cells, and favor neuronal survival while inhibiting apoptosis (31). Finally, insulin can promote neuronal survival by protecting the brain against neuroinflammation (32).

Inflammation plays a critical role in the pathogenesis of AD and T2D. AD pathology could be influenced by tissues involved in T2D (Liver, adipose tissue, pancreas), through the excessive trafficking of neurotoxic molecules such as pro-inflammatory mediators, generated by the diabetic condition, across the blood-brain barrier. Thus, periphery-derived pro-inflammatory molecules could aggravate AD pathogenesis in the central nervous system (**Figure 1**) (15, 16, 33).

However, despite intense research efforts, our knowledge of the cellular and molecular pathways linking AD and metabolic disorders remains incomplete. The understanding of the common molecular mechanisms associated with both AD and diabetes, are crucial because it could ultimately lead to the identification of common therapeutic targets for these two interconnected conditions.

SHARED MOLECULAR PATHWAYS LINKING T2D TO AD

Abnormalities in insulin/IGF signaling pathways have been shown in brains with AD (34, 35). These abnormalities were associated with reduced levels of insulin receptor substrate (IRS) mRNA, tau mRNA, IRS-associated phosphotidylinositol 3-kinase, and phospho-Akt. They also increased glycogen synthase kinase-3β (GSK3β) activity, an enzyme involved in Tau phosphorylation, and APP mRNA expression (36). Hence, the disruption of insulin functions in diabetic condition interrupts insulin signaling involved in the clearance of Aβ plaques and in neurofibrillary tangles (NFTs) pathology. This participates

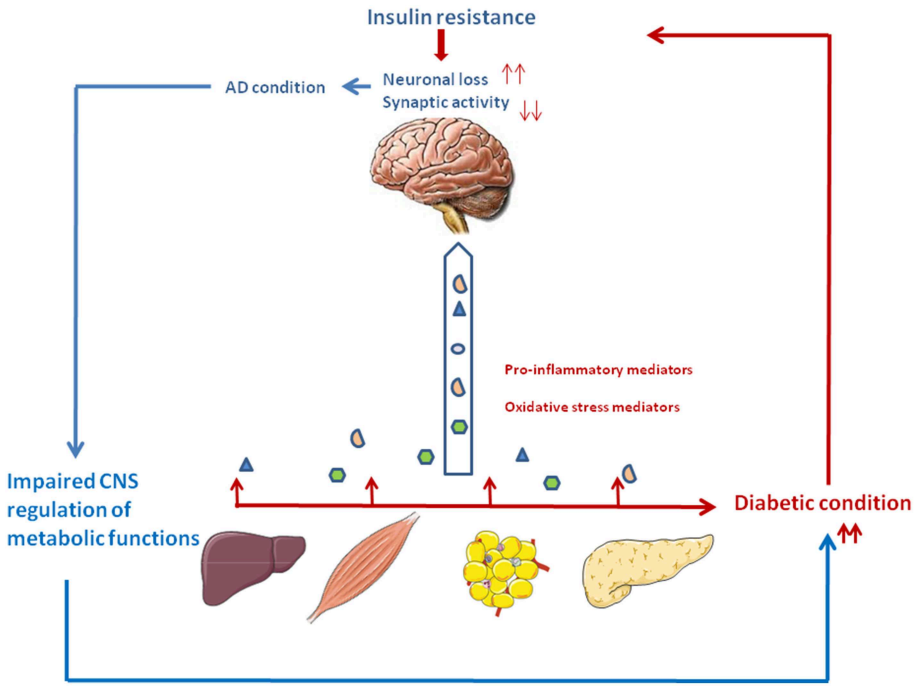

FIGURE 1 | The vicious circle between type 2 diabetes and Alzheimer's disease. Type 2 diabetes is associated with relative insulin deficiency and insulin resistance. Peripheral tissues generate proinflammatory and oxidative stress mediators which can reach the central nervous system. Impaired insulin action and the excess of neurotoxic agents may lead to neuronal loss and impaired synaptic plasticity, thus participating to the cognitive and memory loss characteristics of AD. In turn, deterioration of brain areas important for glucose and energy metabolism such as the hypothalamus and hippocampus may contribute to peripheral metabolic dysfunction, thus aggravating the diabetic condition.

to the accelerated formation of neurotoxic Aβ and NFTs via various mechanisms including GSK3β and the dual-specificity tyrosine-(Y)-phosphorylation regulated kinase DYRK1A (37). Thus, insulin resistance and T2D can interact with key pathways involved in AD pathology.

Hyperphosphorylated Tau protein is the main constituent of NFTs, which alongside amyloid β plaques, have long been considered as key histopathological hallmarks of AD. Interestingly, amyloid deposition (amylin) and abnormal Tau processing may provide yet another link between diabetes and AD. Indeed, studies report a potential role of Aβ and Tau in peripheral tissues. In humans, pancreatic amyloid deposition, similar to its damaging effect in brain during AD, is associated with β cell loss and global dysmetabolism (38). High levels of phosphorylated Tau were found in pancreas from T2D patients (39). GSK3β is involved in the formation of both Aβ deposits and NFTs. GSK3β induces Tau hyperphoshorylation to form NFTs through PI3K/Akt/GSK3β signaling pathway (40). Importantly, GSK3β is also involved in many aspects of T2D pathogenesis. As a negative effector of the insulin signaling pathway, GSK3β is involved in insulin resistance (41). GSK3β expression and activity were shown to be increased in muscle of diabetic patients and was implicated in muscle insulin resistance (42). Furthermore, we and others have reported that beyond its implication in insulin resistance in target tissues, GSK3β acts as a negative regulator of β-cell growth and function, thus further implicating this enzyme in the relative insulin deficiency associated with T2D (43–46).

Peripheral insulin resistance, a hallmark of T2D, can cause brain insulin resistance via a liver/pancreas/brain axis. Elevated plasma homocysteine (Hcy) level is a risk factor for AD pathology and is also closely associated with metabolic syndrome (47–49). Elevated Hcy levels have been linked with gray and white matter volume reduction among individuals with mild cognitive impairment and AD (50). Plasma Hcy is highly dependent on intracellular Hcy metabolism in the liver and kidney, but it may also reflect one-carbon metabolism in a number of other cell types, notably in pancreas and brain. Hepatic insulin resistance during T2D leads to inflammation which could in turn results in excessive Hcy production. Cytotoxic Hcy generated in liver, traffics through the circulation following injury or cell death, and can cross the blood-brain barrier and exert neurotoxic effects by impairing central insulin signaling and activating pro-inflammatory cytokines. These abnormalities establish or help propagate a cascade of neurodegeneration associated with oxidative stress, which exacerbate brain insulin resistance, cell death, and neuro-inflammation (51) (**Figure 1**).

Another potentially important player in AD pathogenesis is DYRK1A (52). DYRK1A interacts with APP and APP processing by direct phosphorylation of APP at Thr-668 and indirect phosphorylation of the presenilin 1 (PS1) at

Thr-354, promoting the pathological Aβ pathway (53, 54). Increased expression of DYRK1A seems to promote brain β-amyloidosis by enhancing the phosphorylation and the amyloidogenic cleavage of APP, increasing the amyloidogenic levels of Aβ40 and Aβ42 (54). It also promotes neurofibrillary degeneration directly through hyperphosphorylation of tau and indirectly through phosphorylation of alternative splicing factor, therefore participating to neurodegeneration and neuronal loss appearing in AD (54–56). Moreover, we have shown that AD patients exhibited a positive correlation between plasma DYRK1A levels and CSF tau and phosphorylated-tau proteins (57).

In recent years, increasing interest has been drawn to the role of DYRK1A in β cell biology, making it another possible molecular link between AD and T2D. Several studies show that inhibition of DYRK1A alone (58, 59) or associated with the inhibition of GSK3β (60), or with SMAD and Trithorax pathways (61) induces human β cell proliferation.

However, other data in mice model of DYRK1A overexpression showed expansion of β cell mass through increased proliferation and cell size (62), suggesting a positive effect of DYRK1A on β cell growth in this model, which contrasts with the data on human β cells cited above.

DYRK1A has been demonstrated to be involved in the cycle of Hcy (63, 64), and its overexpression was linked with BDNF reduction (65). BDNF, the most widely distributed neurotrophin in the central nervous system, has a pivotal role in maturation, synaptic connection, neuronal repair, and plasticity of the central nervous system (66). Loss of BDNF in neurodegenerative disorders is a key mediator of synaptic dysfunction, neurodegeneration and subsequent cognitive decline (67). AD subjects show reduced BDNF levels in the serum and brain as compared with healthy elderly controls (68–70). Interestingly, there was a notable increase in plasma Hcy level and significant decrease in serum BDNF level in amnestic mild cognitive impairment patients that converts to AD, especially in those with the APOE ε4 allele (71).

Higher expression of BDNF slows down cognitive decline in the elderly, especially in the setting of advancing AD neuropathology, indicating that the brain BDNF level could be used as a novel marker for evaluating AD progression (68, 72). Combined assessment of DYRK1A and the related markers BDNF and Hcy has been validated by our team, by logistic regression analysis as diagnostic marker for AD in two unrelated AD patient cohorts with age-matched controls (73).

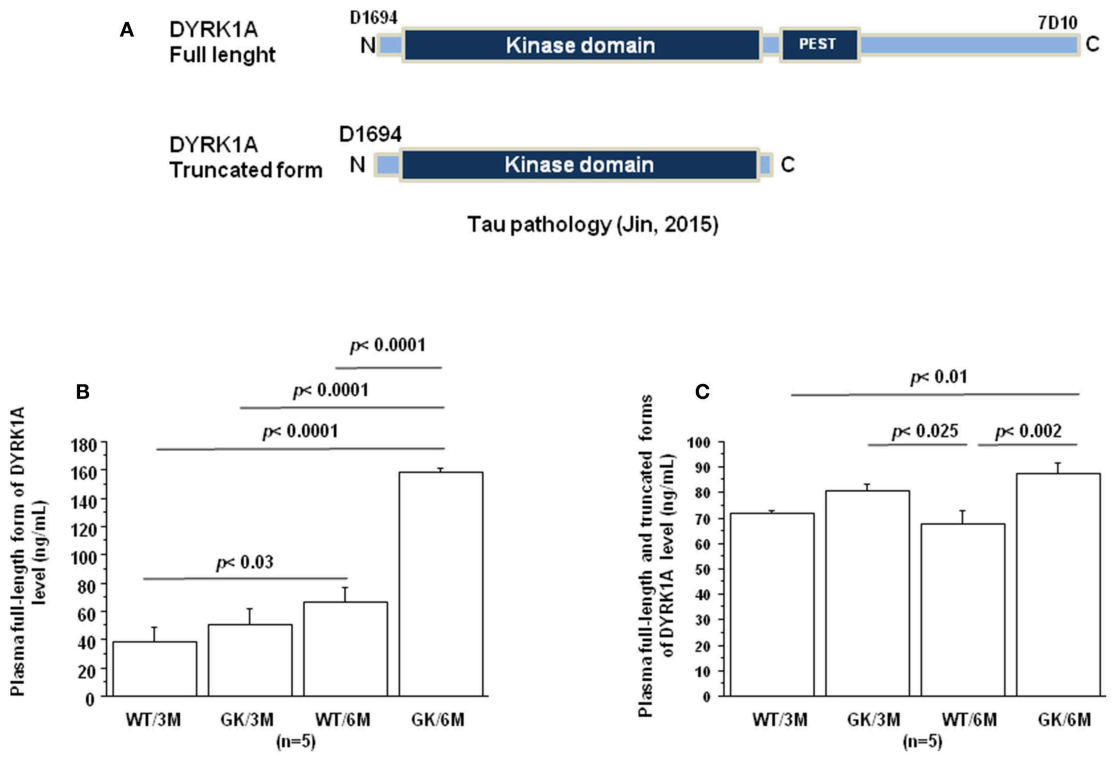

FIGURE 2 | Age-dependent changes in plasma Dyrk1A levels. Blood was collected at the tail vein of Wistar and GK rats of 3 and 6 months of age, at 9:00 a.m. Analyses were performed in plasma. **(A)** Schematic representation of Dyrk1A with distinct epitopes recognized by different antibodies. Plasma levels of full-length Dyrk1A form **(B)** and full-length and truncated forms of Dyrk1A **(C)**. The DYRK1A levels were assessed by a solid phase immobilized epitope- immunoassay set up for antibody 7D10 (Abnova; immunogen: 674 aa ~763 aa) and antibody D1694 (Sigma; immunogen: 32 aa ~51 aa) (73). After removal of unbound conjugates, bound enzyme activity was assessed by use of a chromogenic substrate for measurement at 450 nm by a microplate reader (Flex Station 3, Molecular Device, San Diego, CA, USA). All the assays were performed in duplicate. For multiple pairwise comparisons between genotypes and ages, statistical analysis was done with two-way ANOVA followed by Fisher's *post-hoc* test using Statview software. The results are expressed as means ± SEM (standard error of the mean). n = number of rats. Data were considered significant when $p < 0.05$.

Interestingly, several reports also documented an association between plasma BDNF and systemic or peripheral inflammatory conditions, notably T2D (74). Plasma BDNF levels were found to be decreased in T2D patients (75–78). Interestingly, the relationship between T2D, BDNF and dementia was reported in one study which demonstrated lower plasma BDNF levels in patient group with both T2D and dementia than in non-diabetic patients with dementia (79).

Treatments to alleviate brain insulin resistance, such as intranasal insulin administration, have been evaluated in mice models and AD patients (80–84). Insulinotropic hormones such as glucagon-like peptide-1 (GLP-1), have also been proposed as a treatment for neurodegenerative disorders. Indeed, exenatide, a glucagon-like peptide-1 (GLP-1) agonist used for the treatment of T2D led to improvements in motor assessments in patients with Parkinson's disease (85). The potential relevance of this drug for other neurodegenerative disorders (e.g., AD) has being assessed in pre-clinical studies. Exenatide was tested in different mice models of AD, in 3xTg-AD mice on a high-fat diet, in APP/PS1 mice, and in adult wild-type mice as a model of mid-life brain aging. Results demonstrate a beneficial effect of drug treatment not only on cognition but also on BDNF neurotrophic axis (86–88).

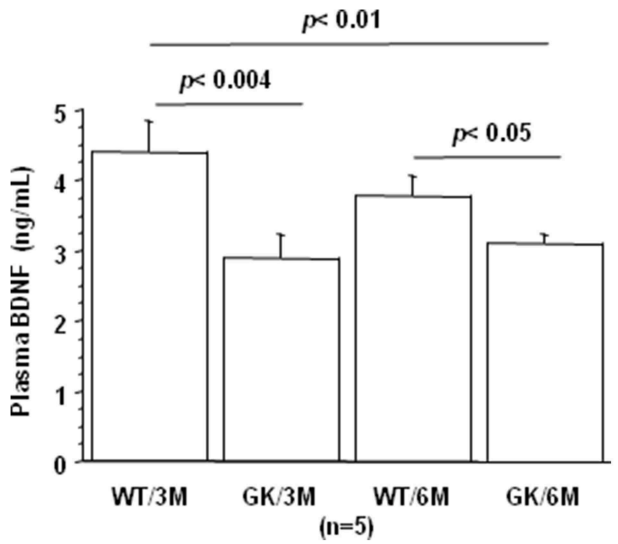

FIGURE 3 | Age-dependent changes in plasma BDNF. Blood was collected at the tail vein of Wistar and GK rats of 3 and 6 months of age, at 9:00 a.m. Analyses were performed in plasma. BDNF was assessed using sandwich ELISA (ELISA E-Max, Promega, Madison, WI, USA). After removal of unbound conjugates, bound enzyme activity was assessed by use of a chromogenic substrate for measurement at 450 nm by a microplate reader (Flex Station 3, Molecular Device, San Diego, CA, USA). All the assays were performed in duplicate. For multiple pairwise comparisons between genotypes and ages, statistical analysis was done with two-way ANOVA followed by Fisher's *post-hoc* test using Statview software. The results are expressed as means ± SEM (standard error of the mean). n = number of rats. Data were considered significant when $p < 0.05$.

ANIMAL MODELS OF COMBINED AD AND T2D

Several animal models, mostly in rodents, have been designed to study the interconnection between AD and T2D. These models include high fat died-induced insulin resistance, streptozotocin-induced diabetes or monosodium glutamate (MSG)-treated rodents (89, 90). A variety of cognitive/behavioral impairments and/or histopathological defects have been reported in these studies, thus providing the experimental basis for the epidemiological data that link T2D to AD. However, most of available models have back draws since they do not replicate the progressive characteristics of T2D with a silent phase followed by the development of insulin resistance and relative insulin deficiency.

The Goto–Kakizaki (GK) rats is a spontaneous model of T2D with close similarities with human T2D (91). The chronology of the infra-clinical and clinical phases in the GK rat, ranging from primary defects in the endocrine pancreas, as early as the fetal stage, followed by a neonatal phase of pre-diabetes, and finally the occurrence of overt hyperglycemia in adult individuals has been extensively described by our team (91, 92). The relevance of the GK rat as a T2D model lies in the fact that it is a spontaneous model without genetic manipulation, in which diabetes develops through a gradual process following a well-characterized phase of pre-diabetes (93), similar to the human T2D pathology. We have previously demonstrated in GK rats, a strong association between Hcy metabolism and insulin via cystathionine beta synthase (CBS) activity, the enzyme implicated in the first step of the trans-sulfuration pathway (94). In addition to several metabolic defects, GK rats also display impairment in their learning abilities and memory capabilities. Interestingly, cognitive dysfunction in GK rats was correlated with their insulin resistance index (95). Another study have reported significant decrease in phosphorylation of Akt, as well as reduced expression of CREB, an important regulator in the expression of functional proteins associated with learning and memory in this model of T2D (96). Studies using transgenic models of AD have generated mounting evidence supporting alteration in neurogenesis (97). Previous studies by our group and other's showed that chronic hyperglycemia impairs hippocampus neurogenesis in adult diabetic GK rats (98, 99), showing similarity with defects reported transgenic mice models of AD.

TABLE 1 | Correlations between plasma levels of Dyrk1A, BDNF, and Tau determined by Spearman's rank correlation.

	Full-length Dyrk1A	Full-length and truncated forms of Dyrk1A	BDNF
BDNF		$r = -0.58$ $p < 0.017$	
Tau		$r = 0.758$ $p < 0.0007$	$r = -0.646$ $p < 0.005$
Tau46	$r = 0.571$ $p < 0.01$	$r = 0.646$ $p < 0.002$	$r = -0.646$ $p < 0.005$

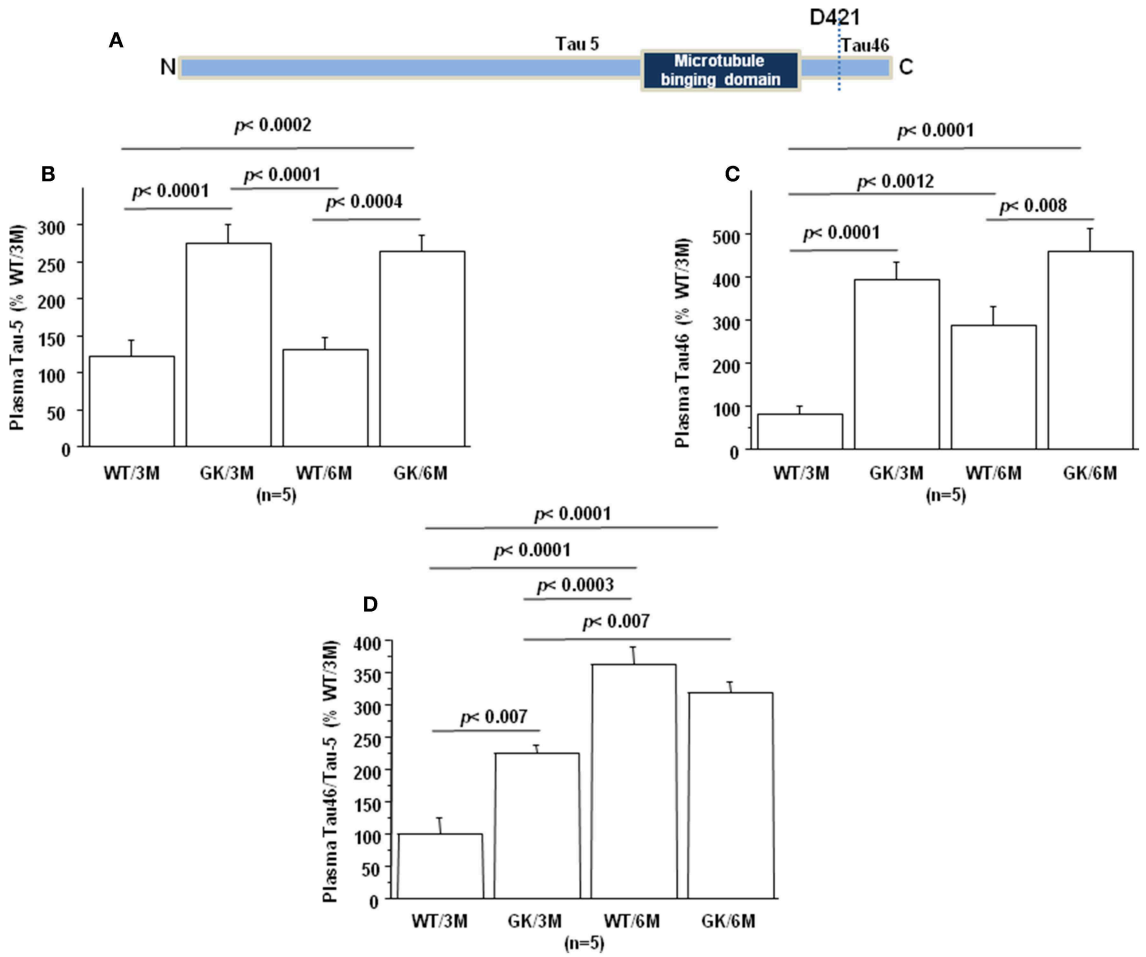

FIGURE 4 | Age-dependent changes in plasma Tau. Blood was collected at the tail vein of Wistar and GK rats of 3 and 6 months of age, at 9:00 a.m. Analyses were performed in plasma. **(A)** Schematic representation of Tau with distinct epitopes recognized by different antibodies (Tau5 and Tau46) used for the truncation detection at D421. Plasma relative intensities of **(B)** Tau5 (1/1000, ThermoFisher/ AHB0042; immunogen 210 aa ~230 aa) and **(C)** Tau46 (1/1000, ThermoFisher/13-6400; immunogen 404 aa ~441 aa). **(D)** The relative intensities of Tau46 were normalized to those of Tau5. Data were normalized to the mean of wild-type rats at 3 months. For multiple pairwise comparisons between genotypes and ages, statistical analysis was done with two-way ANOVA followed by Fisher's *post-hoc* test using Statview software. The results are expressed as means ± SEM (standard error of the mean). n = number of rats. Data were considered significant when $p < 0.05$.

Based on the literature described above, the spontaneous GK model of T2D appears as a valuable tool to investigate the relationship between T2D and AD.

STATEMENT OF HYPOTHESIS: PLASMA LEVELS OF DYRK1A, BDNF, AND TAU ARE MODIFIED IN GOTO-KAKIZAKI RATS

Taking advantage of the characteristics of this model, we sought to analyze the circulating levels of some of the biomarkers of AD, which could potentially be related to T2D, namely DYRK1A, BDNF and Tau, in 3 and 6 months old diabetic GK rats.

Jin et al. have reported that DYRK1A was truncated in the brains of AD patients resulting in formation of truncated forms due to increased calpain activity (100), associated with a decrease of the full-length form (**Figure 2A**). DYRK1A contains a PEST sequence, a signal peptide for protein degradation via calpain (101, 102). Recently, Souchet et al. found that this DYRK1A cleavage is a consequence of the amyloid pathology (103). Resulting truncated forms accumulate in astrocytes and exhibit increased affinity toward a regulator of inflammatory process (103). Here, we analyzed these different forms by the use of two different antibodies, one recognizing the full-length form, and the other the full-length and truncated forms of Dyrk1A (**Figure 2A**) in plasma of control Wistar (WT) and GK rats. No difference was found between WT and GK rats at 3 months (**Figures 2B,C**), while an increase of full-length and the truncated forms was found in GK rats at 6 months (**Figure 2C**). The full-length form was also increased at 6 months in WT rats compared to 3 months old WT rats (**Figure 2B**), suggesting an age-related effect.

BDNF levels were decreased in plasma of GK rats at 3 and 6 months, compared to age-matched WT rats (**Figure 3**). This was in keeping with studies showing decreased plasma

levels of BDNF in diabetic patients (75–78). There is also solid evidence demonstrating a reduction in BDNF mRNA and protein levels in AD cortex and hippocampus (104, 105), and decreased BDNF levels contribute to cognitive dysfunction in AD (66). A significant decrease in BDNF serum concentration has been found in AD patients compared with healthy controls (106). Correlations were determined by using Spearman's rank correlation, as data were not normally distributed according to Shapiro-Wilk test. A negative correlation was found between plasma BDNF and full-length and truncated forms of Dyrk1A levels (**Table 1**). As Dyrk1A is involved in controlling numerous pathways, this result emphasizes the role of this kinase on BDNF signaling pathways, as previously suggested by our team (65, 73).

Tau protein truncated at amino acid D421 has been detected in AD (**Figure 4A**). This C-terminal truncation introduces a conformational change contributing to aggregation (107, 108). We therefore measured the levels of centrally-situated Tau epitope (**Figure 4B**) and levels of Tau 46 (**Figure 4C**), to evaluate the index of truncation. The index of C-terminal truncation was provided by the ratio of Tau46/Tau5 (**Figure 4D**). Tau levels (Tau5 immunoreactivity) increased in plasma of GK rats at 3 and 6 months, compared to age-matched WT rats. There was no difference of Tau levels between WT rats at 3 and 6 months (**Figure 4A**). Tau levels are correlated positively with full-length and truncated forms of Dyrk1A levels (**Table 1**) and negatively with BDNF levels (**Table 1**). Interestingly, we previously found a positive correlation between plasma Dyrk1A levels and CSF Tau proteins in AD patients (57).

An increased Tau46 immunoreactivity and Tau46/Tau 5 was found in plasma of GK rats as early as 3 months and persisted at 6 months of age compared to age-matched WT rats (**Figures 4C,D**). Interestingly, Tau46 immunoreactivity and Tau46/Tau5 ratio increased in an age-dependent manner within the WT group (**Figures 4C,D**). The Tau46 immunoreactivity also correlated positively with full-length form of Dyrk1A levels (**Table 1**), full-length and truncated forms of Dyrk1A levels (**Table 1**), and negatively with BDNF levels (**Table 1**). These results indicate that Tau undergoes increased C-terminal cleavage as early as 3 months in the GK rats, while this effect appears in non-diabetic Wistar rats only at 6 months.

CONCLUSION

In this paper we used the type 2 diabetic GK rat as a tool to assess circulating biomarkers for AD. We show that plasma BDNF and the index of C-terminal truncation of Tau could be considered as early biomarkers, while plasma Dyrk1A could represent a late biomarker. As a spontaneous model of T2D with gradual progression, the GK rat is acknowledged as a valuable tool to study the pathogenesis of diabetes. Here we propose that the GK rat could be a new model to investigate the link between T2D and AD. It could therefore be a useful tool for pre-clinical studies to assess drug efficacy in the onset of the disease. Currently, we are addressing the question of possible abnormalities in the expression/activity of the above markers in the brain and the pancreas of the GK rat, to validate the relevance of this model as a model of T2D-associated AD. These results need to be compared with those described in validated rodent models of AD with different grade pathology. Further longitudinal studies of metabolic and cognitive parameters with pharmacological intervention are warranted to comprehend the causal relationship underlining the progression of AD and T2D.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

All procedures were carried out in accordance with the ethical standards of French and European regulations (European Communities Council Directive, 86/609/EEC). Official authorization from the French Ministry of Agriculture was granted to perform research and experiments on animals (authorization number B-75-13-17) and the experimental protocol was approved by the institutional animal care and use committee of the Paris Diderot University (CEEA40).

AUTHOR CONTRIBUTIONS

JM and NJ designed the study, made the review of the literature, and wrote the manuscript. ED, JL, and YG performed experiments. All authors read and approved the manuscript.

FUNDING

This work was supported by Lagafe Association and Fondation Jérome Lejeune (grant number 2017a-1634).

REFERENCES

1. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *J Alzheimers Dis.* (2005) 7:63–80. doi: 10.3233/JAD-2005-7107
2. Haan MN. Therapy Insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. *Nat Clin Pract Neurol.* (2006) 2:159–66. doi: 10.1038/ncpneuro0124
3. Huang C-C, Chung C-M, Leu H-B, Lin L-Y, Chiu C-C, Hsu C-Y, et al. Diabetes mellitus and the risk of Alzheimer's disease: a nationwide population-based study. *PLoS ONE.* (2014) 9:e87095. doi: 10.1371/journal.pone.0087095
4. Vieira MNN, Lima-Filho RAS, De Felice FG. Connecting Alzheimer's disease to diabetes: underlying mechanisms and potential therapeutic targets. *Neuropharmacology.* (2018) 136:160–71. doi: 10.1016/j.neuropharm.2017.11.014

5. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer's disease. *Lancet Lond Engl.* (2011) 377:1019–31. doi: 10.1016/S0140-6736(10)61349-9
6. Neth BJ, Craft S. Insulin resistance and Alzheimer's disease: bioenergetic linkages. *Front Aging Neurosci.* (2017) 9:345. doi: 10.3389/fnagi.2017.00345
7. Huang H-C, Jiang Z-F. Accumulated amyloid-beta peptide and hyperphosphorylated tau protein: relationship and links in Alzheimer's disease. *J Alzheimers Dis.* (2009) 16:15–27. doi: 10.3233/JAD-2009-0960
8. Saha P, Sen N. Tauopathy: a common mechanism for neurodegeneration and brain aging. *Mech Ageing Dev.* (2019) 178:72–9. doi: 10.1016/j.mad.2019.01.007
9. Bello-Chavolla OY, Antonio-Villa NE, Vargas-Vázquez A, Ávila-Funes JA, Aguilar-Salinas CA. Pathophysiological mechanisms linking type 2 diabetes and dementia: review of evidence from clinical, translational and epidemiological research. *Curr. Diabetes Rev.* (2019). doi: 10.2174/1573399815666190129155654. [Epub ahead of print].
10. de la Monte SM. Contributions of brain insulin resistance and deficiency in amyloid-related neurodegeneration in Alzheimer's disease. *Drugs.* (2012) 72:49–66. doi: 10.2165/11597760-000000000-00000
11. Kandimalla R, Thirumala V, Reddy PH. Is Alzheimer's disease a type 3 diabetes? A critical appraisal. *Biochim Biophys Acta Mol Basis Dis.* (2017) 1863:1078–89. doi: 10.1016/j.bbadis.2016.08.018
12. Wijesekara N, Gonçalves RA, De Felice FG, Fraser PE. Impaired peripheral glucose homeostasis and Alzheimer's disease. *Neuropharmacology.* (2018) 136(Pt B):172–81. doi: 10.1016/j.neuropharm.2017.11.027
13. Cole GM, Frautschy SA. The role of insulin and neurotrophic factor signaling in brain aging and Alzheimer's Disease. *Exp Gerontol.* (2007) 42:10–21. doi: 10.1016/j.exger.2006.08.009
14. Rad SK, Arya A, Karimian H, Madhavan P, Rizwan F, Koshy S, et al. Mechanism involved in insulin resistance via accumulation of β-amyloid and neurofibrillary tangles: link between type 2 diabetes and Alzheimer's disease. *Drug Des Devel Ther.* (2018) 2:3999–4021. doi: 10.2147/DDDT.S173970
15. Ferreira LSS, Fernandes CS, Vieira MNN, De Felice FG. Insulin resistance in Alzheimer's disease. *Front Neurosci.* (2018) 13:830. doi: 10.3389/fnins.2018.00830
16. de la Monte SM. Insulin resistance and neurodegeneration: progress towards the development of new therapeutics for Alzheimer's disease. *Drugs.* (2017) 77:47–65. doi: 10.1007/s40265-016-0674-0
17. Janson J, Laedtke T, Parisi JE, O'Brien P, Petersen RC, Butler PC. Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes.* (2004) 53:474–81. doi: 10.2337/diabetes.53.2.474
18. Matsuzaki T, Sasaki K, Tanizaki Y, Hata J, Fujimi K, Matsui Y, et al. Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama study. *Neurology.* (2010) 75:764–70. doi: 10.1212/WNL.0b013e3181eee25f
19. Jiménez-Palomares M, Ramos-Rodríguez JJ, López-Acosta JF, Pacheco-Herrero M, Lechuga-Sancho AM, Perdomo G, et al. Increased Aβ production prompts the onset of glucose intolerance and insulin resistance. *Am J Physiol Endocrinol Metab.* (2012) 302:E1373–80. doi: 10.1152/ajpendo.00500.2011
20. Ruiz HH, Chi T, Shin AC, Lindtner C, Hsieh W, Ehrlich M, et al. Increased susceptibility to metabolic dysregulation in a mouse model of Alzheimer's disease is associated with impaired hypothalamic insulin signaling and elevated BCAA levels. *Alzheimers Dement J Alzheimers Assoc.* (2016) 12:851–61. doi: 10.1016/j.jalz.2016.01.008
21. Hao K, Di Narzo AF, Ho L, Luo W, Li S, Chen R, et al. Shared genetic etiology underlying Alzheimer's disease and type 2 diabetes. *Mol Aspects Med.* (2015) 43–4:66–76. doi: 10.1016/j.mam.2015.06.006
22. Sims-Robinson C, Kim B, Rosko A, Feldman EL. How does diabetes accelerate Alzheimer disease pathology? *Nat Rev Neurol.* (2010) 6:551–9. doi: 10.1038/nrneurol.2010.130
23. Dankner R, Chetrit A, Shanik MH, Raz I, Roth J. Basal-state hyperinsulinemia in healthy normoglycemic adults is predictive of type 2 diabetes over a 24-year follow-up: a preliminary report. *Diabetes Care.* (2009) 32:1464–6. doi: 10.2337/dc09-0153
24. Portha B, Lacraz G, Kergoat M, Homo-Delarche F, Giroix M-H, Bailbé D, et al. The GK rat beta-cell: a prototype for the diseased human beta-cell in type 2 diabetes? *Mol Cell Endocrinol.* (2009) 297:73–85. doi: 10.1016/j.mce.2008.06.013
25. Lee S-H, Zabolotny JM, Huang H, Lee H, Kim Y-B. Insulin in the nervous system and the mind: functions in metabolism, memory, and mood. *Mol Metab.* (2016) 5:589–601. doi: 10.1016/j.molmet.2016.06.011
26. McNay EC, Recknagel AK. Brain insulin signaling: a key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes. *Neurobiol Learn Mem.* (2011) 96:432–42. doi: 10.1016/j.nlm.2011.08.005
27. Lázár BA, Jancsó G, Pálvölgyi L, Dobos I, Nagy I, Sántha P. Insulin confers differing effects on neurite outgrowth in separate populations of cultured dorsal root ganglion neurons: the role of the insulin receptor. *Front Neurosci.* (2018) 12:732. doi: 10.3389/fnins.2018.00732
28. Broughton S, Partridge L. Insulin/IGF-like signalling, the central nervous system and aging. *Biochem J.* (2009) 418:1–12. doi: 10.1042/BJ20082102
29. Bondy CA, Cheng CM. Signaling by insulin-like growth factor 1 in brain. *Eur J Pharmacol.* (2004) 490:25–31. doi: 10.1016/j.ejphar.2004.02.042
30. Haas CB, Kalinine E, Zimmer ER, Hansel G, Brochier AW, Oses JP, et al. Brain insulin administration triggers distinct cognitive and neurotrophic responses in young and aged rats. *Mol Neurobiol.* (2016) 53:5807–17. doi: 10.1007/s12035-015-9494-6
31. Bianchi VE, Locatelli V, Rizzi L. Neurotrophic and neuroregenerative effects of GH/IGF1. *Int J Mol Sci.* (2017) 18:E2441. doi: 10.3390/ijms18112441
32. Bortoluzzi Canteiro P, Casagrande Antero D, Dos Santos Tramontin N, Ugioni Simon K, Mendes C, Anastácio Borges Correa ME, et al. Insulin treatment protects the brain against neuroinflammation by reducing cerebral cytokines and modulating mitochondrial function. *Brain Res Bull.* (2019) 149:120–8. doi: 10.1016/j.brainresbull.2019.04.011
33. De Felice FG. Alzheimer's disease and insulin resistance: translating basic science into clinical applications. *J Clin Invest.* (2013) 123:531–9. doi: 10.1172/JCI64595
34. Moloney AM, Griffin RJ, Timmons S, O'Connor R, Ravid R, O'Neill C. Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiol Aging.* (2010) 31:224–43. doi: 10.1016/j.neurobiolaging.2008.04.002
35. Talbot K, Wang H-Y, Kazi H, Han L-Y, Bakshi KP, Stucky A, et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest.* (2012) 122:1316–38. doi: 10.1172/JCI59903
36. Chen Z, Zhong C. Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. *Prog Neurobiol.* (2013) 108:21–43. doi: 10.1016/j.pneurobio.2013.06.004
37. Yang Y, Song W. Molecular links between Alzheimer's disease and diabetes mellitus. *Neuroscience.* (2013) 250:140–50. doi: 10.1016/j.neuroscience.2013.07.009
38. Jarosz-Griffiths HH, Noble E, Rushworth JV, Hooper NM. Amyloid-β receptors: the good, the bad, and the prion protein. *J Biol Chem.* (2016) 291:3174–83. doi: 10.1074/jbc.R115.702704
39. Miklossy J, Qing H, Radenovic A, Kis A, Vileno B, Làszló F, et al. Beta amyloid and hyperphosphorylated tau deposits in the pancreas in type 2 diabetes. *Neurobiol Aging.* (2010) 31:1503–15. doi: 10.1016/j.neurobiolaging.2008.08.019
40. Gao C, Hölscher C, Liu Y, Li L. GSK3: a key target for the development of novel treatments for type 2 diabetes mellitus and Alzheimer disease. *Rev Neurosci.* (2011) 23:1–11. doi: 10.1515/rns.2011.061
41. Eldar-Finkelman H. Glycogen synthase kinase 3: an emerging therapeutic target. *Trends Mol Med.* (2002) 8:126–32. doi: 10.1016/S1471-4914(01)02266-3
42. Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. *Diabetes.* (2000) 49:263–71. doi: 10.2337/diabetes.49.2.263
43. Figeac F, Uzan B, Faro M, Chelali N, Portha B, Movassat J. Neonatal growth and regeneration of beta-cells are regulated by the Wnt/beta-catenin signaling in normal and diabetic rats. *Am J Physiol Endocrinol Metab.* (2010) 298:E245–56. doi: 10.1152/ajpendo.00538.2009
44. Figeac F, Ilias A, Bailbe D, Portha B, Movassat J. Local *in vivo* GSK3β knockdown promotes pancreatic β cell and acinar cell regeneration in 90% pancreatectomized rat. *Mol Ther J Am Soc Gene Ther.* (2012) 20:1944–52. doi: 10.1038/mt.2012.112

45. Liu Z, Tanabe K, Bernal-Mizrachi E, Permutt MA. Mice with beta cell overexpression of glycogen synthase kinase-3beta have reduced beta cell mass and proliferation. *Diabetologia.* (2008) 51:623–31. doi: 10.1007/s00125-007-0914-7
46. Tanabe K, Liu Z, Patel S, Doble BW, Li L, Cras-Méneur C, et al. Genetic deficiency of glycogen synthase kinase-3beta corrects diabetes in mouse models of insulin resistance. *PLoS Biol.* (2008) 6:e37. doi: 10.1371/journal.pbio.0060037
47. Ala OA, Akintunde AA, Ikem RT, Kolawole BA, Ala OO, Adedeji TA. Association between insulin resistance and total plasma homocysteine levels in type 2 diabetes mellitus patients in south west Nigeria. *Diabetes Metab Syndr.* (2017) 11(Suppl. 2:S803–9). doi: 10.1016/j.dsx.2017.06.002
48. Farina N, Jernerén F, Turner C, Hart K, Tabet N. Homocysteine concentrations in the cognitive progression of Alzheimer's disease. *Exp Gerontol.* (2017) 99:146–50. doi: 10.1016/j.exger.2017.10.008
49. Sreckovic B, Sreckovic VD, Soldatovic I, Colak E, Sumarac-Dumanovic M, Janeski H, et al. Homocysteine is a marker for metabolic syndrome and atherosclerosis. *Diabetes Metab Syndr.* (2017) 11:179–82. doi: 10.1016/j.dsx.2016.08.026
50. Tan B, Venketasubramanian N, Vrooman H, Cheng C-Y, Wong TY, Ikram MK, et al. Homocysteine and cerebral atrophy: the epidemiology of dementia in singapore study. *J Alzheimers Dis.* (2018) 62:877–85. doi: 10.3233/JAD-170796
51. Kamat PK, Vacek JC, Kalani A, Tyagi N. Homocysteine induced cerebrovascular dysfunction: a link to Alzheimer's disease etiology. *Open Neurol J.* (2015) 9:9–14. doi: 10.2174/1874205X01509010009
52. Lott IT, Dierssen M. Cognitive deficits and associated neurological complications in individuals with Down's syndrome. *Lancet Neurol.* (2010) 9:623–33. doi: 10.1016/S1474-4422(10)70112-5
53. Coutadeur S, Benyamine H, Delalonde L, de Oliveira C, Leblond B, Foucourt A, et al. A novel DYRK1A (dual specificity tyrosine phosphorylation-regulated kinase 1A) inhibitor for the treatment of Alzheimer's disease: effect on Tau and amyloid pathologies *in vitro*. *J Neurochem.* (2015) 133:440–51. doi: 10.1111/jnc.13018
54. Wegiel J, Gong C-X, Hwang Y-W. The role of DYRK1A in neurodegenerative diseases. *FEBS J.* (2011) 278:236–45. doi: 10.1111/j.1742-4658.2010.07955.x
55. Branca C, Shaw DM, Belfiore R, Gokhale V, Shaw AY, Foley C, et al. Dyrk1 inhibition improves Alzheimer's disease-like pathology. *Aging Cell.* (2017) 16:1146–54. doi: 10.1111/acel.12648
56. Ryoo S-R, Jeong HK, Radnaabazar C, Yoo J-J, Cho H-J, Lee H-W, et al. DYRK1A-mediated hyperphosphorylation of Tau. A functional link between Down syndrome and Alzheimer disease. *J Biol Chem.* (2007) 282:34850–7. doi: 10.1074/jbc.M707358200
57. Janel N, Sarazin M, Corlier F, Corne H, de Souza LC, Hamelin L, et al. Plasma DYRK1A as a novel risk factor for Alzheimer's disease. *Transl Psychiatry.* (2014) 4:e425. doi: 10.1038/tp.2014.61
58. Kumar K, Man-Un Ung P, Wang P, Wang H, Li H, Andrews MK, et al. Novel selective thiadiazine DYRK1A inhibitor lead scaffold with human pancreatic β-cell proliferation activity. *Eur J Med Chem.* (2018) 157:1005–16. doi: 10.1016/j.ejmech.2018.08.007
59. Wang P, Alvarez-Perez J-C, Felsenfeld DP, Liu H, Sivendran S, Bender A, et al. A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. *Nat Med.* (2015) 21:383–8. doi: 10.1038/nm.3820
60. Shen W, Taylor B, Jin Q, Nguyen-Tran V, Meeusen S, Zhang Y-Q, et al. Inhibition of DYRK1A and GSK3B induces human β-cell proliferation. *Nat Commun.* (2015) 6:8372. doi: 10.1038/ncomms9372
61. Wang P, Karakose E, Liu H, Swartz E, Ackeifi C, Zlatanic V, et al. Combined inhibition of DYRK1A, SMAD, and Trithorax pathways synergizes to induce robust replication in adult human beta cells. *Cell Metab.* (2018) 29:638–52.e5. doi: 10.1016/j.cmet.2018.12.005
62. Rachdi L, Kariyawasam D, Aïello V, Herault Y, Janel N, Delabar J-M, et al. Dyrk1A induces pancreatic β cell mass expansion and improves glucose tolerance. *Cell Cycle Georget Tex.* (2014) 13:2221–9. doi: 10.4161/cc.29250
63. Delabar J-M, Latour A, Noll C, Renon M, Salameh S, Paul J-L, et al. One-carbon cycle alterations induced by Dyrk1a dosage. *Mol Genet Metab Rep.* (2014) 1:487–92. doi: 10.1016/j.ymgmr.2014.11.004
64. Noll C, Hamelet J, Matulewicz E, Paul J-L, Delabar J-M, Janel N. Effects of red wine polyphenolic compounds on paraoxonase-1 and lectin-like oxidized low-density lipoprotein receptor-1 in hyperhomocysteinemic mice. *J Nutr Biochem.* (2009) 20:586–96. doi: 10.1016/j.jnutbio.2008.06.002
65. Tlili A, Hoischen A, Ripoll C, Benabou E, Badel A, Ronan A, et al. BDNF and DYRK1A are variable and inversely correlated in lymphoblastoid cell lines from Down syndrome patients. *Mol Neurobiol.* (2012) 46:297–303. doi: 10.1007/s12035-012-8284-7
66. Diniz BS, Teixeira AL. Brain-derived neurotrophic factor and Alzheimer's disease: physiopathology and beyond. *Neuromolecular Med.* (2011) 13:217–22. doi: 10.1007/s12017-011-8154-x
67. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci.* (2015) 11:1164–78. doi: 10.5114/aoms.2015.56342
68. Beeri MS, Sonnen J. Brain BDNF expression as a biomarker for cognitive reserve against Alzheimer disease progression. *Neurology.* (2016) 86:702–3. doi: 10.1212/WNL.0000000000002389
69. Laske C, Stransky E, Leyhe T, Eschweiler GW, Maetzler W, Wittorf A, et al. BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. *J Psychiatr Res.* (2007) 41:387–94. doi: 10.1016/j.jpsychires.2006.01.014
70. Lee JG, Shin BS, You YS, Kim JE, Yoon SW, Jeon DW, et al. Decreased serum brain-derived neurotrophic factor levels in elderly korean with dementia. *Psychiatry Invest.* (2009) 6:299–305. doi: 10.4306/pi.2009.6.4.299
71. Zheng L, Kong X, Cui Y, Wei Y, Zhang J, Wei W. Conversion from MCI to AD in patients with the APOE ε4 genotype: prediction by plasma HCY and serum BDNF. *Neurosci Lett.* (2016) 626:19–24. doi: 10.1016/j.neulet.2016.05.018
72. Buchman AS, Yu L, Boyle PA, Schneider JA, De Jager PL, Bennett DA. Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. *Neurology.* (2016) 86:735–41. doi: 10.1212/WNL.0000000000002387
73. Janel N, Alexopoulos P, Badel A, Lamari F, Camproux AC, Lagarde J, et al. Combined assessment of DYRK1A, BDNF and homocysteine levels as diagnostic marker for Alzheimer's disease. *Transl Psychiatry.* (2017) 7:e1154. doi: 10.1038/tp.2017.123
74. Suwa M, Kishimoto H, Nofuji Y, Nakano H, Sasaki H, Radak Z, et al. Serum brain-derived neurotrophic factor level is increased and associated with obesity in newly diagnosed female patients with type 2 diabetes mellitus. *Metabolism.* (2006) 55:852–7. doi: 10.1016/j.metabol.2006.02.012
75. Fujinami A, Ohta K, Obayashi H, Fukui M, Hasegawa G, Nakamura N, et al. Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: relationship to glucose metabolism and biomarkers of insulin resistance. *Clin Biochem.* (2008) 41:812–7. doi: 10.1016/j.clinbiochem.2008.03.003
76. Krabbe KS, Nielsen AR, Krogh-Madsen R, Plomgaard P, Rasmussen P, Erikstrup C, et al. Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia.* (2007) 50:431–8. doi: 10.1007/s00125-006-0537-4
77. Li B, Lang N, Cheng Z-F. Serum levels of brain-derived neurotrophic factor are associated with diabetes risk, complications, and obesity: a Cohort Study from Chinese Patients with Type 2 Diabetes. *Mol Neurobiol.* (2016) 53:5492–9. doi: 10.1007/s12035-015-9461-2
78. Zhen YF, Zhang J, Liu XY, Fang H, Tian LB, Zhou DH, et al. Low BDNF is associated with cognitive deficits in patients with type 2 diabetes. *Psychopharmacology.* (2013) 227:93–100. doi: 10.1007/s00213-012-2942-3
79. Passaro A, Dalla Nora E, Morieri ML, Soavi C, Sanz JM, Zurlo A, et al. Brain-derived neurotrophic factor plasma levels: relationship with dementia and diabetes in the elderly population. *J Gerontol A Biol Sci Med Sci.* (2015) 70:294–302. doi: 10.1093/gerona/glu028
80. Hölscher C. First clinical data ofthe neuroprotective effects of nasal insulin application in patiens with Alzheimer's disease. *Alzheimers Dement.* (2014) 10:S33–7. doi: 10.1016/j.jalz.2013.12.006
81. Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, et al. Long-acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. *J Alzheimers Dis.* (2015) 44:897–906. doi: 10.3233/JAD-141791
82. Craft S, Claxton A, Baker LD, Hanson AJ, Cholerton B, Trittschuh EH, et al. Effects of regular and long-acting insulin on cognition and Alzheimer's

disease biomarkers: a pilot clinical trial. *J Alzheimers Dis*. (2017) 57:1325–34. doi: 10.3233/JAD-161256
83. Barone E, Tramutola A, Triani F, Calcagnini S, Di Domenico F, Ripoli C, et al. Biliverdin Reductase-A mediates the beneficial effects of intranasal insulin in Alzheimer disease. *Mol Neurobiol.* (2019) 56:2922–43. doi: 10.1007/s12035-018-1231-5
84. Hölscher C. Insulin signaling impairment in the brain as a risk factor in Alzheimer's disease. *Front Aging Neurosci.* (2019) 11:88. doi: 10.3389/fnagi.2019.00088
85. Athauda D, Maclagan K, Skene SS, Bajwa-Joseph M, Letchford D, Chowdhury K, et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. *Lancet.* (2017) 390:1664–75. doi: 10.1016/S0140-6736(17)31585-4
86. Bomfim TR, Forny-Germano L, Sathler LB, Brito-Moreira J, Houzel JC, Decker H, et al. An antidiabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Aβ oligomers. *J Clin Invest.* (2012) 122:1339–53. doi: 10.1172/JCI57256
87. Bomba M, Granzotto A, Castelli V, Massetti N, Silvestri E, Canzoniero LMT, et al. Exenatide exerts cognitive effects by modulating the BDNF-TrkB neurotrophic axis in adult mice. *Neurobiol Aging*. (2018) 64:33–43. doi: 10.1016/j.neurobiolaging.2017.12.009
88. Bomba M, Granzotto A, Castelli V, Lattanzio R, Cimini AM, Sensi SL. Exenatide reverts the high-fat-diet-induced impairment of BDNF signaling and inflammatory response in an animal model of Alzheimer's disease. *BioRxiv [Preprint]*. (2018). doi: 10.1101/487629
89. Jin L, Li Y-P, Feng Q, Ren L, Wang F, Bo G-J, et al. Cognitive deficits and Alzheimer-like neuropathological impairments during adolescence in a rat model of type 2 diabetes mellitus. *Neural Regen Res.* (2018) 13:1995–2004. doi: 10.4103/1673-5374.239448
90. Kimura N. Diabetes mellitus induces Alzheimer's disease pathology: histopathological evidence from animal models. *Int J Mol Sci.* (2016) 17:503. doi: 10.3390/ijms17040503
91. Movassat J, Calderari S, Fernández E, Martín MA, Escrivá F, Plachot C, et al. Type 2 diabetes - a matter of failing beta-cell neogenesis? Clues from the GK rat model. *Diabetes Obes Metab.* (2007) 9(Suppl. 2):187–95. doi: 10.1111/j.1463-1326.2007.00786.x
92. Portha B, Giroix M-H, Tourrel-Cuzin C, Le-Stunff H, Movassat J. The GK rat: a prototype for the study of non-overweight type 2 diabetes. *Methods Mol Biol Clifton NJ.* (2012) 933:125–59. doi: 10.1007/978-1-62703-068-7_9
93. Movassat J, Saulnier C, Portha B. Beta-cell mass depletion precedes the onset of hyperglycaemia in the GK rat, a genetic model of non-insulin-dependent diabetes mellitus. *Diabete Metab.* (1995) 21:365–70.
94. Noll C, Lacraz G, Ehses J, Coulaud J, Bailbe D, Paul J-L, et al. Early reduction of circulating homocysteine levels in Goto-Kakizaki rat, a spontaneous nonobese model of type 2 diabetes. *Biochim Biophys Acta.* (2011) 1812:699–702. doi: 10.1016/j.bbadis.2011.03.011
95. Li X-H, Xin X, Wang Y, Wu J, Jin Z, Ma L, et al. Pentamethylquercetin protects against diabetes-related cognitive deficits in diabetic Goto-Kakizaki rats. *J Alzheimers Dis.* (2013) 34:755–67. doi: 10.3233/JAD-122017
96. Xiang Q, Zhang J, Li C-Y, Wang Y, Zeng M-J, Cai Z-X, et al. Insulin resistance-induced hyperglycemia decreased the activation of Akt/CREB in hippocampus neurons: molecular evidence for mechanism of diabetes-induced cognitive dysfunction. *Neuropeptides.* (2015) 54:9–15. doi: 10.1016/j.npep.2015.08.009
97. Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol Neurodegener.* (2011) 6:85. doi: 10.1186/1750-1326-6-85
98. Beauquis J, Homo-Delarche F, Giroix M-H, Ehses J, Coulaud J, Roig P, et al. Hippocampal neurovascular and hypothalamic-pituitary-adrenal axis alterations in spontaneously type 2 diabetic GK rats. *Exp Neurol.* (2010) 222:125–34. doi: 10.1016/j.expneurol.2009.12.022
99. Lang BT, Yan Y, Dempsey RJ, Vemuganti R. Impaired neurogenesis in adult type-2 diabetic rats. *Brain Res.* (2009) 1258:25–33. doi: 10.1016/j.brainres.2008.12.026
100. Jin N, Yin X, Gu J, Zhang X, Shi J, Qian W, et al. Truncation and activation of dual specificity tyrosine phosphorylation-regulated Kinase 1A by Calpain I: a molecular mechanism linked to tau pathology in alzheimer disease. *J Biol Chem.* (2015) 290:15219–37. doi: 10.1074/jbc.M115.645507
101. Hamelet J, Noll C, Ripoll C, Paul J-L, Janel N, Delabar J-M. Effect of hyperhomocysteinemia on the protein kinase DYRK1A in liver of mice. *Biochem Biophys Res Commun.* (2009) 378:673–7. doi: 10.1016/j.bbrc.2008.11.126
102. Latour A, Gu Y, Kassis N, Daubigney F, Colin C, Gausserès B, et al. LPS-induced inflammation abolishes the effect of DYRK1A on IkB stability in the brain of mice. *Mol. Neurobiol.* (2018) 56:963–75. doi: 10.1007/s12035-018-1113-x
103. Souchet B, Audrain M, Billard J, Dairou J, Fol R, Salvatore Orefice N. et al. Inhibition of DYRK1A proteolysis modifies its kinase specificity and rescues Alzheimer phenotype in APP/PS1 mice. *Acta Neuropathol.* (2019) 7:46. doi: 10.1186/s40478-019-0678-6
104. Ferrer I, Marín C, Rey MJ, Ribalta T, Goutan E, Blanco R, et al. BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J Neuropathol Exp Neurol.* (1999) 58:729–39. doi: 10.1097/00005072-199907000-00007
105. Phillips HS, Hains JM, Armanini M, Laramee GR, Johnson SA, Winslow JW. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron.* (1991) 7:695–702. doi: 10.1016/0896-6273(91)90273-3
106. Forlenza OV, Diniz BS, Teixeira AL, Ojopi EB, Talib LL, Mendonça VA, et al. Effect of brain-derived neurotrophic factor Val66Met polymorphism and serum levels on the progression of mild cognitive impairment. *World J Biol Psychiatry Off J World Fed Soc Biol Psychiatry.* (2010) 11:774–80. doi: 10.3109/15622971003797241
107. Guillozet-Bongaarts AL, Garcia-Sierra F, Reynolds MR, Horowitz PM, Fu Y, Wang T, et al. Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. *Neurobiol Aging.* (2005) 26:1015–22. doi: 10.1016/j.neurobiolaging.2004.09.019
108. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, et al. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest.* (2004) 114:121–30. doi: 10.1172/JCI2004 20640

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neurology

HYPOTHESIS AND THEORY
published: 31 July 2019
doi: 10.3389/fneur.2019.00807

Deciphering the Link Between Hyperhomocysteinemia and Ceramide Metabolism in Alzheimer-Type Neurodegeneration

Hervé Le Stunff[1,2], *Julien Véret*[1], *Nadim Kassis*[1], *Jessica Denom*[1], *Kelly Meneyrol*[1], *Jean-Louis Paul*[3], *Céline Cruciani-Guglielmacci*[1], *Christophe Magnan*[1] *and Nathalie Janel*[1]*

[1] Université de Paris, BFA, UMR 8251, CNRS, Paris, France, [2] Institut des Neurosciences Paris-Saclay (Neuro-PSI), Université Paris-Sud, CNRS UMR 9197, Orsay, France, [3] AP-HP, Hôpital Européen Georges Pompidou, Service de Biochimie, Paris, France

Aging is one of the strongest risk factor for Alzheimer's disease (AD). However, several data suggest that dyslipidemia can either contribute or serve as co-factors in AD appearance. AD could be examined as a metabolic disorder mediated by peripheral insulin resistance. Insulin resistance is associated with dyslipidemia, which results in increased hepatic ceramide generation. Hepatic steatosis induces pro-inflammatory cytokine activation which is mediated by the increased ceramides production. Ceramides levels increased in cells due to perturbation in sphingolipid metabolism and upregulated expression of enzymes involved in ceramide synthesis. Cytotoxic ceramides and related molecules generated in liver promote insulin resistance, traffic through the circulation due to injury or cell death caused by local liver inflammation, and because of their hydrophobic nature, they can cross the blood-brain barrier and thereby exert neurotoxic responses as reducing insulin signaling and increasing pro-inflammatory cytokines. These abnormalities propagate a cascade of neurodegeneration associated with oxidative stress and ceramide generation, which potentiate brain insulin resistance, apoptosis, myelin degeneration, and neuro-inflammation. Therefore, excess of toxic lipids generated in liver can cause neurodegeneration. Elevated homocysteine level is also a risk factor for AD pathology and is narrowly associated with metabolic diseases and non-alcoholic fatty liver disease. The existence of a homocysteine/ceramides signaling pathway suggests that homocysteine toxicity could be partly mediated by intracellular ceramide accumulation due to stimulation of ceramide synthase. In this article, we briefly examined the role of homocysteine and ceramide metabolism linking metabolic diseases and non-alcoholic fatty liver disease to AD. We therefore analyzed the expression of mainly enzymes implicated in ceramide and sphingolipid metabolism and demonstrated deregulation of *de novo* ceramide biosynthesis and S1P metabolism in liver and brain of hyperhomocysteinemic mice.

Keywords: NAFLD, Alzheimer's disease, hyperhomocysteinemia, sphingolipid metabolism, ceramides

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Ilaria Piano,
University of Pisa, Italy
Bindu Diana Paul,
Johns Hopkins University,
United States

***Correspondence:**
Nathalie Janel
janel@univ-paris-diderot.fr

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neurology

Received: *05 March 2019*
Accepted: *15 July 2019*
Published: *31 July 2019*

Citation:
Le Stunff H, Véret J, Kassis N, Denom J, Meneyrol K, Paul J-L, Cruciani-Guglielmacci C, Magnan C and Janel N (2019) Deciphering the Link Between Hyperhomocysteinemia and Ceramide Metabolism in Alzheimer-Type Neurodegeneration. Front. Neurol. 10:807. doi: 10.3389/fneur.2019.00807

INTRODUCTION

Insulin resistance is a major public health outcome by its association with the non-alcoholic fatty liver disease (NAFLD), metabolic syndrome, type 2 diabetes mellitus (T2DM), obesity, and Alzheimer's disease (AD)-type neurodegeneration epidemics. Aging is also a powerful AD risk factor. However, many data imply dyslipidemic conditions as contributor or co-factors in pathogenesis of AD. The classical "amyloid cascade" hypothesis in AD, actually deeply debated, demonstrates that cognitive defects and memory loss implicate the development of wide and insoluble beta amyloid plaques in various brain areas, resulting in apoptosis of neurons (1).

Apart from amyloid-β peptide (Aβ), many evidences suggest that impairing of insulin signaling and brain glucose metabolism act an important role in AD development. Human post-mortem studies support this notion, indicating that insulin resistance in AD brain is systematically showed and increased with disease advancement (2–4). Another connection between T2DM and AD could be supplied by Tau processing failures. Neurofibrillary tangles (NFTs) are principally constituted by hyperphosporylated tau proteins. NFTs, such as amyloid beta plaques, account as important histopathological characteristics of AD. Some authors have called AD as "type 3 diabetes" in relation with the alterations, at the very early disease stages, of insulin signaling more specifically in the brain (2). Insulin resistance is linked with inflammation and dyslipidemia which results in increased ceramides generation notably in hepatic function (5, 6). For this reason, AD could be considered like metabolic diseases mediated by disorders due to peripheral insulin resistance. Progressive hepatic steatosis induces inflammation with activation of pro-inflammatory cytokines, leading not only to increased ceramide production, but also alteration in one carbon metabolism. Ceramides build up in cells because of disruptions in sphingolipid metabolism and activation of pro-ceramides genes (7). Cytotoxic ceramides and related molecules generated in liver promote insulin resistance, traffic through the circulation due to injury or apoptosis caused by local liver inflammation, and because of their hydrophobic nature, can pass through the blood-brain barrier (BBB), thereby exerting toxic responses as reducing insulin signaling and increasing pro-inflammatory cytokines. These defects initiate or support propagation of neurodegeneration with an oxidative stress and ceramide production, exacerbating brain insulin resistance, neuronal apoptosis, and neuro-inflammation. Therefore, neurodegeneration can be caused by toxic liver lipid production (5). In mice, increased production of toxic lipid/ceramide can be induced by liver/peripheral tissue-brain axis of neurodegeneration and be moved through the BBB resulting cognitive impairments (8).

Disturbances in one carbon metabolism, determined by enzyme failures integral to this process, comply with abnormally increased plasma homocysteine (Hcy) levels, namely hyperhomocysteinemia (HHcy). HHcy has been frequently linked with T2DM, cardiovascular diseases (CVD), atherosclerosis and present in NAFLD (9–11). Given that the majority of dietary methionine metabolism is made in liver, this organ represents the major place for Hcy metabolism (12). During liver failure, metabolism of Hcy was modified in association with lipid metabolism disturbance (13–15). Increased Hcy level is also associated with AD pathology (16–19). Taken as a whole, results suggest that Hcy/ceramides signaling pathway exists which suggests a link between Hcy toxicity and intracellular ceramide accumulation via the activation of ceramide synthase (20).

HOMOCYSTEINE, A LINK BETWEEN NAFLD, T2DM, AND AD

In the world, NAFLD is the most shared hepatic disorder, its incidence reaching 70–90%. It is also linked with obesity, T2DM and related metabolic diseases (21). NAFLD is linked to hepatic insulin resistance and occurs frequently with obesity/T2DM. Intrahepatic fat accumulation is the feature of NAFLD. Progression of NAFLD is more likely to take place in metabolic diseases patients (22). Oxidative stress associated with insulin resistance have an essential part in NAFLD (23). One-carbon metabolism is involved in methylation of notably proteins, DNA, RNA, and protects cells against oxidation (24). S-adenosylmethionine (SAM) is produced by methionine adenosylation and is the most important methyl-group donor in cellular metabolism (25) (**Figure 1**). DNA methylation capacity of cells can be modified by a reduction of SAM concentration associated with a reduction in SAM: S-adenosylhomocysteine (SAH) ratio (26). One-carbon metabolism perturbation consequently participates to pathogenesis and NAFLD promotion.

In the liver, Hcy, a thiol-containing amino acid, is involved in metabolism of dietary methionine. SAH metabolism produces Hcy, SAH being produced through methylation reactions implicating SAM and methyltransferases. Hcy can be metabolized by conversion to cysteine via the transsulfuration pathway, the first step involving cystathionine beta synthase (CBS) (27) (**Figure 1**). In alcoholic fatty liver disease (28) but also in non-alcoholic steatohepatitis (NASH) (29), serum Hcy levels are elevated and good predictor of disease progression. Hepatic steatosis in human patients and in mice with CBS deficiency is associated with HHcy (29–32). In rats, HHcy due to decreased hepatic CBS activity is elicited by NAFLD induced by high fat diet (15). Therefore, NAFLD is early characterized by HHcy. The involvement of dual-specificity tyrosine-(Y)-phosphorylation regulated kinase (DYRK1A) in one carbon metabolism is strengthened by the positive correlation between hepatic protein expression and CBS activity (33, 34). DYRK1A, a protein involved in development, growth and apoptosis (35), is also implicated in Hcy cycle (36, 37) (**Figure 1**). DYRK1A is also implicated in β-cell mass adjustment and involved in carbohydrate metabolism (38, 39). In mice, DYRK1A is also implicated in liver damage induced by alcohol consumption (34).

Individuals with NAFLD can exhibit neuropsychiatric dysfunction, including anxiety and depression, which frequently precede cognitive impairment and dementia. Factors affecting one-carbon metabolism and consequently elevated Hcy levels

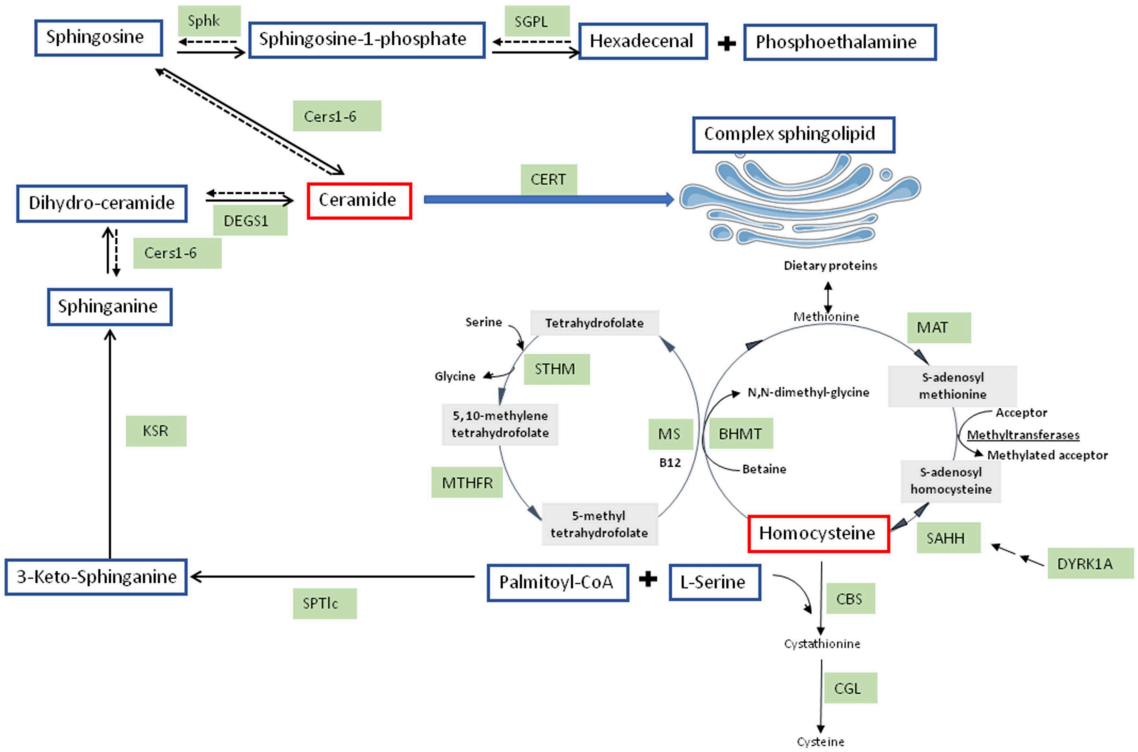

FIGURE 1 | Integrated pathways for homocysteine/cysteine synthesis from methionine and sphingolipid synthesis. BHMT, betaine-homocysteine methyl transferase; CBS, cystathionine beta-synthase; Cers, ceramide synthases; CERT, ceramide transferase; CGL, cystathionine gamma-lyase; DEGS1, dihydroceramide desaturase 1; KSR, 3-keto-sphinganine reductase; MAT, methionine adenosyl transferase; MS, methionine synthase; MTHFR, 5,10-methylene tetrahydrofolate reductase; SAHH, S-adenosyl homocysteine hydrolase; SGPL, sphingosine-1-phosphate lyase; Sphk, sphingosine kinase; SPTlc, serine palmitoyltransferase; STHM, serine transhydroxymethylase.

have been linked to AD. Divers risk factors of sporadic AD have been characterized by long-term and prospective population and cross-sectional retrospective studies, as hypercholesterolemia, T2DM, and HHcy (40). Elevated proinflammatory cytokines have metabolic connotation (40). Older patients suffering from mild hypertension showed association between hippocampal atrophy, and white matter atrophy, with HHcy. Elderly patients with HHcy showed increased rate of hippocampal atrophy and cognitive decline (41, 42). In the aged population, elevated Hcy level can be established as a risk factor for cognitive decline (43). Progression of white matter hyperintensities and faster rates of total brain volume loss have been associated with increased plasma Hcy levels in patients with hypertension (19). Many studies have demonstrated that moderately elevated Hcy levels increased late-onset Alzheimer's disease (LOAD) risk, even if the value is close to the critical threshold (16–19, 44). According to recent international consensus statement (45), moderately elevated Hcy level can increase the relative risk of dementia in the elderly 1.15- to 2.5-fold, with the Population Attributable risk from 4.3 to 31%.

Many mechanisms have been suggested to connect elevated Hcy level with AD. Many experimental studies have demonstrated that elevated Hcy level can produce many neurotoxic effects implying excitotoxicity, oxidative stress, mitochondrial dysfunction, DNA damage and apoptosis. Therefore, HHcy can participate to AD neurodegeneration (46). Preclinical studies show that HHcy generates Aβ accumulation in brain (47–51) and increased hyperphosphorylation of tau (52). The link of Hcy to Aβ can lead to the development of interconnections and consequently to the development of aggregates (53). Elevation of Hcy contributes to the decrease of SAM levels. Demethylation of DNA can be produced by decreased SAM level, leading to overexpression of presenilin (PSEN1) and beta-secretase (BACE1), the β-site amyloid precursor protein (APP)-cleaving enzyme (54). Another study demonstrates that dimerization of apoE3 can be block by Hcy, reducing apoE3-mediated high-density lipoprotein (HDL) generation and consequently reducing microglial degradation of soluble Aβ (55). Patients with HHcy, compared to control subjects, have lower ratio of apoE3 dimers in their cerebrospinal fluid (CSF).

Many studies demonstrate the involvement of DYRK1A in AD (56). DYRK1A interacts with APP, and plays a role in APP processing by direct phosphorylation of APP at Thr-668 and indirect phosphorylation of PSEN1 at Thr-354, promoting the pathological Aβ pathway and the production of Aβ. Increased DYRK1A expression enhances APP phosphorylation and its cleavage, resulting in increased Aβ40 and Aβ42 levels

and thus promoting brain β-amyloidosis (57). Its direct tau hyperphophorylation and indirect phosphorylation of alternative splicing factor promotes neurofibrillary degeneration, showing its involvement in neurodegenerative processes and neuronal depletion appearing in AD (57–60). We have previously shown, on the one hand, that plasma DYRK1A levels correlate positively with CSF tau and phosphorylated-tau proteins in AD (61), and on the other hand that combined assessment of plasma levels of DYRK1A and Hcy can be validate as diagnostic marker for AD (44). Lipidomics analysis showed that not only triglycerides (TG) content but also ceramide content were increased in HHcy mice which have decreased liver DYRK1A protein expression (36, 62). We previously demonstrated altered lipoprotein metabolism in mice overexpressing DYRK1A (63). Note that mice overexpressing DYRK1A have, on the contrary, not only a decreased plasma Hcy level (37) but also a decreased serum TG level (0.369 ± 0.04 vs. 0.519 ± 0.057 mmol/L; $p < 0.05$ by Student's t-test; $n = 8$ for each). It will be important to demonstrate if the effects of Hcy not only in liver but also in brain are mediated by ceramide signaling.

SPHINGOLIPID METABOLISM AND AD

Considered for a long time as structural compounds, several evidences demonstrated that bioactive sphingolipids are involved as signaling molecules in the various tissues including the brain. In these tissues, sphingolipids play important role in several pathologies including neurodegenerative diseases, such as AD. Sphingolipids could be produced by several pathways. *De novo* sphingolipid synthesis is initiated in the cytoplasmic face of the endoplasmic reticulum (ER) which is started with the condensation of palmitoyl-CoA L-serine with to form 3-ketosphinganine (**Figure 1**). Serine palmitoyl-transferase (SPT) catalyzes this reaction (64). Two subunits, SPTLC1 and SPTLC2, showing a similarity at amino acid sequence of around 20%, compose the heterodimer SPT. SPTLC1 and SPTLC2 seem to be both required for enzyme activity. However, the SPTLC2 subunit contains a pyridoxal phosphate binding motif (65) and produces the common and major C18-sphingoid bases. A third SPT subunit, SPTLC3, has been identified, with 68% homology to the SPTLC2 subunit. However, SPTLC3 is involved in the production of C14- and C16-sphingoid bases. SPTLC2 and SPTLC3 are therefore distinct from a specificity point of view (66). The SPT substrate preference toward the requirement of longer acyl-CoA could be modulated by the differential expression of SPTLC2 and SPTLC3. Moreover, C16-sphingoid bases could be transformed into more complex sphingolipids, such as C16-ceramide and glycosphingolipid. Sphingolipids can be implicated in AβPP/Aβ metabolism and therefore AD development due to their structural roles in cellular membranes including lipid rafts (67). Conversely, ceramide formation can be promoted by accumulation of oligomerized Aβ in AD brain. Indeed, Aβ peptides can activate SPT, resulting in neurotoxic ceramide increase by the *de novo* synthesis pathway (68, 69). Interestingly, SPTLC2 was found to be up-regulated in AD (70, 71). However, a regulation of SPTLC3 subunit has not been explored in neurodegenerative diseases development, such as AD.

3-ketosphinganine is formed and rapidly reduced by 3-ketosphinganine reductase into dihydrosphingosine (DH-Sph) (**Figure 1**). The reaction results in DH-Sph, implicated in the production of various species of dihydro-ceramides by ceramide synthases (CerS) (**Figure 1**). This species will differ by the nature of acyl-CoA chain length used for the N-acylation of DH-Sph (72). The dihydroceramide desaturases (DEGS1) metabolizes dihydro-ceramides into ceramides (**Figure 1**). Interestingly, CerS1 and CerS2 are up-regulated in AD brains whereas CerS6 is reduced (70, 71), suggesting a remodeling of ceramide species during the development of AD. The ceramide produced are converted in the Golgi apparatus into sphingomyelin or glucosyl-ceramides by sphingomyelin synthase and glucosyl-ceramide synthase, respectively (73) (**Figure 1**).

Ceramidases could deacylated ceramides to produce sphingosine (**Figure 1**). In cells, sphingosine but also other sphingoid bases can be phosphorylated by two sphingosine kinases (SphK 1 and SphK2) to form sphingosine-1-phopshate (S1P). S1P can be dephosphorylated to sphingosine by specific S1P phosphohydrolases, the reaction being reversible, or can be cleaved by a pyridoxal-dependent S1P lyase (SGPL) into ethanolamine phosphate and hexadecenal, the reaction being irreversible (74) (**Figure 1**). S1P, in contrary to ceramide, is known to be a pro-survival lipid for various cells including neurons (74). Interestingly, S1P metabolism has also been related to AD. Indeed, FTY720, a substrate of SphK2 that can bind S1P receptors has been shown to reduce neuronal Aβ generation (75). Loss of neuroprotective S1P and SPHK activity was found early in AD development prior to AD diagnosis with a decrease of Sphk2 activity in hippocampus (76). However, the role of SphK2 is still controversial in AD since S1P production by SphK2 and Aβ processing seemed to be positively correlated (77). This discrepancy could come from subcellular distribution of SphK2 between cytosol and nucleus which is altered in AD brains (78). These data suggest that the pro-survival cytosolic S1P may be less efficient by a shift in the subcellular localization of the S1P generating by SphK2 which will lead to the production of nuclear S1P associated with deleterious effects in AD pathogenesis. Up to date, SphK2 and SGPL (70) have been shown to be overexpressed whereas SphK1 was found to be down-regulated in AD brain suggesting a deregulation of S1P signaling in this pathology which remained to be clarified.

CERAMIDES, A LINK BETWEEN NAFLD, T2DM, AND AD

Ceramides are also important mediators of insulin resistance in various peripheral tissues but also in pancreatic β cell deregulation induced by obesity (79, 80). Obesity is well-established as a predisposing factor for the appearance of hepatic steatosis, NAFLD being strongly associated with both hepatic and peripheral insulin resistance with a defect in the ability of insulin to suppress endogenous glucose production (81). In addition, NAFLD is linked to important hepatic changes in

lipid metabolites, such as an increase in hepatic cholesterol. When excess of saturated fatty acids are poorly incorporated into hepatocyte triglycerides, they induce lipotoxicity, resulting in liver injuries (82). Excess of saturated fatty acids constitutes a preferential substrate for the *de novo* ceramide biosynthesis (83). It is known that ceramide levels contribute to the development of NAFLD by mediating obesity, inflammation, insulin resistance, and oxidative stress (84).

In addition to intracellular-based actions, circulating extracellular ceramides have roles in insulin resistance. *In vitro* studies showed that hepatocytes treated with palmitate could efficiently secrete newly synthesized ceramide in response to hyperlipidemia, demonstrated by increased extracellular ceramide concentration (85). Ceramide could be either transported by lipoproteins but also by cell-derived membrane shed both basally and under stress conditions called extracellular vesicles (86, 87). Interestingly, advanced pathological signs of AD and also induced neuronal apoptosis could be elicited by chronic NAFLD in mice (88). Therefore, chronic inflammation induced by obesity-associated NAFLD outside from the brain is sufficient to induce neurodegeneration in the absence of genetic predisposition. Since NAFLD is associated with an increase of circulating sphingolipid, such as ceramide, it is tempting to suggest that circulating ceramide originating from liver could also target brain tissues and favor the development of AD.

Some of sphingolipids could constitute biomarkers to identify individuals who are at risk to develop T2DM. Therefore, it will be more important to quantify circulating sphingolipid concentrations. Plasma dihydro-ceramides levels were showed effectively to be significantly elevated up to 9 years before the detection of the disease of individuals from two human cohorts who will progress to diabetes (89). Interestingly, deregulation of ceramide metabolism reflected by an increase of plasma ceramide level could arise in different stages of AD progression (90, 91). Moreover, it would be important to define whether AD is associated with a differential distribution of ceramides in lipoproteins, but also in exosomes, which could serve as biomarkers of disease progression.

S1P has been also shown as a potent regulator of NAFLD, treatment with S1P enhancing hepatic lipid storage (92). A 2-fold increase of SphK1 was determined in livers from humans with NAFLD but also in mice feeded with a high saturated fat diet (92, 93). These mice showed activation of NFκB, elevated cytokine production, and immune cell infiltration. Importantly, a total SphK1-null mice were protected from these outcomes. To date, the role of SphK2 has not been explored in the context of NALFD. In contrast, it has been shown that a total SPL-null mice resulted in a widespread change in lipid metabolism genes expression pattern, with a significant increase in the expression of PPARgamma, a key transcriptional regulator of lipid metabolism (94), suggesting that regulation of SGPL could be a potent regulator of NAFLD. The liver is known to be engaged in regulating the plasma level of S1P, as liver produces a chaperone for S1P transport, the apolipoprotein M (apoM) (95). Interestingly, polarized endothelial cells, composing the lining of the BBB, also express and secrete apoM toward the brain as well as to the circulation suggesting that circulating S1P could target the brain by this way (96). Therefore, as ceramide, secretion of S1P by hepatocytes could constitute a potent regulator of AD by targeting the brain.

STATEMENT OF HYPOTHESIS: CERAMIDE AND SPHINGOLIPID METABOLISM IS MODIFIED IN LIVER AND HYPOTHALAMUS OF HYPERHOMOCYSTEINEMIC MICE ON A HIGH FAT DIET

Based on the results described above, we used mice heterozygous for targeted disruption of the Cbs gene ($Cbs^{+/-}$) (30) and wild type ($Cbs^{+/+}$) mice on the same background, fed on a standard diet supplemented with 0.5% L-methionine (Sigma-Aldrich, France) in drinking water to induce intermediate HHcy in $Cbs^{+/-}$ mice (97), and with a high-fat diabetogenic diet (HFD) (98). As expected, $Cbs^{+/-}$ mice showed a significant increase of plasma Hcy level ($37.1 \pm 3.2\,\mu M$ vs. $6.4 \pm 0.4\,\mu M$; $p < 0.0001$ by Student's t-test $n = 8$ for each). We previously evaluated plasma Hcy levels in transgenic mouse models of AD. No significant difference was observed in blood of transgenic mouse models of AD compared to control mice, indicating that Hcy is not a primary cause of AD (99). Animal studies using AD-like transgenic mouse models, on a methionine enriched diet, were used to provide potential mechanisms by which HHcy might influence AD development. Tg2576 transgenic female mice expressing hAPP with the Swedish mutation (K670N/M671L) on a methionine enriched diet exhibited an increase from 6 to 35 μM (51, 100).

Effect of HHcy and HFD on Enzymes Expression Involved in Ceramide and Sphingolipid Metabolism in Liver

Gene expression of mainly enzymes implicated in ceramide and sphingolipid metabolism has first been analyzed by Q-PCR in mice liver. As expected, $Cbs^{+/-}$ mice showed a significant decrease of liver CBS activity (45.2 ± 11.1 vs. 102 ± 21.6; $p < 0.05$ by Student's t-test $n = 4$ for each), commensurate with a decrease in mRNA expression (13.5 ± 7.3 vs. 100 ± 26; $p < 0.03$ by Student's t-test $n = 4$ for each). $Cbs^{+/-}$ mice also showed a significant decrease of liver Dyrk1A level (40.6 ± 6.3 vs. 100 ± 16; $p < 0.02$ by Student's t-test $n = 4$ for each) (36), associated with a decrease in mRNA expression (18.9 ± 14 vs. 100 ± 21.3; $p < 0.023$ by Student's t-test $n = 4$ for each). SGPL1 and SPTlc2 expression were decreased in liver of $Cbs^{+/-}$ on methionine and HFD (**Table 1**) (**Figures 2A,B**). In agreement with the decreased gene expression of SPTlc2, we also found a decrease in protein level (**Figure 2C**). Hepatic expression of CBS and SGPL1 ($r = 0.96$, $p < 0.02$), Sphk2 ($r = 0.83$, $p < 0.05$), and SPTlc2 ($r = 0.93$, $p < 0.03$) were positively correlated, Dyrk1A and Cers5 expression being correlated negatively ($r = -0.93$, $p < 0.02$).

Ceramide is a potent regulator of cell proliferation, activation, and apoptosis. Ceramide plays an key role in different cellular functions, such as plasma lipoprotein metabolism and cell

TABLE 1 | Relative liver and hypothalamus mRNA expression based upon Q-PCR data obtained from wild-type ($Cbs^{+/+}$ mice) and mice heterozygous for targeted disruption of the Cbs ($Cbs^{+/-}$ mice) on a methionine enriched diet (Met) and high fat diet (HFD).

mRNA (%)	Hypothalamus $Cbs^{+/+}$ mice Met/HFD ($n = 4$)	Hypothalamus $Cbs^{+/-}$ mice Met/HFD ($n = 4$)	Liver $Cbs^{+/+}$ mice Met/HFD ($n = 4$)	Liver $Cbs^{+/-}$ mice Met/HFD ($n = 4$)
Cers1	100 ± 52.2	354 ± 211	100 ± 71	107.7 ± 52.8
Cers2	104 ± 8	247 ± 29**	100 ± 21	124 ± 15
Cers3	100 ± 26	115 ± 48	100 ± 41	95 ± 24
Cers4	99.9 ± 63.9	103.1 ± 58.8	100.9 ± 29.3	101.7 ± 55.1
Cers5	100 ± 18	226 ± 87	100 ± 28	189 ± 43
Cers6	100 ± 33	157 ± 58	100 ± 30	82 ± 21
CERT	100.4 ± 22.5	40.8 ± 17.1	100 ± 35.2	48.2 ± 25.4
DEGS1	100 ± 38.9	61.1 ± 26.1	100 ± 10.3	105.8 ± 29.6
SGPL1	100 ± 51.6	57.3 ± 23	100 ± 15.3	13.5 ± 7.4**
Sphk1	99.3 ± 27.9	71.6 ± 39.2	100.4 ± 44	21 ± 10
Sphk2	100.1 ± 36.4	14.8 ± 6.5*	100.4 ± 24.2	45 ± 21.6
SPTlc1	100 ± 28	10.5 ± 1*	100 ± 22.9	115.4 ± 32.3
SPTlc2	100 ± 2	52 ± 20.6	100 ± 31.6	9.5 ± 6.3*
SPTlc3	98.3 ± 47	371.3 ± 118.3	97.5 ± 24.9	294.5 ± 144.6

*Total RNA was isolated from the hypothalamus and liver, reverse transcribed and real time quantitative PCR amplification reactions were carried as described using the LightCycler FastStart DNA Master plus SYBR Green I kit (Roche) (97). The mRNA transcript level was normalized against the mean of two genes: H1a and TBP (**Table S1**). Data were normalized to the mean of $Cbs^{+/+}$ mice on Met/HFD. Data are presented as mean ± SEM, and analyzed with the Student's t-test by using Statview software. n, number of mice. *p < 0.05; **p < 0.01. Data were considered significant when p < 0.05.*

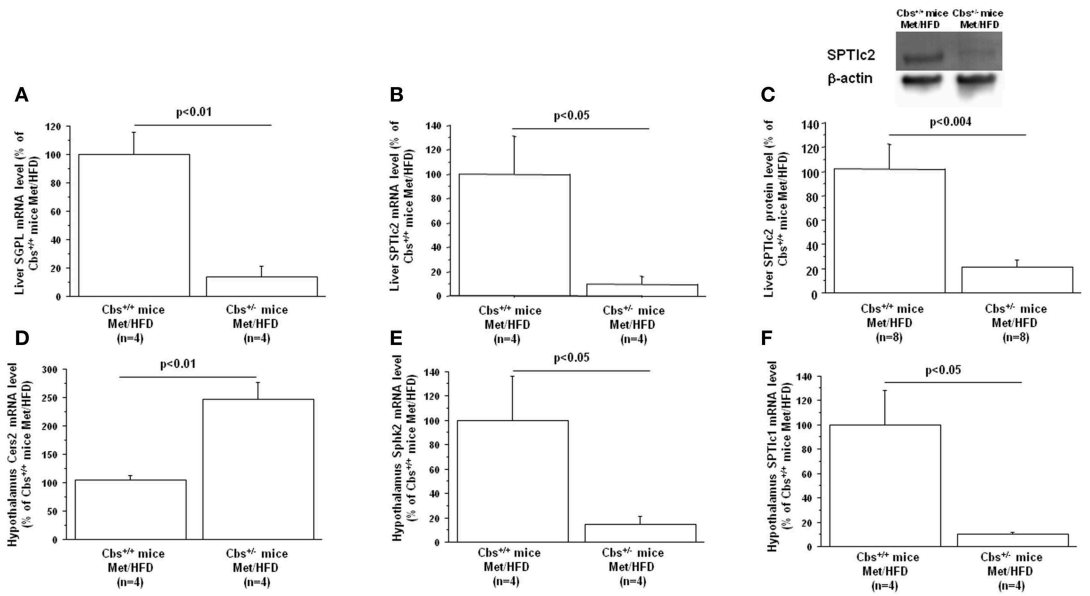

FIGURE 2 | Effect of hyperhomocysteinemia and high fat diet on liver **(A)** SGPL1 mRNA, **(B)** SPTlc2 mRNA, **(C)** SPTlc2 protein, **(D)** Cers2 hypothalamus mRNA, **(E)** Sphk2 hypothalamus mRNA, and **(F)** SPTlc1 hypothalamus mRNA level in wild type ($Cbs^{+/+}$ mice) and mice heterozygous for targeted disruption of the Cbs ($Cbs^{+/-}$ mice) on a methionine enriched diet (Met) and high fat diet (HFD). Relative liver and hypothalamus mRNA expression was described in **Table 1**. Liver SPTlc2 was determined by western blot and quantified by slot blotting [1/1,000 (101)]. β-actin (1/10,000) (Sigma-Aldrich, France) was used as an internal control for Western blot analysis or ponceau-S coloration for slot blot analysis. Data are presented as mean ± SEM, and analyzed with the Student's *t*-test by using Statview software. *n*, number of mice. Data were considered significant when $p < 0.05$.

membrane formation, known to contribute to the development of atherosclerosis and other sclerotic diseases, such as insulin resistance, obesity, and AD (102, 103). Previous results have demonstrated that *de novo* ceramide biosynthesis is implicated in induction of kidney NAD(P)H oxidase activity in HHcy rats fed a folate-free diet and report the important role of redox signaling catalyzed by ceramides in glomerular injury induced by HHcy in rats (104). Acid sphingomyelinase is also involved in the development of glomerular oxidative stress and injury induced by HHcy (105, 106).

In HHcy induced by supplementation of Hcy in drinking water for 6 weeks in mice, hepatic steatosis was found to be associated with a notable increase in ceramide-related metabolites and subsequent upregulation of ceramide synthesis genes including *Sptlc3*, *Degs2*, *Cer4*, and *Smpd4* (62). Moreover, ceramide synthases were suggested to be involved in Hcy-induced ceramide production by the fact that abolishing the expression of *Sptlc3* and *Degs2* by omega-3 significantly ameliorated HHcy-mediated increases of hepatic ceramide (62). In our study, the increase of Cers5 (correlating negatively with Dyrk1A expression), the non-significant increase of *Sptlc3* support an increased ceramide levels in liver of $Cbs^{+/-}$ mice on methionine and HFD mediated through specific ceramide synthases. The SPTLC3 subunit has been identified as generating short chain sphingoid bases (66) compared to SPTLC2. Commensurate with the increased *Sptlc3* level, *Sptlc2* was found to be decreased in liver of $Cbs^{+/-}$ mice on methionine and HFD, correlating with CBS expression. Palmitate-CoA is the predominant substrate for SPTLC2, whereas mysristoyl and lauryl-CoA is the preferential substrates for SPTLC3, which results in the production of different chain length of the sphingoid base. It is possible that these two subunits can be switched in a SPT enzyme complex to replenish the ceramide pool, knocking down all SPTLC subunits being necessary to decrease total ceramides significantly (107). The hepatic decrease of SPTLC2 in $Cbs^{+/-}$ mice on methionine and HFD could therefore lead to a decrease of ceramides with a C18-sphingoid bases backbone and promotes the synthesis of ceramide species through the action of SPLTC3 with a C16-sphingoid base backbone. Interestingly, SPTLC3 expression has been associated with NAFLD (108) and therefore could participate to its development under the context of HHcy. We also found a strong hepatic decrease of SGPL in $Cbs^{+/-}$ on methionine and HFD suggesting an altered catabolism of S1P. Knowing the novel role of S1P in hepatic injury, such as NAFLD (109), it will tempting to propose that S1P metabolism also could contribute to NAFLD in $Cbs^{+/-}$ mice on methionine and HFD (109). More importantly, down-regulation of SGPL could also contribute to increased circulating S1P levels since it has been shown that saturated fatty acids serve to the synthesis of S1P in hepatocytes which is released in the extracellular environment (110). Increasing S1P levels in HHcy mice could have repercussion on glucose homeostasis by targeting peripheral tissues but could also target the brain where S1P is a potent regulator of AD development (111). Hypothalamic insulin resistance and lipotoxicity have been previously reported to be induce by *de novo* ceramide biosynthesis (101). Therefore, we also analyzed the main enzymes implicated in ceramide and sphingolipid metabolism in hypothalamus of mice.

Effect of HHcy and HFD on Enzymes Expression Involved in Ceramide and Sphingolipid Metabolism in Hypothalamus

As expected, $Cbs^{+/-}$ mice showed a significant decrease of CBS mRNA expression in hypothalamus (15 ± 10 vs. 100 ± 29; $p < 0.03$ by Student's *t*-test $n = 4$ for each). A significant increase of hypothalamus Dyrk1A level (259 ± 22.3 vs. 101.4 ± 25.2; $p < 0.003$ by Student's *t*-test $n = 4$ for each) was found as expected, without difference in mRNA level (58.9 ± 21.1 vs. 100 ± 30.7; $n = 4$ for each) (112). Expression of Cers2 was increased, with a decrease of Sphk2 and SPTlc1 in hypothalamus of $Cbs^{+/-}$ mice on methionine and HFD (**Table 1**) (**Figures 2D–F**). A positive correlation was found between hypothalamic expression of CBS and SGPL1 ($r = 0.76$, $p < 0.04$), Sphk2 ($r = 0.81$, $p < 0.03$), and SPTlc1 ($r = 0.94$, $p < 0.04$). We also found a positive correlation between liver expression of CBS and hypothalamic expression of Sphk2 ($r = 0.86$, $p < 0.02$), and SPTlc1 ($r = 0.79$, $p < 0.04$).

It has been demonstrated that Hcy-treatment of cerebral endothelial cells induces acid sphingomyelinase ceramide pathway (113). The increase of *Cers2*, the decrease of *Sphk2* with the non-significant increase of *Sptlc3* and the non-significant decrease of *SGPL* (*Sphk2 and SGPL* correlating positively with CBS expression) support increased hypothalamic ceramide levels in HHcy mice on HFD mediated through the regulation of specific ceramide synthases. Previous results found in human post-mortem brain and mouse transgenic AD model an increased mRNA level of *Cers1*, Cers2, and a decrease in Sphk1 and Sphk2 (68, 76, 114). Loss of neuroprotective S1P and SPHK activity was found early in AD pathogenesis prior to AD diagnosis (76). Altogether, our results suggest that local alteration of S1P metabolism also could contribute to AD development associated with HHcy.

CONCLUSION

In this study, we used HHcy mice due to CBS deficiency to analyze expression of the main enzymes implicated in ceramide and sphingolipid metabolism. Our results support an increased ceramide levels in liver of HHcy mice, particularly implicated in NAFLD, and altered hepatic catabolism of S1P, which could target the brain where S1P is a potent regulator of AD development. Our results also support an increased hypothalamic ceramide levels in HHcy mice, with a local alteration of S1P metabolism. Altogether, our study emphasizes the role of Hcy/ceramides pathway in AD pathology.

ETHICS STATEMENT

All procedures were carried out in accordance with the ethical standards of French and European regulations (European Communities Council Directive, 86/609/EEC). Official authorization from the French Ministry of Agriculture was granted to perform research and experiments on animals (authorization number 75-369) and the experimental protocol was approved by the Institutional Animal Care and Use Committee of the Paris Diderot University (CEEA40), and the agreement # 8728 was given to the project.

AUTHOR CONTRIBUTIONS

HL and NJ made the review of the literature and wrote the manuscript. HL, CC-G, CM, and NJ designed the study. JV, NK,

JD, KM, J-LP, and CC-G performed the experiments. All authors read and approved the manuscript.

ACKNOWLEDGMENTS

This work was supported by Lagafe Association, Foundation Jerome Lejeune (grant number 2017a-1634), grant from National Research Agency (ANR-16-CE14-0026 Fat4Brain), and the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No. 115881 (RHAPSODY).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00807/full#supplementary-material

REFERENCES

1. Ricciarelli R, Fedele E. The amyloid cascade hypothesis in Alzheimer's disease: it's time to change our mind. *Curr Neuropharmacol.* (2017) 15:926–35. doi: 10.2174/1570159X15666170116143743
2. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *J Alzheimers Dis.* (2005) 7:63–80. doi: 10.3233/JAD-2005-7107
3. Leboucher A, Ahmed T, Caron E, Tailleux A, Raison S, Joly-Amado A, et al. Brain insulin response and peripheral metabolic changes in a Tau transgenic mouse model. *Neurobiol Dis.* (2019) 125:14–22. doi: 10.1016/j.nbd.2019.01.008
4. Marciniak E, Leboucher A, Caron E, Ahmed T, Tailleux A, Dumont J, et al. Tau deletion promotes brain insulin resistance. *J Exp Med.* (2017) 214:2257–69. doi: 10.1084/jem.20161731
5. Lyn-Cook LE Jr, Lawton M, Tong M, Silbermann E, Longato L, Jiao P, et al. Hepatic ceramide may mediate brain insulin resistance and neurodegeneration in type 2 diabetes and non-alcoholic steatohepatitis. *J Alzheimers Dis.* (2009) 16:715–29. doi: 10.3233/JAD-2009-0984
6. Sajan MP, Ivey RA, Lee MC, Farese RV. Hepatic insulin resistance in ob/ob mice involves increases in ceramide, aPKC activity, and selective impairment of Akt-dependent FoxO1 phosphorylation. *J Lipid Res.* (2015) 56:70–80. doi: 10.1194/jlr.M052977
7. Cong WN, Tao RY, Tian JY, Liu GT, Ye F. The establishment of a novel non-alcoholic steatohepatitis model accompanied with obesity and insulin resistance in mice. *Life Sci.* (2008) 82:983–90. doi: 10.1016/j.lfs.2008.01.022
8. de la Monte SM, Tong M, Nguyen V, Setshedi M, Longato L, Wands JR. Ceramide-mediated insulin resistance and impairment of cognitive-motor functions. *J Alzheimers Dis.* (2010) 21:967–84. doi: 10.3233/JAD-2010-091726
9. Al-Maskari MY, Waly MI, Ali A, Al-Shuaibi YS, Ouhtit A. Folate and vitamin B12 deficiency and hyperhomocysteinemia promote oxidative stress in adult type 2 diabetes. *Nutrition.* (2012) 28:e23–6. doi: 10.1016/j.nut.2012.01.005
10. Zhou J, Austin RC. Contributions of hyperhomocysteinemia to atherosclerosis: causal relationship and potential mechanisms. *Biofactors.* (2009) 35:120–9. doi: 10.1002/biof.17
11. de Carvalho SC, Muniz MT, Siqueira MD, Siqueira ER, Gomes AV, Silva KA, et al. Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). *Nutr J.* (2013) 12:37. doi: 10.1186/1475-2891-12-37
12. Mato JM, Lu SC. Homocysteine, the bad thiol. *Hepatology.* (2005) 41:976–9. doi: 10.1002/hep.20708
13. Werstuck GH, Lentz SR, Dayal S, Hossain GS, Sood SK, Shi YY, et al. Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways. *J Clin Investig.* (2001) 107:1263–73. doi: 10.1172/JCI11596
14. Obeid R, Herrmann W. Homocysteine and lipids: S-Adenosyl methionine as a key intermediate. *FEBS Lett.* (2009) 583:1215–25. doi: 10.1016/j.febslet.2009.03.038
15. Bravo E, Palleschi S, Aspichueta P, Buqué X, Rossi B, Cano A, et al. High fat diet-induced non alcoholic fatty liver disease in rats is associated with hyperhomocysteinemia caused by down regulation of the transsulphuration pathway. *Lipids Health Dis.* (2011) 10:60. doi: 10.1186/1476-511X-10-60
16. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med.* (2002) 346:476–83. doi: 10.1056/NEJMoa011613
17. Faux NG, Ellis KA, Porter L, Fowler CJ, Laws SM, Martins RN, et al. Homocysteine, vitamin B12, and folic acid levels in Alzheimer's disease, mild cognitive impairment, and healthy elderly: baseline characteristics in subjects of the Australian Imaging Biomarker Lifestyle study. *J Alzheimers Dis.* (2011) 27:909–22. doi: 10.3233/JAD-2011-110752
18. Hooshmand B, Solomon A, Kåreholt I, Leiviskä J, Rusanen M, Ahtiluoto S, et al. Homocysteine and holotranscobalamin and the risk of Alzheimer disease: a longitudinal study. *Neurology.* (2010) 75:1408–14. doi: 10.1212/WNL.0b013e3181f88162
19. Hooshmand B, Mangialasche F, Kalpouzos G, Solomon A, Kåreholt I, Smith AD, et al. Association of vitamin B12, folate, and sulfur amino acids with brain magnetic resonance imaging measures in older adults: a longitudinal population-based study. *JAMA Psychiatry.* (2016) 73:606–13. doi: 10.1001/jamapsychiatry.2016.0274
20. Bismuth J, Lin P, Yao Q, Chen C. Ceramide: a common pathway for atherosclerosis? *Atherosclerosis.* (2008) 196:497–504. doi: 10.1016/j.atherosclerosis.2007.09.018
21. Adams LA, Angulo P. Recent concepts in non-alcoholic fatty liver disease. *Diabet Med.* (2005) 22:1129–33. doi: 10.1111/j.1464-5491.2005.01748.x
22. Svegliati-Baroni G, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, et al. A model of insulin resistance and nonalcoholic steatohepatitis in rats. *Am J Pathol.* (2006) 169:846–60. doi: 10.2353/ajpath.2006.050953
23. Chang M, Zhang T, Han X, Tang Q, Yanagita T, Xu J, et al. Comparative analysis of EPA/DHA-PL forage and liposomes in orotic acid-induced nonalcoholic fatty liver rats and their related mechanisms *J Agric Food Chem.* (2018) 66:1408–18. doi: 10.1021/acs.jafc.7b05173
24. Larsson SC, Giovannucci E, Wolk A. Vitamin B6 intake, alcohol consumption, and colorectal cancer: a longitudinal population-based cohort of women. *Gastroenterology.* (2005) 128:1830–7. doi: 10.1053/j.gastro.2005.03.005
25. Kaelin WG, McKnight SL. Influence of metabolism on epigenetics and disease. *Cell.* (2013) 153:56–69. doi: 10.1016/j.cell.2013.03.004
26. Pascale RM, Feo CF, Calvisi DF, Feo F. Deregulation of methionine metabolism as determinant of progression and prognosis of hepatocellular carcinoma Transl. *Gastroenterol Hepatol.* (2018) 3:36. doi: 10.21037/tgh.2018.06.04
27. Jhee KH, Kruger WD. The role of cystathionine beta-synthase in homocysteine metabolism. *Antioxid Redox Signal.* (2005) 7:813–22. doi: 10.1089/ars.2005.7.813
28. Cravo M, Gloria L, Selhub J, Nadeau M, Camilo M, Resende M, et al. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. *Am J Clin Nutr.* (1996) 63:220–4. doi: 10.1093/ajcn/63.2.220
29. Gulsen M, Yesilova Z, Bagci S, Uygun A, Ozcan A, Ercin CN, et al. Elevated plasma homocysteine concentrations as a predictor of steatohepatitis in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol.* (2005) 20:1448–55. doi: 10.1111/j.1440-1746.2005.03891.x
30. Watanabe M, Osada J, Aratani Y, Kluckman K, Reddick R, Malinow MR, et al. Mice deficient in cystathionine β-synthase: animal models for mild and severe homocyst(e)inemia. *Proc Natl Acad Sci USA.* (1995) 92:1585–9. doi: 10.1073/pnas.92.5.1585
31. Namekata K, Enokido Y, Ishii I, Nagai Y, Harada T, Kimura H. Abnormal lipid metabolism in cystathionine β-synthase-deficient mice, an animal model for hyperhomocysteinemia. *J Biol Chem.* (2004) 279:52961–9. doi: 10.1074/jbc.M406820200

32. Robert K, Nehmé J, Bourdon E, Pivert G, Friguet B, Delcayre C, et al. Cystathionine beta synthase deficiency promotes oxidative stress, fibrosis, and steatosis in mice liver. *Gastroenterology*. (2005) 128:1405–15. doi: 10.1053/j.gastro.2005.02.034
33. Delabar JM, Latour A, Noll C, Renon M, Salameh S, Paul JL, et al. One-carbon cycle alterations induced by Dyrk1a dosage. *Mol Genet Metab Rep*. (2014) 1:487–92. doi: 10.1016/j.ymgmr.2014.11.004
34. Renon M, Legrand B, Blanc E, Daubigney F, Bokobza C, Mortreux M, et al. Impact of Dyrk1A level on alcohol metabolism. *Biochim Biophys Acta*. (2016) 1862:1495–503. doi: 10.1016/j.bbadis.2016.05.011
35. Dierssen M, de Lagrán MM. DYRK1A (dual-specificity tyrosine-phosphorylated and -regulated kinase 1A): a gene with dosage effect during development and neurogenesis. *ScientificWorldJournal*. (2006) 6:1911–22. doi: 10.1100/tsw.2006.319
36. Hamelet J, Noll C, Ripoll C, Paul JL, Janel N, Delabar JM. Effect of hyperhomocysteinemia on the protein kinase DYRK1A in liver of mice. *Biochem Biophys Res Commun*. (2009) 378:673–7. doi: 10.1016/j.bbrc.2008.11.126
37. Noll C, Planque C, Ripoll C, Guedj F, Diez A, Ducros V, et al. DYRK1A, a novel determinant of the methionine-homocysteine cycle in different mouse models overexpressing this Down-syndrome-associated kinase. *PLoS ONE*. (2009) 4:e7540. doi: 10.1371/journal.pone.0007540
38. Wang P, Alvarez-Perez JC, Felsenfeld DP, Liu H, Sivendran S, Bender A, et al. A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. *Nat Med*. (2015) 21:383–8. doi: 10.1038/nm.3820
39. Shen W, Taylor B, Jin Q, Nguyen-Tran V, Meeusen S, Zhang YQ, et al. Inhibition of DYRK1A and GSK3B induces human β-cell proliferation. *Nat Commun*. (2015) 6:8372. doi: 10.1038/ncomms9372
40. Chakrabarti S, Khemka VK, Banerjee A, Chatterjee G, Ganguly A, Biswas A. Metabolic risk factors of sporadic Alzheimer's disease: implications in the pathology, pathogenesis and treatment. *Aging Dis*. (2015) 6:282–99. doi: 10.14336/AD.2014.002
41. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol*. (1998) 55:1449–55. doi: 10.1001/archneur.55.11.1449
42. Firbank MJ, Narayan SK, Saxby BK, Ford GA, O'Brien JT. Homocysteine is associated with hippocampal and white matter atrophy in older subjects with mild hypertension. *Int Psychogeriatr*. (2010) 22:804–11. doi: 10.1017/S1041610210000499
43. Smith AD, Refsum H. Homocysteine, B vitamins, and cognitive impairment. *Annu Rev Nutr*. (2016) 36:211–39. doi: 10.1146/annurev-nutr-071715-050947
44. Janel N, Alexopoulos P, Badel A, Lamari F, Camproux AC, Lagarde J, et al. Combined assessment of DYRK1A, BDNF and homocysteine levels as diagnostic marker for Alzheimer's disease. *Transl Psychiatry*. (2017) 7:e1154. doi: 10.1038/tp.2017.123
45. Smith AD, Refsum H, Bottiglieri T, Fenech M, Hooshmand B, McCaddon A, et al. Homocysteine and dementia: an international consensus statement. *J Alzheimers Dis*. (2018) 62:561–70. doi: 10.3233/JAD-171042
46. Zhuo JM, Wang H, Praticò D. Is hyperhomocysteinemia an Alzheimer's disease (AD) risk factor, an AD marker, or neither? *Trends Pharmacol Sci*. (2001) 32:562–71. doi: 10.1016/j.tips.2011.05.003
47. Sai X, Kawamura Y, Kokame K, Yamaguchi H, Shiraishi H, Suzuki R, et al. Endoplasmic reticulum stress-inducible protein, herp, enhances presenilin-mediated generation of amyloid β-protein. *J Biol Chem*. (2002) 277:12915–20. doi: 10.1074/jbc.M112372200
48. Pacheco-Quinto J, RodriguezdeTurco EB, DeRosa S, Howard A, Cruz-Sanchez F, Sambamurti K, et al. Hyperhomocysteinemic Alzheimer's mouse model of amyloidosis shows increased brain amyloid β peptide levels. *Neurobiol Dis*. (2006) 22:651–6. doi: 10.1016/j.nbd.2006.01.005
49. Fuso A, Nicolia V, Cavallaro RA, Ricceri L, D'Anselmi F, Coluccia P, et al. B-vitamin deprivation induces hyperhomocysteinemia and brain S-adenosylhomocysteine, depletes brain S-adenosylmethionine, and enhances ps1 and bace expression and amyloid-β deposition in mice. *Mol Cell Neurosci*. (2008) 37:731–46. doi: 10.1016/j.mcn.2007.12.018
50. Zhang C-E, Wei W, Liu Y-H, Peng J-H, Tian Q, Liu G-P, et al. Hyperhomocysteinemia increases β-amyloid by enhancing expression of γ-secretase and phosphorylation of amyloid precursor protein in rat brain. *Am J Pathol*. (2009) 174:1481–91. doi: 10.2353/ajpath.2009.081036
51. Zhuo JM, Portugal GS, Kruger WD, Wang H, Gould TJ, Praticò D. Diet-induced hyperhomocysteinemia increases amyloid-β formation and deposition in a mouse model of Alzheimer's disease. *Curr Alzheimer Res*. (2010) 7:140–9. doi: 10.2174/156720510790691326
52. Sontag E, Nunbhakdi-Craig V, Sontag J-M, Diaz-Arrastia R, Ogris E, Dayal S, et al. Protein phosphatase 2A methyltransferase links homocysteine metabolism with tau and amyloid precursor protein regulation. *J Neurosci*. (2007) 27:2751–9. doi: 10.1523/JNEUROSCI.3316-06.2007
53. Agnati LF, Genedani S, Leo G, Forni A, Woods AS, Filaferro M, et al. Aβpeptides as one of the crucial volume transmission signals in the trophic units and their interactions with homocysteine. Physiological implications and relevance for Alzheimer's disease. *J Neural Transm*. (2007) 114:21–31. doi: 10.1007/s00702-006-0564-9
54. Irier HA, Jin P. Dynamics of DNA methylation in aging and Alzheimer's disease. *DNA Cell Biol*. (2012) 31:S42–S48. doi: 10.1089/dna.2011.1565
55. Minagawa H, Watanabe A, Akatsu H, Adachi K, Ohtsuka C, Terayama Y, et al. Homocysteine, another risk factor for Alzheimer disease, impairs apolipoprotein E3 function. *J Biol Chem*. (2010) 285:38382–8. doi: 10.1074/jbc.M110.146258
56. Lott IT, Dierssen M. Cognitive deficits and associated neurological complications in individuals with Down's syndrome. *Lancet Neurol*. (2010) 9:623–33. doi: 10.1016/S1474-4422(10)70112-5
57. Wegiel J, Kaczmarski W, Barua M, Kuchna I, Nowicki K, Wang KC, et al. Link between DYRK1A overexpression and several-fold enhancement of neurofibrillary degeneration with 3-repeat tau protein in Down syndrome. *J Neuropathol Exp Neurol*. (2011) 70:36–50. doi: 10.1097/NEN.0b013e318202bfa1
58. Ryoo SR, Jeong HK, Radnaabazar C, Yoo JJ, Cho HJ, Lee HW, et al. DYRK1A-mediated hyperphosphorylation of Tau. A functional link between Down syndrome and Alzheimer disease. *J Biol Chem*. (2007) 282:34850–7. doi: 10.1074/jbc.M707358200
59. Ryoo SR, Cho HJ, Lee HW, Jeong HK, Radnaabazar C, Kim YS, et al. Dual-specificity tyrosine(Y)-phosphorylation regulated kinase 1A-mediated phosphorylation of amyloid precursor protein: evidence for a functional link between Down syndrome and Alzheimer's disease. *J Neurochem*. (2008) 104:1333–44. doi: 10.1111/j.1471-4159.2007.05075.x
60. Branca C, Shaw DM, Belfiore R, Gokhale V, Shaw AY, Foley C, et al. Dyrk1 inhibition improves Alzheimer's disease-like pathology. *Aging Cell*. (2017) 16:1146–54. doi: 10.1111/acel.12648
61. Janel N, Sarazin M, Corlier F, Corne H, de Souza LC, Hamelin L, et al. Plasma DYRK1A as a novel risk factor for Alzheimer's disease. *Transl Psychiatry*. (2014) 4:e425. doi: 10.1038/tp.2014.61
62. Dong YQ, Zhang XZ, Sun LL, Zhang SY, Liu B, Liu HY, et al. Omega-3 PUFA ameliorates hyperhomocysteinemia-induced hepatic steatosis in mice by inhibiting hepatic ceramide synthesis. *Acta Pharmacol Sin*. (2017) 38:1601–10. doi: 10.1038/aps.2017.127
63. Tlili A, Noll C, Middendorp S, Duchon A, Jouan M, Benabou E, et al. DYRK1A overexpression decreases plasma lecithin:cholesterol acyltransferase activity and apolipoprotein A-I levels. *Mol Genet Metab*. (2013) 110:371–7. doi: 10.1016/j.ymgme.2013.07.014
64. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol*. (2008) 9:139–50. doi: 10.1038/nrm2329
65. Hanada K, Hara T, Nishijima M, Kuge O, Dickson RC, Nagiec MM. A mammalian homolog of the yeast *LCB1* encodes a component of serine palmitoyltransferase, the enzyme catalyzing the first step in sphingolipid synthesis. *J. Biol Chem*. (1997) 272:32108–14. doi: 10.1074/jbc.272.51.32108
66. Hornemann T, Penno A, Rütti MF, Ernst D, Kivrak-Pfiffner F, Rohrer L, et al. The SPTLC3 subunit of serine palmitoyltransferase generates short chain sphingoid bases. *J Biol Chem*. (2009) 284:26322–30. doi: 10.1074/jbc.M109.023192

67. Olsen ASB, Færgeman NJ. Sphingolipids: membrane microdomains in brain development, function and neurological diseases. *Open Biol.* (2017) 7:170069. doi: 10.1098/rsob.170069
68. Cutler RG, Kellyn J, Storien K, Pedersen WA, Tammara A, Hatanpass K, et al. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. *Proc Natl Acad Sci USA.* (2004) 101:2070–5. doi: 10.1073/pnas.0305799101
69. Geekiyanage H, Upadhye A, Chan C. Inhibition of serine palmitoyltransferase reduces Aβ and tau hyperphosphorylation in a murine model: a safe therapeutic strategy for Alzheimer's disease. *Neurobiol Aging.* (2013) 34:2037–51. doi: 10.1016/j.neurobiolaging.2013.02.001
70. Katsel P, Li C, Haroutunian V. Gene expression alterations in the sphingolipid metabolism pathways during progression of dementia and Alzheimer's disease: a shift toward ceramide accumulation at the earliest recognizable stages of Alzheimer's disease? *Neurochem Res.* (2007) 32:845–56. doi: 10.1007/s11064-007-9297-x
71. Grimm MOW, Grösgen S, Rothhaar TL, Burg VK, Hundsdörfer B, Haupenthal VJ, et al. Intracellular APP domain regulates serine-palmitoyl-CoA transferase expression and is affected in Alzheimer's disease. *Int J Alzheimers Dis.* (2011) 2011:695413. doi: 10.4061/2011/695413
72. Mullen TD, Hannun YA, Obeid LM. Ceramide synthases at the centre of sphingolipid metabolism and biology. *Biochem J.* (2012) 441:789–802. doi: 10.1042/BJ20111626
73. Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, Fukasawa M, et al. Molecular machinery for non-vesicular trafficking of ceramide. *Nature.* (2003) 426:803–9. doi: 10.1038/nature02188
74. Le Stunff H, Milstien S, Spiegel S. Generation and metabolism of bioactive sphingosine-1-phosphate. *J Cell Biochem.* (2004) 92:882–99. doi: 10.1002/jcb.20097
75. Takasugi N, Sasaki T, Ebinuma I, Osawa S, Isshiki H, Takeo K, et al. FTY720/fingolimod, a sphingosine analogue, reduces amyloid-β production in neurons. *PLoS ONE.* (2013) 8:e64050. doi: 10.1371/journal.pone.0064050
76. Couttas TA, Kain N, Daniels B, Lim XY, Shepherd C, Kril J, et al. Loss of the neuroprotective factor sphingosine 1-phosphate early in Alzheimer's disease pathogenesis. *Acta Neuropathol Commun.* (2014) 2:9. doi: 10.1186/2051-5960-2-9
77. Takasugi N, Sasaki T, Suzuki K, Osawa S, Isshiki H, Hori Y, et al. BACE1 activity is modulated by cell-associated sphingosine-1-phosphate. *J Neurosci.* (2011) 31:6850–7. doi: 10.1523/JNEUROSCI.6467-10.2011
78. Dominguez G, Maddelein M-L, Pucelle M, Nicaise Y, Maurage CA, Duyckaerts C, et al. Neuronal sphingosine kinase 2 subcellular localization is altered in Alzheimer's disease brain. *Acta Neuropathol Commun.* (2018) 6:25. doi: 10.1186/s40478-018-0527-z
79. Bellini L, Campana M, Mahfouz R, Carlier A, Véret J, Magnan C, et al. Targeting sphingolipid metabolism in the treatment of obesity/type 2 diabetes. *Expert Opin Ther Targets.* (2015) 19:1037–50. doi: 10.1517/14728222.2015.1028359
80. Bandet CL, Tan-Chen S, Bourron O, Le Stunff H, Hajduch E. Sphingolipid metabolism: new insight into ceramide-induced lipotoxicity in muscle cells. *Int J Mol Sci.* (2019) 20:E479. doi: 10.3390/ijms20030479
81. Marchesini, G., Brizi, M., Bianchi, G., Tomassetti, S., Bugianesi, E., Lenzi, M., et al. (2001). Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes.* 50 1844–1850. doi: 10.2337/diabetes.50.8.1844
82. Li ZZ, Berk M, McIntyre TM, Feldstein AE. Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease. *J Biol Chem.* (2009) 284:5637–44. doi: 10.1074/jbc.M807616200
83. Shimabukuro M, Zhou YT, Levi M, Unger RH. Fatty acid-induced beta cell 7 apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci USA.* (1998) 95:2498–502. doi: 10.1073/pnas.95.5.2498
84. Régnier M, Polizzi A, Guillou H, Loiseau N. Sphingolipid metabolism in non-alcoholic fatty liver diseases. *Biochimie.* (2019) 159:9–22. doi: 10.1016/j.biochi.2018.07.021
85. Watt MJ, Barnett AC, Bruce CR, Schenk S, Horowitz JF, Hoy AJ. Regulation of plasma ceramide levels with fatty acid oversupply: evidence that the liver detects and secretes *de novo* synthesised ceramide. *Diabetologia.* (2012) 55:2741–6. doi: 10.1007/s00125-012-2649-3
86. Boon J, Hoy AJ, Stark R, Brown RD, Meex RC, Henstridge DC, et al. Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes.* (2013) 62:401–10. doi: 10.2337/db12-0686
87. Kakazu E, Mauer AS, Yin M, Malhi H. Hepatocytes release ceramide-enriched pro-inflammatory extracellular vesicles in an IRE1alpha-dependent manner. *J Lipid Res.* (2016) 57:233–45. doi: 10.1194/jlr.M063412
88. Kim DG, Krenz A, Toussaint LE, Maurer KJ, Robinson SA, Yan A, et al. Non-alcoholic fatty liver disease induces signs of Alzheimer's disease (AD) in wild-type mice and accelerates pathological signs of AD in an AD model. *J Neuroinflammation.* (2016) 13:1. doi: 10.1186/s12974-015-0467-5
89. Wigger L, Cruciani-Guglielmacci C, Nicolas A, Denom J, Fernandez N, Fumeron F, et al. Plasma dihydroceramides are diabetes susceptibility biomarker candidates in mice and humans. *Cell Rep.* (2017) 18:2269–79. doi: 10.1016/j.celrep.2017.02.019
90. Han X, Rozen S, Boyle SH, Hellegers C, Cheng H, Burke JR, et al. Metabolomics in early Alzheimer's disease: identification of altered plasma sphingolipidome using shotgun lipidomics. *PLoS ONE.* (2011) 6:e21643. doi: 10.1371/journal.pone.0021643
91. Kim M, Nevado-Holgado A, Whiley L, Snowden SG, Soininen H, Kloszewska I, et al. Association between plasma ceramides and phosphatidylcholines and hippocampal brain volume in late onset Alzheimer's disease. *J Alzheimers Dis.* (2017) 60:809–17. doi: 10.3233/JAD-160645
92. Chen J, Wang W, Qi Y, Kaczorowski D, McCaughan GW, Gamble JR, et al. Deletion of sphingosine kinase 1 ameliorates hepatic steatosis in diet-induced obese mice: role of PPARγ. *Biochim Biophys Acta.* (2016) 1861:138–47. doi: 10.1016/j.bbalip.2015.11.006
93. Geng T, Sutter A, Harland MD, Law BA, Ross JS, Lewin D, et al. SphK1 mediates hepatic inflammation in a mouse model of NASH induced by high saturated fat feeding and initiates proinflammatory signaling in hepatocytes. *J Lipid Res.* (2015) 56:2359–71. doi: 10.1194/jlr.M063511
94. Bektas M, Allende ML, Lee BG, Chen W, Amar MJ, Remaley AT, et al. Sphingosine 1-phosphate lyase deficiency disrupts lipid homeostasis in liver. *J Biol Chem.* (2010) 285:10880–9. doi: 10.1074/jbc.M109.081489
95. Christoffersen C, Obinata H, Kumaraswamy SB, Galvani S, Ahnström J, Sevvana M, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. *Proc Natl Acad Sci USA.* (2011) 108:9613–968. doi: 10.1073/pnas.1103187108
96. Hajny S, Christoffersen C. A novel perspective on the ApoM-S1P axis, highlighting the metabolism of ApoM and its role in liver fibrosis and neuroinflammation. *Int J Mol Sci.* (2017) 18:1636. doi: 10.3390/ijms18081636
97. Baloula V, Fructuoso M, Kassis N, Gueddouri D, Paul JL, Janel N. Homocysteine-lowering gene therapy rescues signaling pathways in brain of mice with intermediate hyperhomocysteinemia. *Redox Biol.* (2018) 19:200–9. doi: 10.1016/j.redox.2018.08.015
98. Cruciani-Guglielmacci C, Bellini L, Denom J, Oshima M, Fernandez N, Normandie-Levi P, et al. Molecular phenotyping of multiple mouse strains under metabolic challenge uncovers a role for Elovl2 in glucose-induced insulin secretion. *Mol Metab.* (2017) 6:340–51. doi: 10.1016/j.molmet.2017.01.009
99. Santiard-Baron D, Aupetit J, Janel N. Plasma homocysteine levels are not increased in murine models of Alzheimer's disease. *Neurosci Res.* (2005) 53:447–9. doi: 10.1016/j.neures.2005.09.001
100. Zhuo JM, Praticò D. Normalization of hyperhomocysteinemia improves cognitive deficits and ameliorates brain amyloidosis of a transgenic mouse model of Alzheimer's disease. *FASEB J.* (2010) 24:3895–902. doi: 10.1096/fj.10-161828
101. Campana M, Bellini L, Rouch C, Rachdi L, Coant N, Butin N, et al. Inhibition of central *de novo* ceramide synthesis restores insulin signaling in hypothalamus and enhances β-cell function of obese Zucker rats. *Mol Metab.* (2018) 8:23–36. doi: 10.1016/j.molmet.2017.10.013
102. Summers SA. Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res.* (2006) 45:42–72. doi: 10.1016/j.plipres.2005.11.002
103. Mielke MM, Haughey NJ, Ratnam Bandaru VV, Schech S, Carrick R, Carlson MC, et al. Plasma ceramides are altered in mild cognitive impairment and predict cognitive decline and hippocampal volume loss. *Alzheimers Dement.* (2010) 6:378–85. doi: 10.1016/j.jalz.2010.03.014

104. Yi F, Zhang AY, Li N, Muh RW, Fillet M, Renert AF, et al. Inhibition of ceramide-redox signaling pathway blocks glomerular injury in hyperhomocysteinemic rats. *Kidney Int.* (2006) 70:88–96. doi: 10.1038/sj.ki.5001517
105. Boini KM, Xia M, Li C, Zhang C, Payne LP, Abais JM, et al. Acid sphingomyelinase gene deficiency ameliorates the hyperhomocysteinemia-induced glomerular injury in mice. *Am J Pathol.* (2011) 179:2210–9. doi: 10.1016/j.ajpath.2011.07.019
106. Boini KM, Xia M, Abais JM, Xu M, Li CX, Li PL. Acid sphingomyelinase gene knockout ameliorates hyperhomocysteinemic glomerular injury in mice lacking cystathionine-β-synthase. *PLoS ONE.* (2012) 7:e45020. doi: 10.1371/journal.pone.0045020
107. Ruangsiriluk W, Grosskurth SE, Ziemek D, Kuhn M, des Etages SG, Francone OL. Silencing of enzymes involved in ceramide biosynthesis causes distinct global alterations of lipid homeostasis and gene expression. *J Lipid Res.* (2012) 53:1459–71. doi: 10.1194/jlr.M020941
108. Yoshimine Y, Uto H, Kumagai K, Mawatari S, Arima S, Ibusuki R, et al. Hepatic expression of the Sptlc3 subunit of serine palmitoyltransferase is associated with the development of hepatocellular carcinoma in a mouse model of nonalcoholic steatohepatitis. *Oncol Rep.* (2015) 33:1657–66. doi: 10.3892/or.2015.3745
109. Kleuser B. Divergent role of sphingosine 1-phosphate in liver health and disease. *Int J Mol Sci.* (2018) 3:19. doi: 10.3390/ijms19030722
110. Fayyaz S, Henkel J, Japtok L, Kramer S, Damm G, Seehofer D, et al. Involvement of sphingosine 1-phosphate in palmitate-induced insulin resistance of hepatocytes via the S1P2 receptor subtype. *Diabetologia.* (2014) 57:373–82. doi: 10.1007/s00125-013-3123-6
111. Czubowicz K, Jeśko H, Wencel P, Lukiw WJ, Strosznajder RP. The role of ceramide and sphingosine-1-phosphate in Alzheimer'sdisease and other neurodegenerative disorders. *Mol Neurobiol.* (2019) 56:5436–55. doi: 10.1007/s12035-018-1448-3
112. Planque C, Dairou J, Noll C, Bui LC, Ripoll C, Guedj F, et al. Mice deficient in cystathionine beta synthase display increased Dyrk1A and SAHH activities in brain. *J Mol Neurosci.* (2013) 50:1–6. doi: 10.1007/s12031-012-9835-0
113. Lee JT, Peng GS, Chen SY, Hsu CH, Lin CC, Cheng CA, et al. Homocysteine induces cerebral endothelial cell death by activating the acid sphingomyelinase ceramide pathway. *Prog Neuropsychopharmacol Biol Psychiatry.* (2013) 45:21–7. doi: 10.1016/j.pnpbp.2013.05.003
114. Jeśko H, Wencel PL, Lukiw WJ, Strosznajder RP. Modulatory effects of fingolimod (FTY720) on the expression of sphingolipid metabolism-related genes in an animal model of Alzheimer's disease. *Mol Neurobiol.* (2019) 56:174–85. doi: 10.1007/s12035-018-1040-x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 31 July 2019
doi: 10.3389/fnins.2019.00788

Brain Insulin Resistance and Hippocampal Plasticity: Mechanisms and Biomarkers of Cognitive Decline

Matteo Spinelli[1], *Salvatore Fusco*[2*†] *and Claudio Grassi*[1,2*†]

[1] *Institute of Human Physiology, Università Cattolica del Sacro Cuore, Rome, Italy,* [2] *Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy*

In the last decade, much attention has been devoted to the effects of nutrient-related signals on brain development and cognitive functions. A turning point was the discovery that brain areas other than the hypothalamus expressed receptors for hormones related to metabolism. In particular, insulin signaling has been demonstrated to impact on molecular cascades underlying hippocampal plasticity, learning and memory. Here, we summarize the molecular evidence linking alteration of hippocampal insulin sensitivity with changes of both adult neurogenesis and synaptic plasticity. We also review the epidemiological studies and experimental models emphasizing the critical role of brain insulin resistance at the crossroad between metabolic and neurodegenerative disease. Finally, we brief novel findings suggesting how biomarkers of brain insulin resistance, involving the study of brain-derived extracellular vesicles and brain glucose metabolism, may predict the onset and/or the progression of cognitive decline.

Keywords: brain insulin resistance, hippocampus, Alzheimer's disease, synaptic plasticity, adult neurogenesis

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Andrea Giaccari,
Fondazione Policlinico Universitario A. Gemelli, Università Cattolica del Sacro Cuore, Italy
Viviana Triaca,
Italian National Research Council (CNR), Italy
João M. N. Duarte,
Lund University, Sweden

***Correspondence:**
Salvatore Fusco
salvatore.fusco@unicatt.it
Claudio Grassi
claudio.grassi@unicatt.it

†Shared last authorship

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *30 April 2019*
Accepted: *15 July 2019*
Published: *31 July 2019*

Citation:
Spinelli M, Fusco S and Grassi C (2019) Brain Insulin Resistance and Hippocampal Plasticity: Mechanisms and Biomarkers of Cognitive Decline. Front. Neurosci. 13:788. doi: 10.3389/fnins.2019.00788

INTRODUCTION

Since the discovery of insulin almost a century ago, much efforts have been conducted to study the effects of this hormone on all organs. Insulin was originally shown to act on the brain by stimulating the hypothalamic satiety center and inhibiting the feeding behavior (Debons et al., 1970). For long time the impact of insulin on other brain areas remained unknown because the central nervous system was considered a non-insulin dependent tissue. In the last decades, the discovery of insulin receptor (IR) expression in brain areas involved in functions different from the feeding control, such as learning and memory, has revolutionized this idea and paved the way for the understanding of how the brain is a highly insulin-sensitive organ (Hill et al., 1986; Zhao and Alkon, 2001). Brain plasticity, the capability of this organ to undergo structural and functional changes in response to environmental stimuli, is finely modulated by diet and nutrient-dependent hormones including insulin (Mainardi et al., 2015). Accordingly, alteration of insulin signaling into the central nervous system may accelerate brain aging, affect brain plasticity and promote neurodegeneration (Kullmann et al., 2016).

Here, we review the effects of insulin on hippocampus, a brain area playing a pivotal role in learning and memory and primarily affected in Alzheimer's disease (AD) (Bartsch and Wulff, 2015). First, we will describe the effects of insulin on both hippocampal synaptic plasticity and hippocampal adult neurogenesis. In addition, we will illustrate the crosstalk between insulin and neurotrophin signaling, the impact of insulin on cognitive function and the role of its signaling on brain aging. Moreover, we will describe how alteration of brain insulin signaling develops (i.e., brain insulin resistance, hereinafter named BIR) and we will summarize the effects of BIR on hippocampal plasticity, learning and memory along with the link between BIR and AD.

Finally, we will discuss novel evidence suggesting that both brain-derived extracellular vesicles and brain glucose metabolism may represent novel biomarkers of BIR able to predict and follow up cognitive decline.

INSULIN AND BRAIN PLASTICITY

Neurons are high energy-consuming cells. Most energy is spent to generate action and postsynaptic potentials (Howarth et al., 2012), and for the biosynthesis of neurotransmitters (Dienel, 2012). Glucose is the main energy source used by brain cells and its transport across the plasma membrane is mediated by a specific family of membrane proteins known as glucose transporters (GLUTs) (Shepherd and Kahn, 1999). Though numerous GLUT isoforms (1–14) have been identified and characterized, only some of these transporters are expressed in the brain and can be involved in neuronal homeostasis and brain function (Duelli and Kuschinsky, 2001). Specifically, the insulin-independent transporters GLUT1 and GLUT3 mediate glucose uptake into glial and neuronal cells, respectively (Simpson et al., 2007), suggesting that the impact of insulin on synaptic plasticity should be independent of glucose uptake.

Moreover, GLUT2 and GLUT4 expression has been characterized in specific brain areas: GLUT2 is predominantly localized in the hypothalamus that regulates food intake (Eny et al., 2008), whereas GLUT4 has been identified in cerebellum, neocortex, and hippocampus, suggesting a role of GLUT-driven glucose uptake in neuronal activity (Vannucci et al., 1998; Sankar et al., 2002). GLUT4 is also expressed in astrocytes and insulin stimulation promotes both glucose uptake and glycogen accumulation in astrocyte cultures (Heni et al., 2011). However, not much data are available in the literature on the role of insulin on astrocytic functions. GLUT5 expression is less relevant and mainly detected in human and rat brain microglia (Payne et al., 1997). GLUT6 and GLUT13, which have very low affinity to glucose, are also expressed in the brain, but their role in central nervous system has yet to be clarified (Joost and Thorens, 2001). Conversely, GLUT8 has been shown to drive hippocampal neuron proliferation during embryogenesis (Membrez et al., 2006). However, tissue/cell type specific expression of GLUTs in the brain still remains matter of debate. Further, several growth factors have been reported to modulate the insulin pathway, GLUT plasma membrane translocation and glucose uptake by transactivation of the IR downstream effectors (Assefa et al., 2017).

In this section, we brief the insulin cascade effectors and the effects of this hormone on both synaptic plasticity and adult neurogenesis in the hippocampus along with their impact on cognitive functions.

Insulin Signaling in the Brain

Insulin and the insulin-like growth factor 1 (IGF-1) exert their biological effects through two tyrosine kinase receptors, the IR and the IGF-1 receptors (IGF-1R), which are closely related and highly distributed throughout the brain (Belfiore et al., 2009). In the mouse, IR is predominantly expressed in the olfactory bulb, hippocampus, neocortex, hypothalamus, and cerebellum, whereas IGF-1R is highly expressed in the hippocampus, neocortex, and thalamus, with lower expression in the hypothalamus, cerebellum, olfactory bulb, midbrain, and brainstem (Bruning et al., 2000; Fernandez and Torres-Aleman, 2012). As reported in other tissues, IRs and IGF-1Rs can heterodimerize in the brain and partially transactivate their signaling (Bailyes et al., 1997). Moreover, IR and IGF-1R share intracellular signaling machinery, and all major components of brain signaling cascades are similar to those present in peripheral tissues, including IR substrate 1 and 2 (IRS1 and IRS2, respectively), the major downstream phosphoinositide-3-kinase–protein kinase B/Akt (PI3K/Akt) pathway, the downstream effectors target of rapamycin (mTOR) and glycogen synthase kinase 3 beta (GSK3β), and the transcription factors cAMP response element-binding protein (CREB) and forkhead box O (FOXO) family (Fernandez and Torres-Aleman, 2012; **Figure 1**).

Many molecules involved in these signaling cascades have been demonstrated to have key roles in brain functions. Activation of PI3-kinase is required for glutamate receptor insertion at plasma membranes during synaptic plasticity (Man et al., 2003). GSK3β regulates neural progenitor cell proliferation and neuroplasticity, and its activation induces hyper-phosphorylation of tau protein that is considered a major determinant of AD pathogenesis (Salcedo-Tello et al., 2011). Insulin induces phosphorylation of GSK3β on inhibitory serine 9 residue, thus reducing its enzymatic activity. Moreover, mTOR complex 1 (mTORC1) is fundamental for both protein synthesis and autophagy, which are molecular processes involved in the regulation of long-term synaptic plasticity and degradation of misfolded proteins in neurons, respectively (Stoica et al., 2011; Son et al., 2012). Insulin/IGF-1 signaling also stimulates the growth factor receptor-bound protein 2- Son of Sevenless-Rat sarcoma-mitogen activated protein kinase (Grb2-SOS-Ras-MAPK) cascade, which plays a pivotal role in cytoskeletal modifications underlying the dendritic spine reorganization and memory formation (Adams and Sweatt, 2002).

The origin of insulin in the central nervous system is controversial. Insulin crosses the blood-brain barrier (BBB) via an IR-dependent transport operated by vascular endothelium and its concentration increases after meals (Woods et al., 2003). However, insulin can be also synthesized and secreted by neurons and adult neural progenitor cells of the hippocampus (Devaskar et al., 1994; Kuwabara et al., 2011), although no evidence clearly demonstrated that insulin synthesis in the brain is physiologically relevant.

However, as described in the next paragraphs, physiological levels of insulin play a neurotrophic action on both differentiated neurons and neural stem cells (NSCs).

Insulin, Synaptogenesis and Hippocampal Synaptic Plasticity

Modifications of both activity and number of synapses are the functional and structural substrates, respectively, of brain plasticity underlying learning and memory

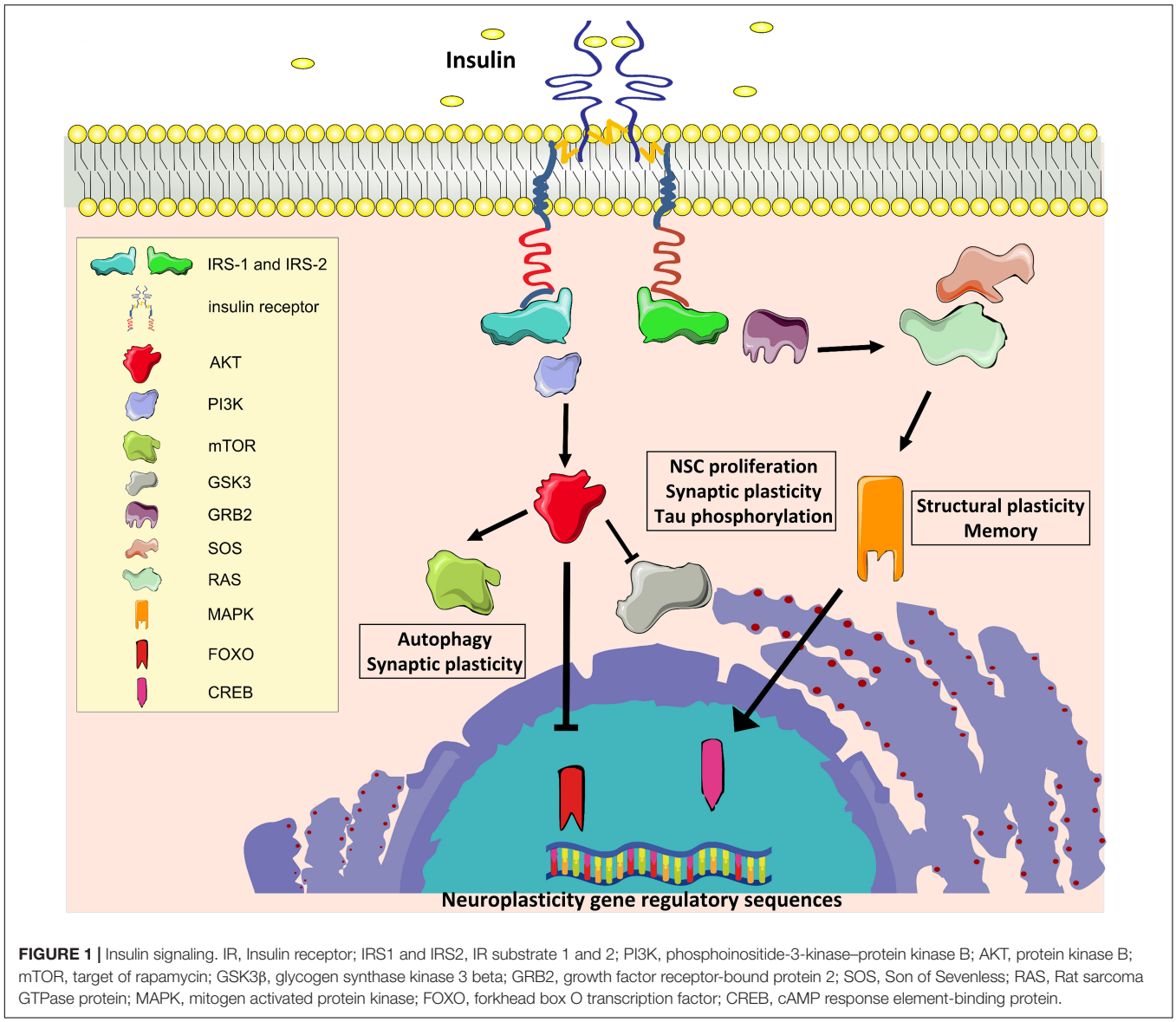

FIGURE 1 | Insulin signaling. IR, Insulin receptor; IRS1 and IRS2, IR substrate 1 and 2; PI3K, phosphoinositide-3-kinase–protein kinase B; AKT, protein kinase B; mTOR, target of rapamycin; GSK3β, glycogen synthase kinase 3 beta; GRB2, growth factor receptor-bound protein 2; SOS, Son of Sevenless; RAS, Rat sarcoma GTPase protein; MAPK, mitogen activated protein kinase; FOXO, forkhead box O transcription factor; CREB, cAMP response element-binding protein.

(Nakahata and Yasuda, 2018). Changes of the synaptic strength, either potentiation or depression, and generation of new dendritic spines are causally related to the acquisition and consolidation of behavioral modifications.

Insulin stimulation of hippocampal neurons induces both presynaptic and postsynaptic effects. Insulin increases basal neurotransmitter release from presynaptic terminals, as revealed by enhanced frequency of miniature excitatory postsynaptic currents (mEPSCs) (Lee et al., 2011). This effect is paralleled by a Rac1-mediated cytoskeleton rearrangement leading to increased density of dendritic spines (Lee et al., 2011). Moreover, insulin promotes synaptic plasticity by modulating long-term potentiation (LTP) or long-term depression (LTD) at hippocampal synapses through a metaplastic mechanism. Indeed, insulin administration reduces the stimulation frequency threshold required for inducing both LTP and LTD (Van Der Heide et al., 2005). Postsynaptic effects are mediated by PI3K activation (Van Der Heide et al., 2005) and increased membrane recruitment of *N*-methyl-D-aspartate receptors (NMDARs) (Skeberdis et al., 2001). Insulin impacts on glutamate receptor activity by multiple mechanisms. It increases NMDAR-mediated currents by enhancing phosphorylation of both NR2A and NR2B subunits (Christie et al., 1999; Liu et al., 1995). Insulin treatment of hippocampal cultures also increases phosphorylation and clathrin-dependent endocytosis of the GluA1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) (Adzovic and Domenici, 2014; Chen et al., 2014). Downregulation of AMPAR activity in excitatory synapse of hippocampal CA1 neurons is fundamental for insulin-induced LTD, which is a key step for memory consolidation and flexibility (Ge et al., 2010). IRs have also been demonstrated to modulate type A γ-aminobutyric acid (GABA) receptor activity by regulating both its membrane localization and expression in inhibitory synapses (Wan et al., 1997).

Furthermore, insulin may impinge on structural features of synapses. For instance, in thalamocortical organotypic slices this hormone stimulates maturation of silent synapses (Plitzko et al., 2001). Moreover, IR substrate p53 (IRSp53) interacts with the postsynaptic protein PSD-95 and enhances dendritic spine formation (Choi et al., 2005). Interestingly, IRS2 knockout mice show lower activation of NR2B subunits (Martin et al., 2012) and decreased LTP at the CA3–CA1 synapses in parallel with higher density of CA1 dendritic spines (Irvine et al., 2011). It is important to underline that these studies did not evaluate dendritic spine morphology, therefore our knowledge of the effects of IRSs manipulation on structural and functional plasticity still remains incomplete. Considering the physical and functional interaction between IR and IGF-1R, it is not surprising that IGF-1 stimulation can promote plasticity in the hippocampus by increasing spine density of CA1 basal dendrites in response to physical exercise (Glasper et al., 2010). Accordingly, IGF-I knockout mice show reduced density of glutamatergic synapses (Trejo et al., 2007). Importantly, IR expression and insulin activity in the brain are not restricted to neurons. Insulin has been demonstrated to influence proliferation and metabolism in insulin sensitive glial cells (Heni et al., 2011). Collectively, all the above mentioned evidence supports the positive effects of insulin on hippocampal synaptic and structural plasticity.

Insulin and Hippocampal Adult Neurogenesis

Hippocampus is one of the brain areas where newborn neurons are generated throughout adulthood (Braun and Jessberger, 2014). Specifically, adult neurogenesis occurs in the subgranular zone of the hippocampus of all mammals including humans (Eriksson et al., 1998). NSCs populating this neurogenic niche proliferate and differentiate to generate new neurons (Kempermann et al., 2003). A proper balance between NSC proliferation and their differentiation/maturation underlies the maintenance of both the hippocampal stem cell niche and the supply of newborn neurons that integrate into existing circuits thus supporting cognitive functions under physiological conditions and brain repair after injury (Castilla-Ortega et al., 2011). Indeed, a growing number of studies indicates that hippocampal neurogenesis plays a critical role in learning, memory, and its impairment has been associated with cognitive dysfunction in neurodegenerative disorders including AD (van Praag et al., 2002; Taylor et al., 2013).

Insulin is a key trophic factor for brain development and control of neurogenic niches. Indeed, neuroblast exit from quiescence is regulated by insulin/IGF-I pathway activation (Chell and Brand, 2010; Sousa-Nunes et al., 2011). Evidence from *in vitro* and *in vivo* experiments indicate that insulin and IGF-I promote neurogenesis by modulating NSC proliferation, differentiation, and survival (Brooker et al., 2000; Åberg et al., 2003). However, a chronic hyper-activation of insulin/IGF-I signaling cascades can cause premature depletion of the NSC reservoir (Sun, 2006). Thus, insulin may produce either trophic or detrimental effects on neural stem niche based on the timing and the duration of stimulation.

Furthermore, calorie restriction has been clearly demonstrated to reduce plasma levels of both glucose and insulin, in parallel with increasing neurogenesis in the dentate gyrus (Lee et al., 2002) and counteracting the age-related decline of stem cell niche (Park et al., 2013). Nutrient deprivation may impact on NSC compartment by inducing the expression of the brain-derived neurotrophic factor (Bdnf) gene (Maswood et al., 2004). In addition, calorie restriction may preserve the NSC capacity to self-renew and differentiate by cell-autonomous mechanisms involving metabolic sensors such as CREB and the NAD-dependent histone deacetylase Sirtuin 1 (SIRT1). In this regard, CREB is a nutrient-dependent transcription factor regulating genes promoting neuronal differentiation and survival (Lonze et al., 2002; Fusco et al., 2012b). Moreover, SIRT1 is as an epigenetic repressor that modulates adult neurogenesis in the subventricular zone and hippocampus (Saharan et al., 2013). Calorie restriction also induces the expression of SIRT1, which has been shown to functionally impinge on CREB-dependent gene expression, thus highlighting a novel molecular link between nutrient-dependent signaling and brain health (Fusco et al., 2012a). Under metabolic and oxidative stress, SIRT1 inhibits NSC self-renewal and induces their differentiation (Prozorovski et al., 2008; Ma et al., 2014). In summary, SIRT1 and CREB work as metabolic sensors regulating proliferation and self-renewal of NSCs and controlling their reservoir in the hippocampus (Fusco et al., 2016). Conversely, abolishing the expression of genes encoding the insulin-regulated FOXO transcription factors induces hyper-proliferation of neural progenitors and rapid exhaustion of stem cell niche (Renault et al., 2009). Similarly, aberrant stimulation of the nutrient-dependent mTOR pathway causes reduced self-renewal and accelerates NSC differentiation (Magri et al., 2011). Together, this evidence confirms that nutrient related signals control NSC fate under both physiological and pathological conditions.

In addition, it is worth mentioning the close similarity between the intracellular signaling pathways activated by insulin and neurotrophins (Reichardt, 2006). In particular, CREB has been shown to play a critical role in the neurotrophin-triggered effects on neuronal differentiation, survival, and plasticity, and it has been also characterized as metabolic sensor modulated by fasting-related stimuli (Finkbeiner, 2000; Altarejos and Montminy, 2011). Moreover, neurotrophic factors as BDNF, ciliary neurotrophic factor (CNTF), and glial cell-derived neurotrophic factor (GDNF) regulate adult neurogenesis in multiple stages of NSC maturation and their expression is affected by overnutrition and metabolic stress (Lindsay et al., 1994). These evidence emphasizes the role of insulin as growth factor for neural niche especially during the early stages of life.

Insulin Signaling and Hippocampus-Dependent Cognitive Task

The evidence summarized above suggest that all aspects of hippocampal plasticity (i.e., functional and structural synaptic

plasticity, and adult neurogenesis) are strongly sensitive to the modulation of insulin signaling into the brain. According to the key role of hippocampal plasticity in learning and memory, changes of insulin cascade in the hippocampus markedly affects cognitive functions.

Heterozygous knockout mice for IR display lower preference index in the novel object recognition (NOR) test (Nisticò et al., 2012). Accordingly, Zucker rats show lower performance in the Morris Water Maze (MWM) in parallel with impaired insulin sensitivity (Kamal et al., 2013). Moreover, a recent study performed on Goto-Kakizaki (GK) rats, a model of non-obese type 2 diabetes (T2D), display spatial memory impairment in Y-maze task and hippocampal synaptic dysfunction evaluated by LTP (Duarte et al., 2019). Further, GK rats show a reduction of SNAP25 and synaptophysin levels suggesting synapse degeneration (Duarte et al., 2019). In addition, IRSp53 knockout mice show impaired learning and memory when evaluated in both MWM and NOR tests (Kim et al., 2009). However, forebrain-specific IRS2 deficiency improved memory retention in the MWM task (Irvine et al., 2011), suggesting that molecular hubs of insulin signaling may differentially interfere on cognitive behavior.

In agreement with this finding, chronic brain stimulation by 8-week intranasal insulin administration improved memory in humans (Benedict et al., 2004). Moreover, lower values of both glycaemia and glycosylated hemoglobin (HbA1c) are associated with better performance in memory tasks in humans (Kerti et al., 2013). It has been shown that peripheral changes of insulin signaling and sensitivity may affect brain health and function although the results of these studies are controversial. Learning and memory deficits in MWM have been demonstrated in liver-specific IGF1 knockout mice (Trejo et al., 2007). In addition, IGF-I antiserum administration to young rats impairs learning in passive avoidance task (Lupien et al., 2003). Conversely, intraperitoneal injection of insulin impairs retention and spatial working memory in a dose-dependent manner (Kopf and Baratti, 1999; Akanmu et al., 2009). It seems that the negative impact of peripheral insulin administration on cognitive functions may be due to the lowering of blood glucose levels, as indicated by the positive effect of the simultaneous glucose infusion (Kopf et al., 1998). In line with this hypothesis, peripheral administration of insulin increases verbal memory and attention in healthy subjects under euglycemic conditions (Kern et al., 2001).

To avoid the side effects of systemic insulin administration, Park et al. (2000) studied the impact of intracerebroventricular injection of insulin in rats and found that insulin improved cognitive performance in passive avoidance test. Accordingly, intrahippocampal injection of insulin into the CA1 region has been reported to enhance memory of rats in passive avoidance test (Babri et al., 2007). More importantly, the effects of intrahippocampal injection of insulin on cognitive functions seem to be related to its dose. High doses of insulin significantly ameliorate spatial learning and memory in the MWM test, whereas low doses reduce cognitive performance (Moosavi et al., 2006). It has been hypothesized that the negative effects of insulin on spatial memory may be dependent on either upregulation of $GABA_A$ receptors or downregulation of AMPA receptors upon insulin treatment (Moosavi et al., 2006). Finally, McNay et al. (2010) demonstrated that endogenous intrahippocampal insulin signaling was required for memory processing. These authors showed that acute injection of insulin into the hippocampus at physiological doses enhanced spatial memory via a PI3K-dependent mechanism (McNay et al., 2010). Collectively, the results obtained in humans and rodents suggest that insulin is fundamental for both memory formation and retention.

EFFECTS OF BRAIN INSULIN RESISTANCE ON HIPPOCAMPUS-DEPENDENT FUNCTIONS

Data reviewed in the previous paragraphs support the view that changes of either insulin signaling or insulin sensitivity in the hippocampus may alter molecular pathways involved in synaptic plasticity and adult neurogenesis, thereby leading to reduced "mindspan" (the maintenance of mental abilities throughout life) and increased risk of neurodegeneration (Kodl and Seaquist, 2008). Accordingly, while calorie restriction furthers neuronal survival and improves cognitive function (Fusco and Pani, 2013), the excess of nutrients harms the brain health and accelerates cognitive decline (Elias et al., 2012; Sellbom and Gunstad, 2012). Nutrient excess causes hyper-activation of insulin signaling in all tissues expressing IR, leading to the desensitization of IR-dependent molecular cascades. BIR decreases the ability of brain cells to respond to insulin and abolishes both metabolic and cognitive effects of this hormone (Kullmann et al., 2016). Specifically, this deficiency could be caused by lower expression of IR or poor activation of insulin signaling. IR downstream effectors may become insensitive to the insulin stimulation, resulting in inability of brain cells to respond to the hormone and leading to impairment of brain plasticity. In the Western world, the incidence of metabolic disorders, including insulin resistance, obesity and T2D, is increasing at alarming rates in parallel with the prevalence of cognitive decline (Cukierman-Yaffee, 2009). Obesity and inflammation affect the insulin transport to the brain (Ketterer et al., 2011) and low expression of IR has been reported in patients with T2D (Kullmann et al., 2016). However, patients with T2DM and/or obesity showed decreased insulin levels in the cerebrospinal fluid despite higher levels of this hormone in their plasma (Heni et al., 2014). In the following paragraphs, we will summarize the mechanisms underlying the detrimental effects of BIR on hippocampal plasticity and cognition, and the epidemiological and experimental evidence supporting a link between BIR and AD.

Alterations of Hippocampal Plasticity in BIR Models

High-fat diet (HFD) is a well-established animal model of metabolic disorders (Wong et al., 2016). HFD induces obesity by compromising β-cell functions, promoting hyper-glycaemia,

whole-body insulin resistance, and dyslipidemia, and increasing free fatty acids in the blood. Many studies have investigated the structural and functional changes of neuroplasticity in experimental models of insulin resistance (Fadel and Reagan, 2016).

More specifically, HFD produces detrimental effects on brain functions including decreased neurogenesis in the dentate gyrus (Lindqvist et al., 2006), alteration of BBB integrity (Freeman and Granholm, 2012) and changes in both spine density and synapse formation (Stranahan et al., 2008a). HFD also impairs insulin signaling in the hippocampus and reduces the expression of synaptic proteins PSD-95 and synaptopodin (Arnold et al., 2014). However, the most significant effects occur on activity-dependent synaptic plasticity. Indeed, Zucker rats show impairment in LTP at CA3–CA1 synapses in parallel with loss of insulin sensitivity (Kamal et al., 2013). Moreover, IR heterozygous knockout mice display normal levels of both basal synaptic transmission and LTP that, however, fails to be consolidated due to reduced Akt activation (Nisticò et al., 2012).

Obesity and T2D have been demonstrated to induce hippocampal insulin resistance through different metabolic changes including alteration of hypothalamic-pituitary-adrenal (HPA) axis leading to elevated levels of glucocorticoids (Plotsky et al., 1992). Accordingly, glucocorticoids stimulation inhibits translocation of GLUT4 to the plasma membrane in the rat hippocampus (Piroli et al., 2007). Moreover, Stranahan et al. showed that restoring physiological levels of glucocorticoids in insulin resistant db/db mice rescued the impairment of hippocampal synaptic plasticity (Stranahan et al., 2008b). A different model of BIR is obtained by intracerebral injection of streptozotocin, which impairs cognitive function by reducing the activity of the neuroprotective protein SIRT1 (Du et al., 2014). As mentioned before, SIRT1 cooperates with the transcription factor CREB promoting the CREB-dependent expression of the neuroplasticity-related gene Bdnf (Jeong et al., 2012). To better clarify the functional role of hippocampal insulin resistance, Grillo et al. (2015) silenced the expression of IR in the hippocampus by injecting lentiviral particles harboring IR antisense sequence. This experimental model showed deficits in hippocampal synaptic transmission and spatial learning, in parallel with downregulation of NMDA subunit GluN2B expression and lower phosphorylation of AMPA subunit GluA1, without altering peripheral metabolic parameters (i.e., body weight, adiposity, and glucose homeostasis) (Grillo et al., 2015).

Recently, we described a novel link between BIR and altered glutamate receptor function underlying the HFD-dependent impairment of hippocampal synaptic plasticity (Spinelli et al., 2017). In particular, we found that HFD induced accumulation of palmitic acid and increased FOXO3a-dependent expression of palmitoyl-transferase zDHHC3 leading to GluA1 hyper-palmitoylation in the hippocampus. Accordingly, *in vitro* stimulation of hippocampal neurons with a cocktail of insulin and palmitic acid replicated the *in vivo* molecular changes, inhibiting the GluA1 localization at the synaptic membrane and AMPA currents at glutamatergic synapses. Finally, either silencing of zDHHC3 or overexpression of the palmitoylation-deficient GluA1 mutant in the hippocampus abolished the insulin resistance-dependent impairment of synaptic plasticity (Spinelli et al., 2017). Of course, aberrant palmitoylation of other zDHHC3 targets (e.g., $GABA_A$ Rγ2) may contribute to the detrimental effects of HFD on hippocampus-dependent learning and memory. However, our study adds a new layer to the hippocampal synaptic plasticity regulation by insulin signaling deterioration and proposes a novel molecular mechanism potentially linking BIR and cognitive decline. Other mechanisms underlying fatty acid-driven learning deficits involve cholesterol dysmetabolism, oxidative stress, endothelial dysfunctions, and neurotrophin depletion. Mice fed with HFD show higher levels of reactive oxygen species (ROS), superoxide, and peroxynitrite into the brain, leading to lower level of brain-derived neurotrophic factor (BDNF) and impaired cognition performance evaluated by spatial task (Wu et al., 2004). Moreover, epidemiological studies showed that diets enriched in cholesterol (HCD) were associated with poor cognitive performance in humans (Requejo et al., 2003). HCD diet also induced impairment of spatial and working memory due to microglial activation and alteration of the BBB integrity in rats (Chen et al., 2018). Interestingly, feeding obese rodents with HFD inhibited the transport through the BBB of neuroendocrine molecules, such as ghrelin and leptin, which promote synaptic plasticity and cognitive functions (Banks et al., 2008; Kanoski et al., 2013; Mainardi et al., 2017). Finally, HFD has been shown to induce activation of microglia and astrocytes, and increase of pro-inflammatory cytokines/mediators such as cyclooxygenase 2, TNF-α, IL-1-β, and IL-6 in the hippocampus of mice (Thirumangalakudi et al., 2008; Duffy et al., 2019).

In summary, metabolic diseases affecting insulin signaling may impair the synaptic function through a plethora of molecular mechanisms targeting neurons, astrocytes, endothelial or inflammatory cells.

Cognitive Impairment in BIR Models

Epidemiological evidence indicate that metabolic alterations occurring in T2D, such as hyper-glycaemia and hyper-insulinaemia, positively correlate with cognitive impairment and diabetic patients exhibit higher susceptibility to develop dementia (Cukierman-Yaffee, 2009). Dysregulation of glucose homeostasis increases the risk of dementia in both diabetic and non-diabetic patients (Crane et al., 2013) and is associated with reduced hippocampal volume and cognitive decline (Kerti et al., 2013). Furthermore, longitudinal studies demonstrated that also Type 1 diabetes (T1D) patients were affected by mild-severe cognitive impairment related to the age of onset of the disease and the microvascular complications (Moheet et al., 2015; Nunley et al., 2015). Insulin administration is crucial to promote glucose homeostasis in these patients and to reduce the vascular complications but it increases the risk for hypoglycemic episodes, which negatively impact on cognitive functions (Desrocher and Rovet, 2004). However, the role of hypo- or hyper-insulinemia in T1D-related cognitive alterations has still to be clarified.

Numerous clinical studies revealed worse cognitive performance and earlier age incidence of all-cause dementia in subjects with T2D (Davis et al., 2017; Callisaya et al., 2019). Accordingly, meta-analysis studies showed that in diabetic

patients the risk for all types of dementia is increased by 60–73% (Gudala et al., 2013; Chatterjee et al., 2016).

However, alteration of brain insulin signaling may negatively impact on brain function also in the absence of T2D and before the onset of obesity. Several studies have demonstrated deficits in hippocampal-dependent learning and spatial memory associated with Western diet intake (Molteni et al., 2002; Kanoski and Davidson, 2010). Interestingly, when Kanoski and Davidson (2010) investigated both hippocampus-dependent and hippocampus-independent memory retention ability after different Western diet treatments, they found that only spatial memory impairments occurred after short-term consumption. This suggests that hippocampus is a brain area very sensitive to metabolic stress, and memory impairment may arise before the development of diet-induced metabolic alterations in peripheral tissues. Accordingly, few days of HFD regimen were sufficient to cause cognitive impairment in rats evaluated with MWM test (Murray et al., 2009). High caloric intake also affected hippocampus-dependent non-spatial learning and memory tasks and these results were related to changes of the BBB integrity. Specifically, high energy diet consumption reduced the expression of tight junction proteins selectively causing increased blood-to-brain permeability in the hippocampus (Kanoski and Davidson, 2010). The observed learning and memory deficits are strikingly similar to the poor task performance and cognitive impairment observed in patients with mild or severe metabolic derangements, which strengthens the hypothesis that hippocampal insulin resistance is a key mediator of diet-dependent cognitive alterations. Therefore, cognitive dysfunction related to HFD or obesity in otherwise healthy individuals may be due to decreased insulin signaling and development of BIR in the hippocampus (McNay et al., 2010).

Nevertheless, peripheral insulin resistance and diet-induced obesity are correlated with some other changes that may cause neurocognitive dysfunction. Indeed, they induce systemic and central inflammation with high levels of circulating pro-inflammatory interleukins that have been linked to impaired executive function (Trollor et al., 2012). Obesity also alters HPA axis causing enhanced secretion of glucocorticoids, which have been associated with reduced hippocampal volume, memory impairment and mood alterations (MacQueen and Frodl, 2011). Moreover, mice specifically lacking the IR into the brain (NIRKO mice) display changes in dopamine turnover associated with anxiety and depressive-like behaviors (Kleinridders et al., 2015). In addition, diet-induced microbiota dysbiosis can impact on the gut-brain axis, thus promoting insulin resistance and cognitive impairment (Daulatzai, 2014). Finally, HFD exposure during early stages of life is associated with impaired learning and spatial memory (Boitard et al., 2012), suggesting that alteration of insulin signaling may negatively influence cognitive function at each stage of life.

Brain Insulin Resistance, Brain Aging and Neurodegenerative Diseases

While diabetes is known to increase the risk for dementia, the underlying mechanisms linking insulin resistance, T2D and AD are poorly understood. Undoubtedly, micro- and macro-vascular complications of T2D may increase the risk of cerebrovascular disease, cognitive impairment and vascular dementia (Gorelick et al., 2011). Moreover, white matter disease, alteration of the BBB and neuro-inflammation may play a pathophysiologic role (Hsu and Kanoski, 2014). However, hyper-insulinaemia promotes the formation of advanced glucose end products and ROS causing neurotoxicity and brain damage (Brownlee, 2001). Despite insulin exerts a neurotrophic role at moderate concentrations, higher levels of the hormone may be associated with increased deposition of amyloid-β (Aβ) in the brain due to competition for their common and main clearance mechanism, the insulin-degrading enzyme (Farris et al., 2003). In this regard, AD has been defined a form of type 3 diabetes, based on the evidence of BIR development in the AD brain (Steen et al., 2005; Bedse et al., 2015; Sposato et al., 2019). The insulin synthesis decreases during aging and AD progression in brain areas such as frontal cortex, hippocampus, and hypothalamus (Frolich et al., 1998). In addition, Aβ inhibits insulin expression in astrocytes (Pitt et al., 2017). Together, these studies indicate a crosstalk between brain insulin signaling alteration and Aβ accumulation in neurodegenerative diseases. Accordingly, experimental data obtained from neuroimaging and biomarker studies revealed that T2D patients showed alterations of both brain glucose metabolism and cerebrospinal fluid including phosphorylated tau, which are reminiscent of changes observed in AD (Baker et al., 2011; Moran et al., 2015). In addition, analysis of AD postmortem brains revealed insulin signaling alterations in hippocampal tissues resembling the biochemical features of insulin resistance in parallel with histopathological hallmarks of neurodegeneration (Talbot et al., 2012; Tramutola et al., 2015). Moreover, tau is hyper-phosphorylated in the brain of NIRKO mice (Schubert et al., 2004) and BIR has been associated with tau pathology in AD human brains (Yarchoan and Arnold, 2014).

Interestingly, T2D and AD also share several metabolic derangements promoting brain aging. AD patients show hyper-insulinaemia and decreased peripheral insulin sensitivity (Craft et al., 1996), whereas insulin levels in cerebrospinal fluid are reduced (Craft et al., 1998). Accordingly, sustained peripheral hyper-insulinaemia can reduce the transport of insulin into the brain due to the lower expression of IR at the BBB (Schwartz et al., 1990). Brain insulin uptake is also impaired in both aging and AD independently by T2D (Frolich et al., 1998). Recent evidence suggests that insulin may influence Aβ deposition and AD-dependent impairment of both synaptic plasticity and memory formation (Cholerton et al., 2013). Intranasal insulin administration has been demonstrated to improve cognitive function in humans (Hallschmid et al., 2007; Reger et al., 2008). However, recent data about a clinical trial with mild cognitive impairment (MCI) or moderate AD patients revealed no significant effects of long-term intranasal insulin delivery on cognitive performance in memory task (Craft et al., 2017).

Finally, genetic and experimental data about insulin degrading enzyme and, more recently, the Aβ metabolism regulation by sortilin related VPS10 domain containing receptor 1 (SorCS1) gene suggest novel mechanistic links between BIR

and AD (Lane et al., 2010; Wang et al., 2015). Thus, BIR seems to play a pivotal role at the crossroad between metabolic and neurodegenerative diseases, independently from the cerebrovascular mechanisms.

BIOMARKERS OF BRAIN INSULIN RESISTANCE

In view of the close relationship among metabolic diseases, BIR and cognitive decline, it is emerging the need to identify biomarkers able to detect BIR before, or possibly even in the absence of, peripheral insulin resistance, that may be predictive of age- and dementia-related cognitive impairment. Ideal biomarkers should be reliable, simple to measure, non-invasive and inexpensive (Noel-Storr et al., 2013). In this regard, the dosage of both Aβ and tau proteins in the cerebrospinal fluid is invasive and most likely indicative of a pathology already under development. For these reasons, in the last years several studies focused on evaluation of brain glucose metabolism and analysis of brain-derived extracellular vesicles extracted from the blood as biomarkers of BIR and early-phase cognitive decline.

Cerebral glucose metabolism is tightly correlated with neuronal activity (Simpson et al., 2007). Therefore, imaging of local brain hypo-metabolism can be used to visualize areas of reduced synaptic activity. The most frequently used method of brain metabolic imaging is positron emission tomography (PET) with (^{18}F)fluorodeoxyglucose (FDG) (Cohen and Klunk, 2014). Reduced cerebral glucose metabolism represents one of the earliest signs of AD, and studies in both humans and experimental models suggest that altered brain glucose metabolism is associated with AD progression (Kapogiannis and Mattson, 2011; Ishibashi et al., 2015).

Recent work have identified in GK rats reduced glutamine synthesis and impairment of the glutamate-glutamine cycle between astrocytes and neurons, driving to diabetes-induced neurodegeneration and cognitive dysfunction (Girault et al., 2017). In a mouse model of AD, impaired glucose transport through the BBB and decreased cerebral lactate release during neuronal activity occur at early stages of the phenotype (Merlini et al., 2011). Dysregulated brain glucose metabolism resembling changes observed in AD patients has been observed in metabolic disorders such as obesity or T2D (Tschritter et al., 2006, 2007). However, whether neuroimaging changes of brain glucose metabolism anticipate the onset of neurodegeneration or are related to the development of BIR in the same brain areas remain still poorly understood.

More recently, molecular strategies have been developed to selectively isolate brain-derived exosomes (BDE) from biological fluids (Tschritter et al., 2006, 2007; Fiandaca et al., 2015; Goetzl et al., 2016). Exosomes are extracellular vesicles carrying information (e.g., proteins, lipids, and nucleic acids) to distant cells, which are emerging as novel potential biomarkers for human diseases (Tkach and Thery, 2016). Several pathogenic proteins that are involved in neurodegenerative diseases, including AD, are loaded into vesicles and then extracellularly secreted via exosomes (Rajendran et al., 2006; Sharples et al., 2008). More importantly, changes of insulin resistance molecular markers (i.e., higher serine phosphorylation and lower tyrosine phosphorylation of IRS-1) have been found in neural-derived exosomes extracted from blood of AD patients compared to age- and gender-matched patients with frontotemporal dementia or T2D (Kapogiannis et al., 2015). These differences were detectable up to 10 years before the onset of AD symptoms. Finally, exosomal biomarkers of BIR were associated with higher brain atrophy in AD patients (Mullins et al., 2017) emphasizing the potential role of brain derived microvesicles as detectors of brain insulin signaling and biomarkers of brain damage due to metabolic and neurodegenerative disorders.

CONCLUSION

Molecules involved in metabolic homeostasis are now recognized to exert a great influence on hippocampal plasticity, and alteration of their equilibrium has a strong impact at the functional and behavioral levels. Insulin exerts a trophic role into the brain and it may also act as a signal of positive metabolic homeostasis promoting neuroplasticity, which is a high energy demanding process. Insulin plays a pivotal role in the regulation of central nervous system homeostasis and higher functions such as learning and memory, by controlling both NSC fate and the activity of neuronal network. In this regard, identifying the molecular targets that underlie the effects of insulin on brain plasticity may contribute to understand the mechanisms regulating neural plasticity in health and metabolic diseases and reveal novel targets in pathologies characterized by impaired neural plasticity, especially AD.

Actually, we do not yet have an exhaustive understanding of how systemic and brain insulin resistance are related to brain aging and AD, but clinical and experimental evidence indicates that insulin supplementation can be a therapeutic tool for patients with cognitive impairment and an added value in the treatment of dementia (Chapman et al., 2018; Santiago and Hallschmid, 2019). The availability of BDE, such as other biomarkers of brain metabolism detectable in the plasma, will foster clinical studies to identify novel therapeutic approaches for personalized medicine in neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

All authors conceived the work, took part to the scientific discussion, and wrote the manuscript.

FUNDING

This research was supported by the Italian Ministry of University and Research (SIR 2014 RBSI14ZV59 to SF) and Universitá Cattolica del Sacro Cuore intramural grants (Linea D.3.2 2015 and Linea D.3.2 2017 to CG).

REFERENCES

Åberg, M. A., Åberg, N. D., Palmer, T. D., Alborn, A. M., Carlsson-Skwirut, C., Bang, P., et al. (2003). IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. *Mol. Cell. Neurosci.* 24, 23–40. doi: 10.1016/s1044-7431(03)00082-4

Adams, J. P., and Sweatt, J. D. (2002). Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annu. Rev. Pharmacol. Toxicol.* 42, 135–163. doi: 10.1146/annurev.pharmtox.42.082701.145401

Adzovic, L., and Domenici, L. (2014). Insulin induces phosphorylation of the AMPA receptor subunit GluR1, reversed by ZIP, and over-expression of Protein Kinase M zeta, reversed by amyloid beta. *J. Neurochem.* 131, 582–587. doi: 10.1111/jnc.12947

Akanmu, M. A., Nwabudike, N. L., and Ilesanmi, O. R. (2009). Analgesic, learning and memory and anxiolytic effects of insulin in mice. *Behav. Brain Res.* 196, 237–241. doi: 10.1016/j.bbr.2008.09.008

Altarejos, J. Y., and Montminy, M. (2011). CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat. Rev. Mol. Cell Biol.* 12, 141–151. doi: 10.1038/nrm3072

Arnold, S. E., Lucki, I., Brookshire, B. R., Carlson, G. C., Browne, C. A., Kazi, H., et al. (2014). High fat diet produces brain insulin resistance, synaptodendritic abnormalities and altered behavior in mice. *Neurobiol. Dis.* 67, 79–87. doi: 10.1016/j.nbd.2014.03.011

Assefa, B., Mahmoud, A. M., Pfeiffer, A. F. H., Birkenfeld, A. L., Spranger, J., and Arafat, A. M. (2017). Insulin-like growth factor (IGF) binding protein-2, independently of IGF-1, induces GLUT-4 translocation and glucose uptake in 3T3-L1 adipocytes. *Oxid. Med. Cell Longev.* 2017:3035184. doi: 10.1155/2017/3035184

Babri, S., Badie, H. G., Khamenei, S., and Seyedlar, M. O. (2007). Intrahippocampal insulin improves memory in a passive-avoidance task in male wistar rats. *Brain Cogn.* 64, 86–91. doi: 10.1016/j.bandc.2007.01.002

Bailyes, E. M., Nave, B. T., Soos, M. A., Orr, S. R., Hayward, A. C., and Siddle, K. (1997). Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. *Biochem. J.* 327, 209–215. doi: 10.1042/bj3270209

Baker, L. D., Cross, D. J., Minoshima, S., Belongia, D., Watson, G. S., and Craft, S. (2011). Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch. Neurol.* 68, 51–57. doi: 10.1001/archneurol.2010.225

Banks, W. A., Burney, B. O., and Robinson, S. M. (2008). Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. *Peptides* 29, 2061–2065. doi: 10.1016/j.peptides.2008.07.001

Bartsch, T., and Wulff, P. (2015). The hippocampus in aging and disease: from plasticity to vulnerability. *Neuroscience* 309, 1–16. doi: 10.1016/j.neuroscience.2015.07.084

Bedse, G., Di Domenico, F., Serviddio, G., and Cassano, T. (2015). Aberrant insulin signaling in Alzheimer's disease: current knowledge. *Front. Neurosci.* 9:204. doi: 10.3389/fnins.2015.00204

Belfiore, A., Frasca, F., Pandini, G., Sciacca, L., and Vigneri, R. (2009). Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr. Rev.* 30, 586–623. doi: 10.1210/er.2008-0047

Benedict, C., Hallschmid, M., Hatke, A., Schultes, B., Fehm, H. L., Born, J., et al. (2004). Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* 29, 1326–1334. doi: 10.1016/j.psyneuen.2004.04.003

Boitard, C., Etchamendy, N., Sauvant, J., Aubert, A., Tronel, S., Marighetto, A., et al. (2012). Juvenile, but not adult exposure to high-fat diet impairs relational memory and hippocampal neurogenesis in mice. *Hippocampus* 22, 2095–2100. doi: 10.1002/hipo.22032

Braun, S. M., and Jessberger, S. (2014). Adult neurogenesis: mechanisms and functional significance. *Development* 141, 1983–1986. doi: 10.1242/dev.104596

Brooker, G. J., Kalloniatis, M., Russo, V. C., Murphy, M., Werther, G. A., and Bartlett, P. F. (2000). Endogenous IGF-1 regulates the neuronal differentiation of adult stem cells. *J. Neurosci. Res.* 59, 332–341. doi: 10.1002/(sici)1097-4547(20000201)59:3<332::aid-jnr6>3.0.co;2-2

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature* 414, 813–820. doi: 10.1038/414813a

Bruning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., et al. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science* 289, 2122–2125. doi: 10.1126/science.289.5487.2122

Callisaya, M. L., Beare, R., Moran, C., Phan, T., Wang, W., and Srikanth, V. K. (2019). Type 2 diabetes mellitus, brain atrophy and cognitive decline: a longitudinal study. *Diabetologia* 62, 448–458. doi: 10.1007/s00125-018-4778-9

Castilla-Ortega, E., Pedraza, C., Estivill-Torrús, G., and Santín, L. (2011). When is adult hippocampal neurogenesis necessary for learning? Evidence from animal research. *Rev. Neurosci.* 22, 267–283. doi: 10.1515/RNS.2011.027

Chapman, C. D., Schioth, H. B., Grillo, C. A., and Benedict, C. (2018). Intranasal insulin in Alzheimer's disease: food for thought. *Neuropharmacology* 136, 196–201. doi: 10.1016/j.neuropharm.2017.11.037

Chatterjee, S., Peters, S. A., Woodward, M., Mejia Arango, S., Batty, G. D., Beckett, N., et al. (2016). Type 2 diabetes as a risk factor for dementia in women compared with men: a pooled analysis of 2.3 million people comprising more than 100,000 cases of dementia. *Diabetes Care* 39, 300–307. doi: 10.2337/dc15-1588

Chell, J. M., and Brand, A. H. (2010). Nutrition-responsive glia control exit of neural stem cells from quiescence. *Cell* 143, 1161–1173. doi: 10.1016/j.cell.2010.12.007

Chen, T.-J., Dean-Chuan, W., Hui-Shan, H., and Hsuan-Fang, H. (2014). Insulin can induce the expression of a memory-related synaptic protein through facilitating AMPA receptor endocytosis in rat cortical neurons. *Cell. Mol. Life Sci.* 71, 4069–4080. doi: 10.1007/s00018-014-1620-5

Chen, Y., Yin, M., Cao, X., Hu, G., and Xiao, M. (2018). Pro- and anti-inflammatory effects of high cholesterol diet on aged brain. *Aging Dis.* 9, 374–390. doi: 10.14336/AD.2017.0706

Choi, J., Ko, J., Racz, B., Burette, A., Lee, J. R., Kim, S., et al. (2005). Regulation of dendritic spine morphogenesis by insulin receptor substrate 53, a downstream effector of Rac1 and Cdc42 small GTPases. *J. Neurosci.* 25, 869–879. doi: 10.1523/jneurosci.3212-04.2005

Cholerton, B., Baker, L. D., and Craft, S. (2013). Insulin, cognition, and dementia. *Eur. J. Pharmacol.* 719, 170–179. doi: 10.1016/j.ejphar.2013.08.008

Christie, J. M., Wenthold, R. J., and Monaghan, D. T. (1999). Insulin causes a transient tyrosine phosphorylation of NR2A and NR2B NMDA receptor subunits in rat hippocampus. *J. Neurochem.* 72, 1523–1528. doi: 10.1046/j.1471-4159.1999.721523.x

Cohen, A. D., and Klunk, W. E. (2014). Early detection of Alzheimer's disease using PiB and FDG PET. *Neurobiol. Dis.* 72, 117–122. doi: 10.1016/j.nbd.2014.05.001

Craft, S., Claxton, A., Baker, L. D., Hanson, A. J., Cholerton, B., Trittschuh, E. H., et al. (2017). Effects of regular and long-acting insulin on cognition and Alzheimer's disease biomarkers: a pilot clinical trial. *J. Alzheimers Dis.* 57, 1325–1334. doi: 10.3233/JAD-161256

Craft, S., Newcomer, J., Kanne, S., Dagogo-Jack, S., Cryer, P., Sheline, Y., et al. (1996). Memory improvement following induced hyperinsulinemia in Alzheimer's disease. *Neurobiol. Aging* 17, 123–130. doi: 10.1016/0197-4580(95)02002-0

Craft, S., Peskind, E., Schwartz, M. W., Schellenberg, G. D., Raskind, M., Porte, D. Jr., et al. (1998). Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology* 50, 164–168. doi: 10.1212/wnl.50.1.164

Crane, P. K., Walker, R., and Larson, E. B. (2013). Glucose levels and risk of dementia. *N Engl. J. Med.* 369, 1863–1864. doi: 10.1056/nejmc1311765

Cukierman-Yaffee, T. (2009). The relationship between dysglycemia and cognitive dysfunction. *Curr. Opin. Invest. Drugs* 10, 70–74.

Daulatzai, M. A. (2014). Chronic functional bowel syndrome enhances gut-brain axis dysfunction, neuroinflammation, cognitive impairment, and vulnerability to dementia. *Neurochem. Res.* 39, 624–644. doi: 10.1007/s11064-014-1266-6

Davis, W. A., Zilkens, R. R., Starkstein, S. E., Davis, T. M., and Bruce, D. G. (2017). Dementia onset, incidence and risk in type 2 diabetes: a matched cohort study with the fremantle diabetes study phase I. *Diabetologia* 60, 89–97. doi: 10.1007/s00125-016-4127-9

Debons, A. F., Krimsky, I., and From, A. (1970). A direct action of insulin on the hypothalamic satiety center. *Am. J. Physiol.* 219, 938–943. doi: 10.1152/ajplegacy.1970.219.4.938

Desrocher, M., and Rovet, J. (2004). Neurocognitive correlates of type 1 diabetes mellitus in childhood. *Child Neuropsychol.* 10, 36–52. doi: 10.1076/chin.10.1.36.26241

Devaskar, S. U., Giddings, S. J., Rajakumar, P. A., Carnaghi, L. R., Menon, R. K., Zahm, D. S., et al. (1994). Insulin gene expression and insulin synthesis in mammalian neuronal cell. *J. Biol. Chem.* 269, 8445–8454.

Dienel, G. A. (2012). Fueling and imaging brain activation. *ASN Neuro.* 4:e00093. doi: 10.1042/AN20120021

Du, L. L., Xie, J. Z., Cheng, X. S., Li, X. H., Kong, F. L., Jiang, X., et al. (2014). Activation of sirtuin 1 attenuates cerebral ventricular streptozotocin-induced tau hyperphosphorylation and cognitive injuries in rat hippocampi. *Age* 36, 613–623. doi: 10.1007/s11357-013-9592-1

Duarte, J. M. N., Skoug, C., Silva, H. B., Carvalho, R. A., Gruetter, R., and Cunha, R. A. (2019). Impact of caffeine consumption on Type 2 diabetes-induced spatial memory impairment and neurochemical alterations in the Hippocampus. *Front. Neurosci.* 12:1015. doi: 10.3389/fnins.2018.01015

Duelli, R., and Kuschinsky, W. (2001). Brain glucose transporters: relationship to local energy demand. *News Physiol. Sci.* 16, 71–76. doi: 10.1152/physiologyonline.2001.16.2.71

Duffy, C. M., Hofmeister, J. J., Nixon, J. P., and Butterick, T. A. (2019). High fat diet increases cognitive decline and neuroinflammation in a model of orexin loss. *Neurobiol. Learn. Mem.* 157, 41–47. doi: 10.1016/j.nlm.2018.11.008

Elias, M. F., Goodell, A. L., and Waldstein, S. R. (2012). Obesity, cognitive functioning and dementia: back to the future. *J. Alzheimers Dis.* 30, S113–S125. doi: 10.3233/JAD-2011-111175

Eny, K. M., Wolever, T. M., Fontaine-Bisson, B., and El-Sohemy, A. (2008). Genetic variant in the glucose transporter type 2 is associated with higher intakes of sugars in two distinct populations. *Physiol. Genomics* 33, 355–360. doi: 10.1152/physiolgenomics.00148.2007

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., et al. (1998). Neurogenesis in the adult human hippocampus. *Nat. Med.* 4, 1313–1317.

Fadel, J. R., and Reagan, L. P. (2016). Stop signs in hippocampal insulin signaling: the role of insulin resistance in structural, functional and behavioral deficits. *Curr. Opin. Behav. Sci.* 9, 47–54. doi: 10.1016/j.cobeha.2015.12.004

Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E. A., Frosch, M. P., et al. (2003). Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 100, 4162–4167. doi: 10.1073/pnas.0230450100

Fernandez, A. M., and Torres-Aleman, I. (2012). The many faces of insulin-like peptide signalling in the brain. *Nat. Rev. Neurosci.* 13, 225–239. doi: 10.1038/nrn3209

Fiandaca, M. S., Kapogiannis, D., Mapstone, M., Boxer, A., Eitan, E., Schwartz, J. B., et al. (2015). Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. *Alzheimers Dement.* 11, 600–607. doi: 10.1016/j.jalz.2014.06.008

Finkbeiner, S. (2000). CREB couples neurotrophin signals to survival messages. *Neuron* 25, 11–14. doi: 10.1016/s0896-6273(00)80866-1

Freeman, L. R., and Granholm, A. C. (2012). Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet. *J. Cereb. Blood Flow Metab.* 32, 643–653. doi: 10.1038/jcbfm.2011.168

Frolich, L., Blum-Degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., et al. (1998). Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J. Neural. Transm.* 105, 423–438.

Fusco, S., Leone, L., Barbati, S. A., Samengo, D., Piacentini, R., Maulucci, G., et al. (2016). A CREB-Sirt1-Hes1 circuitry mediates neural stem cell response to glucose availability. *Cell Rep.* 14, 1195–1205. doi: 10.1016/j.celrep.2015.12.092

Fusco, S., Maulucci, G., and Pani, G. (2012a). Sirt1: def-eating senescence? *Cell Cycle* 11, 4135–4146. doi: 10.4161/cc.22074

Fusco, S., Ripoli, C., Podda, M. V., Ranieri, S. C., Leone, L., Toietta, G., et al. (2012b). A role for neuronal cAMP responsive-element binding (CREB)-1 in brain responses to calorie restriction. *Proc. Natl. Acad. Sci. U.S.A.* 109, 621–626. doi: 10.1073/pnas.1109237109

Fusco, S., and Pani, G. (2013). Brain response to calorie restriction. *Cell Mol. Life. Sci.* 70, 3157–3170. doi: 10.1007/s00018-012-1223-y

Ge, Y., Dong, Z., Bagot, R. C., Howland, J. G., Phillips, A. G., Wong, T. P., et al. (2010). Hippocampal long-term depression is required for the consolidation of spatial memory. *Proc. Natl. Acad. Sci. U.S.A.* 107, 16697–16702. doi: 10.1073/pnas.1008200107

Girault, F. M., Sonnay, S., Gruetter, R., and Duarte, J. M. N. (2017). Alterations of brain energy metabolism in Type 2 diabetic goto-kakizaki rats measured in vivo by 13C magnetic resonance spectroscopy. *Neurotox. Res.* 36, 268–278. doi: 10.1007/s12640-017-9821-y

Glasper, E. R., Llorens-Martin, M. V., Leuner, B., Gould, E., and Trejo, J. L. (2010). Blockade of insulin-like growth factor-I has complex effects on structural plasticity in the hippocampus. *Hippocampus* 20, 706–712. doi: 10.1002/hipo.20672

Goetzl, E. J., Mustapic, M., Kapogiannis, D., Eitan, E., Lobach, I. V., Goetzl, L., et al. (2016). Cargo proteins of plasma astrocyte-derived exosomes in Alzheimer's disease. *FASEB J.* 30, 3853–3859. doi: 10.1096/fj.201600756r

Gorelick, P. B., Scuteri, A., Black, S. E., Decarli, C., Greenberg, S. M., Iadecola, C., et al. (2011). Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* 42, 2672–2713. doi: 10.1161/str.0b013e3182299496

Grillo, C. A., Piroli, G. G., Lawrence, R. C., Wrighten, S. A., Green, A. J., Wilson, S. P., et al. (2015). Hippocampal insulin resistance impairs spatial learning and synaptic plasticity. *Diabetes Metab. Res. Rev.* 64, 3927–3936. doi: 10.2337/db15-0596

Gudala, K., Bansal, D., Schifano, F., and Bhansali, A. (2013). Diabetes mellitus and risk of dementia: a meta-analysis of prospective observational studies. *J. Diabetes Invest.* 4, 640–650. doi: 10.1111/jdi.12087

Hallschmid, M., Benedict, C., Born, J., and Kern, W. (2007). Targeting metabolic and cognitive pathways of the CNS by intranasal insulin administration. *Expert Opin. Drug Deliv.* 4, 319–322. doi: 10.1517/17425247.4.4.319

Heni, M., Hennige, A. M., Peter, A., Siegel-Axel, D., Ordelheide, A. M., Krebs, N., et al. (2011). Insulin promotes glycogen storage and cell proliferation in primary human astrocytes. *PLoS One* 6:e21594. doi: 10.1371/journal.pone.0021594

Heni, M., Schöpfer, P., Peter, A., Sartorius, T., Fritsche, A., Synofzik, M., et al. (2014). Evidence for altered transport of insulin across the blood-brain barrier in insulin-resistant humans. *Acta Diabetol.* 51, 679–681. doi: 10.1007/s00592-013-0546-y

Hill, J. M., Lesniak, M. A., Pert, C. B., and Roth, J. (1986). Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience* 17, 1127–1138. doi: 10.1016/0306-4522(86)90082-5

Howarth, C., Gleeson, P., and Attwell, D. (2012). Updated energy budgets for neural computation in the neocortex and cerebellum. *J. Cereb. Blood Flow Metab.* 32, 1222–1232. doi: 10.1038/jcbfm.2012.35

Hsu, T. M., and Kanoski, S. E. (2014). Blood-brain barrier disruption: mechanistic links between Western diet consumption and dementia. *Front. Aging Neurosci.* 6:88. doi: 10.3389/fnagi.2014.00088

Irvine, E. E., Drinkwater, L., Radwanska, K., Al-Qassab, H., Smith, M. A., O'Brien, M., et al. (2011). Insulin receptor substrate 2 is a negative regulator of memory formation. *Learn. Mem.* 18, 375–383. doi: 10.1101/lm.2111311

Ishibashi, K., Kawasaki, K., Ishiwata, K., and Ishii, K. (2015). Reduced uptake of 18F-FDG and 15O-H2O in Alzheimer's disease-related regions after glucose loading. *J. Cereb. Blood Flow Metab.* 35, 1380–1385. doi: 10.1038/jcbfm.2015.127

Jeong, H., Cohen, D. E., Cui, L., Supinski, A., Savas, J. N., Mazzulli, J. R., et al. (2012). Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. *Nat. Med.* 18:159. doi: 10.1038/nm.2559

Joost, H. G., and Thorens, B. (2001). The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members. *Mol. Membr. Biol.* 18, 247–256. doi: 10.1080/09687680110090456

Kamal, A., Ramakers, G. M., Gispen, W. H., and Biessels, G. J. (2013). Hyperinsulinemia in rats causes impairment of spatial memory and learning with defects in hippocampal synaptic plasticity by involvement of postsynaptic mechanisms. *Exp. Brain Res.* 226, 45–51. doi: 10.1007/s00221-013-3409-4

Kanoski, S. E., and Davidson, T. L. (2010). Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet. *J. Exp. Psychol. Anim. Behav. Process* 36, 313–319. doi: 10.1037/a0017228

Kanoski, S. E., Fortin, S. M., Ricks, K. M., and Grill, H. J. (2013). Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biol. Psychiatry* 73, 915–923. doi: 10.1016/j.biopsych.2012.07.002

Kapogiannis, D., Boxer, A., Schwartz, J. B., Abner, E. L., Biragyn, A., Masharani, U., et al. (2015). Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. *FASEB J.* 29, 589–596. doi: 10.1096/fj.14-262048

Kapogiannis, D., and Mattson, M. P. (2011). Disrupted energy metabolism and neuronal circuit dysfunction in cognitive impairment and Alzheimer's disease. *Lancet Neurol.* 10, 187–198. doi: 10.1016/S1474-4422(10)70277-5

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F. H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 130, 391–399. doi: 10.1242/dev.00203

Kern, W., Peters, A., Fruehwald-Schultes, B., Deininger, E., Born, J., Fehm, H. L., et al. (2001). Improving influence of insulin on cognitive functions in humans. *Neuroendocrinology* 74, 270–280. doi: 10.1159/000054694

Kerti, L., Witte, A. V., Winkler, A., Grittner, U., Rujescu, D., and Floel, A. (2013). Higher glucose levels associated with lower memory and reduced hippocampal microstructure. *Neurology* 81, 1746–1752. doi: 10.1212/01.wnl.0000435561.00234.ee

Ketterer, C., Tschritter, O., Preissl, H., Heni, M., Häring, H. U., and Fritsche, A. (2011). Insulin sensitivity of the human brain. *Diabetes Res. Clin. Pract.* 93, S47–S51. doi: 10.1016/S0168-8227(11)70013-4

Kim, M. H., Choi, J., Yang, J., Chung, W., Kim, J. H., Paik, S. K., et al. (2009). Enhanced NMDA receptor-mediated synaptic transmission, enhanced long-term potentiation, and impaired learning and memory in mice lacking IRSp53. *J. Neurosci.* 29, 1586–1595. doi: 10.1523/JNEUROSCI.4306-08.2009

Kleinridders, A., Cai, W., Cappellucci, L., Ghazarian, A., Collins, W. R., Vienberg, S. G., et al. (2015). Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3463–3468. doi: 10.1073/pnas.1500877112

Kodl, C. T., and Seaquist, E. R. (2008). Cognitive dysfunction and diabetes mellitus. *Endocr. Rev.* 29, 494–511. doi: 10.1210/er.2007-0034

Kopf, S. R., and Baratti, C. M. (1999). Effects of posttraining administration of insulin on retention of a habituation response in mice: participation of a central cholinergic mechanism. *Neurobiol. Learn. Mem.* 71, 50–61. doi: 10.1006/nlme.1998.3831

Kopf, S. R., Boccia, M. M., and Baratti, C. M. (1998). AF-DX 116, a presynaptic muscarinic receptor antagonist, potentiates the effects of glucose and reverses the effects of insulin on memory. *Neurobiol. Learn. Mem.* 70, 305–313. doi: 10.1006/nlme.1998.3855

Kullmann, S., Heni, M., Hallschmid, M., Fritsche, A., Preissl, H., and Haring, H. U. (2016). 'Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. *Physiol. Rev.* 96, 1169–1209. doi: 10.1152/physrev.00032.2015

Kuwabara, T., Kagalwala, M. N., Onuma, Y., Ito, Y., Warashina, M., Terashima, K., et al. (2011). Insulin biosynthesis in neuronal progenitors derived from adult hippocampus and the olfactory bulb. *EMBO Mol. Med.* 3, 742–754. doi: 10.1002/emmm.201100177

Lane, R. F., Raines, S. M., Steele, J. W., Ehrlich, M. E., Lah, J. A., Small, S. A., et al. (2010). Diabetes-associated SorCS1 regulates Alzheimer's amyloid-beta metabolism: evidence for involvement of SorL1 and the retromer complex. *J. Neurosci.* 30, 13110–13115. doi: 10.1523/JNEUROSCI.3872-10.2010

Lee, C. C., Huang, C. C., and Hsu, K. S. (2011). Insulin promotes dendritic spine and synapse formation by the PI3K/Akt/mTOR and Rac1 signaling pathways. *Neuropharmacology* 61, 867–879. doi: 10.1016/j.neuropharm.2011.06.003

Lee, J., Seroogy, K. B., and Mattson, M. P. (2002). Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. *J. Neurochem.* 80, 539–547. doi: 10.1046/j.0022-3042.2001.00747.x

Lindqvist, A., Mohapel, P., Bouter, B., Frielingsdorf, H., Pizzo, D., Brundin, P., et al. (2006). High-fat diet impairs hippocampal neurogenesis in male rats. *Eur. J. Neurol.* 13, 1385–1388. doi: 10.1111/j.1468-1331.2006.01500.x

Lindsay, R. M., Wiegand, S. J., Altar, C. A., and DiStefano, P. S. (1994). Neurotrophic factors: from molecule to man. *Trends Neurosci.* 17, 182–190. doi: 10.1016/0166-2236(94)90099-x

Liu, L., Brown, J. C., Webster, W. W., Morrisett, R. A., and Monaghan, D. T. (1995). Insulin potentiates N-methyl-D-aspartate receptor activity in *Xenopus oocytes* and rat hippocampus. *J. Neurosci. Lett.* 192, 5–8. doi: 10.1016/0304-3940(95)11593-l

Lonze, B. E., Riccio, A., Cohen, S., and Ginty, D. D. (2002). Apoptosis, axonal growth defects, and degeneration of peripheral neurons in mice lacking CREB. *Neuron* 34, 371–385. doi: 10.1016/s0896-6273(02)00686-4

Lupien, S. B., Bluhm, E. J., and Ishii, D. N. (2003). Systemic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. *J. Neurosci. Res.* 74, 512–523. doi: 10.1002/jnr.10791

Ma, C. Y., Yao, M. J., Zhai, Q. W., Jiao, J. W., Yuan, X. B., and Poo, M. M. (2014). SIRT1 suppresses self-renewal of adult hippocampal neural stem cells. *Development* 141, 4697–4709. doi: 10.1242/dev.117937

MacQueen, G., and Frodl, T. (2011). The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol. Psychiatry* 16, 252–264. doi: 10.1038/mp.2010.80

Magri, L., Cambiaghi, M., Cominelli, M., Alfaro-Cervello, C., Cursi, M., Pala, M., et al. (2011). Sustained activation of mTOR pathway in embryonic neural stem cells leads to development of tuberous sclerosis complex-associated lesions. *Cell Stem Cell.* 9, 447–462. doi: 10.1016/j.stem.2011.09.008

Mainardi, M., Fusco, S., and Grassi, C. (2015). Modulation of hippocampal neural plasticity by glucose-related signaling. *Neural Plast.* 2015:657928. doi: 10.1155/2015/657928

Mainardi, M., Spinelli, M., Scala, F., Mattera, A., Fusco, S., D'Ascenzo, M., et al. (2017). Loss of leptin-induced modulation of hippocampal synaptic trasmission and signal transduction in high-fat diet-fed mice. *Front. Cell Neurosci.* 11:225. doi: 10.3389/fncel.2017.00225

Man, H.-Y., Wang, Q., Lu, W. Y., Ju, W., Ahmadian, G., Liu, L., et al. (2003). Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. *Neuron* 38, 611–624. doi: 10.1016/s0896-6273(03)00228-9

Martin, E. D., Sánchez-Perez, A., Trejo, J. L., Martin-Aldana, J. A., Cano Jaimez, M., Pons, S., et al. (2012). IRS-2 Deficiency impairs NMDA receptor-dependent long-term potentiation. *Cereb. Cortex* 22, 1717–1727. doi: 10.1093/cercor/bhr216

Maswood, N., Young, J., Tilmont, E., Zhang, Z., Gash, D. M., Gerhardt, G. A., et al. (2004). Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. *Proc. Natl. Acad. Sci. U.S.A.* 101, 18171–18176. doi: 10.1073/pnas.0405831102

McNay, E. C., Ong, C. T., McCrimmon, R. J., Cresswell, J., Bogan, J. S., Sherwin, R. S., et al. (2010). Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. *Neurobiol. Learn. Mem.* 93, 546–553. doi: 10.1016/j.nlm.2010.02.002

Membrez, M., Hummler, E., Beermann, F., Haefliger, J. A., Savioz, R., Pedrazzini, T., et al. (2006). GLUT8 is dispensable for embryonic development but influences hippocampal neurogenesis and heart function. *Mol. Cell. Biol.* 26, 4268–4276. doi: 10.1128/mcb.00081-06

Merlini, M., Meyer, E. P., Ulmann-Schuler, A., and Nitsch, R. M. (2011). Vascular beta-amyloid and early astrocyte alterations impair cerebrovascular function and cerebral metabolism in transgenic arcAbeta mice. *Acta Neuropathol.* 122, 293–311. doi: 10.1007/s00401-011-0834-y

Moheet, A., Mangia, S., and Seaquist, E. R. (2015). Impact of diabetes on cognitive function and brain structure. *Ann. N. Y. Acad. Sci.* 1353, 60–71. doi: 10.1111/nyas.12807

Molteni, R., Barnard, R. J., Ying, Z., Roberts, C. K., and Gomez-Pinilla, F. (2002). A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* 112, 803–814. doi: 10.1016/s0306-4522(02)00123-9

Moosavi, M., Naghdi, N., Maghsoudi, N., and Zahedi, S. (2006). The effect of intrahippocampal insulin microinjection on spatial learning and memory. *Horm. Behav.* 50, 748–752. doi: 10.1016/j.yhbeh.2006.06.025

Moran, C., Beare, R., Phan, T. G., Bruce, D. G., Callisaya, M. L., and Srikanth, V. (2015). Type 2 diabetes mellitus and biomarkers of neurodegeneration. *Neurology* 85, 1123–1130. doi: 10.1212/WNL.0000000000001982

Mullins, R. J., Mustapic, M., Goetzl, E. J., and Kapogiannis, D. (2017). Exosomal biomarkers of brain insulin resistance associated with regional atrophy in Alzheimer's disease. *Hum. Brain Mapp.* 38, 1933–1940. doi: 10.1002/hbm.23494

Murray, A. J., Knight, N. S., Cochlin, L. E., McAleese, S., Deacon, R. M., Rawlins, J. N., et al. (2009). Deterioration of physical performance and cognitive function

in rats with short-term high-fat feeding. *FASEB J.* 23, 4353–4360. doi: 10.1096/fj.09-139691

Nakahata, Y., and Yasuda, R. (2018). Plasticity of spine structure: local signaling, translation and cytoskeletal reorganization. *Front. Synaptic Neurosci.* 10:29. doi: 10.3389/fnsyn.2018.00029

Nisticò, R., Cavallucci, V., Piccinin, S., Macrì, S., Pignatelli, M., Mehdawy, B., et al. (2012). Insulin receptor beta-subunit haploinsufficiency impairs hippocampal late-phase LTP and recognition memory. *Neuromol. Med.* 14, 262–269. doi: 10.1007/s12017-012-8184-z

Noel-Storr, A. H., Flicker, L., Ritchie, C. W., Nguyen, G. H., Gupta, T., Wood, P., et al. (2013). Systematic review of the body of evidence for the use of biomarkers in the diagnosis of dementia. *Alzheimers Dement.* 9, e96–e105. doi: 10.1016/j.jalz.2012.01.014

Nunley, K. A., Rosano, C., Ryan, C. M., Jennings, J. R., Aizenstein, H. J., Zgibor, J. C., et al. (2015). Clinically relevant cognitive impairment in middle-aged adults with childhood-onset type 1 diabetes. *Diabetes Care* 38, 1768–1776. doi: 10.2337/dc15-0041

Park, C. R., Seeley, R. J., Craft, S., and Woods, S. C. (2000). Intracerebroventricular insulin enhances memory in a passive-avoidance task. *Physiol. Behav.* 68, 509–514. doi: 10.1016/s0031-9384(99)00220-6

Park, J. H., Glass, Z., Sayed, K., Michurina, T. V., Lazutkin, A., Mineyeva, O., et al. (2013). Calorie restriction alleviates the age-related decrease in neural progenitor cell division in the aging brain. *Eur. J. Neurosci.* 37, 1987–1993. doi: 10.1111/ejn.12249

Payne, J., Maher, F., Simpson, I., Mattice, L., and Davies, P. (1997). Glucose transporter Glut 5 expression in microglial cells. *Glia* 21, 327–331. doi: 10.1002/(sici)1098-1136(199711)21:3<327::aid-glia7>3.0.co;2-1

Piroli, G. G., Grillo, C. A., Reznikov, L. R., Adams, S., McEwen, B. S., Charron, M. J., et al. (2007). Corticosterone impairs insulin-stimulated translocation of GLUT4 in the rat hippocampus. *Neuroendocrinology* 85, 71–80. doi: 10.1159/000101694

Pitt, J., Wilcox, K. C., Tortelli, V., Diniz, L. P., Oliveira, M. S., Dobbins, C., et al. (2017). Neuroprotective astrocyte-derived insulin/insulin-like growth factor 1 stimulates endocytic processing and extracellular release of neuron-bound abeta oligomers. *Mol. Biol. Cell* 28, 2623–2636. doi: 10.1091/mbc.E17-06-0416

Plitzko, D., Rumpel, S., and Gottmann, K. (2001). Insulin promotes functional induction of silent synapses in differentiating rat neocortical neurons. *Eur. J. Neurosci.* 14, 1412–1415. doi: 10.1046/j.0953-816x.2001.01740.x

Plotsky, P. M., Thrivikraman, K. V., Watts, A. G., and Hauger, R. L. (1992). Hypothalamic-pituitary-adrenal axis function in the zucker obese rat. *Endocrinology* 130, 1931–1941. doi: 10.1210/en.130.4.1931

Prozorovski, T., Schulze-Topphoff, U., Glumm, R., Baumgart, J., Schröter, F., Ninnemann, O., et al. (2008). Sirt1 contributes critically to the redox-dependent fate of neural progenitors. *Nat. Cell Biol.* 10, 385–394. doi: 10.1038/ncb1700

Rajendran, L., Honsho, M., Zahn, T. R., Keller, P., Geiger, K. D., Verkade, P., et al. (2006). Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11172–11177. doi: 10.1073/pnas.0603838103

Reger, M. A., Watson, G. S., Green, P. S., Baker, L. D., Cholerton, B., Fishel, M. A., et al. (2008). Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. *J. Alzheimers Dis.* 13, 323–331. doi: 10.3233/jad-2008-13309

Reichardt, L. F. (2006). Neurotrophin-regulated signalling pathways. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 361, 1545–1564. doi: 10.1098/rstb.2006.1894

Renault, V. M., Rafalski, V. A., Morgan, A. A., Salih, D. A., Brett, J. O., Webb, A. E., et al. (2009). FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell* 5, 527–539. doi: 10.1016/j.stem.2009.09.014

Requejo, A. M., Ortega, R. M., Robles, F., Navia, B., Faci, M., and Aparicio, A. (2003). Influence of nutrition on cognitive function in a group of elderly, independently living people. *Eur. J. Clin. Nutr.* 57(Suppl. 1), S54–S57.

Saharan, S., Jhaveri, D. J., and Bartlett, P. F. (2013). SIRT1 regulates the neurogenic potential of neural precursors in the adult subventricular zone and hippocampus. *J. Neurosci. Res.* 91, 642–659. doi: 10.1002/jnr.23199

Salcedo-Tello, P., Ortiz-Matamoros, A., and Arias, C. (2011). GSK3 function in the brain during development, neuronal plasticity, and neurodegeneration. *Int. J. Alzheimers Dis.* 2011:189728.

Sankar, R., Thamotharan, S., Shin, D., Moley, K. H., and Devaskar, S. U. (2002). Insulin-responsive glucose transporters-GLUT8 and GLUT4 are expressed in the developing mammalian brain. *Brain Res. Mol. Brain Res.* 107, 157–165. doi: 10.1016/s0169-328x(02)00487-4

Santiago, J. C. P., and Hallschmid, M. (2019). Outcomes and clinical implications of intranasal insulin administration to the central nervous system. *Exp. Neurol.* 317, 180–190. doi: 10.1016/j.expneurol.2019.03.007

Schubert, M., Gautam, D., Surjo, D., Ueki, K., Baudler, S., Schubert, D., et al. (2004). Role for neuronal insulin resistance in neurodegenerative diseases. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3100–3105.

Schwartz, M. W., Figlewicz, D. F., Kahn, S. E., Baskin, D. G., Greenwood, M. R., Porte, D. Jr., et al. (1990). Insulin binding to brain capillaries is reduced in genetically obese, hyperinsulinemic Zucker rats. *Peptides* 11, 467–472. doi: 10.1016/0196-9781(90)90044-6

Sellbom, K. S., and Gunstad, J. (2012). Cognitive function and decline in obesity. *J. Alzheimers Dis.* 30(Suppl. 2), S89–S95. doi: 10.3233/JAD-2011-111073

Sharples, R. A., Vella, L. J., Nisbet, R. M., Naylor, R., Perez, K., Barnham, K. J., et al. (2008). Inhibition of gamma-secretase causes increased secretion of amyloid precursor protein C-terminal fragments in association with exosomes. *FASEB J.* 22, 1469–1478. doi: 10.1096/fj.07-9357com

Shepherd, P. R., and Kahn, B. B. (1999). Glucose transporters and insulin action. Implications for insulin resistance and diabetes mellitus. *New Engl. J. Med.* 341, 248–257. doi: 10.1056/nejm199907223410406

Simpson, I. A., Carruthers, A., and Vannucci, S. J. (2007). Supply and demand in cerebral energy metabolism: the role of nutrient transporters. *J. Cereb. Blood Flow Metab.* 27, 1766–1791. doi: 10.1038/sj.jcbfm.9600521

Skeberdis, V. A., Lan, J.-Y., Zheng, X., Zukin, S. R., and Bennett, M. V. (2001). Insulin promotes rapid delivery of N-methyl-D-aspartate receptors to the cell surface by exocytosis. *Proc. Natl. Acad. Sci. U.S.A.* 98, 3561–3566. doi: 10.1073/pnas.051634698

Son, J. H., Shim, J. H., Kim, K.-H., Ha, J.-Y., and Han, J. Y. (2012). Neuronal autophagy and neurodegenerative diseases. *J Exp. Mol. Med.* 44, 89–98.

Sousa-Nunes, R., Yee, L. L., and Gould, A. P. (2011). Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in *Drosophila*. *Nature* 471, 508–512. doi: 10.1038/nature09867

Spinelli, M., Fusco, S., Mainardi, M., Scala, F., Natale, F., Lapenta, R., et al. (2017). Brain insulin resistance impairs hippocampal synaptic plasticity and memory by increasing GluA1 palmitoylation through FoxO3a. *Nat. Commun.* 8:2009. doi: 10.1038/s41467-017-02221-9

Sposato, V., Canu, N., Fico, E., Fusco, S., Bolasco, G., Ciotti, M. T., et al. (2019). The medial septum is insulin resistant in the AD presymptomatic phase: rescue by nerve growth factor-driven IRS1 activation. *Mol. Neurobiol.* 56, 535–552. doi: 10.1007/s12035-018-1038-4

Steen, E., Terry, B. M., Rivera, E. J., Cannon, J. L., Neely, T. R., Tavares, R., et al. (2005). Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *J. Alzheimers Dis.* 7, 63–80. doi: 10.3233/jad-2005-7107

Stoica, L., Zhu, P. J., Huang, W., Zhou, H., Kozma, S. C., Costa-Mattioli, M., et al. (2011). Selective pharmacogenetic inhibition of mammalian target of Rapamycin complex I (mTORC1) blocks long-term synaptic plasticity and memory storage. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3791–3796. doi: 10.1073/pnas.1014715108

Stranahan, A. M., Arumugam, T. V., Cutler, R. G., Lee, K., Egan, J. M., and Mattson, M. P. (2008a). Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nat. Neurosci.* 11, 309–317. doi: 10.1038/nn2055

Stranahan, A. M., Norman, E. D., Lee, K., Cutler, R. G., Telljohann, R. S., Egan, J. M., et al. (2008b). Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus* 18, 1085–1088. doi: 10.1002/hipo.20470

Sun, L. Y. (2006). Hippocampal IGF-1 expression, neurogenesis and slowed aging: clues to longevity from mutant mice. *Age* 28, 181–189. doi: 10.1007/s11357-006-9009-5

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., et al. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J. Clin. Invest.* 122, 1316–1338. doi: 10.1172/JCI59903

Taylor, C. J., Jhaveri, D. J., and Bartlett, P. F. (2013). The therapeutic potential of endogenous hippocampal stem cells for the treatment of neurological disorders. *Front. Cell Neurosci.* 7:5. doi: 10.3389/fncel.2013.00005

Thirumangalakudi, L., Prakasam, A., Zhang, R., Bimonte-Nelson, H., Sambamurti, K., Kindy, M. S., et al. (2008). High cholesterol-induced neuroinflammation and

amyloid precursor protein processing correlate with loss of working memory in mice. *J. Neurochem.* 106, 475–485. doi: 10.1111/j.1471-4159.2008.05415.x

Tkach, M., and Thery, C. (2016). Communication by extracellular vesicles: where we are and where we need to go. *Cell* 164, 1226–1232. doi: 10.1016/j.cell.2016.01.043

Tramutola, A., Triplett, J. C., Di Domenico, F., Niedowicz, D. M., Murphy, M. P., Coccia, R., et al. (2015). Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD. *J. Neurochem.* 133, 739–749. doi: 10.1111/jnc.13037

Trejo, J. L., Piriz, J., Llorens-Martin, M. V., Fernandez, A. M., Bolós, M., LeRoith, D., et al. (2007). Central actions of liver-derived insulin-like growth factor I underlying its pro-cognitive effects. *Mol. Psychiatry* 12, 1118–1128. doi: 10.1038/sj.mp.4002076

Trollor, J. N., Smith, E., Agars, E., Kuan, S. A., Baune, B. T., Campbell, L., et al. (2012). The association between systemic inflammation and cognitive performance in the elderly: the sydney memory and ageing study. *Age* 34, 1295–1308. doi: 10.1007/s11357-011-9301-x

Tschritter, O., Preissl, H., Hennige, A. M., Stumvoll, M., Porubska, K., Frost, R., et al. (2006). The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: a magnetoencephalographic study. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12103–12108. doi: 10.1073/pnas.0604404103

Tschritter, O., Preissl, H., Yokoyama, Y., Machicao, F., Haring, H. U., and Fritsche, A. (2007). Variation in the FTO gene locus is associated with cerebrocortical insulin resistance in humans. *Diabetologia* 50, 2602–2603. doi: 10.1007/s00125-007-0839-1

Van Der Heide, L. P., Kamal, A., Artola, A., Gispen, W. H., and Ramakers, G. M. J. (2005). Insulin modulates hippocampal activity-dependent synaptic plasticity in a N-methyl-d-aspartate receptor and phosphatidyl-inositol-3-kinase-dependent manner. *J. Neurochem.* 94, 1158–1166. doi: 10.1111/j.1471-4159.2005.03269.x

van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D., and Gage, F. H. (2002). Functional neurogenesis in the adult hippocampus. *Nature* 415, 1030–1034.

Vannucci, S. J., Koehler-Stec, E. M., Li, K., Reynolds, T. H., Clark, R., and Simpson, I. A. (1998). GLUT4 glucose transporter expression in rodent brain: effect of diabetes. *Brain Res.* 797, 1–11. doi: 10.1016/s0006-8993(98)00103-6

Wan, Q., Xiong, Z. G., Man, H. Y., Ackerley, C. A., Braunton, J., Lu, W. Y., et al. (1997). Recruitment of functional GABA(A) receptors to postsynaptic domains by insulin. *Nature* 388, 686–690. doi: 10.1038/41792

Wang, S., He, F., and Wang, Y. (2015). Association between polymorphisms of the insulin-degrading enzyme gene and late-onset Alzheimer disease. *J. Geriatr. Psychiatry Neurol.* 28, 94–98. doi: 10.1177/0891988714554707

Wong, S. K., Chin, K. Y., Suhaimi, F. H., Fairus, A., and Ima-Nirwana, S. (2016). Animal models of metabolic syndrome: a review. *Nutr. Metab.* 13:65.

Woods, S. C., Randy, J. S., Baskin, D. G., and Schwartz, M. W. (2003). Insulin and the blood-brain barrier. *Curr. Pharm. Des.* 9, 795–800.

Wu, A., Ying, Z., and Gomez-Pinilla, F. (2004). The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. *Eur. J. Neurosci.* 19, 1699–1707. doi: 10.1111/j.1460-9568.2004.03246.x

Yarchoan, M., and Arnold, S. E. (2014). Repurposing diabetes drugs for brain insulin resistance in Alzheimer disease. *Diabetes Metab. Res. Rev.* 63, 2253–2261. doi: 10.2337/db14-0287

Zhao, W. Q., and Alkon, D. L. (2001). Role of insulin and insulin receptor in learning and memory. *Mol. Cell. Endocrinol.* 177, 125–134. doi: 10.1016/s0303-7207(01)00455-5

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer VT, declared a past co-authorship with one of the authors CG, and the reviewer AG, declared a shared affiliation, with no collaboration, with several of the authors, SF and CG, to the handling Editor at the time of review.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 02 August 2019
doi: 10.3389/fnins.2019.00801

Insulin-Mediated Changes in Tau Hyperphosphorylation and Autophagy in a *Drosophila* Model of Tauopathy and Neuroblastoma Cells

Shreyasi Chatterjee[1,2], Suren. S. Ambegaokar[1,3], George R. Jackson[1,4]* and Amritpal Mudher[2]*

[1] Department of Neurology, Mitchell Center for Neurodegenerative Diseases, The University of Texas Medical Branch at Galveston, Galveston, TX, United States, [2] Department of Biological Sciences, University of Southampton, Southampton, United Kingdom, [3] Department of Botany and Microbiology, Ohio Wesleyan University, Delaware, OH, United States, [4] Department of Neurology, Michael E. DeBakey VA Medical Center, Parkinson's Disease Research Education and Clinical Center, Baylor College of Medicine, Houston, TX, United States

OPEN ACCESS

Edited by:
Eugenio Barone, Sapienza University of Rome, Italy

Reviewed by:
Ines Moreno-Gonzalez, The University of Texas Health Science Center at Houston, United States
Antonella Tramutola, Sapienza University of Rome, Italy

***Correspondence:**
Shreyasi Chatterjee
s.chatterjee@soton.ac.uk
George R. Jackson
George.Jackson6@va.gov

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *15 April 2019*
Accepted: *17 July 2019*
Published: *02 August 2019*

Citation:
Chatterjee S, Ambegaokar SS, Jackson GR and Mudher A (2019) Insulin-Mediated Changes in Tau Hyperphosphorylation and Autophagy in a Drosophila Model of Tauopathy and Neuroblastoma Cells. Front. Neurosci. 13:801. doi: 10.3389/fnins.2019.00801

Almost 50 million people in the world are affected by dementia; the most prevalent form of which is Alzheimer's disease (AD). Although aging is considered to be the main risk factor for AD, growing evidence from epidemiological studies suggests that type 2 diabetes mellitus (T2DM) increases the risk of dementia including AD. Defective brain insulin signaling has been suggested as an early event in AD and other tauopathies but the mechanisms that link these diseases are largely unknown. Tau hyperphosphorylation is a hallmark of neurofibrillary pathology and insulin resistance increases the number of neuritic plaques particularly in AD. Utilizing a combination of our *Drosophila* models of tauopathy (expressing the 2N4R-Tau) and neuroblastoma cells, we have attempted to decipher the pathways downstream of the insulin signaling cascade that lead to tau hyperphosphorylation, aggregation and autophagic defects. Using cell-based, genetic, and biochemical approaches we have demonstrated that tau phosphorylation at AT8 and PHF1 residues is enhanced in an insulin-resistant environment. We also show that insulin-induced changes in total and phospho-tau are mediated by the crosstalk of AKT, glycogen synthase kinase-3β, and extracellular regulating kinase located downstream of the insulin receptor pathway. Finally, we demonstrate a significant change in the levels of the key proteins in the mammalian target of rapamycin/autophagy pathway, implying an increased impairment of aggregated protein clearance in our transgenic *Drosophila* models and cultured neuroblastoma cells.

Keywords: Alzheimer's disease, type 2 diabetes, tau aggregation, autophagy, tau hyper-phosphorylation

Abbreviations: 4EIBP, eukaryotic initiation factor 4B; AD, Alzheimer's disease; ERK, extracellular regulating kinase; GMR, glass multimer reporter; GOF, gain of function; GSK, glycogen synthase kinase; HD, Huntington's disease; IRS, insulin receptor substrate; LOF, loss of function; mTOR, mammalian target of rapamycin; NFT, neurofibrillary tangle; p70S6K, p70S6kinase; PD, Parkinson's disease; T2DM, type 2 diabetes mellitus; UAS, GAL4-responsive upstream activating sequence; wt, wild type.

INTRODUCTION

Alzheimer's disease is a widely prevalent form of dementia that is characterized by loss of memory and other cognitive functions required to perform complex daily activities (Citron, 2004). Aging remains the most important risk factor for AD, but population-based studies have identified T2DM, an age-associated chronic metabolic disorder, as a major risk factor for developing cognitive impairment and dementia (Barbagallo and Dominguez, 2014; Sridhar et al., 2015). Insulin resistance in peripheral tissues is a key feature of T2DM, and accumulating evidence suggests that insulin resistance also develops in AD and other tauopathies in early stages of the disease (Goncalves et al., 2019). Recent studies have also shown that brain insulin resistance can develop independently from systemic insulin resistance. However, the underlying mechanisms that lead to persistent brain insulin resistance and needs to be investigated (Triani et al., 2018). Several studies have demonstrated that both insulin and insulin receptors (IRs) are found in the brain, and IR are highly expressed in neurons of the central nervous system (CNS) (Talbot et al., 2012; O'Neill, 2013). FDG-PET studies in AD patients have shown a progressive impairment of cerebral glucose uptake and metabolism particularly in the parieto-temporal lobes and posterior cingulate cortical regions of the brain that correlates strongly with disease progression (Moloney et al., 2010). Moreover, insulin and insulin-sensitizing drugs improve cognitive performance in people at early stages of AD (Freiherr et al., 2013; Roberts et al., 2014). The physiological and pathological role of IR signaling in the CNS is still unknown, but a strong correlation between AD and dysfunction of the insulin-signaling pathway with regard to glucose metabolism in the brain has prompted some investigators to refer to AD as type 3 diabetes or an "insulin resistant" condition of the brain (de la Monte, 2012; Barone et al., 2019).

Pathophysiologically, NFTs composed of aggregated and hyperphosphorylated tau are a hallmark of AD and other tauopathies (Augustinack et al., 2002; Steen et al., 2005). GSK-3β is a key serine/threonine kinase that phosphorylates tau at pathological epitopes at proline directed serine and threonine residues (Jackson et al., 2002; Johnson and Bailey, 2002). Additionally, GSK-3β inactivates glycogen synthesis and forms an important component of the insulin signaling pathway (Doble and Woodgett, 2003; Jolivalt et al., 2008). Another non-proline directed kinase that acts downstream of the insulin signaling pathway and generates pathological phospho-tau epitopes under conditions of oxidative stress is ERK (Colucci-D'Amato et al., 2003; Diehl et al., 2017). Although evidence of tau hyperphosphorylation by GSK-3β and ERK strongly indicates an impairment of the IR pathway as a risk factor in AD, the underlying mechanisms that link insulin resistance to tau hyperphosphorylation remain unclear.

The disease and severity of AD correlates strongly with the spatial and temporal progression of the insoluble aggregated tau fragments (NFTs) in the vulnerable brain regions (Didonna and Legname, 2010). Recent studies have shown that the clearance of these misfolded tau aggregates from the neurons is accomplished by autophagy (Smith, 2002; Lee, 2012). Thus, a prominent feature of AD is the massive accumulation of lysosomal vesicular structures in degenerating neurons, and pathological studies have suggested an impairment of macroautophagy in neurodegenerative disorders including AD and other tauopathies (Codogno and Meijer, 2010; Barnett and Brewer, 2011; Nassif and Hetz, 2012). Macroautophagy involves the formation of double membrane bound structures around cytosolic protein aggregates and forming autophagic vacuoles, which will subsequently fuse with the lysosomes for the degradation of protein aggregates and cellular organelles. The mTOR plays an important role in cellular homeostasis and is an inhibitor of autophagy (Jung et al., 2010). There are reports from a large body of literature, that insulin signaling leads to the activation of AKT and mTOR via a relay of phosphorylation events (Partridge et al., 2011). Phosphorylation of mTOR via the PI3K-AKT signaling pathway induces protein synthesis and downregulates autophagy, whereas dephosphorylation of mTOR has the opposite effect (Avet-Rochex et al., 2012). Several groups have shown that the PI3K/AKT/mTOR pathways are activated in the early stages of AD, while other groups have shown an upregulation of autophagy in the AD brains (Cai et al., 2012; O'Neill, 2013). However, the exact mechanism of autophagic induction and misfolded protein clearance in neurodegeneration especially with regard to insulin resistance, still remains uncertain.

Drosophila models misexpressing the full-length human tau have been used successfully by us and many others to recapitulate prominent features of human tauopathies that include progressive neurodegeneration and tau aggregation/phosphorylation at the disease-associated phospho-epitopes mediated by the key kinases (Jackson et al., 2002; Chatterjee et al., 2009). Likewise, human neuroblastoma cells (SHSY5Y) have been used as *in vitro* models for the study of AD and other neurodegenerative diseases (Tanaka et al., 1995; Lesort et al., 1999; Jamsa et al., 2004).

We have previously demonstrated that the misexpression of full-length (2N4R) human tau under the control of a glass promoter in the fly retina causes a marked "rough eye" phenotype with reduced eye size and missing bristles (Chatterjee et al., 2009). This "rough eye" phenotype has been instrumental in performing genetic screens to identify the modifiers of tau toxicity. Interestingly, an unbiased genetic screen conducted with the *gl*-Tau flies identified "Chico" – the single fly homolog of the mammalian IRS as one of the suppressors of the tau-induced "rough eye" phenotype (Ambegaokar and Jackson, 2011).

We therefore investigated the effect of insulin on tau phosphorylation *in vivo* by co-expressing Chico with Tau in the *Drosophila* retina. Our findings demonstrate that Chico has a strong genetic interaction with Tau, causing a suppression of tau-induced toxicity in our model. We also observe that Chico decreases the level of total tau (T46) as well as phosphorylated tau at AT8 (phospho-Ser202/Thr205) and PHF1 (phospho-Thr231/Ser235) sites.

This effect correlates strongly with an elevation of inactive p-GSK-3βS9, while decreasing the level of active p-ERK (p-p44/42). We further demonstrate that these traits are reversed by Chico loss-of-function (Chico-LOF) indicating a Chico-specific effect on tau pathology. Finally, we show an increased proportion of soluble and insoluble tau aggregates in Tau transgenics that is accompanied by an induction of autophagy though not necessarily autophagic clearance. Interestingly, these effects are rescued by Chico but aggravated by Chico-LOF. We further validated our findings *in vitro* in human neuroblastoma cells under insulin resistant conditions. Collectively, these results show that the mechanisms by which insulin resistance impacts tau pathology are conserved in *Drosophila* and mammalian cell lines.

MATERIALS AND METHODS

Stocks and Genetics

Flies were grown on Jazz mix medium (Applied Scientific Jazz Mix, Fisher Scientific, Pittsburgh, PA, United States) at 25°C. The GAL4 driver used in this study is *GMR*-GAL4 on the X chromosome. *GMR*-GAL4 (X) was placed in *trans* to *gl*-tau (A direct fusion construct of the human full-length tau cDNA to the eye specific glass promoter as described by (Jackson et al., 2002) to generate *GMR*-GAL4-*gl*tau on the X chromosome. Transgenic fly lines carrying *UAS*Chico were established by amplifying target sequences by PCR using *chico* specific primers (chico forward: 5′-ATAATTCCGCACTGGCAAAG-3′; chico reverse: 5′-CCATGCCATTAAGATGCTCA-3′) and the resulting constructs were subcloned using Exelixis (San Francisco, CA, United States) modification of *Drosophila* upstream activation sequence (UAS) expression vector (pEx-*UAS*) and microinjected into fly embryos (BestGene, Chino Hills, CA, United States). *UAS*Chico-RNAi and Chico-LOF flies were a gift from Dr. Minoru Saitoe (Tokyo Metropolitan Institute of Medical Science). *UAS*EGFP was ordered from Bloomington Fly Stock, Indiana, Stock no. 5431). The EGFP gene is located on the second chromosome.

Light Microscopy

Light microscopy retinal images were acquired using a Nikon AZ100M light microscope (Melville, NY, United States). Extended depth of focus and volumetric images were taken using a Nikon DSFi1 camera and Nikon NIS-Elements AR 3.0 software as described previously (Chatterjee et al., 2009; Ambegaokar and Jackson, 2011).

Histology and Immunohistochemistry

For SEM, flies were dehydrated in ethanol, incubated overnight in hexamethyldisilazane, dried under vacuum, attached to stubs with black nail polish and analyzed using a JSM-6510LV SEM (JEOL USA, Peabody, MA, United States) (Chatterjee et al., 2009). TRITC phalloidin (Sigma, St. Louis, MO, United States) whole-mount staining of adult retina was carried out as described previously (Sang and Ready, 2002). Briefly, adult retinas were dissected in cold PBS 1X pH 7.4, fixed in 4% formaldehyde/PBS for 45 min, then washed three times in PBT (0.5% Triton in PBS) and incubated for 40 min with TRITC-Phalloidin. After three washes in PBT, samples were mounted in vectashield for analysis with SP8 AOBS Laser Scanning Confocal Microscope (Leica Microsystems, Germany). 63× objective was used for scanning the retinas using a 561 nm laser spectral detector of detection bandwidth 550–650 nm.

Immunoblotting and Reagents in *Drosophila*

Immunoblotting experiments were performed according to protocols previously described (Chatterjee et al., 2009). Briefly, about thirty freshly eclosed flies were collected and heads were decapitated and homogenized using an Argos battery-operated pestle mortar mixer for 1 min on ice in lysis buffer supplemented with complete protease inhibitors and PhosphoSTOP phosphatase inhibitor (Roche Diagnostics, Indianapolis, IN, United States). Extracts were then centrifuged at 4°C for 10 min at 11,000 × *g* and the supernatants were collected while the pellets were discarded. Samples were then mixed with an equal volume of Laemmle sample buffer with β-mercaptomethanol (Bio-Rad Laboratories, Hercules, CA, United States) and resolved by appropriate SDS-PAGE gels before transfer to nitrocellulose membranes for antibody labeling. The membranes were then incubated with appropriate secondary antibodies and developed using enchanced chemiluminiscence (ECL). Following western blot detection the membranes were re-probed with anti-β-tubulin or anti-β-actin that was used as a loading control for each experiment. The following antibodies were used, anti-tau monoclonal antibody (1:1000; T46, Invitrogen), AT8 (1:500; Thermo Scientific), anti-P-AKT505, anti-AKT, anti-mTOR, anti-P-p70S6K, and anti-P-4E-BP (1:500; Cell Signaling), anti-P-GSK-3βS9 (1:500; Genetex), GSK-3β (1:1000; United States Biologicals), anti-P-p44mapk, and p44mapK (1:500; Promega), anti-Re f(2P) (1:500; Abcam), anti-ATG8 (1:400; EMD Millipore), anti-β-tubulin, and anti-β-actin (1:2000; Sigma). The blots were quantified by ImageJ (NIH). Western blots were repeated at least three times with different sets of animals.

Cell Culture and Treatments

SY5Y human neuroblastoma cells were ordered from ATCC and grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2 atmosphere at 37°C. Cells were plated at a density of 5×10^3 cells/cm^2 in 60 mm-diameter culture dishes with 10% FBS. From day 1 after plating, cells were differentiated in the presence of 10 μm all-trans retinoic acid for a week in the cell medium containing 1% FBS. For experiments, 4×10^6 cells were seeded on 60 mm dishes and cultured for 48 h (Jamsa et al., 2004). Briefly, the cells were serum-starved for 24 h and then incubated in the absence and presence of

insulin (100 nm) for time periods of 10 min, 30 min, 1, 2, and 4 h, respectively.

In parallel, the cells were serum starved for 24 h and then incubated with 100 nm insulin for a period of 48 h. Post-incubation the medium was removed and supplemented progressively with 20 nm insulin for a period of 4 h, respectively. For the study of autophagic flux the cells were incubated with 100 nm bafilomycin for 4 h prior to processing. DMEM, fetal bovine serum, L-glutamine, penicillin, streptomycin, and other cell culture reagents were obtained from Life Technologies, Inc. (Invitrogen), Recombinant human insulin and *trans*–retinoic acid was from Sigma.

Immunoblotting in SY5Y Cells and Reagents

Following insulin treatment, cells were lysed in buffer containing 50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Triton-X-100, 1 mM EDTA, 10 mM NaF, 1 mM Na_3VO_4, and one complete protease inhibitor cocktail tablet (Roche Diagnostics)/10 ml buffer. Cell lysates were centrifuged at 15,000 × *g* (Eppendorf 5417R) for 10 min. The protein content of the supernatants was measured using BCA Protein Assay kit (Pierce). An equal amount of total protein was resolved on 4–20% SDS-polyacrylamide gel electrophoresis for immunoblotting analysis using standard protocols. Primary antibodies were incubated overnight at 4°C at the following dilutions, T46 (1:1000; Invitrogen), AT8 (1:500; Thermo Scientific), pGSK-3βS9, pAKT473, mTOR, P-p70S6K, P-4E-BP1, GSK-3β, AKT, anti-IRS1-pSer616 (1:500; Cell Signaling), pERK, ERK (1:500; Promega), anti-LC3 (1:400; Novus Biologicals), and anti-GAPDH (1:500, Abcam), anti-β-actin (1:5000; Sigma). Horseradish peroxidase (HRP) conjugated secondary antibodies (Amersham Biosciences) were incubated for 1 h at room temperature in 5% milk at the dilution of 1:2000 for anti-mouse and anti-rabbit antibodies. The blots were developed by ECL.

Immunocytochemistry and Reagents

Serum-starved SY5Y cells were stimulated at different time points with or without insulin (100 nm). After a brief PBS wash, cells were fixed for 30 min in 4% paraformaldehyde-PBS, washed three times in PBS, and blocked in 3% BSA for 1 h. Cells were then incubated with total tau antibody (A0024, DAKO) and p62 (NBL) at 1:200 dilution overnight at 4°C. After extensive washing with PBS, cells were incubated with Alexa Fluor 568 nm conjugated goat anti-mouse IgG (Invitrogen) and Alexa Fluor 488 nm conjugated goat anti-rabbit IgG (Invitrogen) and counterstained with 4, 6-diamino-2-phenylindole (1:3000 dilution) to count the number of nuclei per field of view. Cells were mounted in coverslips with vectashield (Vector laboratories). Cell analysis was carried out using a Zeiss Axioplan 2 MOT upright fluorescence microscope equipped with filter sets for DAPI, FITC, and TRITC. The microscope settings were consistent for experimental and control sets.

Statistical Analyses

Comparison between multiple groups in flies was done by one way ANOVA followed by Tukey-Kramer HSD test. Two experimental groups were analyzed using Student's *t* test. Comparison between multiple groups in SY5Y cells were carried out by one way ANOVA followed by Fisher's LSD *post hoc* test. Comparison between two experimental groups was done by Student's *t* test. Statistical analyses were performed using Graphpad Prism or Sigma Plot software.

RESULTS

Several groups have shown that Chico plays a prominent role in the insulin-signaling pathway in *Drosophila* with Chico-LOF generating pronounced insulin resistance (Murillo-Maldonado et al., 2011; Naganos et al., 2012). Our previous studies from an unbiased genetic screen in *Drosophila* tauopathy models, have identified Chico as a strong modifier of the Tau phenotype. Hence, in the next sections we have investigated further the mechanistic pathways by which this interaction occurs.

Misexpression of Chico in the Fly Eye Ameliorates Human Tau Induced Rough-Eye Phenotype

To investigate how insulin signaling impacts tau pathology, we utilized our well-established model of Tauopathy in which we expressed full-length wild type human Tau (2N4R) in *Drosophila* eyes using the pan-retinal *GMR*-GAL4 driver (Jackson et al., 2002; Chatterjee et al., 2009). As reported previously, Tau misexpression in the eye resulted in smaller eye size with a rough anterior and missing bristles (**Figure 1C**). Interestingly, co-expression of Chico with Tau ameliorated the "roughness" of the eye phenotype, resulting in larger eyes with fewer missing bristles (**Figure 1D**) while ChicoRNAi or Chico-LOF (null allele chico[1]) with Tau, resulted in a more severe worsening of the "rough-eye" phenotype (**Figures 1F,G**). As compared to the double transgenics, the Control eyes of (*GMR*/+), *GMR*/*UAS*Chico and *GMR*/*UAS*ChicoRNAi appeared perfectly normal (**Figures 1A,B,E**). Magnification of the SEM insets (400X) clearly demonstrates fused ommatidia and missing bristles in Tau flies (**Figure 1C**, lower panel) that are partially rescued by Chico (**Figure 1D**, lower panel) and exacerbated by Chico-LOF (**Figure 1F**, lower panel). Quantification of the percentage of rough area per eye in each genotype revealed an 80% rough-eye area in Tau and Tau+Chico-LOF dual transgenics as compared to 45% rough-eye area in Tau+Chico transgenics compared to Controls (**Figure 1H**, lower panel). In contrast, *UAS*EGFP co-expressed with Tau, failed to suppress the "rough-eye" phenotype confirming that the effect of Chico on Tau is specific (**Supplementary Figure S1A**).

In order to compare the internal retinal morphology, confocal imaging of adult retinas stained with TRITC-phalloidin was performed. Tangential optical sections display a normal

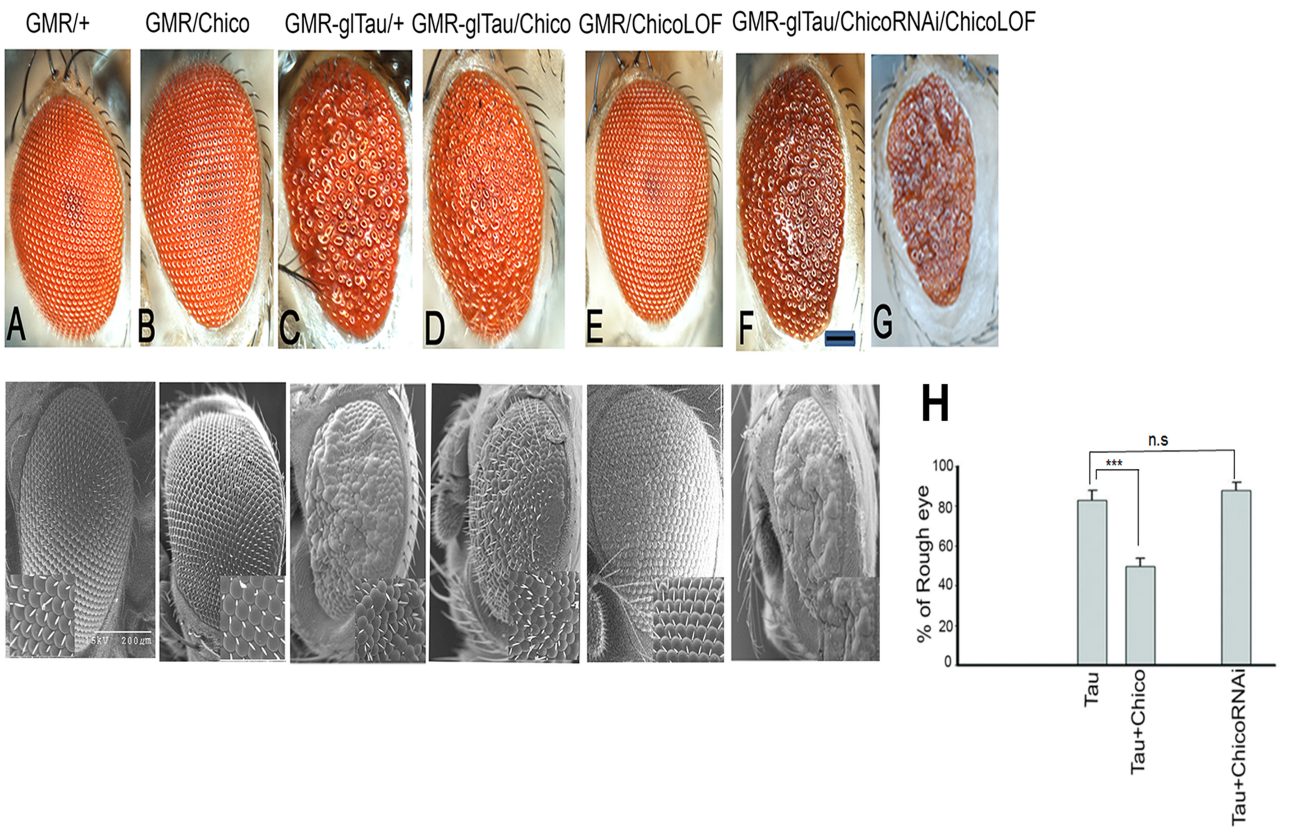

FIGURE 1 | Overexpression of Tau induces a rough eye phenotype that is partially suppressed by Chico coexpression; conversely Tau coexpressed with Chico-RNAi or Chico-LOF restores the rough eye phenotype. **(A–G)** Displayed are photomicrographs and scanning electron microscopy of progeny eyes taken 24–48 h post-eclosion; **(A)** driver alone, **(B)** Chico, **(C)** Tau, **(D)** Tau+Chico, **(E)** Chico-RNAi, **(F)** Tau+Chico-RNAi, **(G)** Tau+Chico-LOF (null allele). Scale bars, 50 μm for the light micrographs and 200 μm for the scanning electron micrographs. **(H)** Quantitation of the percentage of "Rough eye" phenotypes in Tau, Tau+Chico, and Tau+ChicoRNAi transgenics. Error bars represent SEM. N = 15 eyes/genotype, $^{***}P < 0.001$ relative to Tau flies. Student's t test was done for pairwise comparison between two groups.

trapezoidal array of rhabdomeres in the driver-alone controls (**Supplementary Figure S2A**). In contrast, Tau flies produced disorganized ommatidia with massive loss of photoreceptor neurons (**Supplementary Figure S2B**). The retinas of transgenics co-expressing Chico (**Supplementary Figure S2C**) showed a relatively normal array of rhabdomeres other than some abnormal polarity.

Taken together our results show that the co-expression of Chico with Tau atleast partially rescues tau-induced neuronal loss and protects against tau-induced neurotoxicity.

Misexpression of Chico With Tau Reduces the Levels of Total and Hyperphosphorylated Tau

We and others have previously shown that the "roughness" of the eye phenotype in Tau transgenics is associated with elevated tau expression as well as hyperphosphorylation at the disease epitopes (Jackson et al., 2002; Chatterjee et al., 2009). Therefore, in order to analyze the rescue of the "rough-eye" phenotype in Tau+Chico flies, we examined if there were any alterations in the levels of total and hyperphosphorylated tau in these transgenics.

Our results show that coexpression of Chico and Tau significantly decreased the total tau (T46) and phospho-tau at AT8 and PHF1 residues leading to >50% reduction of AT8/T46 and PHF1/T46 ratios as compared to Tau-alone flies. We further observed that these effects were reversed when Tau was coexpressed with Chico-LOF or ChicoRNAi (**Figure 2A** and **Supplementary Figure S3A**). Our data also showed that the coexpression of EGFP produced no alterations in the tau levels confirming that this is a Chico-specific effect (**Supplementary Figure S1B**). In summary, our results suggests that the ability of Chico to ameliorate the tau-induced "rough-eye" phenotype occurs by decreasing the level of total and phospho-tau at pathological epitopes.

Misexpression of Chico With Tau Decreases the Formation of Soluble and Insoluble Tau Aggregates Compared to Tau and Tau+ChicoRNAi

Aggregation of hyperphosphorylated tau is one of the key features of AD and related tauopathies (Simic et al., 2016).

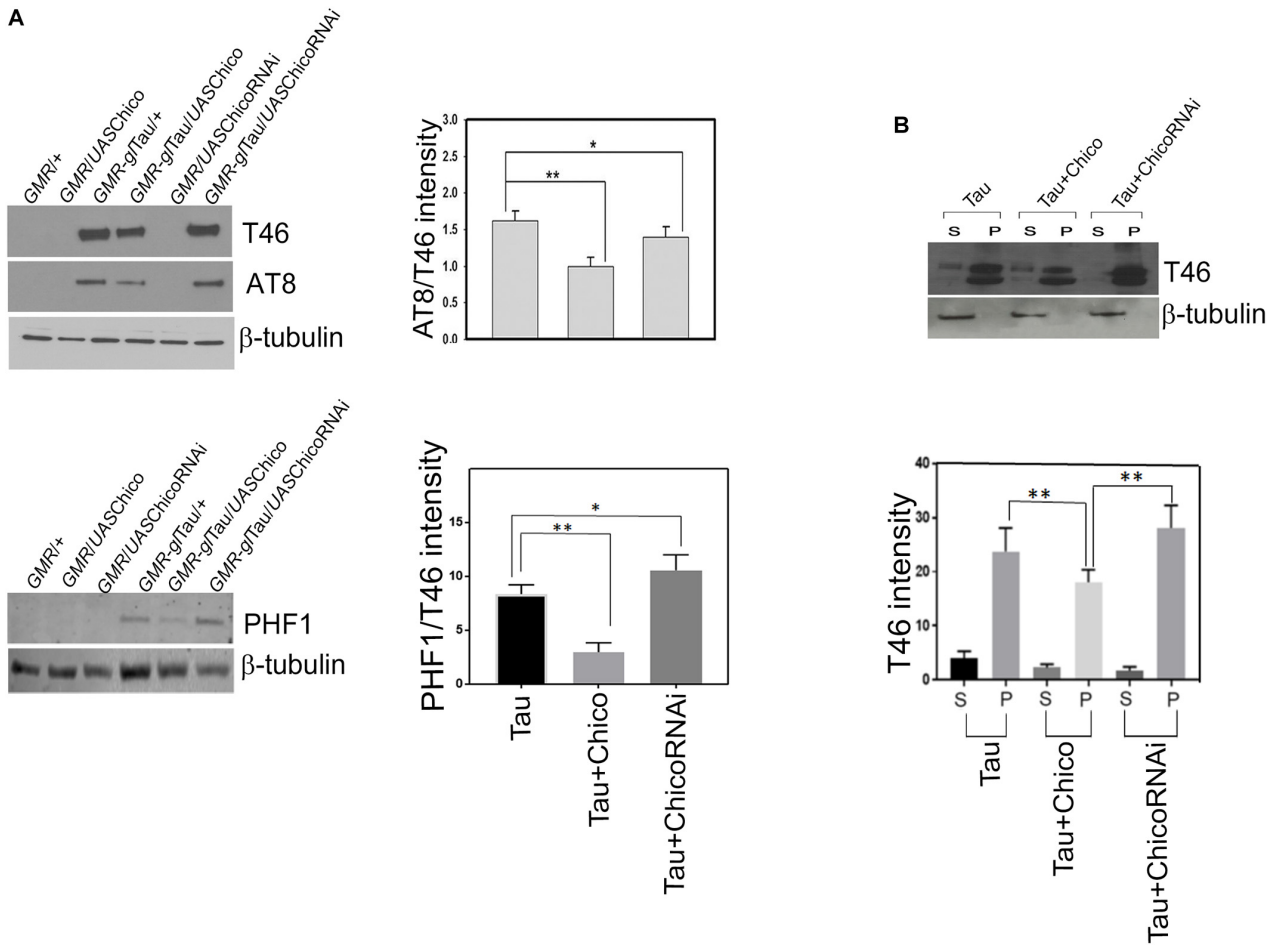

FIGURE 2 | Western blot data shows a decrease in the levels of total (T46) and phosphorylated tau (AT8, PHF1) when Chico is coexpressed with Tau; the T46 and AT8 levels are reversed when Chico-RNAi is coexpressed with Tau. **(A)** Representative blots are probed with T46 (total tau), AT8, PHF1, (phospho-tau), and β-tubulin antibodies, respectively. All flies were 3 days after eclosion. Genotypes are as follows: *GMR*-GAL4/+; *GMR*-GAL4/*UAS*Chico; *GMR*-GAL4-*gl*-Tau/+; *GMR*-GAL4-*gl*-Tau/*UAS*Chico; *GMR*-GAL4/*UAS*Chico-RNAi; *GMR*-GAL4-*gl*-Tau/*UAS*Chico-RNAi. **(B)** Tau and Chico coexpression decreases the level of sarcosyl soluble and insoluble tau aggregates in the supernatant and pellet as compared to Tau alone and Tau+Chico-LOF dual transgenics. Western blot analysis from flies probed with T46 antibody against total tau. Genotypes: *GMR*-GAL4-*gl*-Tau/+; *GMR*-GAL4-*gl*-Tau/*UAS*Chico; *GMR*-GAL4-*gl*-Tau/*UAS*Chico-LOF. Densitometric analyses of AT8/T46 and PHF1/T46 levels and total tau distribution in soluble and pellet fractions are normalized to β-tubulin. (One way ANOVA with Tukey-Kramer HSD was used for multiple groups comparison or *post hoc* Dunnett's test for comparison against Tau, $n = 3$ sets of biological replicates for each genotype.) Data represents the mean ± SEM, $^{**}P < 0.01$ and $^{*}P < 0.05$ relative to Tau.

Our previous studies in tauopathy models demonstrated the accumulation of sarcosyl soluble and –insoluble tau aggregates that contributed toward tau-induced neurotoxicity (Chatterjee et al., 2009). In line with these reports, we investigated whether loss or GOF of Chico has any effect on sarcosyl solubility of tau. Co-expression of Chico with Tau reduced the accumulation of sarcosyl-soluble and insoluble tau species in the supernatant and pellet fractions, respectively. However, co-expression of ChicoRNAi significantly altered the pattern of tau solubility and increased accumulation of sarcosyl-insoluble tau (**Figure 2B**). Although recent studies have demonstrated that soluble tau aggregates are toxic for neuronal cells, sarcosyl-insoluble tau aggregates eventually form the NFTs detected in AD brains (Ren and Sahara, 2013). Taken together, our data shows that in addition to decreasing tau expression and hyperphosphorylation, Chico reduces the formation of sarcosyl-soluble and insoluble tau aggregates in Tau transgenics.

Effect of Chico on Tau Hyperphosphorylation Is Mediated by GSK-3β

Given that the insulin signaling pathway in *Drosophila* is highly conserved with that of higher vertebrates, and the kinases GSK-3β and ERK lie downstream of Chico in the insulin signaling cascade; we investigated whether there is a direct effect of Chico on the regulation of these kinases in our model.

Our data showed a >50% reduction in the level of inactive p-GSK-3βS9 in Tau genotypes compared to controls (GMR/+

and GMR/*UAS*Chico) that matched with the elevated tau phosphorylation at AT8 and PHF1 epitopes. Co-expression of Chico with Tau rescued the levels of phospho-GSK-3βS9 while Tau+ChicoRNAi lines reversed the effect (**Figure 3A**). Conversely, when active GSK-3β (phospho-GSK-3βY216) levels were assessed; Tau+Chico transgenics showed a marked reduction in active GSK-3β compared to Tau-only and Tau+ChicoRNAi transgenics (data not shown).

Next, we analyzed the phosphorylation status of active ERK (p42/44) and observed <10% reduction in Tau transgenics compared to Controls. Interestingly, this effect was not rescued by either Chico overexpressed or knock-down lines (**Figure 3A**). Despite the alterations in phosphorylated GSK-3β and ERK, total protein levels remained constant in all the genotypes.

Collectively, our data suggests that Chico-mediated effects of tau hyperphosphorylation display a direct correlation with GSK-3β but not with ERK.

To assess whether insulin resistance was generated by the gain or LOF of Chico, we analyzed the ability of Chico to stimulate AKT phosphorylation at position Serine505. This phospho-AKT site in flies is analogous to mammalian AKT phosphorylated at Serine473 and a marker of insulin sensitivity (Musselman et al., 2011). We observe that compared with other genotypes, the Tau+ChicoRNAi lines show a 50% reduction in phospho-AKTSerine505 levels suggesting an insulin-resistant phenotype (**Figure 3B**).

Tau-Induced Down-Regulation of TOR Pathway Components Is Rescued in Tau+Chico Double Transgenics

As in higher vertebrates, in *Drosophila*, the insulin signaling pathway is upstream of IRS/PI3K pathway, which in turn positively regulates target of rapamycin (TOR) activity. In a healthy environment, TOR activates protein synthesis and promotes cellular growth and proliferation by enhancement of important translational components, including the translation initiation factor 4E binding proteins (4E-BP1-3) and ribosomal protein S6 kinases. However, under stressful conditions, TOR signaling is impaired and autophagy is upregulated (Cai et al., 2012; O'Neill, 2013). Interestingly in our studies, we observed a 40% reduction of the TOR pathway components phospho-p70S6K and phospho-4E-BP1 in the Tau flies with a partial rescue observed in Tau+Chico transgenics (**Figure 4A**). In addition, we assessed the TOR protein levels in the Tau flies. A significant reduction of phospho-TOR/Total TOR was found in Tau flies compared to Control and Chico-only flies. This effect was rescued partially by Chico co-expression (**Supplementary Figure S4A**).

Upregulation of Autophagy Is Observed in Tau and Tau+ChicoRNAi Transgenics

Previous studies have demonstrated that the inactivation of the TOR pathway stimulates basal autophagy (Hands et al., 2009; Metcalf et al., 2012; O'Neill, 2013). The *Drosophila* homologs of mammalian autophagy marker LC3 are Atg8a and Atg8b. Atg8a particular, has been extensively used as an autophagy marker in *Drosophila*. The lipidation of Atg8a produces Atg8a-II that is directed to the autophagosomes as opposed to Atg8a-I, which is the unprocessed Atg8a protein (Nagy et al., 2015). Compared to other genotypes Tau transgenics showed significant upregulation of autophagy that was partially reduced by Chico. Interestingly, Tau+Chico-LOF flies displayed a similar enhancement of autophagy (**Figure 4B** and **Supplementary Figure S5**).

However, an increase in Atg8a-II signal implies an enhanced autophagic induction but not necessarily an autophagic clearance (Rusten and Stenmark, 2010). Hence we decided to investigate if there was an alteration in the levels of Ref(2P) – the fly homolog of mammalian p62 which accumulates due to an impairment in autophagy (Son et al., 2012). Although there was a moderate increase of Ref(2P) accumulation in Tau and Tau+Chico-LOF, it was not significant compared to other genotypes (data not shown). Thus, in summary, our data suggests that Tau overexpression induces autophagy without an autophagic blockage.

Progressive Insulin Treatment of SY5Y Cells Causes a Time-Dependent Increase in Total and Phosphorylated Tau That Correlates With the Decrease of Inactive GSK-3β

To determine whether the insulin-induced alteration in the total and phospho-tau levels that we observe in our *Drosophila* tauopathy models, is conserved in human cells, we next investigated the effect of insulin treatment in neuroblastoma cells. SY5Y cells were serum-starved for a period of 24 h and then treated with 100 nm insulin for 8 h (Son et al., 2012). We observed an immediate decrease in T46 (total Tau) and AT8 (Serine 202/Threonine 205) that lasted for a period of 30 min of insulin treatment, followed by a gradual increase in the levels of total and AT8-tau over a period of 4 h after which it remained constant. However, as the insulin treatment continued for a period of 4 h, AT8-tau levels were significantly elevated as compared to total tau (T46), thereby increasing the ratio of AT8/T46 by almost 2.5-fold (>50%) at 4 h as compared to controls (0 min post-treatment) (**Figure 5A** and **Supplementary Figure S3B**).

Next, we examined the change in phospho-IRS-1(Ser636) at specified time intervals to monitor insulin resistance in the cells. From 30 min to 4 h we observed a gradual increase in the ratio of phospho-IRS1(Ser636)/Total IRS1 levels consistent with an insulin-resistant phase in our cellular model (**Figure 5B**).

Insulin resistance in animal and cellular models of type 2 diabetes is also marked by a reduction in phospho-AKTSer473 while total AKT remains constant (Shao et al., 2000; Standaert et al., 2002). Our data showed that phospho-AKTS473 levels did not change significantly compared to control till a period of 30 min. However, post-insulin treatment there was a gradual decrease of phospho-AKT/Total AKT signal from 1 h (50% reduction) to 4 h (80% reduction) signifying progressive insulin resistance (**Figure 5C**, left panel). Taken together our results indicate a moderate increase in the level of total tau and a more substantial increase in phospho-tau in a progressively insulin-resistant environment.

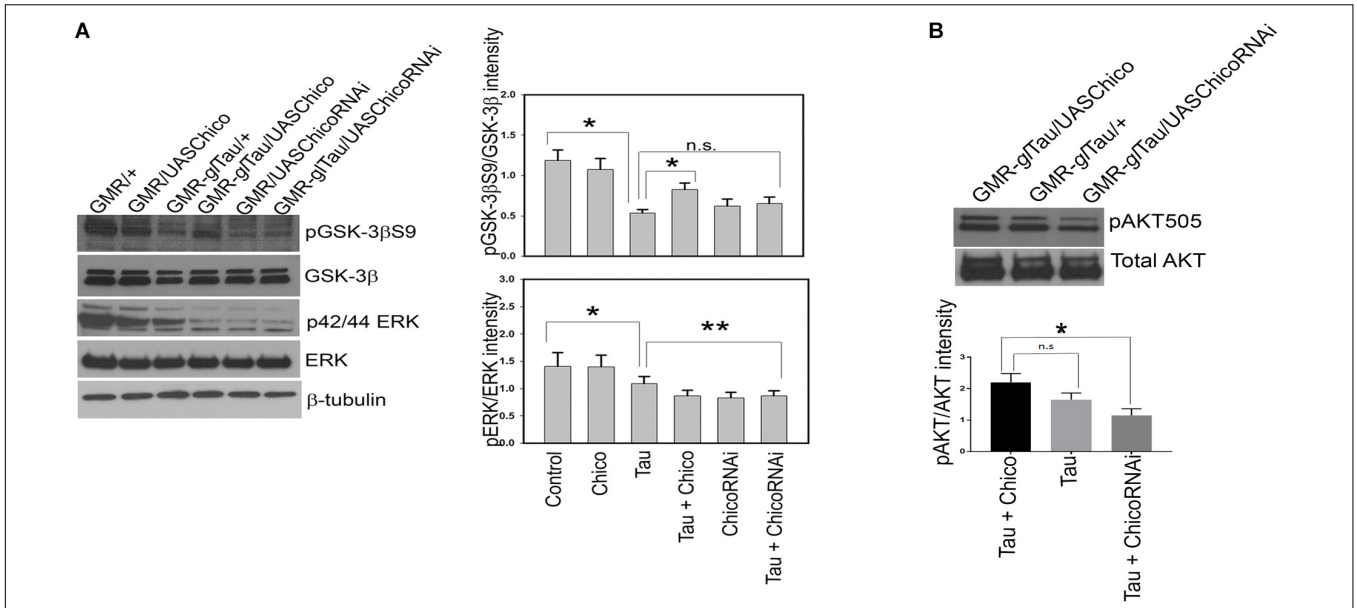

FIGURE 3 | Overexpression of Tau decreases pGSK-3βS9 and pERK in representative blots. Coexpression of Chico restores the levels of pGSK-3βS9 but not of pERK. **(A)** Western blot analysis from flies probed with pGSK-3βS9, pERK, total GSK-3β, total ERK, and β-tubulin antibodies, respectively. **(B)** Western blot analysis from flies probed with pAKT and total AKT antibodies, respectively. Quantification of phospho-antibody levels are normalized to total protein levels. (One way ANOVA with Tukey-Kramer HSD was done for multiple group comparisons or *post hoc* Dunnett's test was used for comparison with Tau, $n = 3$ sets of biological replicates for each genotype.) Data represents the mean ± SEM, $^{**}P < 0.01$ and $^{*}P < 0.05$ relative to Tau in panel **(A)** and to Tau+Chico in panel **(B)**. All flies were 3 days before eclosion. Genotypes: *GMR*-GAL4/+; *GMR*-GAL4/*UAS*Chico; *GMR*-GAL4-*gl*-Tau/+; *GMR*-GAL4-*gl*-Tau/*UAS*Chico; *GMR*-GAL4/*UAS*Chico-RNAi; *GMR*-GAL4-*gl*-Tau/*UAS*Chico-RNAi in panel **(A)**. Genotypes: *GMR*-GAL4-*gl*-Tau/*UAS*Chico; *GMR-gl*-Tau; and *GMR-gl*-Tau/*UAS*Chico-RNAi in panel **(B)**.

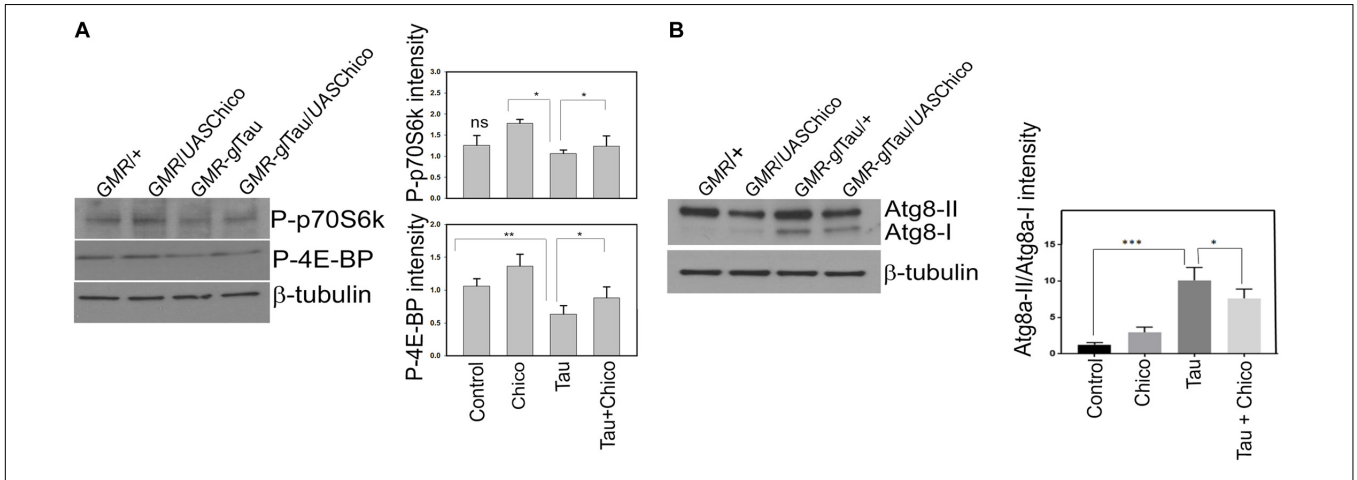

FIGURE 4 | Tau overexpression effects a reduction in the level TOR pathway markers but an increase in autophagy pathway markers (Atg8a-II/Atg8a-I) that are rescued by Chico coexpression with Tau. **(A,B)** Western blot analysis from flies probed with P-p70S6K, P-4E-BP1, and Atg8 antibodies. β-tubulin is the loading control. Quantification of the levels of the antibodies normalized to β-tubulin levels. (One way ANOVA with Tukey-Kramer HSD was done for multiple group comparisons or *post hoc* Dunnett's test was done for comparison with Tau, $n = 3$ sets of biological replicates for each genotype.) Data represents the mean ± SEM, $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ relative to Tau. All flies were 3 days after eclosion. Genotypes: *GMR*-GAL4/+; *GMR*-GAL4/*UAS*Chico; *GMR*-GAL4-*gl*-Tau/+; and *GMR*-GAL4-*gl*-Tau/*UAS*Chico.

To determine whether alterations in tau phospho-epitopes correlate with changes in the key kinases downstream of the IR pathway, we next examined the levels of GSK-3β and ERK using phospho-specific antibodies over a period of 4 h. We observed a progressive decrease in phospho-GSK-3βS9/Total GSK-3β levels to 80% compared to controls at the end of 4 h. This correlated with the gradual increase in the AT8 immunoreactivity that we observed earlier. On the contrary, the phospho-ERK/Total ERK immunoreactivity did not change significantly at the end of 4 h. In addition, we did not detect any change in the level of total GSK-3β or ERK, indicating that the change in the pattern of phosphorylated kinases is an

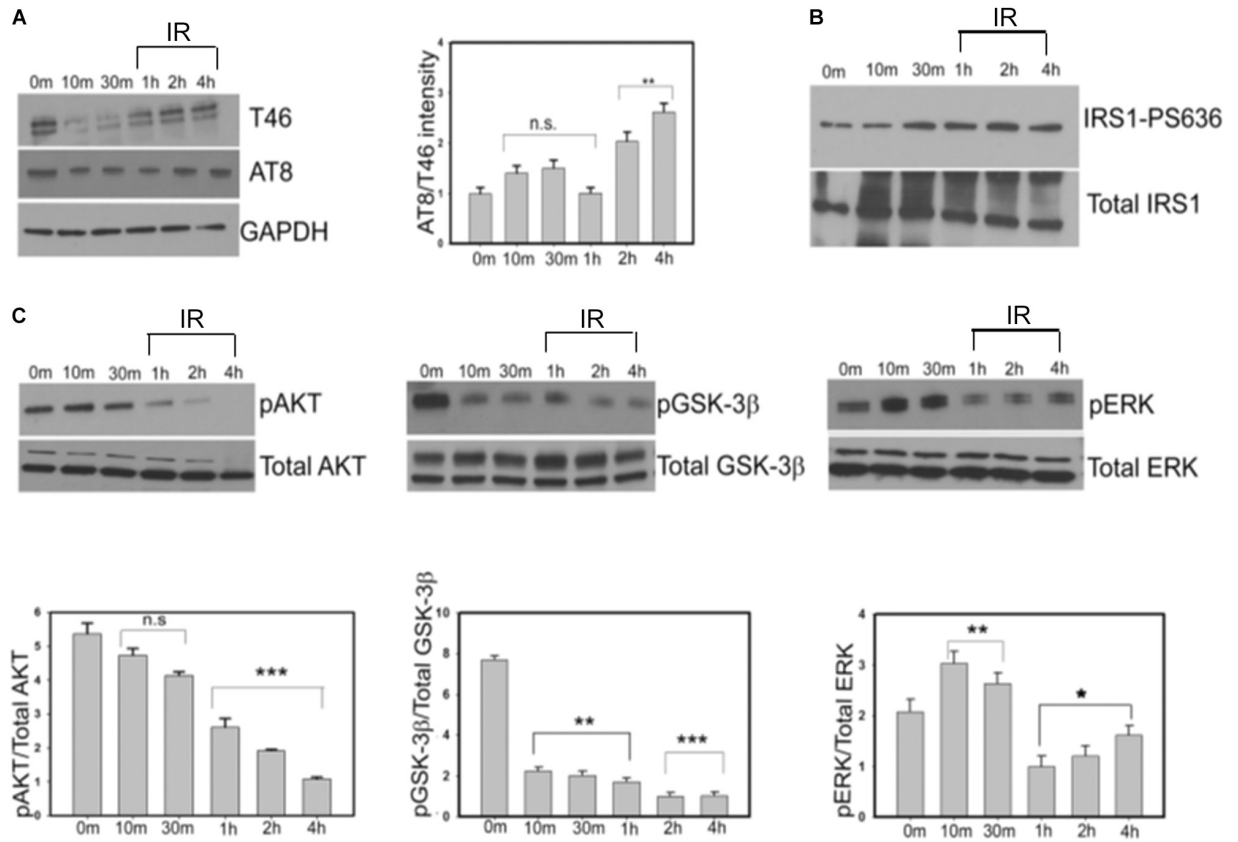

FIGURE 5 | Progressive insulin treatment increases total and phosphorylated tau in SY5Y cells in an insulin resistant state. This is accompanied by the gradual decrease in the levels of pAKT and pGSK-3βS9. SY5Y cells were serum-starved for 24 h and then treated with 100 nm insulin for various time points (0, 10, 30, 60, 120, and 240 min). After treatment, cells were harvested, lysed and immunoblotted with either **(A)** anti-tau (T46) and AT8 antibodies **(B)** IRS1-pS636 and total IRS1 or **(C)** pAKT, pGSK-3β, pERK, and total kinase antibodies in the representative blots. GAPDH was used as a loading control. Densitometric analyses of phosphorylated tau (AT8) over total tau (T46) and phosphorylated kinases (pAKT, pGSK-3β, and pERK) over total kinases normalized to GAPDH were performed based on four independent experiments. Data represent the mean ± SEM, $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ versus control (0 min), $n = 4$ biological replicates. (One way ANOVA followed by Fisher's test for multiple comparisons and Student's *t* test for pairwise comparisons.) IR, insulin resistance.

insulin signaling pathway-specific response (**Figure 5C**, middle and right panels).

In summary, our data indicates that tau hyperphosphorylation maybe contributed by active GSK-3β but not by active ERK in insulin-treated cells.

Insulin Treatment of SY5Y Cells Progressively Affects the Levels of Protein Synthesis Regulators in the mTOR Pathway

It has been previously reported that in the normal physiological environment and in the presence of sufficient nutrients, insulin or insulin-like growth factors activate PI3K/AKT/mTOR pathways subsequently promoting protein synthesis and inhibiting autophagy (Hands et al., 2009; O'Neill, 2013). We observed that insulin treatment of SY5Y cells initially increased the levels of phospho-mTOR (Ser2448), phospho-p70S6K and phospho-4E-BP1 for a period of 1 h followed by a progressive decrease in the levels of protein biosynthesis markers as the cells gradually entered an insulin-resistant phase at the end of the 4-h time-period. Conversely, the ratio of autophagic marker LC3II/LC3I increased rapidly within the first 30 min of insulin treatment, indicating an activation of autophagy. However, at the end of 4 h of insulin treatment, the LC3II/LC3I ratio significantly reduced compared to earlier time points (10 and 30 min), suggesting a blockage in the autophagic pathway (**Figure 6** and **Supplementary Figure S4B**). These results indicate that progressive insulin stimulation not only alters the levels of tau and key kinases but also mediates changes in protein synthesis and key autophagic markers in our model.

Insulin Pre-treatment of SY5Y Cells Increases the Tau Levels by Autophagic Impairment

To further investigate whether tau accumulation in insulin-treated SY5Y cells was linked to an impairment of autophagy, we pre-treated these cells with 100 nm insulin for a period of 48 h after serum starvation and exposed them to 20 nm insulin

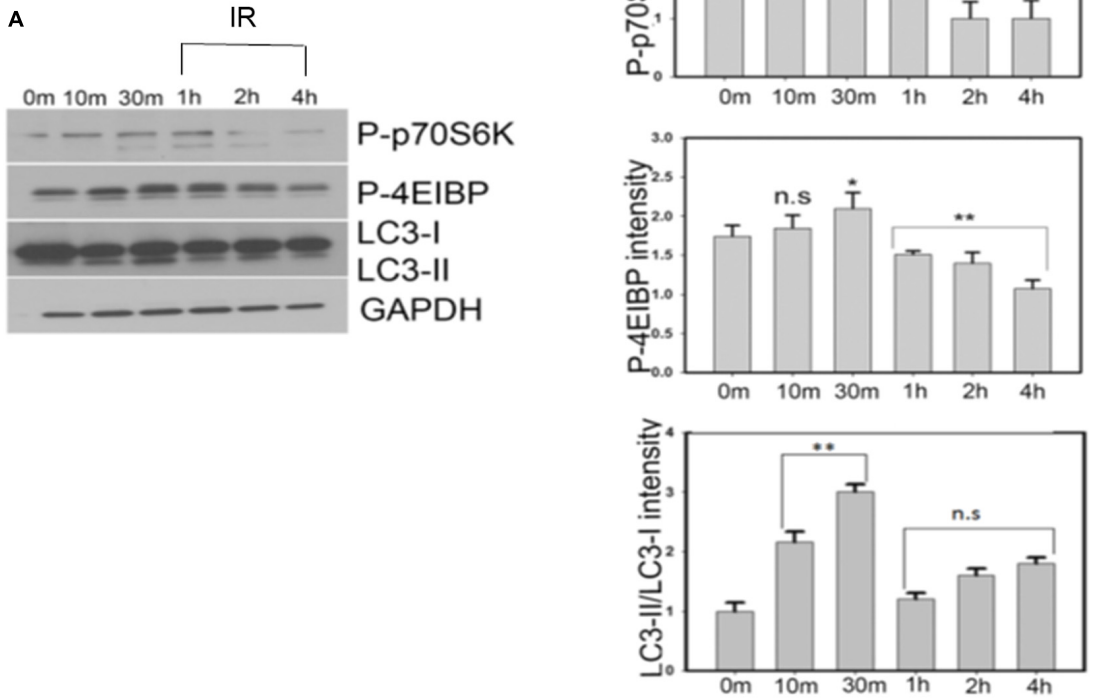

FIGURE 6 | Insulin treatment of SY5Y cells affects the levels of autophagic markers via mTOR pathway demonstrated by a time dependent decrease in the levels of p-p70 S6K and p-4E-BP1 proteins. A simultaneous increase in the level of LC3-II/LC3-I ratio is observed till 30 min after which there is a decrease of autophagy in the insulin-resistant phase. **(A)** SY5Y cells were treated with 100 nm insulin for different time points (0, 10, 30, 60, 120, 240 min) and lysates were analyzed by immunoblotting with antibodies against P-p70S6K, P-4E-BP1, and LC3. GAPDH was used as a loading control. **(B)** Densitometric analysis of these antibodies relative to GAPDH was performed based on four independent experiments. Data represent the mean ± SEM, $^{**}P < 0.01$, $^{*}P < 0.05$ versus control (0 min), $n = 4$ biological replicates (One way ANOVA followed by Fisher's test for multiple comparisons and Student's *t* test for pairwise comparisons.) IR, insulin resistance.

progressively for a period of 4 h (Son et al., 2012). In parallel, these SY5Y cells were also exposed to bafilomycin treatment. Bafilomycin is one of the well-known compounds that inhibit late-stage autophagy and prevents maturation of autophagic vacuoles by impairing the fusion between autophagosomes and lysosomes (Mauvezin and Neufeld, 2015). Interestingly, we observed that pre-treatment of the SY5Y cells with insulin and bafilomycin enhanced tau staining (A0024) compared to untreated controls (**Figures 7B,C** vs. **Figure 7A**).

Previous studies have also shown that the lack of autophagy or blockage of autophagic flux leads to the accumulation of p62 protein in cells (Rusten and Stenmark, 2010). Immunohistochemical studies in SY5Y cells also showed an increased accumulation of p62 (red) in the bafilomycin-treated cells implying an impairment of autophagy. A similar p62 enhancement was observed in the insulin-treated cells confirming an inhibition of autophagy or a blockage of autophagic flux (**Figures 8B,C** vs. **Figure 8A**).

Taken together our results show that pre-treatment of SY5Y cells with insulin results in an inhibition of autophagy and increased tau protein accumulation. It is worthwhile to note that SY5Y cells treated with both insulin and bafilomycin were toxic resulting in massive cell death possibly due to a combination of tau aggregation and autophagic impairment triggering apoptotic pathways.

DISCUSSION

Alzheimer's disease is the most common cause of dementia in humans (Citron, 2004). While plaques and tangles are designated as pathological signatures of AD, it is now speculated that these factors are consequential rather than causal of the neurodegenerative cascade (Ishiguro et al., 1992; Armstrong, 2006). Despite intense investigation, the etiology and pathogenesis of sporadic AD remain unknown. Brain glucose metabolism is impaired in AD, and growing evidence supports the concept that AD is fundamentally a metabolic disease with progressive dysfunction in brain glucose utilization and responsiveness to insulin and insulin-like growth factor

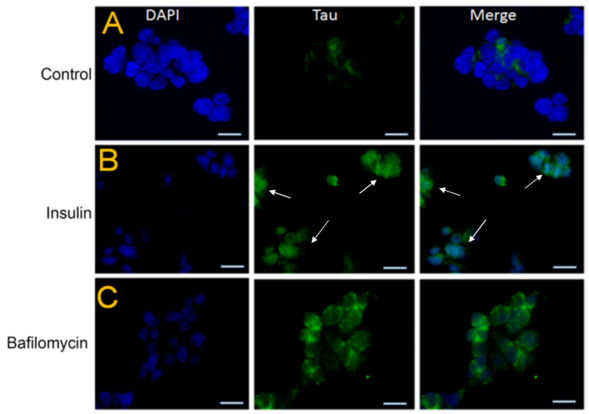

FIGURE 7 | Pre-treatment with insulin increases the tau protein levels in SY5Y cells relative to untreated controls. **(A–C)** SY5Y cells were serum-starved for 24 h and then treated with 100 nm insulin for 48 h followed by progressive treatment with 20 nm insulin at various time points (0, 1, 5, 10, 30, 60, 120, and 240 min) In parallel, SHSY5Y cells were subjected to autophagy-blocker Bafilomycin treatment for 4 h. This was followed by immunostaining by total Tau antibody (green) and DAPI (blue). Tau immunoreactivity increased in the insulin and bafilomycin-treated cells compared to untreated controls (bar = 20 μm). White arrowheads represent total Tau in insulin-treated cells.

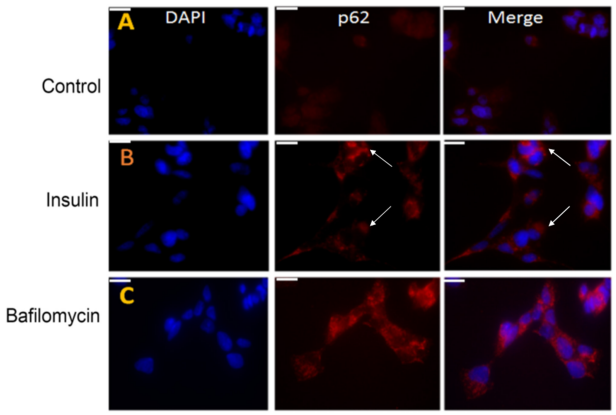

FIGURE 8 | Pre-treatment with insulin increases the p62 protein levels in SY5Y cells relative to untreated controls. **(A–C)** SY5Y cells were serum-starved for 24 h and then pre-treated with 100 nm insulin for 48 h, followed by immunostaining with p62 (red) antibody. In parallel, SHSY5Y cells were subjected to autophagy-blocker bafilomycin treatment for 4 h. The intensity of p62 was greater in insulin and bafilomycin-treated cells **(B,C)** compared to untreated cells **(A)** as observed by fluorescence microscopy implying an autophagic blockage. DAPI (blue) was used as a nuclear stain (bar = 20 μm). White arrowheads represent p62 in insulin-treated cells.

stimulation (Freude et al., 2009; Schuh et al., 2011). Although, there have been numerous studies on peripheral insulin resistance, the mechanism of central insulin resistance and particularly its impact on tau, one of the hallmarks of AD, is relatively unknown.

Our laboratory has generated a model of tauopathy in *Drosophila* by expressing human wild-type full-length tau (2N/4R) in the retina, that results in a "rough-eye" phenotype (Jackson et al., 2002; Chatterjee et al., 2009). This tau-induced eye phenotype is highly useful for conducting unbiased enhancer/suppressor screens through which Chico-the single fly homolog of mammalian IRS was identified as a suppressor of tau-induced "rough-eye" phenotype (Ambegaokar and Jackson, 2011). Recent studies show that loss-of-function mutation in Chico causes insulin resistance by increasing the amount of circulating trehalose and lipids in *Drosophila* (Murillo-Maldonado et al., 2011; Naganos et al., 2012). Our data demonstrates that Chico ameliorates the tau-induced neurotoxicity by reducing tau aggregation and hyperphosphorylation at the disease epitopes while Chico-LOF exacerbates these effects. The exacerbation of tau pathology by Chico-LOF was attributed to insulin resistance as these flies displayed 50% reduction in *Drosophila* AKT phosphorylated at Serine505 residue that corresponds with insulin resistance in mammalian AKT phosphorylated at Serine473 residue (Standaert et al., 2002). Our results further show that this augmentation is achieved by modulating the kinase GSK-3β, which lies downstream of the IR signaling pathway. Finally, we demonstrate that Chico impacts mTOR/autophagy pathway, thus playing a significant role in clearance of tau aggregates. These observations in the *Drosophila* tauopathy model were further validated in mammalian neuroblastoma cells, implying a conservation of these pathways across diverse systems.

In post-mortem brains from AD patients, tau phosphorylation was found to increase at several GSK-3β directed epitopes such as AT8, AT180, AT100, and PHF1 (Ishiguro et al., 1992; Armstrong, 2006). GSK-3β is one of the key tau kinases that also colocalizes with tau tangles and microtubules in the brains from patients with AD (Hooper et al., 2008; Avila et al., 2012). Interestingly, GSK-3β is regulated by the insulin signaling pathway. Briefly, insulin signaling is initiated by the binding of insulin to its receptor, located in the cytoplasmic membrane. This leads to rapid phosphorylation of IRSs that activate PI3K/AKT signaling in the brain (Diehl et al., 2017). AKT phosphorylates GSK-3β at Serine 9, thereby inhibiting its activity of tau hyperphosphorylation and facilitating binding to microtubules. Conversely, when GSK-3β is in an active state (due to phosphorylation at Tyrosine 216 residue), it phosphorylates tau to generate AT8 and PHF1 disease epitopes (Chatterjee et al., 2009). In another independent study Barone et al. have shown that reduction of Biliverdin reductase-A (BVR-A) in the hippocampus of 3xTg-AD mice impairs AKT-mediated inhibition of GSK-3β and increases tau phosphorylation in response to oxidative stress. They have also shown that increased GSK-3β activation (decreased Serine 9 phosphorylation) is detected in the early stages of AD (Sharma et al., 2019).

In our study we observe, that co-expression of Chico with Tau significantly increases the level of inactive GSK-3β phosphorylated at Serine9 residue and reduces tau hyperphosphorylation. Conversely, the coexpression of insulin-resistant Chico-LOF activates GSK-3β and exacerbates tau hyperphosphorylation. Our results are supported by the studies

of Jolivalt et al. (2008) and Kim et al. (2009) in which STZ-induced type-1 diabetic mouse models display an increased tau hyperphosphorylation mediated by active GSK-3β (Doble and Woodgett, 2003; Kim et al., 2009). Interestingly this effect was not observed in type 2 diabetic mouse models. Our study differs significantly in this respect since the genetically manipulated Chico-LOF mutants recapitulate features of type 2 diabetes and not of type 1 diabetes.

To examine whether insulin-resistance – induced tau enhancement was conserved in the mammalian system, these experiments were repeated in insulin treated SY5Y cells. It has been observed in human hippocampal tissue that insulin resistance is accompanied by an increased phosphorylation at the Serine636 residues of IRS-1 (Copps and White, 2012; Diehl et al., 2017). Interestingly we observed a progressive increase in the ratio of phospho-IRS1/Total IRS1 from 30 min to 4 h time period signifying insulin resistance. This was also confirmed by an 80% reduction of phospho-AKT/Total AKT at the 4 h time-period confirming insulin resistance in SY5Y cells.

Interestingly, while we observed an initial decrease in total and AT8-tau levels, there was a sharp increase in the AT8/T46 ratios as the cells entered an insulin resistant condition. Our studies are in contrast to the studies done by Lesort et al. (1999) who treated the SY5Y cells with 10 nm insulin and observed a transient increase in AT8-tau hyperphosphorylation at the early time points (0–60 min) and dephosphorylation post 1 h. In comparison, we have used 100 nm insulin which generated insulin resistance after 30 min of treatment subsequently increasing levels of total and phospho-tau. In this respect, the early time points in SY5Y cells recapitulate our observations from Chico gain-of-function while the later time points pertaining to insulin-resistance represent Chico loss-of-function or knock-down flies.

Several studies have shown that hyperphosphorylated tau dislodges from the microtubules and binds with tau monomers to form tau aggregates. These abnormal tau aggregates are considered to be a critical pathological feature of tauopathy (Cowan et al., 2015; Goedert and Spillantini, 2017). It is hypothesized that small molecular weight, sarcosyl-soluble tau aggregates gradually consolidate to form NFTs that are sarcosyl-insoluble. However, the toxicity of the sarcosyl soluble and insoluble aggregates remains controversial. A study by Lasagna-Reeves et al. (2011) showed that the injection of soluble tau oligomers but not monomers or fibrils were sufficient in inducing synaptic dysfunctions and cognitive impairments in wild type mouse. There are others studies that show the spread of soluble tau across the synapses for the propagation of tau pathology while the insoluble tau fibrils within the neurons act as a "sink" to sequester the toxic and soluble tau species (Kopeikina et al., 2012). Although, Chico-LOF exacerbates the tau-induced toxicity in the "rough-eye" phenotype, we detect a significant amount of insoluble tau species in these transgenics. Taking into account the prevailing literature in this area, there is a possibility that the shift of tau from soluble to insoluble aggregates in an insulin-resistant state is a defense mechanism of neurons in response to progressive tau accumulation.

Aggregates of tau are removed by macroautophagy (Inoue et al., 2012; Neufeld, 2012). The protein kinase TOR, an evolutionarily conserved protein, plays a central role in regulating macroautophagy in response to nutrient availability and stress factors (Miron et al., 2003). The involvement of TOR pathway in *Drosophila* neurodegeneration models have been controversial depending on disease model in question. Feany and colleagues and Berger et al. (2006) have previously reported that TOR pathways components enhance mutant tau (R406W) induced toxicity in their fly models that is ameliorated by treatment with TOR inhibitor rapamycin (Khurana et al., 2006; Khurana et al., 2010). These observations have also been supported by Caccamo et al. (2013) in P301S mouse models where increasing mTOR activity has been shown to increase tau pathology whereas decreasing mTOR ameliorates tau-induced neuronal dysfunctions and pathology. In contrast, we observe a down-regulation of the TOR pathway in our tauopathy models that is rescued by coexpression of Chico. One possibility is that we have used wild type full-length human tau in our fly models as opposed to mutant tau used in the studies above. An interesting study by Tramutola et al. (2015) in post-mortem AD brains reports significant upregulation of PI3K/AKT/mTOR pathways in both MCI and late stage AD but not in the early stage AD. Since we have used 0–3 days old adult flies for this study, it is possible that our tauopathy model represents an early stage of pathology during which the PI3K/AKT/mTOR signaling pathway is not yet activated.

In keeping with decreased TOR function, we observe a simultaneous upregulation of autophagy in Tau transgenics. This was measured by elevated levels of membrane bound Atg8a-II relative to unbound Atg8a-I. We also observe a similar upregulation of autophagy in Tau+Chico-LOF transgenics. However, an upregulation of autophagy does not necessarily mean autophagic clearance. A recent study by Bakhoum et al. (2014) demonstrates the formation of giant autophagic bodies (GABS) in retinas of tauopathy models that arise due to incomplete acidification of autophagolysosomes and culminates in an impairment of autophagic flux. Although we did not see an autophagic blockage in our freshly eclosed progeny, further experiments need to be done with aged Tau flies. The relationship between insulin resistance and autophagy is a relatively new field with contradicting observations depending on animal or cellular model in question. Recent studies in cellular models of carcinoma show that insulin resistance activates autophagy (Zhou et al., 2009). In contrast, studies done in APP/PS1 mice show that insulin sensitivity improves autophagy in neurodegenerative disease models (Macklin et al., 2017). Thus, in our Tauopathy model, the impact of hyperphosphorylated and aggregated tau on autophagic clearance in an insulin-resistant environment cannot be ruled out (Rodriguez-Rodriguez et al., 2017).

In SY5Y cells, prolonged treatment with insulin resulted in downregulation of the mTOR pathway in an insulin-resistant environment. Interestingly, our data matches with the observations of Barone et al. (2016) who report mTOR activation at lower concentrations of insulin but a subsequent decrease in higher insulin concentrations in SY5Y cells. However, instead of an upregulation of autophagy,

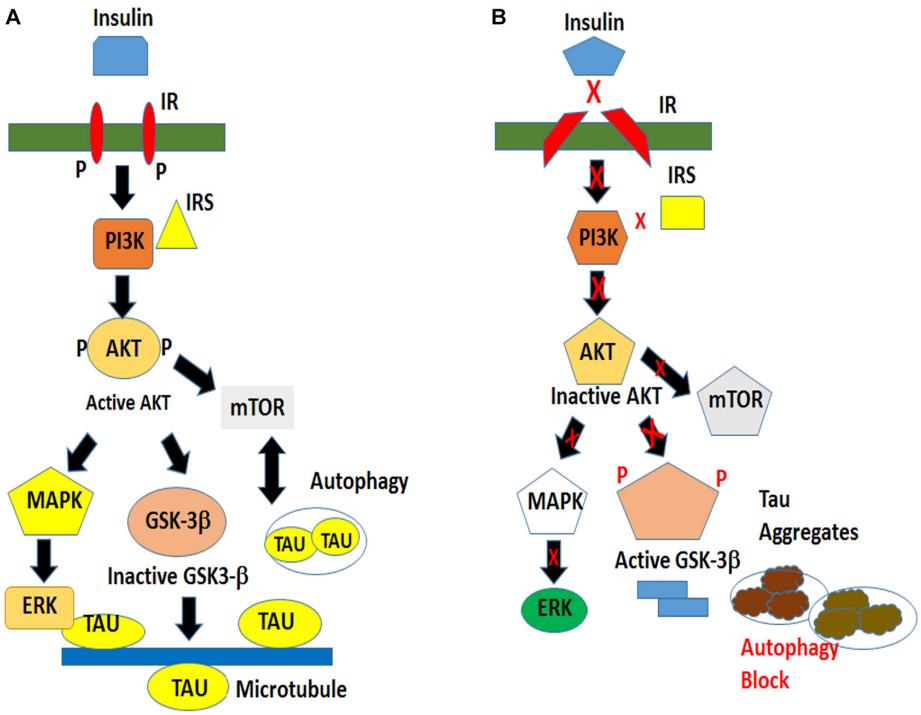

FIGURE 9 | Schematic of a proposed model of tau hyperphosphorylation under the conditions of normal and impaired insulin signaling. **(A)** Insulin signaling in a physiological environment activates AKT, which in turn inactivates GSK-3β and prevents tau hyperphosphorylation. Insulin also activates TOR pathway for cellular growth and maintains a basal level of autophagy for cell survival. **(B)** An impaired insulin signaling results in an inactivation of AKT and an activation of GSK-3βY216 resulting in tau hyperphosphorylation leading to the formation of insoluble tau aggregates. The pathological condition is exacerbated by an impaired clearance of toxic protein.

we observed an autophagic blockage that was evident by decreased LC3-II signaling in insulin-treated cells compared to controls. These results were further validated by the accumulation of p62 protein in insulin-treated cells indicating an autophagic blockage akin to the treatment of the cells with known autophagy blocker Bafilomycin (Bartlett et al., 2011; Cai et al., 2012). It is possible that the autophagic upregulation observed at the early time points was atleast partly contributed by the serum starvation before subjecting the cells to insulin treatment.

Taken together our results imply that insulin resistance increases the amount hyperphosphorylated tau not only by activating the key kinases but also by modulating the TOR/autophagy pathways.

Therefore, we propose a speculative mechanism of insulin sensitivity and resistance in our fly and cellular models. In an insulin-sensitive state there exists an optimal physiological level of total and phospho-tau partially controlled by the principal tau kinase GSK-3β located downstream of the insulin signaling pathway. This situation is reversed in an insulin-resistant state with dysregulation of the downstream kinases that result in the increased production of hyperphosphorylated and aggregated tau. Despite increased autophagic induction, abnormal tau accumulation is aggravated by impaired autophagic clearance ultimately causing neuronal death (**Figure 9**).

This study, for the first time highlights the role of Chico – the ortholog of mammalian IRSs in playing a crucial role in reducing tau hyperphosphorylation and aggregation in our tauopathy models by controlling the downstream tau kinases GSK-3β and ERK and modulating the mTOR/autophagy pathways. The recapitulation of the *in vivo* data in mammalian cells stresses the fact that the insulin signaling pathway is conserved in both systems. Our study deciphers a compelling linkage between insulin resistance in type 2 diabetes and tau pathology observed in 4R-tauopathies including AD. Further elucidation of the mechanisms may pave the way for the early detection of risk factors such as insulin resistance and designing of novel drug targets (Huang and Mucke, 2012).

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

AUTHOR CONTRIBUTIONS

SC conducted the experiments and wrote the manuscript. SA performed the initial genetic screening with tau overexpressing

Drosophila lines and identified Chico as a suppressor of the tau-induced "rough-eye" phenotype. AM and GJ helped in reviewing the manuscript. This study was initiated at GJ's laboratory at the University of Texas Medical Branch, Galveston and completed at AM's laboratory at the University of Southampton by SC as a Marie-Curie fellow.

FUNDING

This work was supported by the Cullen Foundation Grants, Department of Neurology, University of Texas Medical Branch, Galveston and Marie-Curie Fellowship Project 705417 from the European Commission, Horizon 2020 at the University of Southampton, United Kingdom.

ACKNOWLEDGMENTS

We thank Drs. Shintaro Naganos and Minoru Saitoe for *Drosophila* Chico antibody. We also thank Dr. Mark Willett from the Imaging and Microscopy Unit and Mr. Ben Batchelor at the University of Southampton for assisting in retinal dissections and confocal microscopy imaging.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00801/full#supplementary-material

REFERENCES

Ambegaokar, S. S., and Jackson, G. R. (2011). Functional genomic screen and network analysis reveal novel modifiers of tauopathy dissociated from tau phosphorylation. *Hum. Mol. Genet.* 20, 4947–4977. doi: 10.1093/hmg/ddr432

Armstrong, R. A. (2006). Plaques and tangles and the pathogenesis of Alzheimer's disease. *Folia Neuropathol.* 44, 1–11.

Augustinack, J. C., Schneider, A., Mandelkow, E. M., and Hyman, B. T. (2002). Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* 103, 26–35. doi: 10.1007/s004010100423

Avet-Rochex, A., Kaul, A. K., Gatt, A. P., McNeill, H., and Bateman, J. M. (2012). Concerted control of gliogenesis by InR/TOR and FGF signalling in the drosophila post-embryonic brain. *Development* 139, 2763–2772. doi: 10.1242/dev.074179

Avila, J., Leon-Espinosa, G., Garcia, E., Garcia-Escudero, V., Hernandez, F., and Defelipe, J. (2012). Tau phosphorylation by GSK3 in different conditions. *Intern. J. Alzheimer's Dis.* 2012:578373. doi: 10.1155/2012/578373

Bakhoum, M. F., Bakhoum, C. Y., Ding, Z., Carlton, S. M., Campbell, G. A., and Jackson, G. R. (2014). Evidence of autophagic gridlock in aging and neurodegeneration. *Transl. Res.* 164, 1–12. doi: 10.1016/j.trsl.2014.01.016

Barbagallo, M., and Dominguez, L. J. (2014). Type 2 diabetes mellitus and Alzheimer's disease. *World J. Diabetes* 5, 889–893. doi: 10.4239/wjd.v5.i6.889

Barnett, A., and Brewer, G. J. (2011). Autophagy in aging and Alzheimer's disease: pathologic or protective? *J. Alzheimer's Dis.* 25, 385–394. doi: 10.3233/JAD-2011-101989

Barone, E., Di Domenico, F., Cassano, T., Arena, A., Tramutola, A., Lavecchia, M. A., et al. (2016). "Impairment of biliverdin reductase-A promotes brain insulin resistance in Alzheimer disease: a new paradigm." *Free Radic. Biol. Med.* 91, 127–142. doi: 10.1016/j.freeradbiomed.2015.12.012

Barone, E., Tramutola, A., Triani, F., Calcagnini, S., Di Domenico, F., Ripoli, C., et al. (2019). "Biliverdin reductase-a mediates the beneficial effects of intranasal insulin in alzheimer disease." *Mol. Neurobiol.* 56, 2922–2943. doi: 10.1007/s12035-018-1231-5

Bartlett, B. J., Isakson, P., Lewerenz, J., Sanchez, H., Kotzebue, R. W., Cumming, R. C., et al. (2011). p62, Ref(2)P and ubiquitinated proteins are conserved markers of neuronal aging, aggregate formation and progressive autophagic defects. *Autophagy* 7, 572–583. doi: 10.4161/auto.7.6.14943

Berger, Z., Ravikumar, B., Menzles, F. M., Oroz, L. G., Underwood, B. R., Pangalos, M. N., et al. (2006). Rapamycin alleviates toxicity of different aggregate-prone proteins. *Hum. Mol. Genet.* 15, 433–442. doi: 10.1093/hmg/ddi458

Caccamo, A., Magri, A., Medina, D. X., Wisely, E. V., Lopez-Aranda, M. F., Silva, A. J., et al. (2013). mTOR regulates tau phosphorylation and degradation: implications for Alzheimer's disease and other tauopathies. *Aging Cell* 12, 370–380. doi: 10.1111/acel.12057

Cai, Z., Zhao, B., Li, K., Zhang, L., Li, C., Quazi, S. H., et al. (2012). Mammalian target of rapamycin: a valid therapeutic target through the autophagy pathway for Alzheimer's disease? *J. Neurosci. Res.* 90, 1105–1118. doi: 10.1002/jnr.23011

Chatterjee, S., Sang, T. K., Lawless, G. M., and Jackson, G. R. (2009). Dissociation of tau toxicity and phosphorylation: role of GSK-3beta, MARK and Cdk5 in a drosophila model. *Hum. Mol. Genet.* 18, 164–177. doi: 10.1093/hmg/ddn326

Citron, M. (2004). Strategies for disease modification in Alzheimer's disease. *Nat. Rev. Neurosci.* 5, 677–685.

Codogno, P., and Meijer, A. J. (2010). Autophagy: a potential link between obesity and insulin resistance. *Cell Metab.* 11, 449–451. doi: 10.1016/j.cmet.2010.05.006

Colucci-D'Amato, L., Perrone-Capano, C., and di Porzio, U. (2003). Chronic activation of ERK and neurodegenerative diseases. *Bioessays* 25, 1085–1095. doi: 10.1002/bies.10355

Copps, K. D., and White, M. F. (2012). Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* 55, 2565–2582. doi: 10.1007/s00125-012-2644-8

Cowan, M. C., Quraishe, S., Hands, S., Sealey, M., Mahajan, S., Allan, D. W., et al. (2015). Rescue from tau-induced neuronal dysfunction produces insoluble tau oligomers. *Sci. Rep.* 5, 1–14. doi: 10.1038/srep17191

de la Monte, S. M. (2012). Therapeutic targets of brain insulin resistance in sporadic Alzheimer's disease. *Front. Biosci.* 4:1582–1605. doi: 10.2741/e482

Didonna, A., and Legname, G. (2010). Aberrant ERK 1/2 complex activation and localization in scrapie-infected GT1-1 cells. *Mol. Neurodegener.* 5:29. doi: 10.1186/1750-1326-5-29

Diehl, T., Mullins, R., and Kapogiannis, D. (2017). Insulin resistance in Alzheimer's disease. *Transl. Res.* 183, 26–40. doi: 10.1016/j.trsl.2016.12.005

Doble, B. W., and Woodgett, J. R. (2003). GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell Sci.* 116, 1175–1186. doi: 10.1242/jcs.00384

Freiherr, J., Hallschmid, M., Frey, W. H. II, Brunner, Y. F., Chapman, C. D., Holscher, C., et al. (2013). Intranasal insulin as a treatment for Alzheimer's disease: a review of basic research and clinical evidence. *CNS Drugs* 27, 505–514. doi: 10.1007/s40263-013-0076-8

Freude, S., Schilbach, K., and Schubert, M. (2009). The role of IGF-1 receptor and insulin receptor signaling for the pathogenesis of Alzheimer's disease: from model organisms to human disease. *Curr. Alzheimer Res.* 6, 213–223. doi: 10.2174/156720509788486527

Goedert, M., and Spillantini, M. G. (2017). Propagation of tau aggregates. *Mol. Brain* 10:18. doi: 10.1186/s13041-017-0298-7

Goncalves, R. A., Wijesekara, N., Fraser, P. E., and De Felice, F. G. (2019). The link between tau and insulin signaling: implications for Alzheimer's disease and other tauopathies. *Front. Cell Neurosci.* 13:17. doi: 10.3389/fncel.2019.00017

Hands, S. L., Proud, C. G., and Wyttenbach, A. (2009). mTOR's role in ageing: protein synthesis or autophagy? *Aging* 1, 586–597. doi: 10.18632/aging.100070

Hooper, C., Killick, R., and Lovestone, S. (2008). "The GSK3 hypothesis of Alzheimer's disease." *J. Neurochem.* 104, 1433–1439. doi: 10.1111/j.1471-4159.2007.05194.x

Huang, Y., and Mucke, L. (2012). Alzheimer mechanisms and therapeutic strategies. *Cell* 148, 1204–1222. doi: 10.1016/j.cell.2012.02.040

Inoue, K., Rispoli, J., Kaphzan, H., Klann, E., Chen, E. I., Kim, J., et al. (2012). Macroautophagy deficiency mediates age-dependent neurodegeneration through a phospho-tau pathway. *Mol. Neurodegener.* 7:48. doi: 10.1186/1750-1326-7-48

Ishiguro, K., Omori, A., Takamatsu, M., Sato, K., Arioka, M., Uchida, T., et al. (1992). Phosphorylation sites on tau by tau protein kinase I, a bovine derived kinase generating an epitope of paired helical filaments. *Neurosci. Lett.* 148, 202–206. doi: 10.1016/0304-3940(92)90839-y

Jackson, G. R., Wiedau-Pazos, M., Sang, T. K., Wagle, N., Brown, C. A., Massachi, S., et al. (2002). Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in drosophila. *Neuron* 34, 509–519. doi: 10.1016/s0896-6273(02)00706-7

Jamsa, A., Hasslund, K., Cowburn, R. F., Backstrom, A., and Vasange, M. (2004). The retinoic acid and brain-derived neurotrophic factor differentiated SH-SY5Y cell line as a model for Alzheimer's disease-like tau phosphorylation. *Biochem. Biophys. Res. Commun.* 319, 993–1000. doi: 10.1016/j.bbrc.2004.05.075

Johnson, G. V., and Bailey, C. D. (2002). Tau, where are we now? *J. Alzheimer Dis.* 4, 375–398. doi: 10.3233/jad-2002-4505

Jolivalt, C. G., Lee, C. A., Beiswenger, K. K., Smith, J. L., Orlov, M., Torrance, M. A., et al. (2008). Defective insulin signaling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: parallels with Alzheimer's disease and correction by insulin. *J. Neurosci. Res.* 86, 3265–3274. doi: 10.1002/jnr.21787

Jung, C. H., Ro, S. H., Cao, J., Otto, N. M., and Kim, D. H. (2010). mTOR regulation of autophagy. *FEBS Lett.* 584, 1287–1295. doi: 10.1016/j.febslet.2010.01.017

Khurana, V., Elson-Schwab, I., Fulga, T. A., Sharp, K. A., Loewen, C. A., Mulkearns, E., et al. (2010). Lysosomal dysfunction promotes cleavage and neurotoxicity of tau in vivo. *PLoS Genet.* 6:e1001026. doi: 10.1371/journal.pgen.1001026

Khurana, V., Lu, Y., Steinhilb, M. L., Oldham, S., Shulman, J., and Feany, M. B. (2006). TOR-mediated cell-cycle activation causes neurodegeneration in a drosophila tauopathy model. *Curr. Biol.* 16, 230–241. doi: 10.1016/j.cub.2005.12.042

Kim, B., Backus, C., Oh, S., Hayes, J. M., and Feldman, E. L. (2009). Increased tau phosphorylation and cleavage in mouse models of type 1 and type 2 diabetes. *Endocrinology* 150, 5294–5301. doi: 10.1210/en.2009-0695

Kopeikina, K. J., Hyman, B. T., and Spires-Jones, T. L. (2012). Soluble forms of tau are toxic in Alzheimer's disease. *Transl. Neurosci.* 3, 223–233.

Lasagna-Reeves, C. A., Castillo-Carranza, D. L., Sengupta, U., Clos, A. L., Jackson, G. R., and Kayed, R. (2011). Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol. Neurodegener.* 6:39. doi: 10.1186/1750-1326-6-39

Lee, J. A. (2012). Neuronal autophagy: a housekeeper or a fighter in neuronal cell survival? *Exp. Neurobiol.* 21, 1–8. doi: 10.5607/en.2012.21.1.1

Lesort, M., Jope, R. S., and Johnson, G. V. (1999). "Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase." *J. Neurochem.* 72, 576–584. doi: 10.1046/j.1471-4159.1999.0720576.x

Macklin, L., Griffith, C. M., Cai, Y., Rose, G. M., Yan, X. X., and Patrylo, P. R. (2017). "Glucose tolerance and insulin sensitivity are impaired in APP/PS1 transgenic mice prior to amyloid plaque pathogenesis and cognitive decline." *Exp. Gerontol.* 88, 9–18. doi: 10.1016/j.exger.2016.12.019

Mauvezin, C., and Neufeld, T. P. (2015). Bafilomycin A1 disrupts autophagic flux by inhibiting both V-ATPase-dependent acidification and Ca-P60A/SERCA-dependent autophagosome-lysosome fusion. *Autophagy* 11, 1437–1438. doi: 10.1080/15548627.2015.1066957

Metcalf, D. J., Garcia-Arencibia, M., Hochfeld, W. E., and Rubinsztein, D. C. (2012). "Autophagy and misfolded proteins in neurodegeneration." *Exp. Neurol.* 238, 22–28. doi: 10.1016/j.expneurol.2010.11.003

Miron, M., Lasko, P., and Sonenberg, N. (2003). Signaling from Akt to FRAP/TOR targets both 4E-BP and S6K in drosophila melanogaster. *Mol. Cell. Biol.* 23, 9117–9126. doi: 10.1128/mcb.23.24.9117-9126.2003

Moloney, A. M., Griffin, R. J., Timmons, S., O'Connor, R., Ravid, R., and O'Neill, C. (2010). Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiol. Aging* 31, 224–243. doi: 10.1016/j.neurobiolaging.2008.04.002

Murillo-Maldonado, J. M., Sanchez-Chavez, G., Salgado, L. M., Salceda, R., and Riesgo-Escovar, J. R. (2011). "Drosophila insulin pathway mutants affect visual physiology and brain function besides growth, lipid, and carbohydrate metabolism." *Diabetes Metab. Res. Rev* 60, 1632–1636. doi: 10.2337/db10-1288

Musselman, L. P., Fink, J. L., Narzinski, K., Ramachandran, P. V., Hathiramani, S. S., Cagan, R. L., et al. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type drosophila. *Dis. Model. Mech.* 4, 842–849. doi: 10.1242/dmm.007948

Naganos, S., Horiuchi, J., and Saitoe, M. (2012). Mutations in the drosophila insulin receptor substrate, CHICO, impair olfactory associative learning. *Neurosci. Res.* 73, 49–55. doi: 10.1016/j.neures.2012.02.001

Nagy, P., Varga, A., Kovacs, A. L., Takats, S., and Gabor, J. (2015). How and why to study autophagy in drosophila : it's more than just a garbage chute. *Methods* 75, 151–161. doi: 10.1016/j.ymeth.2014.11.016

Nassif, M., and Hetz, C. (2012). Autophagy impairment: a crossroad between neurodegeneration and tauopathies. *BMC Biol.* 10:78. doi: 10.1186/1741-7007-10-78

Neufeld, T. P. (2012). Autophagy and cell growth–the yin and yang of nutrient responses. *J. Cell Sci.* 125, 2359–2368. doi: 10.1242/jcs.103333

O'Neill, C. (2013). PI3-kinase/Akt/mTOR signaling: impaired on/off switches in aging, cognitive decline and Alzheimer's disease. *Exp. Gerontol.* 48, 647–653. doi: 10.1016/j.exger.2013.02.025

Partridge, L., Alic, N., Bjedov, I., and Piper, M. D. (2011). Ageing in drosophila: the role of the insulin/Igf and TOR signalling network. *Exp. Gerontol.* 46, 376–381. doi: 10.1016/j.exger.2010.09.003

Ren, Y., and Sahara, N. (2013). Characteristics of tau oligomers. *Front. Neurol.* 4:102. doi: 10.3389/fneur.2013.00102

Roberts, R. O., Knopman, D. S., Przybelski, S. A., Mielke, M. M., Kantarci, K., Preboske, G. M., et al. (2014). Association of type 2 diabetes with brain atrophy and cognitive impairment. *Neurology* 82, 1132–1141. doi: 10.1212/WNL.0000000000000269

Rodriguez-Rodriguez, P., Sandebring-Matton, A., Merino-Serrais, P., Parrado-Fernandez, C., Rabano, A., Winblad, B., et al. (2017). Tau hyperphosphorylation induces oligomeric insulin accumulation and insulin resistance in neurons. *Brain* 140, 3269–3285. doi: 10.1093/brain/awx256

Rusten, T. E., and Stenmark, H. (2010). p62, an autophagy hero or culprit? *Nat. Cell Biol.* 12, 207–209. doi: 10.1038/ncb0310-207

Sang, T. K., and Ready, D. F. (2002). Eyes closed, a drosophila p47 homolog, is essential for photoreceptor morphogenesis. *Development* 129, 143–154.

Schuh, A. F., Rieder, C. M., Rizzi, L., Chaves, M., and Roriz-Cruz, M. (2011). Mechanisms of brain aging regulation by insulin: implications for neurodegeneration in late-onset Alzheimer's disease. *ISRN Neurol.* 2011:306905. doi: 10.5402/2011/306905

Shao, J., Yamashita, H., Qiao, L., and Friedman, J. E. (2000). "Decreased akt kinase activity and insulin resistance in C57BL/KsJ-Leprdb/db mice." *J. Endocrinol.* 167, 107–115. doi: 10.1677/joe.0.1670107

Sharma, N., Tramutola, A., Lanzillotta, C., Arena, A., Blarzino, C., Cassano, T., et al. (2019). "Loss of biliverdin reductase-A favors Tau hyper-phosphorylation in Alzheimer's disease." *Neurobiol. Dis.* 125, 176–189. doi: 10.1016/j.nbd.2019.02.003

Simic, G., Babic Leko, M., Wray, S., Harrington, C., Delalle, I., Jovanov-Milosevic, N., et al. (2016). Tau protein hyperphosphorylation and aggregation in Alzheimer's disease and other tauopathies, and possible neuroprotective strategies. *Biomolecules* 6:6. doi: 10.3390/biom6010006

Smith, A. D. (2002). "Imaging the progression of Alzheimer pathology through the brain." *Proc. Natl. Acad. Sci. U.S.A.* 99, 4135–4137. doi: 10.1073/pnas.082107399

Son, S. M., Song, H., Byun, J., Park, K. S., Jang, H. C., Park, Y. J., et al. (2012). "Altered APP processing in insulin-resistant conditions is mediated by autophagosome accumulation via the inhibition of mammalian target of rapamycin pathway." *Diabetes Metab. Res. Rev.* 61, 3126–3138. doi: 10.2337/db11-1735

Sridhar, G. R., Lakshmi, G., and Nagamani, G. (2015). Emerging links between type 2 diabetes and Alzheimer's disease. *World J. Diabetes* 6, 744–751. doi: 10.4239/wjd.v6.i5.744

Standaert, M. L., Ortmeyer, H. K., Sajan, M. P., Kanoh, Y., Bandyopadhyay, G., Hansen, B. C., et al. (2002). "Skeletal muscle insulin resistance in obesity-associated type 2 diabetes in monkeys is linked to a defect in insulin activation of protein kinase C-zeta/lambda/iota." *Diabetes Metab. Res. Rev.* 51, 2936–2943. doi: 10.2337/diabetes.51.10.2936

Steen, E., Terry, B. M., Rivera, E. J., Cannon, J. L., Neely, T. R., Tavares, R., et al. (2005). Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease–is this type 3 diabetes? *J. Alzheimers Dis.* 7, 63–80. doi: 10.3233/jad-2005-7107

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., et al. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J. Clin. Invest.* 122, 1316–1338. doi: 10.1172/JCI59903

Tanaka, T., Iqbal, K., Trenkner, E., Liu, D. J., and Grundke-Iqbal, I. (1995). Abnormally phosphorylated tau in SY5Y human neuroblastoma cells. *FEBS Lett.* 360, 5–9. doi: 10.1016/0014-5793(95)00061-d

Tramutola, A., Triplett, J. C., Di Domenico, F., Niedowicz, D. M., Murphy, M. P., Coccia, R., et al. (2015). "Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD." *J. Neurochem.* 133, 739–749. doi: 10.1111/jnc.13037

Triani, F., Tramutola, A., Di Domenico, F., Sharma, N., Butterfield, D. A., Head, E., et al. (2018). Biliverdin reductase-a impairment links brain insulin resistance with increased abeta production in an animal model of aging: implications for Alzheimer disease. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 3181–3194. doi: 10.1016/j.bbadis.2018.07.005

Zhou, L., Zhang, J., Fang, Q., Liu, M., Liu, X., Jia, W., et al. (2009). Autophagy-mediated insulin receptor down-regulation contributes to endoplasmic reticulum stress-induced insulin resistance. *Mol. Pharmacol.* 76, 596–603. doi: 10.1124/mol.109.057067

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

REVIEW
published: 16 August 2019
doi: 10.3389/fnins.2019.00868

The Relevance of Insulin Action in the Dopaminergic System

Francesca Fiory[1,2†], Giuseppe Perruolo[1,2†], Ilaria Cimmino[1,2], Serena Cabaro[1,2], Francesca Chiara Pignalosa[1,2], Claudia Miele[1,2], Francesco Beguinot[1,2], Pietro Formisano[1,2] and Francesco Oriente[1,2]*

[1] Department of Translational Medicine, University of Naples Federico II, Naples, Italy, [2] URT "Genomic of Diabetes," Institute of Experimental Endocrinology and Oncology, National Research Council, Naples, Italy

The advances in medicine, together with lifestyle modifications, led to a rising life expectancy. Unfortunately, however, aging is accompanied by an alarming boost of age-associated chronic pathologies, including neurodegenerative and metabolic diseases. Interestingly, a non-negligible interplay between alterations of glucose homeostasis and brain dysfunction has clearly emerged. In particular, epidemiological studies have pointed out a possible association between Type 2 Diabetes (T2D) and Parkinson's Disease (PD). Insulin resistance, one of the major hallmark for etiology of T2D, has a detrimental influence on PD, negatively affecting PD phenotype, accelerating its progression and worsening cognitive impairment. This review aims to provide an exhaustive analysis of the most recent evidences supporting the key role of insulin resistance in PD pathogenesis. It will focus on the relevance of insulin in the brain, working as pro-survival neurotrophic factor and as a master regulator of neuronal mitochondrial function and oxidative stress. Insulin action as a modulator of dopamine signaling and of alpha-synuclein degradation will be described in details, too. The intriguing idea that shared deregulated pathogenic pathways represent a link between PD and insulin resistance has clinical and therapeutic implications. Thus, ongoing studies about the promising healing potential of common antidiabetic drugs such as metformin, exenatide, DPP IV inhibitors, thiazolidinediones and bromocriptine, will be summarized and the rationale for their use to decelerate neurodegeneration will be critically assessed.

Keywords: type 2 diabetes mellitus, insulin resistance, Parkinson's disease, dopamine, neurodegeneration

OPEN ACCESS

Edited by:
Eugenio Barone,
Sapienza University of Rome, Italy

Reviewed by:
Elizabeth M. Rhea,
VA Puget Sound Health Care System, United States
Maria D'Erme,
Sapienza University of Rome, Italy

****Correspondence:***
Pietro Formisano
fpietro@unina.it

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *27 February 2019*
Accepted: *02 August 2019*
Published: *16 August 2019*

Citation:
Fiory F, Perruolo G, Cimmino I, Cabaro S, Pignalosa FC, Miele C, Beguinot F, Formisano P and Oriente F (2019) The Relevance of Insulin Action in the Dopaminergic System. Front. Neurosci. 13:868. doi: 10.3389/fnins.2019.00868

Abbreviations: 6-OHDA, 6-hydroxydopmin; AMPK, Adenosine Monophosphate-Activated Protein Kinase; BAX, (B Cell Lymphoma)-Associated X; BCL, B Cell Lymphoma; COX, Cyclooxygenase; DPP-4, dipeptidyl-peptidase IV; ERK, Extracellular Receptor Kinase; Ex-4, Exenatide; GLP, Glucagon-like peptide; GSK, Glycogen Synthase Kinase; HFD, high fat diet-treated; IL, Interleukin; iNOS, Inducible nitric oxide synthase; IR, Insulin receptor; IRS, Insulin receptor substrate; JNK, Jun N-terminal Kinase; MD, Mediterranean-style diet; Met, Metformin; MMP-3, matrix metalloprotease 3; MPP+, 1-methyl-4-phenyl pyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NIRKO, neuron-specific insulin receptor knockout; PD, Parkinson's disease; PED/PEA, Phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PI3K, Phosphoinositide 3-kinases; PPARγ, Peroxisome proliferator-activated receptor gamma; RA, Retinoic acid; ROS, Reactive oxygen species; T2DM, Type 2 Diabetes Mellitus; TORC, cytochrome c-type protein; TNF, Tumor Necrosis Factor; TZD, Thiazoledinediones.

INTRODUCTION

The prevalence of aging-associated chronic pathologies, such as neurodegenerative and metabolic diseases, has dramatically increased along with life expectancy (Zochodne and Malik, 2014). Type 2 Diabetes (T2D) embraces almost 90% of all cases of diabetes and actually represents a major public health problem worldwide. Chronic hyperglycemia is the hallmark of T2D, resulting from insulin resistance and beta cell dysfunction, and is associated with long-term complications, including retinopathy, nephropathy, micro and macro vascular diseases. More recently, experimental, clinical and neuroimaging data provided evidence of a connection between T2D and brain injury (Hamed, 2017). T2D-associated brain injury is largely linked to hyperglycemia and involves several different pathological events, such as oxidative stress (Muriach et al., 2014), mitochondrial dysfunction (Shokrzadeh et al., 2018), neuroinflammation (Rom et al., 2018), decrease of neurotrophins (Franco-Robles et al., 2014), modification of neurotransmitters (Datusalia and Sharma, 2014), vascular derangements (Kooistra et al., 2013), amyloid β deposition (Wang X. et al., 2014), increased tau phosphorylation (Platt et al., 2016) and progressive cognitive dysfunction (Simo et al., 2017). Epidemiological studies also support the evidence of a crosstalk linking T2D and neurodegenerative disorders (Morsi et al., 2018). In particular, an interesting association between T2D and PD has recently emerged from clinical, experimental and genome-wide association studies (Biosa et al., 2018; De Pablo-Fernandez et al., 2018).

Deterioration of dopaminergic neurons in the extrapyramidal tract of the midbrain is the trigger for PD pathogenesis, resulting from an interplay of genetic and environmental factors (Olanow et al., 2009). Most of the time PD is a sporadic disease, but few cases have genetic origin and several genes associated to PD have been found (Hernandez et al., 2016). Impairment of the dopaminergic neurons leads to a reduction in dopamine signaling and may lead to a relative increase in acetylcholine release from cholinergic neurons in the striatum, thereby contributing to dyskinesia (Heumann et al., 2014). Other typical motor symptoms of PD are bradykinesia, resting tremor, muscular rigidity and abnormal posture and gait (Olanow et al., 2009). In many cases of PD, loss of dopaminergic neurons in the substantia nigra is accompanied by the formation of intracellular neuronal inclusions composed of alpha-synuclein, known as Lewy bodies, in the central, autonomic, and peripheral nervous system. The diagnosis of PD is essentially based on the neurological examination, aimed to identification of characteristic motor signs, deriving from the loss of nigral dopaminergic neurons. The presence of a sustained response to dopamine drugs (dopamine agonists or levodopa) is also commonly used in diagnosis. Several non-motor symptoms are associated to PD, too. They include hyposmia, sleep behavior disorder, loss of olfaction, constipation, depression and global cognitive decline and precede the clinical effects of dopamine deficiency, sometimes for several years (Schapira et al., 2017). Unlike motor symptoms, non-motor symptoms of PD are not improved by dopamine replacement therapy and seem to derive from the formation of Lewy bodies beyond midbrain dopaminergic neurons (Dickson et al., 2009). Cognitive impairment and dementia are the most disabling non-motor symptoms of PD, resulting from microvascular disease (Kim J.S. et al., 2014), deposition of Lewy bodies in neocortical and limbic areas, hyperphosphorylated tau-containing neurofibrillary tangles and formation of amyloid-beta-peptide plaques (Irwin et al., 2013).

The onset of diabetes appears to increase severity of symptoms in PD patients (Sandyk, 1993), and epidemiological studies suggest that diabetes is a risk factor for PD (Hu et al., 2007; Cereda et al., 2012). Several studies have tried to explain how T2D affects pathogenesis and progression of PD. In 1993, Sandyk (1993) found a relationship between PD and T2D, evidencing that up to 50–80% of patients with PD featured an altered glucose tolerance in response to a glucose load. Some years later, Schernhammer et al. (2011) evaluated a population of 1.931 cases and 9.651 controls, evidencing a 36% increased risk of developing PD among patients with T2D. Similarly, a major risk of developing PD among individuals with T2D was found in the study conducted by Sun et al. (2012). In this case-control study, by examining a Chinese population of 603.416 diabetics and comparing it with a diabetes-free control, they found that diabetic women had a higher incidence of PD compared to men. Moreover, young diabetic men aged 21–40 years or diabetic women aged 41–60 years were more susceptible to the risk of Parkinsonism. Additional studies have suggested a positive association between PD risk and T2D. In particular, Hu et al. (2007) have studied a Finnish population of 51.552 individuals, both men and women, aged between 25 and 74, without a history of PD at baseline, concluding that T2D is associated with an increased risk of PD. Very recently, De Pablo-Fernandez et al. (2018) have found an association between diabetes and PD in a retrospective study, where a cohort of 2,017,115 individuals admitted for hospital treatment with a codified diagnosis of type 2 diabetes was compared with a reference cohort of 6,173,208 people without diabetes.

Nevertheless, there is also opposite evidence, pointing out a lower risk of PD incidence in subjects with T2D (Powers et al., 2006) and an inverse association of hyperglycemia with the onset of PD in individuals without any neurodegenerative disease (Miyake et al., 2010). These conflicting results could be due to confounding sampling of the different populations. For instance, in the report performed by Miyake et al. (2010), T2D diagnosis is based on the filling up of self-reported questionnaires. An additional source of confusion may be that the considered populations are too small to obtain significant results. Differences in study design and methodology and the difficulty to rule out confounders (such as microvascular damage and diabetic treatment) as risk factors for PD negatively affect data reproducibility, too. However, notwithstanding the heterogeneity of the data, the existence of a positive association between T2D and PD has been recently supported by interventional studies showing a reduction in incidence of PD in T2D patients treated with antidiabetic drugs such as metformin, sulfonylureas and exenatide, which exert neuro-protection (Wahlqvist et al., 2012; Aviles-Olmos et al., 2013). Several lines of evidence suggest that impairment of insulin signaling increase the risk of PD (Morris et al., 2008; Bosco et al., 2012; Ashraghi et al., 2016;

Pang et al., 2016). Indeed, it has been recently found that insulin resistance, the impaired responsiveness to insulin, typical of T2D, occurs in PD brains and plays a key role in the progressive development of PD pathological hallmarks. In this review, we examine the relevance of insulin signaling in brain, especially for dopaminergic function, the relationship between insulin resistance and PD and finally we give an overview of the rationale underlying the use of drugs currently used for T2D in PD patients.

INSULIN SIGNALING IN BRAIN

Insulin is a peptide hormone secreted in response to postprandial hyperglycemia from pancreatic beta−cells in blood circulation. Historically, insulin was essentially known as the main regulator of peripheral glucose homeostasis, since it induces glucose uptake in adipose tissue and skeletal muscle and glycogen synthesis in the liver, inhibiting in parallel hepatic glycogenolysis and gluconeogenesis (Haeusler et al., 2018).

In addition to these peripheral targets, insulin also undertakes a neuroregulatory function, although the physiological significance of its role in the brain has only recently started to emerge in both murine models and humans (Schubert et al., 2004; Duarte et al., 2012; Grote and Wright, 2016). Detectable concentrations of insulin have been found in several brain regions, including hypothalamus, olfactory bulb and midbrain since many years (Baskin et al., 1983), but it is not yet clear whether insulin is locally produced in CNS. Experimental evidence supports the hypothesis of insulin biosynthesis in adult neuronal cells derived from the hippocampus and olfactory bulb (Kuwabara et al., 2011) and by pyramidal neurons in the cortex (Dorn et al., 1982). Immunoreactive insulin and C-peptide were found in the brain from human cadavers, and, *in situ* hybridization showed the presence of insulin mRNA in the periventricular nucleus of the rat hypothalamus (Blazquez et al., 2014). Furthermore, Havrankova et al. (1978) showed the presence of insulin in rat brain at concentrations between 10 and 100 times higher than that in plasma. On the contrary, other studies did not confirm these results, and conclusive evidence for significant amounts of insulin synthesized in brain is lacking (Gray et al., 2014). However, insulin may enter brain parenchyma and precapillary space via a receptor-mediated transport (Duffy and Pardridge, 1987; Banks et al., 1997). Studies performed in an experimental model of human blood brain barrier (BBB) formed by isolated capillaries deriving from fresh human brain autopsy have shown that BBB insulin receptor has physicochemical properties similar to the IRs present in peripheral tissues such as adipocytes and hepatocytes (Pardridge et al., 1985; Plata-Salaman, 1991). Insulin transport to the CNS is reduced in high-fat diet-induced obesity (Kaiyala et al., 2000) and suppressed by hyperglycemia (Banks et al., 1997). In addition, Alzheimer's disease and aging are associated with a reduction in insulin transport across the BBB (Craft et al., 1998; Frolich et al., 1998). Several studies have been performed in order to assess the integrity of BBB in PD although the results are still unclear. The observation that peripheral decarboxylase inhibitors, such as carbidopa and benserazide, do not reduce levodopa efficacy in brain indicate that BBB integrity is not compromised in parkinsonian patients (Rinne and Molsa, 1979). In support of this hypothesis, current and future therapeutic strategies for PD treatment are based on lipophilic substances or on a direct injection of proteins, genes and cellular therapies into the brain (Christine et al., 2009). Nevertheless, recent studies have also indicated that BBB is damaged in PD patients. Indeed, compromised BBB integrity in the striatum has been observed in postmortem brain tissue from PD patients (Gray and Woulfe, 2015). Furthermore, Dohgu et al. have indicated that monomeric alpha-synuclein induces BBB dysfunction by activating pericytes which, in turn, release inflammatory mediators (Dohgu et al., 2019). In conclusion, it is not possible to establish if insulin resistance in the PD brain arise from altered insulin transport across BBB. Hopefully, in the next future, advances in imaging techniques will allow to more carefully identify the source of insulin in the brain.

Interestingly, Jimenez-Jimenez et al. have compared cerebrospinal fluid (CSF) insulin levels in PD patients and in healthy subjects without finding significant differences between them (Jimenez-Jimenez et al., 2000). In contrast, other experimental evidence has shown that non-diabetic PD patients have increased blood glucose after oral glucose tolerance test without the concomitant rise in insulin levels, probably due to an impaired adaptive insulin secretion (Marques et al., 2018). Thus, the relationship between CSF/brain and serum insulin levels in PD needs to be elucidated. However, the specific role of this hormone in the different brain areas remains undeniable. Indeed, insulin elicits its effects by binding a specific tyrosine kinase receptor, expressed in different brain regions (Plum et al., 2005), including dopaminergic neurons (Figlewicz et al., 2003; Konner et al., 2011). Glucose uptake into neurons is insulin independent, thus in the brain insulin signaling regulates olfaction, mood and memory (McNay et al., 2010; Ketterer et al., 2011; Aime et al., 2012; Kleinridders et al., 2014; Biessels and Reagan, 2015; Heni et al., 2015). In addition, acting on glucosensing neurons of the hypothalamus, insulin modulates peripheral metabolism, hepatic glucose output, food intake, body weight, lipolysis and white adipose tissue browning (Blazquez et al., 2014; Dodd et al., 2015).

REGULATION OF SURVIVAL OF DOPAMINERGIC NEURONS

Well-characterized insulin functions in the central nervous system are the regulation of apoptosis during neuronal development and the enhancing of neuronal survival. This is not surprising since insulin binding to its receptor (IR) activates several intracellular effectors relevant to cell survival, such as PI3K/Akt pathway. Insulin, indeed, negatively modulates the expression of pro-apoptotic proteins protecting embryonic retinal cells during development from cell death (Diaz et al., 1999). Regarding the increase of neuronal survival, it is known that insulin signaling rescues rat hippocampal cells

in culture injured by oxygen or glucose deprivation (Mielke and Wang, 2005) and has neuroprotective effects on H2O2-induced toxicity of retinoic acid (RA)-differentiated SH-SY5Y cells (Ramalingam and Kim, 2014). During the pathogenesis of PD, characterized by death of dopaminergic neurons in the substantia nigra pars compacta, insulin pro-survival ability is particularly relevant and clearly emerged in studies performed in SH-SY5Y cells pretreated with the neurotoxin MPP+ and in animal models (Moroo et al., 1994). In this cellular model of experimental PD, insulin prevented cell death in a dose dependent manner. It inhibits MPP + -induced iNOS and ERK activation, lowering in turn nitric oxide release, reactive oxygen species (ROS), calcium ion influx and finally decreasing the ratio of Bax to Bcl-2 through activating anti-apoptotic PI3K/Akt/GSK3 pathways (Ramalingam and Kim, 2016b).

MODULATION OF ALPHA-SYNUCLEIN EXPRESSION AND AGGREGATION

Another characteristic neuropathologic feature in the PD brain is the accumulation of cytosolic inclusions of fibrillary forms of alpha-synuclein, called Lewy bodies. In C6 astrocytoma cells, a 24 h MPP + treatment induces a significant increase of a helically folded tetramer of alpha-synuclein accompanied by an augmentation of SNCA mRNA levels. Interestingly insulin affects alpha-synuclein expression and aggregation, too, by a mechanism involving the PI3K/Akt pathway (Ramalingam and Kim, 2017; Yang et al., 2018). Indeed, pretreatment with insulin induced a marked decrease in the tetrameric alpha-synuclein, preventing the cytotoxic effect of MPP + (Ramalingam and Kim, 2017). The molecular mechanisms underlying insulin protective action against MPP + neurotoxicity have been better clarified in SH-SY5Y cells, where insulin decreases alpha-synuclein and Cox-2 levels and blocks ROS-induced membrane damage. In parallel, it activates autophagy, integrins and syndecans signaling (Ramalingam and Kim, 2016a). Autophagy modulation by insulin is particularly relevant for PD pathogenesis, since it is crucial for elimination of abnormal and toxic protein aggregates. Insulin, indeed, blocking mTORC1 activity, stimulates autophagy of toxic proteins and activates Akt survival protein, through an mTORC2-mediated mechanism (Heras-Sandoval et al., 2014). The crucial importance of autophagy regulation by insulin is highlighted by the fact that the specific pharmacological inhibition of mTORC1 by rapamycin reduces alpha-synuclein aggregation (Sarkar et al., 2007) and prevents dopaminergic neuron loss (Tain et al., 2009). An additional plausible mechanism by which insulin promotes autophagy and negatively modulates alpha-synuclein toxicity is the inhibitory phosphorylation of GSK3beta by Akt. GSK3beta, indeed, co-localizes with alpha-synuclein in Lewy bodies and its expression is increased in postmortem brain from PD patients (Nagao and Hayashi, 2009) and in experimental models of PD associated with alpha-synuclein accumulation (Golpich et al., 2015). Recent evidences have revealed the presence of the microtubule associated protein tau in Lewy bodies, which is essentially known for its pathological role in Alzheimer disease, but it has recently been shown to participate in PD pathogenesis as well. GSK3beta inactivation by insulin is also involved in insulin-induced inhibition of tau phosphorylation which reduces neurotoxicity, increasing its binding to microtubules (Tokutake et al., 2012). Interestingly, insulin can directly affect alpha-synuclein turnover, reducing its aggregation and toxicity (Kao, 2009). Insulin action on alpha-synuclein aggregation is mediated by activation of IDE (insulin degrading enzyme), a highly conserved Zinc metallopeptidase which degrades amyloidogenic proteins. IDE, in turns, binds to alpha-synuclein oligomers, preventing them from further assembly into amyloid fibers that cause degeneration of dopaminergic neurons in PD patients (Sharma et al., 2015; **Figure 1**). Experiments performed in specific alpha-synuclein knockout mice have provided contrasting results. Indeed, while Rodriguez-Araujo et al. (2015) suggest that absence of alpha-synuclein in mice is associated with impairment in glucose metabolism during HFD-induced insulin-resistance, Geng et al. (2011) show an increased rate of insulin secretion in alpha-synuclein knockout mice, indicating alpha-synuclein as negative regulator of insulin secretion.

REGULATION OF MITOCHONDRIAL FUNCTION AND INFLAMMATION

In addition to modulate alpha-synuclein amount, insulin is able to regulate mitochondrial biogenesis and to directly affect mitochondrial electron transport chain activity through stimulation of the IR/PI3K/Akt pathway, which suppresses FoxO1/HMOX1 induction (Cheng et al., 2010). Importantly, in hippocampal neurons, compounds activating IR also activate the AMPK-SIRT1-PGC1alpha signaling axis, enhancing in parallel mitochondrial function (Barhwal et al., 2015). Insulin's ability to modulate mitochondrial membrane potential has long been characterized (Huang et al., 2003, 2005), but Aghanoori et al. (2017) recently revealed that insulin also controls mitochondrial function up-regulating mitochondrial electron transport system protein expression and complex activity.

Conversely, experimental models of insulin resistance feature altered levels of mitochondrial proteins in the substantia nigra (Khang et al., 2015), reduced levels of mitochondrial complex I and dysregulated calcium homeostasis (Moreira et al., 2006; Duarte et al., 2012). These phenomena impair mitochondrial biogenesis, inducing membrane depolarization and generation of excessive ROS, oxidative stress and increased cell death (Huang et al., 2003; Kleinridders et al., 2015). The link between mitochondrial dysfunction, insulin resistance and dopaminergic neuronal degeneration probably relies in the disruption of the Parkin-PARIS-PGC1alpha pathway. In chronic insulin resistance condition, indeed, reduced levels of Parkin have been observed in parallel with accumulation of a zinc finger protein, named PARIS, able to repress PGC1alpha expression and highly expressed in the substantia nigra of sporadic PD patients (Khang et al., 2015).

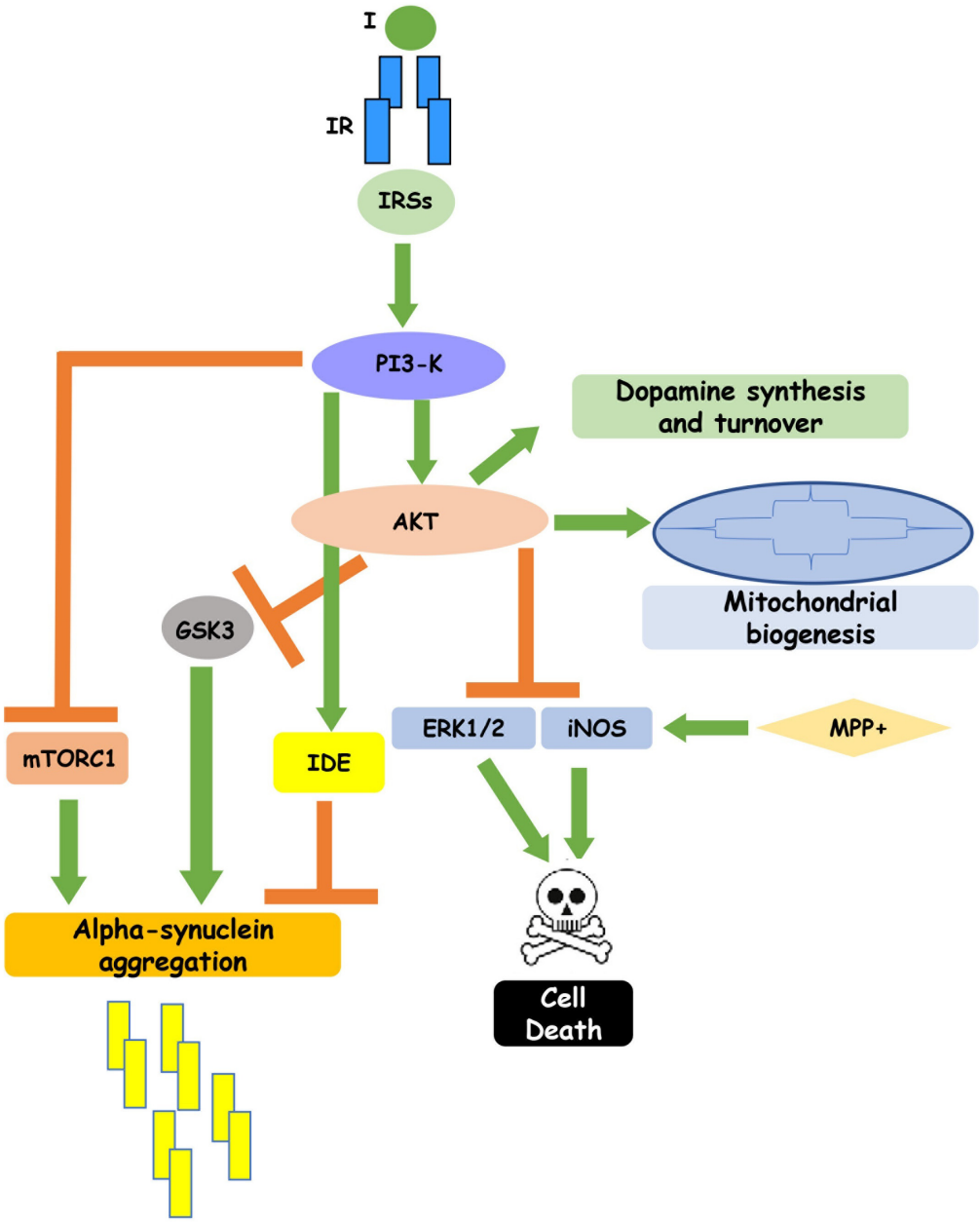

FIGURE 1 | Insulin signaling regulates neuronal function. Insulin binding to its receptor, through the intracellular substrates IRSs, leads to activation of PI3-K pathway which, in turn, inhibits GSK3, mTORC1 and IDE, reducing alpha-synuclein aggregation, and enhances cell survival. In addition, insulin-induced PI3K activation stimulates dopamine synthesis and turnover and mitochondrial biogenesis. This figure includes experimental results obtained in cell cultures and partially confirmed in rodent and human brain.

Insulin represents also a master regulator of extracellular events involved in PD pathogenesis, such as microglial activation and increase of pro-inflammatory mediators that contributes to ROS generation. Interestingly, several different pathways downstream IR activation such as PI3K/Akt and p38/MAPK pathways, are involved in TGF-beta1 neuroprotective effect against MPP + -induced neurodegeneration (Liu et al., 2016). Moreover, PI3K/Akt pathway decreases neuroinflammation up-regulating IkBalpha, a selective endogenous blocker of NF-kB, the main transcription factor responsible for expression of inflammatory genes (Khasnavis et al., 2012).

EFFECT ON DOPAMINE SYNTHESIS AND TURNOVER

Insulin itself represents also a physiological regulator of dopamine synthesis and clearance. A convincing demonstration

for insulin relevance in modulation of dopamine signaling has been provided by the phenotype of NIRKO mice, featuring a neuron-specific knockout of IR. NIRKO mice, indeed, exhibit manifestations of anxiety and depressive-like behaviors. These hallmarks are accompanied by increased dopamine turnover, which in turn leads to decreased dopamine signaling in the striatum and nucleus accumbens. *In vitro* data indicate that in neuronal cells these alterations arise from a loss of insulin effect on expression of MAO A and MAO B, involved in inactivation of monoamine neurotransmitters (Kleinridders et al., 2015). Moreover, there is evidence in literature that insulin is able to regulate expression of tyrosine hydroxylase (TH), the rate-limiting step in the biosynthesis of dopamine. Insulin in rats was shown to induce a transient increase in TH mRNA in adrenal medulla (Rusnak et al., 1998; Xu et al., 2007). Conversely, pathological states characterized by impaired insulin signaling are associated with alterations of TH expression and/or activity. For instance, in experimental diabetes decreased TH activity in terminal fields for noradrenergic and dopaminergic neurons has been observed (Chu et al., 1986; Glanville and Anderson, 1986; Kono and Takada, 1994) and genetically diabetic Wistar rats show decreased immunoreactive TH (Nascimento et al., 2011). Moreover, in streptozotocin-treated rats, TH mRNA was increased in the locus coeruleus but decreased in the ventral tegmental area/substantia nigra pars compacta (Figlewicz et al., 1996). Part of the mechanism underlying TH modulation by insulin has been recently clarified in PC12 cells, where insulin regulates TH expression through the transcription factors HIF-1alpha and Nur77 (Fiory et al., 2018). These data have evidenced the critical role of insulin signaling in maintaining an appropriate dopaminergic tone by regulating TH expression in the central nervous system. In addition, studies in brain slices, in striatal synaptosomes, and *in vivo* have shown that insulin activation of IR increases dopamine uptake by the dopamine transporter (DAT). In particular, direct intracerebroventricular infusion of insulin results in increased DAT mRNA levels. Accordingly, when CNS insulin levels were reduced by 24- to 36-h food deprivation, DAT mRNA levels, assessed by *in situ* hybridization, were significantly decreased in the ventral tegmental area/substantia nigra pars compacta and the Vmax of dopamine uptake was significantly decreased in striatum from fasted rats. Interestingly, *in vitro* incubation with a physiological concentration of insulin augmented striatal dopamine uptake to control levels (Patterson et al., 1998). Similarly, insulin increases dopamine uptake and modulates DAT trafficking via PI3K in rat striatal synaptosomes (Carvelli et al., 2002). In particular, the key regulator downstream PI3K, responsible for DAT regulation by insulin, is Akt2 (Speed et al., 2010). These results suggest that synaptic dopamine signaling may be altered by reducing the available cell surface DATs in states of chronic hypoinsulinemia, such as diabetes (Carvelli et al., 2002). For instance, high fat feeding, impairs striatal insulin-induced activation of Akt, reducing in turns DAT cell surface expression and function and locomotor responses to amphetamine (Speed et al., 2011). Finally, it has been recently shown that insulin influences food choice amplifying action potential-dependent dopamine release in the nucleus accumbens and caudate-putamen through an indirect mechanism involving striatal cholinergic interneurons that express IR. Furthermore, the sensitivity of striatal dopamine release to insulin in rats is oppositely altered by chronic diet manipulations; indeed, food restriction enhances and obesogenic diet decreases responsiveness to insulin, respectively (Patel et al., 2018). On the other end, there is no known information about insulin-regulated food choice effect on PD onset and/or progression.

ROLE IN COGNITIVE FUNCTION

Insulin plays an acknowledged role in regulation of memory and cognitive function, too. This is particularly relevant for PD progression, since cognitive impairment represents a significant non-motor symptom of PD. PD patients, indeed, feature more rapid decline in cognitive domains and in memory (Aarsland et al., 2017), exhibiting a cognitive impairment which embraces a spectrum of severity from relatively mild symptoms to end-stage dementia (Davis and Racette, 2016). However, mild cognitive impairment can occur early in the course of PD, while dementia commonly characterizes advanced stages of PD (Hely et al., 2008). Interestingly, the prevalence of cognitive deficit is significantly higher in PD patients with diabetes mellitus than in patients with PD only, suggesting that diabetes may be one risk factor for cognitive dysfunction in PD patients (Yang et al., 2017). However, specific role of insulin in safeguarding cognitive function has been more clearly confirmed by studies showing that PD patients with dementia are prone to comorbid insulin resistance (Bosco et al., 2012; Ashraghi et al., 2016), even when they were unaffected by diabetes. Cognitive decline in PD and progression to dementia derive from alterations in hippocampal structure and function (Bouchard et al., 2008; Costa et al., 2012; Pan et al., 2013). This is plausible, since hippocampal neurons are particularly susceptible to alterations in insulin sensitivity (Fehm et al., 2006). Importantly, a high density of IRs has been found in the hippocampus, cortex and amygdala, where they participate in cognitive functions (Singh et al., 1997; Gerozissis, 2003). Furthermore, acute administration of insulin, through activation of hippocampal IRs, ameliorates performance on memory tasks in rats (Park et al., 2000) and enhances verbal memory and cognition in humans (Kern et al., 2001; Benedict et al., 2004). Insulin effects on cognition involves the PI3K/Akt pathway (McNay and Recknagel, 2011) and is probably mediated by its ability to affect synaptic plasticity. Activation of the PI3K/Akt pathway, indeed, maintains dendritic spine stabilization, necessary for memory consolidation (Goldin and Segal, 2003; Zhao and Townsend, 2009). The crucial insulin effector downstream PI3K/Akt pathway involved in preservation of cognitive function is GSK3beta. Insulin increases GSK3beta inhibitory phosphorylation through PI3K/Akt signaling. The phosphorylation of GSK3 beta, in turn, improves long-term memory in hippocampal-associated tasks, decreases tau and alpha-synuclein accumulation and neurotoxicity and reduces neuroinflammation and apoptosis. In conclusion, insulin alleviates cognitive impairment in

PD via the inactivation of GSK3beta mediated by PI3K/Akt (Yang et al., 2018).

RELEVANCE OF INSULIN RESISTANCE FOR PARKINSON'S DISEASE

Interestingly, patients with PD feature augmented autoimmune reactivity to insulin (Wilhelm et al., 2007). Moreover, in the substantia nigra pars compacta of patients with PD, death of dopaminergic neurons is often anticipated by marked loss of IR mRNA and enhanced levels of IRS phosphorylation at serine residues, with inhibitory action on insulin signaling and subsequent increased insulin resistance (Moroo et al., 1994; Takahashi et al., 1996; Duarte et al., 2012; Morris et al., 2014). In particular, increased levels of IRS-1 pSer312 in the putamen and of pSer616 in hippocampal tissue of PD patients were found (Athauda and Foltynie, 2016). Likewise, both 6-OHDA-induced PD models and alpha-synuclein overexpressing mice show increased IRS phosphorylation at serine residues in the dopamine-depleted striatum (Morris et al., 2008, 2011a,b; Gao et al., 2015). In addition, increased nuclear translocation of PTEN and GSK3beta, paralleled by an impaired insulin signaling cascade, was observed in postmortem substantia nigra from PD patients (Sekar and Taghibiglou, 2018). Similarly, other authors have found decreased Akt phosphorylation in sections of substantia nigra from parkinsonian and control subjects (Malagelada et al., 2008; Timmons et al., 2009). These alterations may contribute to the pathogenesis and/or progression of PD. However, all of these results have been obtained in absence of "*ex vivo*" stimulation with insulin and, at the best of our knowledge, there is no evidence about the ability of PD postmortem brains to respond to insulin. Thus, the physiological decline in insulin signaling, which represents a typical hallmark of aging (Zhao et al., 2004; Kushner, 2013), is clearly accelerated in PD. On the other hand, the alterations of insulin signaling exacerbate PD clinical-pathological symptoms, enhancing dopaminergic degeneration and worsening disease progression and, in parallel, both motor and cognitive decline (Papapetropoulos et al., 2004). Several studies performed in animal models confirmed the onset of this deleterious crosstalk between insulin resistance and PD. In 2014, Wang and collaborators highlighted the relevance of insulin resistance for PD etiology using ob/ob and db/db mice as T2D model. These mice show insulin signaling impairment, ER stress and inflammation not only in peripheral tissue, but also in midbrain. It is worth of notice that they feature accumulation of alpha-synuclein and microglia activation along with increased production of pro-inflammatory cytokines. All these events were shown to enhance the vulnerability of dopaminergic neurons to MPTP neurotoxicity in the substantia nigra of db/db mice (Wang L. et al., 2014; Wang S. et al., 2014). Similar results were obtained in mice become insulin resistant upon a high-fat diet (HFD), which are more susceptible to PD inducing toxins, such as 6-OHDA and MPTP, characterized by a significant increase in nigrostriatal neurodegeneration and by a reduced dopaminergic signaling. This leads to a more severe motor deficits compared to matched controls (Choi et al., 2005; Morris et al., 2010, 2011a,b).

Recently, Sharma and Taliyan (2018) standardized an animal model suitable to mimic the comorbidity between insulin resistance and PD. To this aim, male Wistar rats were administrated 6-OHDA in medial forebrain bundle after 8 weeks feeding with high fat diet. The phenotype of these rats confirmed the capacity of insulin resistance to exacerbate PD pathology. In HFD-fed rats, indeed, 6-OHDA induced more pronounced neuronal damage and loss of striatal dopamine, leading, in parallel, to worst performance in behavioral tasks such as rotarod, narrow beam walk test and locomotor activity, compared to rats fed with standard diet.

The relevance of insulin resistance for PD has been further confirmed by the phenotype of transgenic mice overexpressing PED/PEA-15, a scaffold protein highly expressed in the brain and overexpressed in T2D subjects. These insulin resistant mice, indeed, show loss of dopaminergic neurons in the striatum and hypokinetic movements resembling PD motor alterations (Perruolo et al., 2016). Not least, NIRKO mice with neuron-specific IR knockout are the proof that insulin resistance is involved also in the onset of PD non-motor symptoms, since these mice develop increased dopamine turnover responsible for anxiety and depressive behaviors (Kleinridders et al., 2015).

Surprisingly, several studies found that alpha-synuclein increases inhibitory phosphorylation of IRS at serine residues, negatively regulating insulin signaling (Gao et al., 2015). Different mechanisms have been proposed to explain the deleterious effect of alpha-synuclein on insulin signaling. First, alpha-synuclein increases degradation of IRS-1, inhibiting protein phosphatase 2A through mTORC1 activation (Gao et al., 2015). In addition, alpha-synuclein induces microglial production of pro-inflammatory cytokines (Beraud et al., 2013; Blandini, 2013; Gallegos et al., 2015).

Overproduction of pro-inflammatory cytokines such as TNF-alpha in the CSF and CNS of PD patients was, indeed, evidenced in postmortem studies (Reale et al., 2009a,b). Similarly, peripheral concentrations of IL-6, TNF-alpha, IL-1beta, IL-2, IL-10, and C-reactive protein in PD patients are significantly higher compared with age-matched controls (Qin et al., 2016). Moreover, among newly diagnosed PD patients, those with higher levels of pro-inflammatory markers feature lower cognitive assessment scores (MMSE) and more rapid motor decline (Williams-Gray et al., 2009). Pro-inflammatory cytokines are probably responsible for increased activity of IRS serine kinases such as JNK, involved in the onset of neuronal insulin resistance (Peng and Andersen, 2003; Klintworth et al., 2007; Morris et al., 2008).

Interestingly, in HFD fed mice, restoring of IR signaling by inhibition of protein tyrosine phosphatase 1B or by treatment with the small molecule IR sensitizing agent, TCS 401, re-establishes insulin positive action on dopamine release and reuptake at dopamine terminals in the nucleus accumbens (Fordahl and Jones, 2017). Similarly, treatment with insulin sensitizing drugs and normalizing HFD ameliorate depressive behaviors in rodents (Yamada et al., 2011; Sharma et al., 2012). This evidence further suggests that insulin resistance could

represent a common risk factor involved in both T2D and PD pathogenesis.

INSULIN RESISTANCE TREATMENTS IN PARKINSON'S DISEASE

Both insulin resistance and PD can be defined as multifactorial disorders due to the interaction of environmental factors with a genetic susceptibility. Thus, modification of lifestyle and health behaviors, such as diet, can improve and prevent the onset of these diseases. It is well established that the MD, a nutritional model widespread in some countries of the Mediterranean sea such as southern Italy, Spain and Greece, which is based on a relatively higher intake of cereals, fruit, vegetables, seeds, olive oil (unsaturated fat) compared to a more rare use of red meat and animal fats (saturated fats), has many beneficial effects on insulin-resistance and T2D (Giugliano and Esposito, 2008; Grosso et al., 2014; Garcia et al., 2016). More recently, some authors have shown that MD seems to play also a neuroprotective role, although not all of epidemiologic studies report a positive function of MD on neurodegenerative diseases and further studies are required to validate these evidences (Alcalay et al., 2012; Okubo et al., 2012; Martinez-Lapiscina et al., 2013a,b; Cassani et al., 2017; **Table 1**).

In parallel with the healthy lifestyle, insulin and several drugs currently used for the treatment of insulin resistance have been suggested to have therapeutic effects in patients with PD (**Figure 2** and **Table 1**). These substances include metformin, exenatide, thiazolidinediones, and bromocriptine.

The role of insulin in PD treatment has been firstly evidenced by several studies indicating the presence of frequent amnesic defects in T2D patients (Perlmuter et al., 1984; Helkala et al., 1995; Vanhanen et al., 1999). Subsequently, these observations have been confirmed by the fact that intranasal administration of insulin in the hippocampus can improve memory deficits in humans (Benedict et al., 2004). However, the importance of insulin is not limited to the learning and memory processes, but also extends to its ability to induce anti-inflammatory and neuroprotective responses. Indeed, intranasal insulin administration protects against substantia nigra dopaminergic neuronal loss and alleviates motor deficits induced by 6-OHDA in rats (Pang et al., 2016). The positive effects mediated by insulin can be probably related to the capacity of this hormone to improve brain oxidative stress, apoptosis, autophagy and neuroinflammation and to the presence of its receptor in the CNS, in particular in the hippocampus and medial temporal cortex and amygdala, as described before (Singh et al., 1997; Gerozissis, 2003).

Metformin belongs to the biguanide family and is the most frequently used oral antidiabetic drug (Viollet et al., 2012). Met, by activating AMPK or increasing the IR expression and tyrosine kinase activity (Musi et al., 2002; Viollet et al., 2012; Rena et al., 2017), reduces hepatic gluconeogenesis and increases insulin-stimulated glucose uptake in skeletal muscle and adipocytes. In addition, Met decreases free fatty acid oxidation, improving insulin sensitivity (Musi et al., 2002; Rena et al., 2017) and insulin secretion from pancreatic beta-cells (Sreenan et al., 1996). Further studies suggest that metformin can cross BBB and activate AMPK in the CNS (Nath et al., 2009). In addition, this drug has been shown to rescue dopaminergic dysfunction and mitochondrial abnormalities in Drosophila models of PD (Ng et al., 2012) and to reduce the phospho-Ser129 alpha-synuclein, the modified form of alpha-synuclein that occurs most frequently within PD, both *in vitro* and *in vivo* (Perez-Revuelta et al., 2014). Nevertheless, studies performed in humans revealed contrasting results. Indeed, while Hsu et al. have suggested that can reduce the risk of dementia (Hsu et al., 2011) in diabetic patients, other authors indicate that metformin exposure in patients with T2D

TABLE 1 | Role of insulin resistance treatments in Parkinson's disease.

Insulin resistance treatments	Role in Parkinson's disease	References
Mediterranean-style diet	neuroprotection ?	Alcalay et al., 2012 Okubo et al., 2012 Martinez-Lapiscina et al., 2013a Martinez-Lapiscina et al., 2013b Cassani et al., 2017
Insulin	neuroprotection memory improvement ↓ neuroinflammation ↓ oxidative stress	Perlmuter et al., 1984 Helkala et al., 1995 Vanhanen et al., 1999 Benedict et al., 2004 Pang et al., 2016 Singh et al., 1997 Gerozissis, 2003
Metformin	neuroprotection	Ng et al., 2012 Perez-Revuelta et al., 2014 Hsu et al., 2011 Imfeld et al., 2012 Moore et al., 2013 Kuan et al., 2017
GLP-1 receptor agonists	neuroprotection amelioration of motor function	Li et al., 2009 Bertilsson et al., 2008 Kim et al., 2009 Chen et al., 2015 Aviles-Olmos et al., 2013 Aviles-Olmos et al., 2014 MacConell et al., 2015 Athauda et al., 2017
DPP-4 inhibitors	neuroprotection ↓ neuroinflammation ↓ oxidative stress	Svenningsson et al., 2016 Matteucci and Giampietro, 2015 Yazbeck et al., 2009 Abdelsalam and Safar, 2015 Nassar et al., 2015
Thiazoledinediones	neuroprotection ?	Rabchevsky et al., 2017 Dehmer et al., 2004 NINDS Exploratory Trials in Parkinson Disease (NET-PD) FS-ZONE Investigators, 2015 Chang et al., 2015

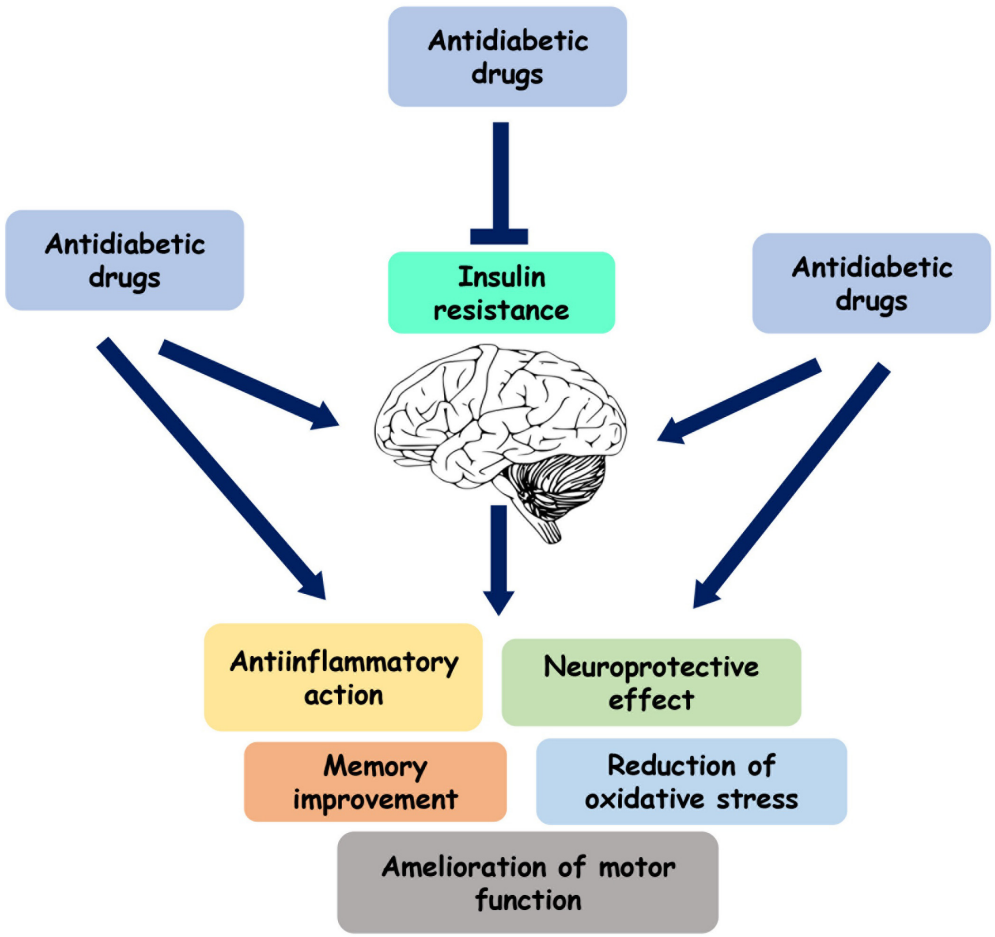

FIGURE 2 | Role of the antidiabetic drugs on brain function. Several antihyperglycemic agents play a neuroprotective and anti-inflammatory role by directly acting on brain or by reducing insulin resistance.

may lead to the development of neuronal diseases, including dementia and PD (Imfeld et al., 2012; Moore et al., 2013; Kuan et al., 2017).

Glucagon-like peptide-1 (GLP-1) receptor agonists, by mimicking the effects of the incretin hormone GLP-1, increase glucose-mediated insulin secretion and reduce postprandial glucagon levels, gastric emptying rate, food intake and body weight. Differentially from GLP-1 hormone, having a short half-life, GLP-1 agonists have two important properties that include the longer duration of action after subcutaneous administration respect to GLP-1 and the resistance to degradation mediated by dipeptidyl-peptidase 4 (DPP-4) enzymes (Prasad-Reddy and Isaacs, 2015). Several GLP-1 receptor agonists, including lixisenatide, exenatide and liraglutide, induce neuroprotective effects, and, in particular, exenatide (Ex-4), a synthetic version of exendin 4, has been suggested to have an important role in PD. Indeed, both "*in vitro*" and "*in vivo*" studies have demonstrated the ability of exenatide to mediate neurotrophic and neuro-protective effects. In particular, Li et al. (2009) have shown that Ex-4 treatment protects dopaminergic neurons against degeneration, preserves dopamine levels and improves motor function in the MPTP mouse model of PD. Similar results have been obtained by Bertilsson et al. (2008) who suggest that that Ex-4 significantly increases the number of neurons positive for TH and vesicular MAO transporter 2 in the substantia nigra of animals lesioned with 6-OHDA. Several mechanisms by which exenatide protects form neurodegeneration have been hypothesized. Kim et al. (2009) have showed that this drug protects dopaminergic neurons by preventing MPTP-induced microglial activation and MMP-3 expression. Other authors have demonstrated that GLP-1 receptor stimulation reduces apoptosis by promoting Bcl-2 expression and inhibiting the activation of caspase 3 and preserves mitochondrial function in dopaminergic neurons (Chen et al., 2015). Toxin-based models of PD, despite their limited translational value, have allowed to clarify mechanisms of action of GLP-1 agonists. Nevertheless, it is still unclear which of the previously described pathways are crucial for the GLP-1 agonists therapeutic effects for PD (Foltynie and Athauda, 2018). Clinical trials have also validated the positive effects of GLP-1R agonists in PD, underlining the safety and tolerability of this drug (Aviles-Olmos et al., 2013, 2014; MacConell et al., 2015;

Athauda et al., 2017). Nevertheless, the difficulty to compare each GLP-1R agonists under the same conditions limits, in part, the reproducibility of these studies.

DPP-4 is an enzyme which rapidly inactivates GLP-1 and GIP incretins, limiting their hypoglycemic action (Hansen et al., 1999). Furthermore, increased serological levels of DPP-4 have been observed in diabetic patients (Kim N.H. et al., 2014) and, thus, several DPP-4 inhibitors are used in the effective treatment of T2D. Treatment with DPP-4 inhibitors improves metabolism, insulin secretion and reduces glucagon secretion. Compared to GLP1 analogs, DPP-4 inhibitors are not able to induce weight loss, but in any case they do not lead to an increase in body weight, which instead occurs with sulfonylurea or insulin treatment.

Since the discovery of neurotrophic and immune regulating functions of DPP-4 inhibitors in the CNS, increasing studies supports the idea that DPP-4 might also be involved in the development of neurological disorders with a neuroinflammatory component.

Svenningsson et al. (2016) in a nationwide case-control study, found a significantly decreased incidence of PD among individuals with a record of DPP-4 inhibitor intake. The authors hypothesize that the this positive effect can be due not only to the increase of GLP-1/GLP-1R binding, but also by reducing the degradation of some neurotrophic neuropeptides, including pituitary adenylate cyclase-activating polypeptide (PACAP), substance P, neuropeptide Y, and gastrin-releasing peptide (Matteucci and Giampietro, 2015). Furthermore, DPP-4 inhibitors may have direct immunosuppressive effects, providing interesting insights for the future therapeutic development of treatments of neurological conditions with recognizable immune-related dysfunctions (Yazbeck et al., 2009; Svenningsson et al., 2016).

Other studies have shown the antiparkinsonian effect of vildagliptin, a dipeptidyl peptidase (DPP)-4 inhibitor, in rotenone−induced PD model in rats. Indeed, in these animals, vildagliptin by blocking the RAGE/NFκB cascade, suppresses inflammatory, oxidative stress, and apoptotic mediators reducing death of dopaminergic neurons and motor impairment (Abdelsalam and Safar, 2015). Similar results have been obtained by Nassar et al. (2015) using saxagliptin, another DPP-4 inhibitor.

Thiazoledinediones, that include rosiglitazone and pioglitazone, are oral hypoglycemic agents which bind and activate the nuclear receptor PPARγ. This protein is expressed not only in many insulin target tissues, but also in the substantia nigra and in the putamen nucleus (Swanson and Emborg, 2014). TZDs improve insulin-resistance in several ways, including the reduction of circulating fatty acids, the activation of the Glut4-mediated glucose transport and the decrease of the levels of inflammatory cytokines (Davidson et al., 2018).

Moreover, pioglitazone mediates its neuro protective effect, by binding a protein residing in the mitochondrial outer membrane, called MitoNEEt and regulating the activity of complex I in neuronal cells (Rabchevsky et al., 2017). In addition, this drug blocks the nitric oxide-mediated toxicity in MPTP-treated mice (Dehmer et al., 2004). As for the other anti-diabetic medications, studies performed in humans about the efficacy of TZDs in PD are still disappointing (NINDS Exploratory Trials in Parkinson Disease (NET-PD) FS-ZONE Investigators, 2015). A possible explanation for difficulty to obtain significant data in neurological disorder such as parkinsonism can be probably related to the poor capacity of TZDs to cross the BBB. Indeed, pioglitazone and rosiglitazone are substrates of the P-glycoprotein. This protein increases during the inflammatory state that occurs in PD and acts as a stereoselective barrier preventing the entry of TZDs into the brain (Chang et al., 2015).

A class of drugs capable of activating D2R dopaminergic receptors is represented by the dopaminergic agonists. In particular, an ergoline derivative, bromocriptine, indicated for the treatment of patients with parkinsonism who no longer respond to treatment with levodopa, improves glycemic homeostasis and is used in the treatment of T2D since 2009. The mechanism of action of bromocriptine is still not very clear. This drug, by activating D2 and blocking D1 receptors, is able to reduce blood glucose and serum triglycerides levels and to decrease body weight (Kalra et al., 2011; Lopez Vicchi et al., 2016). Furthermore, bromocriptine directly activates the alpha 2-adrenergic receptors, inhibiting glucose-stimulated insulin secretion in pancreatic beta cells (Kalra et al., 2011; Lopez Vicchi et al., 2016).

Studies performed on animal models, in particular on ob/ob mice and Syrian hamsters suggest that bromocriptine treatment improves obesity and associated metabolic dysfunctions and inhibits the seasonally occurring obesity, hyperinsulinemia, insulin resistance and impaired glucose tolerance (Cincotta and Meier, 1995; Liang et al., 1998; Luo et al., 1998). In addition, several clinical trials have demonstrated the beneficial effect of bromocriptine on glycemia and weight in obese non-diabetic and diabetic subjects (Meier et al., 1992; Pijl et al., 2000; Aminorroaya et al., 2004; Gaziano et al., 2010). Thus, despite bromocriptine has been used since the 1960s for treatment of PD, acromegaly and prolactinomas, only recently its relevance has been demonstrated in T2D, encouraging its future application.

From these data, it is clear that the correction of metabolic disorders is of fundamental importance in the care of PD. However, actually, there is no resolutive antihyperglycemic treatment able to improve PD, slow down its progression and alleviate its symptoms. Thus, the future challenge of the PD research aims to identify new molecules that are more effective and tolerable both in PD and in insulin-resistance than the traditional ones.

CONCLUSION

Several clinical and experimental studies indicate a higher prevalence of PD in patients diagnosed with diabetes. Indeed, it is now clear that the loss of insulin signaling may cause neuronal mitochondrial dysfunction and oxidative stress followed by loss of dopaminergic neurons and impaired memory functioning. These results have been corroborated by studies performed in animal models and by the positive action that some antidiabetic drugs induce with significant benefits in patients diagnosed

with PD. However, although the scientific research has reached several promising results, further and more detailed investigations are necessary to validate these studies in order to discover new therapeutic avenues.

AUTHOR CONTRIBUTIONS

FF and GP prepared the first draft of the manuscript. IC, SC, FP, and CM were involved in the literature search. FB and PF critically revised the manuscript. PF and FO supervised the work and wrote the final version of the manuscript.

FUNDING

This work was supported by grants from the University of Naples "Federico II" (Progetto di Ricerca di Ateneo) to FO and Associazione Italiana per la Ricerca sul Cancro - AIRC (IG19001) to PF.

REFERENCES

Aarsland, D., Creese, B., Politis, M., Chaudhuri, K. R., Ffytche, D. H., Weintraub, D., et al. (2017). Cognitive decline in Parkinson disease. *Nat. Rev. Neurol.* 13, 217–231. doi: 10.1038/nrneurol.2017.27

Abdelsalam, R. M., and Safar, M. M. (2015). Neuroprotective effects of vildagliptin in rat rotenone Parkinson's disease model: role of RAGE-NFkappaB and Nrf2-antioxidant signaling pathways. *J. Neurochem.* 133, 700–707. doi: 10.1111/jnc.13087

Aghanoori, M. R., Smith, D. R., Roy Chowdhury, S., Sabbir, M. G., Calcutt, N. A., and Fernyhough, P. (2017). Insulin prevents aberrant mitochondrial phenotype in sensory neurons of type 1 diabetic rats. *Exp. Neurol.* 297, 148–157. doi: 10.1016/j.expneurol.2017.08.005

Aime, P., Hegoburu, C., Jaillard, T., Degletagne, C., Garcia, S., Messaoudi, B., et al. (2012). 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* 7:e51227. doi: 10.1371/journal.pone.0051227

Alcalay, R. N., Gu, Y., Mejia-Santana, H., Cote, L., Marder, K. S., and Scarmeas, N. (2012). The association between mediterranean diet adherence and Parkinson's disease. *Mov. Disord.* 27, 771–774. doi: 10.1002/mds.24918

Aminorroaya, A., Janghorbani, M., Ramezani, M., Haghighi, S., and Amini, M. (2004). Does bromocriptine improve glycemic control of obese type-2 diabetics? *Horm. Res.* 62, 55–59. doi: 10.1159/000078932

Ashraghi, M. R., Pagano, G., Polychronis, S., Niccolini, F., and Politis, M. (2016). Parkinson's disease, diabetes and cognitive impairment. *Recent Pat. Endocr. Metab. Immune Drug Discov.* 10, 11–21. doi: 10.2174/1872214810999160628105549

Athauda, D., and Foltynie, T. (2016). Insulin resistance and Parkinson's disease: a new target for disease modification? *Prog. Neurobiol.* 145-146, 98–120. doi: 10.1016/j.pneurobio.2016.10.001

Athauda, D., Maclagan, K., Skene, S. S., Bajwa-Joseph, M., Letchford, D., Chowdhury, K., et al. (2017). Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. *Lancet* 390, 1664–1675. doi: 10.1016/S0140-6736(17)31585-31584

Aviles-Olmos, I., Dickson, J., Kefalopoulou, Z., Djamshidian, A., Ell, P., Soderlund, T., et al. (2013). Exenatide and the treatment of patients with Parkinson's disease. *J. Clin. Invest.* 123, 2730–2736. doi: 10.1172/JCI68295

Aviles-Olmos, I., Dickson, J., Kefalopoulou, Z., Djamshidian, A., Kahan, J., Ell, P., et al. (2014). Motor and cognitive advantages persist 12 months after exenatide exposure in Parkinson's disease. *J. Parkinsons Dis.* 4, 337–344. doi: 10.3233/JPD-140364

Banks, W. A., Jaspan, J. B., and Kastin, A. J. (1997). Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. *Peptides* 18, 1577–1584. doi: 10.1016/s0196-9781(97)00238-6

Barhwal, K., Das, S. K., Kumar, A., Hota, S. K., and Srivastava, R. B. (2015). Insulin receptor A and Sirtuin 1 synergistically improve learning and spatial memory following chronic salidroside treatment during hypoxia. *J. Neurochem.* 135, 332–346. doi: 10.1111/jnc.13225

Baskin, D. G., Porte, D. Jr., Guest, K., and Dorsa, D. M. (1983). Regional concentrations of insulin in the rat brain. *Endocrinology* 112, 898–903. doi: 10.1210/endo-112-3-898

Benedict, C., Hallschmid, M., Hatke, A., Schultes, B., Fehm, H. L., Born, J., et al. (2004). Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* 29, 1326–1334. doi: 10.1016/j.psyneuen.2004.04.003

Beraud, D., Hathaway, H. A., Trecki, J., Chasovskikh, S., Johnson, D. A., Johnson, J. A., et al. (2013). Microglial activation and antioxidant responses induced by the Parkinson's disease protein alpha-synuclein. *J. Neuroimmune Pharmacol.* 8, 94–117. doi: 10.1007/s11481-012-9401-9400

Bertilsson, G., Patrone, C., Zachrisson, O., Andersson, A., Dannaeus, K., Heidrich, J., et al. (2008). Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. *J. Neurosci. Res.* 86, 326–338. doi: 10.1002/jnr.21483

Biessels, G. J., and Reagan, L. P. (2015). Hippocampal insulin resistance and cognitive dysfunction. *Nat. Rev. Neurosci.* 16, 660–671. doi: 10.1038/nrn4019

Biosa, A., Outeiro, T. F., Bubacco, L., and Bisaglia, M. (2018). Diabetes mellitus as a risk factor for Parkinson's disease: a molecular point of view. *Mol. Neurobiol.* 55, 8754–8763. doi: 10.1007/s12035-018-1025-1029

Blandini, F. (2013). Neural and immune mechanisms in the pathogenesis of Parkinson's disease. *J. Neuroimmune Pharmacol.* 8, 189–201. doi: 10.1007/s11481-013-9435-y

Blazquez, E., Velazquez, E., Hurtado-Carneiro, V., and Ruiz-Albusac, J. M. (2014). Insulin in the brain: its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front. Endocrinol.* 5:161. doi: 10.3389/fendo.2014.00161

Bosco, D., Plastino, M., Cristiano, D., Colica, C., Ermio, C., De Bartolo, M., et al. (2012). Dementia is associated with insulin resistance in patients with Parkinson's disease. *J. Neurol. Sci.* 315, 39–43. doi: 10.1016/j.jns.2011.12.008

Bouchard, T. P., Malykhin, N., Martin, W. R., Hanstock, C. C., Emery, D. J., Fisher, N. J., et al. (2008). Age and dementia-associated atrophy predominates in the hippocampal head and amygdala in Parkinson's disease. *Neurobiol. Aging* 29, 1027–1039. doi: 10.1016/j.neurobiolaging.2007.02.002

Carvelli, L., Moron, J. A., Kahlig, K. M., Ferrer, J. V., Sen, N., Lechleiter, J. D., et al. (2002). PI 3-kinase regulation of dopamine uptake. *J. Neurochem.* 81, 859–869. doi: 10.1046/j.1471-4159.2002.00892.x

Cassani, E., Barichella, M., Ferri, V., Pinelli, G., Iorio, L., Bolliri, C., et al. (2017). Dietary habits in Parkinson's disease: adherence to mediterranean diet. *Parkinsonism Relat. Disord.* 42, 40–46. doi: 10.1016/j.parkreldis.2017.06.007

Cereda, E., Barichella, M., Cassani, E., Caccialanza, R., and Pezzoli, G. (2012). Clinical features of Parkinson disease when onset of diabetes came first: a case-control study. *Neurology* 78, 1507–1511. doi: 10.1212/WNL.0b013e3182553cc9

Chang, K. L., Pee, H. N., Yang, S., and Ho, P. C. (2015). Influence of drug transporters and stereoselectivity on the brain penetration of pioglitazone as a potential medicine against Alzheimer's disease. *Sci. Rep.* 5:9000. doi: 10.1038/srep09000

Chen, Y., Zhang, Y., Li, L., and Holscher, C. (2015). Neuroprotective effects of geniposide in the MPTP mouse model of Parkinson's disease. *Eur. J. Pharmacol.* 768, 21–27. doi: 10.1016/j.ejphar.2015.09.029

Cheng, Z., Tseng, Y., and White, M. F. (2010). Insulin signaling meets mitochondria in metabolism. *Trends Endocrinol. Metab.* 21, 589–598. doi: 10.1016/j.tem.2010.06.005

Choi, J. Y., Jang, E. H., Park, C. S., and Kang, J. H. (2005). Enhanced susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in high-fat diet-induced obesity. *Free Radic. Biol. Med.* 38, 806–816. doi: 10.1016/j.freeradbiomed.2004.12.008

Christine, C. W., Starr, P. A., Larson, P. S., Eberling, J. L., Jagust, W. J., Hawkins, R. A., et al. (2009). Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology* 73, 1662–1669. doi: 10.1212/WNL.0b013e3181c29356

Chu, P. C., Lin, M. T., Shian, L. R., and Leu, S. Y. (1986). Alterations in physiologic functions and in brain monoamine content in streptozocin-diabetic rats. *Diabetes* 35, 481–485. doi: 10.2337/diabetes.35.4.481

Cincotta, A. H., and Meier, A. H. (1995). Bromocriptine inhibits in vivo free fatty acid oxidation and hepatic glucose output in seasonally obese hamsters (Mesocricetus auratus). *Metabolism* 44, 1349–1355. doi: 10.1016/0026-0495(95)90041-1

Costa, C., Sgobio, C., Siliquini, S., Tozzi, A., Tantucci, M., Ghiglieri, V., et al. (2012). Mechanisms underlying the impairment of hippocampal long-term potentiation and memory in experimental Parkinson's disease. *Brain* 135(Pt 6), 1884–1899. doi: 10.1093/brain/aws101

Craft, S., Peskind, E., Schwartz, M. W., Schellenberg, G. D., Raskind, M., Porte, D. Jr., et al. (1998). Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. *Neurology* 50, 164–168. doi: 10.1212/wnl.50.1.164

Datusalia, A. K., and Sharma, S. S. (2014). Amelioration of diabetes-induced cognitive deficits by GSK-3beta inhibition is attributed to modulation of neurotransmitters and neuroinflammation. *Mol. Neurobiol.* 50, 390–405. doi: 10.1007/s12035-014-8632-x

Davidson, M. A., Mattison, D. R., Azoulay, L., and Krewski, D. (2018). Thiazolidinedione drugs in the treatment of type 2 diabetes mellitus: past, present and future. *Crit. Rev. Toxicol.* 48, 52–108. doi: 10.1080/10408444.2017.1351420

Davis, A. A., and Racette, B. (2016). Parkinson disease and cognitive impairment: five new things. *Neurol. Clin. Pract.* 6, 452–458. doi: 10.1212/CPJ.0000000000000285

De Pablo-Fernandez, E., Goldacre, R., Pakpoor, J., Noyce, A. J., and Warner, T. T. (2018). Association between diabetes and subsequent Parkinson disease: a record-linkage cohort study. *Neurology* 91, e139–e142. doi: 10.1212/WNL.0000000000005771

Dehmer, T., Heneka, M. T., Sastre, M., Dichgans, J., and Schulz, J. B. (2004). Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with I kappa B alpha induction and block of NF kappa B and iNOS activation. *J. Neurochem.* 88, 494–501. doi: 10.1046/j.1471-4159.2003.02210.x

Diaz, B., Pimentel, B., de Pablo, F., and de La Rosa, E. J. (1999). Apoptotic cell death of proliferating neuroepithelial cells in the embryonic retina is prevented by insulin. *Eur. J. Neurosci.* 11, 1624–1632. doi: 10.1046/j.1460-9568.1999.00577.x

Dickson, D. W., Fujishiro, H., Orr, C., DelleDonne, A., Josephs, K. A., Frigerio, R., et al. (2009). Neuropathology of non-motor features of Parkinson disease. *Parkinsonism Relat. Disord.* 15(Suppl. 3), S1–S5. doi: 10.1016/S1353-8020(09)70769-70762

Dodd, G. T., Decherf, S., Loh, K., Simonds, S. E., Wiede, F., Balland, E., et al. (2015). Leptin and insulin act on POMC neurons to promote the browning of white fat. *Cell* 160, 88–104. doi: 10.1016/j.cell.2014.12.022

Dohgu, S., Takata, F., Matsumoto, J., Kimura, I., Yamauchi, A., and Kataoka, Y. (2019). Monomeric alpha-synuclein induces blood-brain barrier dysfunction through activated brain pericytes releasing inflammatory mediators in vitro. *Microvasc. Res.* 124, 61–66. doi: 10.1016/j.mvr.2019.03.005

Dorn, A., Rinne, A., Hahn, H. J., Bernstein, H. G., and Ziegler, M. (1982). C-peptide immunoreactive neurons in human brain. *Acta Histochem.* 70, 326–330. doi: 10.1016/S0065-1281(82)80080-80089

Duarte, A. I., Moreira, P. I., and Oliveira, C. R. (2012). Insulin in central nervous system: more than just a peripheral hormone. *J. Aging Res.* 2012:384017. doi: 10.1155/2012/384017

Duffy, K. R., and Pardridge, W. M. (1987). Blood-brain barrier transcytosis of insulin in developing rabbits. *Brain Res.* 420, 32–38. doi: 10.1016/0006-8993(87)90236-90238

Fehm, H. L., Kern, W., and Peters, A. (2006). The selfish brain: competition for energy resources. *Prog. Brain Res.* 153, 129–140. doi: 10.1016/S0079-6123(06)53007-53009

Figlewicz, D. P., Brot, M. D., McCall, A. L., and Szot, P. (1996). Diabetes causes differential changes in CNS noradrenergic and dopaminergic neurons in the rat: a molecular study. *Brain Res.* 736, 54–60. doi: 10.1016/s0006-8993(96)00727-5

Figlewicz, D. P., Evans, S. B., Murphy, J., Hoen, M., and Baskin, D. G. (2003). Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Res.* 964, 107–115. doi: 10.1016/s0006-8993(02)04087-8

Fiory, F., Mirra, P., Nigro, C., Pignalosa, F. C., Zatterale, F., Ulianich, L., et al. (2018). Role of the HIF-1alpha/Nur77 axis in the regulation of the tyrosine hydroxylase expression by insulin in PC12 cells. *J. Cell Physiol.* 234, 11861–11870. doi: 10.1002/jcp.27898

Foltynie, T., and Athauda, D. (2018). Glucagon-like peptides (GLP-1) perspectives in synucleinopathies treatment. *Mov. Disord. Clin. Pract.* 5, 255–258. doi: 10.1002/mdc3.12611

Fordahl, S. C., and Jones, S. R. (2017). High-Fat-Diet-Induced deficits in dopamine terminal function are reversed by restoring insulin signaling. *ACS Chem. Neurosci.* 8, 290–299. doi: 10.1021/acschemneuro.6b00308

Franco-Robles, E., Campos-Cervantes, A., Murillo-Ortiz, B. O., Segovia, J., Lopez-Briones, S., Vergara, P., et al. (2014). Effects of curcumin on brain-derived neurotrophic factor levels and oxidative damage in obesity and diabetes. *Appl. Physiol. Nutr. Metab.* 39, 211–218. doi: 10.1139/apnm-2013-2133

Frolich, L., Blum-Degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., et al. (1998). Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J. Neural Transm.* 105, 423–438. doi: 10.1007/s007020050068

Gallegos, S., Pacheco, C., Peters, C., Opazo, C. M., and Aguayo, L. G. (2015). Features of alpha-synuclein that could explain the progression and irreversibility of Parkinson's disease. *Front. Neurosci.* 9:59. doi: 10.3389/fnins.2015.00059

Gao, S., Duan, C., Gao, G., Wang, X., and Yang, H. (2015). Alpha-synuclein overexpression negatively regulates insulin receptor substrate 1 by activating mTORC1/S6K1 signaling. *Int. J. Biochem. Cell Biol.* 64, 25–33. doi: 10.1016/j.biocel.2015.03.006

Garcia, M., Bihuniak, J. D., Shook, J., Kenny, A., Kerstetter, J., and Huedo-Medina, T. B. (2016). The effect of the traditional mediterranean-style diet on metabolic risk factors: a meta-analysis. *Nutrients* 8:168. doi: 10.3390/nu8030168

Gaziano, J. M., Cincotta, A. H., O'Connor, C. M., Ezrokhi, M., Rutty, D., Ma, Z. J., et al. (2010). Randomized clinical trial of quick-release bromocriptine among patients with type 2 diabetes on overall safety and cardiovascular outcomes. *Diabetes Care* 33, 1503–1508. doi: 10.2337/dc09-2009

Geng, X., Lou, H., Wang, J., Li, L., Swanson, A. L., Sun, M., et al. (2011). alpha-Synuclein binds the K(ATP) channel at insulin-secretory granules and inhibits insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* 300, E276–E286. doi: 10.1152/ajpendo.00262.2010

Gerozissis, K. (2003). Brain insulin: regulation, mechanisms of action and functions. *Cell Mol. Neurobiol.* 23, 1–25.

Giugliano, D., and Esposito, K. (2008). Mediterranean diet and metabolic diseases. *Curr. Opin. Lipidol.* 19, 63–68. doi: 10.1097/MOL.0b013e3282f2fa4d

Glanville, N. T., and Anderson, G. H. (1986). Hypothalamic catecholamine metabolism in diabetic rats: the effect of insulin deficiency and meal ingestion. *J. Neurochem.* 46, 753–759. doi: 10.1111/j.1471-4159.1986.tb13036.x

Goldin, M., and Segal, M. (2003). Protein kinase C and ERK involvement in dendritic spine plasticity in cultured rodent hippocampal neurons. *Eur. J. Neurosci.* 17, 2529–2539. doi: 10.1046/j.1460-9568.2003.02694.x

Golpich, M., Amini, E., Hemmati, F., Ibrahim, N. M., Rahmani, B., Mohamed, Z., et al. (2015). Glycogen synthase kinase-3 beta (GSK-3beta) signaling: implications for Parkinson's disease. *Pharmacol. Res.* 97, 16–26. doi: 10.1016/j.phrs.2015.03.010

Gray, M. T., and Woulfe, J. M. (2015). Striatal blood-brain barrier permeability in Parkinson's disease. *J. Cereb. Blood Flow Metab.* 35, 747–750. doi: 10.1038/jcbfm.2015.32

Gray, S. M., Meijer, R. I., and Barrett, E. J. (2014). Insulin regulates brain function, but how does it get there? *Diabetes* 63, 3992–3997. doi: 10.2337/db14-0340

Grosso, G., Mistretta, A., Marventano, S., Purrello, A., Vitaglione, P., Calabrese, G., et al. (2014). Beneficial effects of the mediterranean diet on metabolic syndrome. *Curr. Pharm. Des.* 20, 5039–5044. doi: 10.2174/1381612819666131206112144

Grote, C. W., and Wright, D. E. (2016). A role for insulin in diabetic neuropathy. *Front. Neurosci.* 10:581. doi: 10.3389/fnins.2016.00581

Haeusler, R. A., McGraw, T. E., and Accili, D. (2018). Biochemical and cellular properties of insulin receptor signalling. *Nat. Rev. Mol. Cell Biol.* 19, 31–44. doi: 10.1038/nrm.2017.89

Hamed, S. A. (2017). Brain injury with diabetes mellitus: evidence, mechanisms and treatment implications. *Expert Rev. Clin. Pharmacol.* 10, 409–428. doi: 10.1080/17512433.2017.1293521

Hansen, L., Deacon, C. F., Orskov, C., and Holst, J. J. (1999). Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. *Endocrinology* 140, 5356–5363. doi: 10.1210/endo.140.11.7143

Havrankova, J., Schmechel, D., Roth, J., and Brownstein, M. (1978). Identification of insulin in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 75, 5737–5741. doi: 10.1073/pnas.75.11.5737

Helkala, E. L., Niskanen, L., Viinamaki, H., Partanen, J., and Uusitupa, M. (1995). Short-term and long-term memory in elderly patients with NIDDM. *Diabetes Care* 18, 681–685. doi: 10.2337/diacare.18.5.681

Hely, M. A., Reid, W. G., Adena, M. A., Halliday, G. M., and Morris, J. G. (2008). The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. *Mov. Disord.* 23, 837–844. doi: 10.1002/mds.21956

Heni, M., Kullmann, S., Preissl, H., Fritsche, A., and Haring, H. U. (2015). Impaired insulin action in the human brain: causes and metabolic consequences. *Nat. Rev. Endocrinol.* 11, 701–711. doi: 10.1038/nrendo.2015.173

Heras-Sandoval, D., Perez-Rojas, J. M., Hernandez-Damian, J., and Pedraza-Chaverri, J. (2014). The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. *Cell Signal.* 26, 2694–2701. doi: 10.1016/j.cellsig.2014.08.019

Hernandez, D. G., Reed, X., and Singleton, A. B. (2016). Genetics in Parkinson disease: mendelian versus non-Mendelian inheritance. *J. Neurochem.* 139(Suppl. 1), 59–74. doi: 10.1111/jnc.13593

Heumann, R., Moratalla, R., Herrero, M. T., Chakrabarty, K., Drucker-Colin, R., Garcia-Montes, J. R., et al. (2014). Dyskinesia in Parkinson's disease: mechanisms and current non-pharmacological interventions. *J. Neurochem.* 130, 472–489. doi: 10.1111/jnc.12751

Hsu, C. C., Wahlqvist, M. L., Lee, M. S., and Tsai, H. N. (2011). Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J. Alzheimers Dis.* 24, 485–493. doi: 10.3233/JAD-2011-101524

Hu, G., Jousilahti, P., Bidel, S., Antikainen, R., and Tuomilehto, J. (2007). Type 2 diabetes and the risk of Parkinson's disease. *Diabetes Care* 30, 842–847. doi: 10.2337/dc06-2011

Huang, T. J., Price, S. A., Chilton, L., Calcutt, N. A., Tomlinson, D. R., Verkhratsky, A., et al. (2003). Insulin prevents depolarization of the mitochondrial inner membrane in sensory neurons of type 1 diabetic rats in the presence of sustained hyperglycemia. *Diabetes* 52, 2129–2136. doi: 10.2337/diabetes.52.8.2129

Huang, T. J., Verkhratsky, A., and Fernyhough, P. (2005). Insulin enhances mitochondrial inner membrane potential and increases ATP levels through phosphoinositide 3-kinase in adult sensory neurons. *Mol. Cell Neurosci.* 28, 42–54. doi: 10.1016/j.mcn.2004.08.009

Imfeld, P., Bodmer, M., Jick, S. S., and Meier, C. R. (2012). Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. *J. Am. Geriatr. Soc.* 60, 916–921. doi: 10.1111/j.1532-5415.2012.03916.x

Irwin, D. J., Lee, V. M., and Trojanowski, J. Q. (2013). Parkinson's disease dementia: convergence of alpha-synuclein, tau and amyloid-beta pathologies. *Nat. Rev. Neurosci.* 14, 626–636. doi: 10.1038/nrn3549

Jimenez-Jimenez, F. J., Molina, J. A., Vargas, C., Gomez, P., De Bustos, F., Zurdo, M., et al. (2000). Normal cerebrospinal fluid levels of insulin in patients with Parkinson's disease. *J. Neural Transm.* 107, 445–449. doi: 10.1007/s007020070086

Kaiyala, K. J., Prigeon, R. L., Kahn, S. E., Woods, S. C., and Schwartz, M. W. (2000). Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes* 49, 1525–1533. doi: 10.2337/diabetes.49.9.1525

Kalra, S., Kalra, B., Agrawal, N., and Kumar, S. (2011). Dopamine: the forgotten felon in type 2 diabetes. *Recent Pat. Endocr. Metab. Immune Drug Discov.* 5, 61–65. doi: 10.2174/187221411794351842

Kao, S. Y. (2009). Rescue of alpha-synuclein cytotoxicity by insulin-like growth factors. *Biochem. Biophys. Res. Commun.* 385, 434–438. doi: 10.1016/j.bbrc.2009.05.089

Kern, W., Peters, A., Fruehwald-Schultes, B., Deininger, E., Born, J., and Fehm, H. L. (2001). Improving influence of insulin on cognitive functions in humans. *Neuroendocrinology* 74, 270–280. doi: 10.1159/000054694

Ketterer, C., Heni, M., Thamer, C., Herzberg-Schafer, S. A., Haring, H. U., and Fritsche, A. (2011). Acute, short-term hyperinsulinemia increases olfactory threshold in healthy subjects. *Int. J. Obes.* 35, 1135–1138. doi: 10.1038/ijo.2010.251

Khang, R., Park, C., and Shin, J. H. (2015). Dysregulation of parkin in the substantia nigra of db/db and high-fat diet mice. *Neuroscience* 294, 182–192. doi: 10.1016/j.neuroscience.2015.03.017

Khasnavis, S., Jana, A., Roy, A., Mazumder, M., Bhushan, B., Wood, T., et al. (2012). Suppression of nuclear factor-kappaB activation and inflammation in microglia by physically modified saline. *J. Biol. Chem.* 287, 29529–29542. doi: 10.1074/jbc.M111.338012

Kim, J.S., Oh, Y. S., Lee, K. S., Song, I. U., Park, I. S., Yang, D. W., et al. (2014). Carotid artery thickening and neurocirculatory abnormalities in de novo Parkinson disease. *J. Neural Transm.* 121, 1259–1268. doi: 10.1007/s00702-014-1203-1205

Kim, N.H., Yu, T., and Lee, D. H. (2014). The nonglycemic actions of dipeptidyl peptidase-4 inhibitors. *Biomed. Res. Int.* 2014:368703. doi: 10.1155/2014/368703

Kim, S., Moon, M., and Park, S. (2009). Exendin-4 protects dopaminergic neurons by inhibition of microglial activation and matrix metalloproteinase-3 expression in an animal model of Parkinson's disease. *J. Endocrinol.* 202, 431–439. doi: 10.1677/JOE-09-0132

Kleinridders, A., Cai, W., Cappellucci, L., Ghazarian, A., Collins, W. R., Vienberg, S. G., et al. (2015). Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. *Proc. Natl. Acad. Sci. U. S. A.* 112, 3463–3468. doi: 10.1073/pnas.1500877112

Kleinridders, A., Ferris, H. A., Cai, W., and Kahn, C. R. (2014). Insulin action in brain regulates systemic metabolism and brain function. *Diabetes* 63, 2232–2243. doi: 10.2337/db14-0568

Klintworth, H., Newhouse, K., Li, T., Choi, W. S., Faigle, R., and Xia, Z. (2007). Activation of c-Jun N-terminal protein kinase is a common mechanism underlying paraquat- and rotenone-induced dopaminergic cell apoptosis. *Toxicol. Sci.* 97, 149–162. doi: 10.1093/toxsci/kfm029

Konner, A. C., Hess, S., Tovar, S., Mesaros, A., Sanchez-Lasheras, C., Evers, N., et al. (2011). Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. *Cell Metab.* 13, 720–728. doi: 10.1016/j.cmet.2011.03.021

Kono, T., and Takada, M. (1994). Dopamine depletion in nigrostriatal neurons in the genetically diabetic rat. *Brain Res.* 634, 155–158. doi: 10.1016/0006-8993(94)90269-0

Kooistra, M., Geerlings, M. I., Mali, W. P., Vincken, K. L., van der Graaf, Y., Biessels, G. J., et al. (2013). Diabetes mellitus and progression of vascular brain lesions and brain atrophy in patients with symptomatic atherosclerotic disease. The SMART-MR study. *J. Neurol. Sci.* 332, 69–74. doi: 10.1016/j.jns.2013.06.019

Kuan, Y. C., Huang, K. W., Lin, C. L., Hu, C. J., and Kao, C. H. (2017). Effects of metformin exposure on neurodegenerative diseases in elderly patients with type 2 diabetes mellitus. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 79(Pt B), 77–83. doi: 10.1016/j.pnpbp.2017.06.002

Kushner, J. A. (2013). The role of aging upon beta cell turnover. *J. Clin. Invest.* 123, 990–995. doi: 10.1172/JCI64095

Kuwabara, T., Kagalwala, M. N., Onuma, Y., Ito, Y., Warashina, M., Terashima, K., et al. (2011). Insulin biosynthesis in neuronal progenitors derived from adult hippocampus and the olfactory bulb. *EMBO Mol. Med.* 3, 742–754. doi: 10.1002/emmm.201100177

Li, Y., Perry, T., Kindy, M. S., Harvey, B. K., Tweedie, D., Holloway, H. W., et al. (2009). GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1285–1290. doi: 10.1073/pnas.0806720106

Liang, Y., Jetton, T. L., Lubkin, M., Meier, A. H., and Cincotta, A. H. (1998). Bromocriptine/SKF38393 ameliorates islet dysfunction in the diabetic (db/db) mouse. *Cell Mol. Life Sci.* 54, 703–711. doi: 10.1007/s000180050197

Liu, Z., Chen, H. Q., Huang, Y., Qiu, Y. H., and Peng, Y. P. (2016). Transforming growth factor-beta1 acts via TbetaR-I on microglia to protect against MPP(+)-induced dopaminergic neuronal loss. *Brain Behav. Immun.* 51, 131–143. doi: 10.1016/j.bbi.2015.08.006

Lopez Vicchi, F., Luque, G. M., Brie, B., Nogueira, J. P., Garcia Tornadu, I., and Becu-Villalobos, D. (2016). Dopaminergic drugs in type 2 diabetes and glucose homeostasis. *Pharmacol. Res.* 109, 74–80. doi: 10.1016/j.phrs.2015.12.029

Luo, S., Meier, A. H., and Cincotta, A. H. (1998). Bromocriptine reduces obesity, glucose intolerance and extracellular monoamine metabolite levels in the ventromedial hypothalamus of Syrian hamsters. *Neuroendocrinology* 68, 1–10. doi: 10.1159/000054344

MacConell, L., Gurney, K., Malloy, J., Zhou, M., and Kolterman, O. (2015). Safety and tolerability of exenatide once weekly in patients with type 2 diabetes: an integrated analysis of 4,328 patients. *Diabetes Metab. Syndr. Obes.* 8, 241–253. doi: 10.2147/DMSO.S77290

Malagelada, C., Jin, Z. H., and Greene, L. A. (2008). RTP801 is induced in Parkinson's disease and mediates neuron death by inhibiting Akt phosphorylation/activation. *J. Neurosci.* 28, 14363–14371. doi: 10.1523/JNEUROSCI.3928-08.2008

Marques, A., Dutheil, F., Durand, E., Rieu, I., Mulliez, A., Fantini, M. L., et al. (2018). Glucose dysregulation in Parkinson's disease: too much glucose or not enough insulin? *Parkinsonism Relat. Disord.* 55, 122–127. doi: 10.1016/j.parkreldis.2018.05.026

Martinez-Lapiscina, E. H., Clavero, P., Toledo, E., Estruch, R., Salas-Salvado, J., San Julian, B., et al. (2013a). Mediterranean diet improves cognition: the PREDIMED-NAVARRA randomised trial. *J. Neurol. Neurosurg. Psychiatry* 84, 1318–1325. doi: 10.1136/jnnp-2012-304792

Martinez-Lapiscina, E. H., Clavero, P., Toledo, E., San Julian, B., Sanchez-Tainta, A., Corella, D., et al. (2013b). Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. *J. Nutr. Health Aging* 17, 544–552. doi: 10.1007/s12603-013-0027-26

Matteucci, E., and Giampietro, O. (2015). Mechanisms of neurodegeration in type 2 diabetes and the neuroprotective potential of dipeptidyl peptidase 4 inhibitors. *Curr. Med. Chem.* 22, 1573–1581. doi: 10.2174/0929867322666150227153308

McNay, E. C., Ong, C. T., McCrimmon, R. J., Cresswell, J., Bogan, J. S., and Sherwin, R. S. (2010). Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. *Neurobiol. Learn. Mem.* 93, 546–553. doi: 10.1016/j.nlm.2010.02.002

McNay, E. C., and Recknagel, A. K. (2011). Brain insulin signaling: a key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes. *Neurobiol. Learn. Mem.* 96, 432–442. doi: 10.1016/j.nlm.2011.08.005

Meier, A. H., Cincotta, A. H., and Lovell, W. C. (1992). Timed bromocriptine administration reduces body fat stores in obese subjects and hyperglycemia in type II diabetics. *Experientia* 48, 248–253. doi: 10.1007/bf01930467

Mielke, J. G., and Wang, Y. T. (2005). Insulin exerts neuroprotection by counteracting the decrease in cell-surface GABA receptors following oxygen-glucose deprivation in cultured cortical neurons. *J. Neurochem.* 92, 103–113. doi: 10.1111/j.1471-4159.2004.02841.x

Miyake, Y., Tanaka, K., Fukushima, W., Sasaki, S., Kiyohara, C., Tsuboi, Y., et al. (2010). Case-control study of risk of Parkinson's disease in relation to hypertension, hypercholesterolemia, and diabetes in Japan. *J. Neurol. Sci.* 293, 82–86. doi: 10.1016/j.jns.2010.03.002

Moore, E. M., Mander, A. G., Ames, D., Kotowicz, M. A., Carne, R. P., Brodaty, H., et al. (2013). Increased risk of cognitive impairment in patients with diabetes is associated with metformin. *Diabetes Care* 36, 2981–2987. doi: 10.2337/dc13-0229

Moreira, P. I., Cardoso, S. M., Santos, M. S., and Oliveira, C. R. (2006). The key role of mitochondria in Alzheimer's disease. *J. Alzheimers Dis.* 9, 101–110.

Moroo, I., Yamada, T., Makino, H., Tooyama, I., McGeer, P. L., McGeer, E. G., et al. (1994). Loss of insulin receptor immunoreactivity from the substantia nigra pars compacta neurons in Parkinson's disease. *Acta Neuropathol.* 87, 343–348. doi: 10.1007/bf00313602

Morris, J. K., Bomhoff, G. L., Gorres, B. K., Davis, V. A., Kim, J., Lee, P. P., et al. (2011a). Insulin resistance impairs nigrostriatal dopamine function. *Exp. Neurol.* 231, 171–180. doi: 10.1016/j.expneurol.2011.06.005

Morris, J. K., Seim, N. B., Bomhoff, G. L., Geiger, P. C., and Stanford, J. A. (2011b). Effects of unilateral nigrostriatal dopamine depletion on peripheral glucose tolerance and insulin signaling in middle aged rats. *Neurosci. Lett.* 504, 219–222. doi: 10.1016/j.neulet.2011.09.027

Morris, J. K., Bomhoff, G. L., Stanford, J. A., and Geiger, P. C. (2010). Neurodegeneration in an animal model of Parkinson's disease is exacerbated by a high-fat diet. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R1082–R1090. doi: 10.1152/ajpregu.00449.2010

Morris, J. K., Vidoni, E. D., Perea, R. D., Rada, R., Johnson, D. K., Lyons, K., et al. (2014). Insulin resistance and gray matter volume in neurodegenerative disease. *Neuroscience* 270, 139–147. doi: 10.1016/j.neuroscience.2014.04.006

Morris, J. K., Zhang, H., Gupte, A. A., Bomhoff, G. L., Stanford, J. A., and Geiger, P. C. (2008). Measures of striatal insulin resistance in a 6-hydroxydopamine model of Parkinson's disease. *Brain Res.* 1240, 185–195. doi: 10.1016/j.brainres.2008.08.089

Morsi, M., Maher, A., Aboelmagd, O., Johar, D., and Bernstein, L. (2018). A shared comparison of diabetes mellitus and neurodegenerative disorders. *J. Cell Biochem.* 119, 1249–1256. doi: 10.1002/jcb.26261

Muriach, M., Flores-Bellver, M., Romero, F. J., and Barcia, J. M. (2014). Diabetes and the brain: oxidative stress, inflammation, and autophagy. *Oxid. Med. Cell Longev.* 2014:102158. doi: 10.1155/2014/102158

Musi, N., Hirshman, M. F., Nygren, J., Svanfeldt, M., Bavenholm, P., Rooyackers, O., et al. (2002). Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. *Diabetes* 51, 2074–2081. doi: 10.2337/diabetes.51.7.2074

Nagao, M., and Hayashi, H. (2009). Glycogen synthase kinase-3beta is associated with Parkinson's disease. *Neurosci. Lett.* 449, 103–107. doi: 10.1016/j.neulet.2008.10.104

Nascimento, P. S., Lovatel, G. A., Barbosa, S., Ilha, J., Centenaro, L. A., Malysz, T., et al. (2011). Treadmill training improves motor skills and increases tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta in diabetic rats. *Brain Res.* 1382, 173–180. doi: 10.1016/j.brainres.2011.01.063

Nassar, N. N., Al-Shorbagy, M. Y., Arab, H. H., and Abdallah, D. M. (2015). Saxagliptin: a novel antiparkinsonian approach. *Neuropharmacology* 89, 308–317. doi: 10.1016/j.neuropharm.2014.10.007

Nath, N., Khan, M., Paintlia, M. K., Singh, I., Hoda, M. N., and Giri, S. (2009). Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. *J. Immunol.* 182, 8005–8014. doi: 10.4049/jimmunol.0803563

Ng, C. H., Guan, M. S., Koh, C., Ouyang, X., Yu, F., Tan, E. K., et al. (2012). AMP kinase activation mitigates dopaminergic dysfunction and mitochondrial abnormalities in Drosophila models of Parkinson's disease. *J. Neurosci.* 32, 14311–14317. doi: 10.1523/JNEUROSCI.0499-12.2012

NINDS Exploratory Trials in Parkinson Disease (NET-PD) FS-ZONE Investigators (2015). Pioglitazone in. (early)Parkinson's disease: a phase 2, multicentre, double-blind, randomised trial. *Lancet Neurol.* 14, 795–803. doi: 10.1016/S1474-4422(15)00144-141

Okubo, H., Miyake, Y., Sasaki, S., Murakami, K., Tanaka, K., Fukushima, W., et al. (2012). Dietary patterns and risk of Parkinson's disease: a case-control study in Japan. *Eur. J. Neurol.* 19, 681–688. doi: 10.1111/j.1468-1331.2011.03600.x

Olanow, C. W., Stern, M. B., and Sethi, K. (2009). The scientific and clinical basis for the treatment of Parkinson disease (2009). *Neurology* 72(21 Suppl. 4), S1–S136. doi: 10.1212/WNL.0b013e3181a1d44c

Pan, P. L., Shi, H. C., Zhong, J. G., Xiao, P. R., Shen, Y., Wu, L. J., et al. (2013). Gray matter atrophy in Parkinson's disease with dementia: evidence from meta-analysis of voxel-based morphometry studies. *Neurol. Sci.* 34, 613–619. doi: 10.1007/s10072-012-1250-1253

Pang, Y., Lin, S., Wright, C., Shen, J., Carter, K., Bhatt, A., et al. (2016). Intranasal insulin protects against substantia nigra dopaminergic neuronal loss and alleviates motor deficits induced by 6-OHDA in rats. *Neuroscience* 318, 157–165. doi: 10.1016/j.neuroscience.2016.01.020

Papapetropoulos, S., Ellul, J., Argyriou, A. A., Talelli, P., Chroni, E., and Papapetropoulos, T. (2004). The effect of vascular disease on late onset Parkinson's disease. *Eur. J. Neurol.* 11, 231–235. doi: 10.1046/j.1468-1331.2003.00748.x

Pardridge, W. M., Eisenberg, J., and Yang, J. (1985). Human blood-brain barrier insulin receptor. *J. Neurochem.* 44, 1771–1778. doi: 10.1111/j.1471-4159.1985.tb07167.x

Park, C. R., Seeley, R. J., Craft, S., and Woods, S. C. (2000). Intracerebroventricular insulin enhances memory in a passive-avoidance task. *Physiol. Behav.* 68, 509–514. doi: 10.1016/S0031-9384(99)00220-6

Patel, J. C., Stouffer, M. A., Mancini, M., Nicholson, C., Carr, K. D., and Rice, M. E. (2018). Interactions between insulin and diet on striatal dopamine uptake kinetics in rodent brain slices. *Eur. J. Neurosci.* 49, 794–804. doi: 10.1111/ejn.13958

Patterson, T. A., Brot, M. D., Zavosh, A., Schenk, J. O., Szot, P., and Figlewicz, D. P. (1998). Food deprivation decreases mRNA and activity of the rat dopamine transporter. *Neuroendocrinology* 68, 11–20. doi: 10.1159/000054345

Peng, J., and Andersen, J. K. (2003). The role of c-Jun N-terminal kinase (JNK) in Parkinson's disease. *IUBMB Life* 55, 267–271. doi: 10.1080/1521654031000121666

Perez-Revuelta, B. I., Hettich, M. M., Ciociaro, A., Rotermund, C., Kahle, P. J., Krauss, S., et al. (2014). Metformin lowers Ser-129 phosphorylated alpha-synuclein levels via mTOR-dependent protein phosphatase 2A activation. *Cell Death Dis.* 5:e1209. doi: 10.1038/cddis.2014.175

Perlmuter, L. C., Hakami, M. K., Hodgson-Harrington, C., Ginsberg, J., Katz, J., Singer, D. E., et al. (1984). Decreased cognitive function in aging non-insulin-dependent diabetic patients. *Am. J. Med.* 77, 1043–1048. doi: 10.1016/0002-9343(84)90186-4

Perruolo, G., Viggiano, D., Fiory, F., Cassese, A., Nigro, C., Liotti, A., et al. (2016). Parkinson-like phenotype in insulin-resistant PED/PEA-15 transgenic mice. *Sci. Rep.* 6:29967. doi: 10.1038/srep29967

Pijl, H., Ohashi, S., Matsuda, M., Miyazaki, Y., Mahankali, A., Kumar, V., et al. (2000). Bromocriptine: a novel approach to the treatment of type 2 diabetes. *Diabetes Care* 23, 1154–1161. doi: 10.2337/diacare.23.8.1154

Plata-Salaman, C. R. (1991). Insulin in the cerebrospinal fluid. *Neurosci. Biobehav. Rev.* 15, 243–258.

Platt, T. L., Beckett, T. L., Kohler, K., Niedowicz, D. M., and Murphy, M. P. (2016). Obesity, diabetes, and leptin resistance promote tau pathology in a mouse model of disease. *Neuroscience* 315, 162–174. doi: 10.1016/j.neuroscience.2015.12.011

Plum, L., Schubert, M., and Bruning, J. C. (2005). The role of insulin receptor signaling in the brain. *Trends Endocrinol. Metab.* 16, 59–65. doi: 10.1016/j.tem.2005.01.008

Powers, K. M., Smith-Weller, T., Franklin, G. M., Longstreth, W. T. Jr., Swanson, P. D., and Checkoway, H. (2006). Diabetes, smoking, and other medical conditions in relation to Parkinson's disease risk. *Parkinsonism Relat. Disord.* 12, 185–189. doi: 10.1016/j.parkreldis.2005.09.004

Prasad-Reddy, L., and Isaacs, D. (2015). A clinical review of GLP-1 receptor agonists: efficacy and safety in diabetes and beyond. *Drugs Context* 4:212283. doi: 10.7573/dic.212283

Qin, X. Y., Zhang, S. P., Cao, C., Loh, Y. P., and Cheng, Y. (2016). Aberrations in peripheral inflammatory cytokine levels in parkinson disease: a systematic review and meta-analysis. *JAMA Neurol.* 73, 1316–1324. doi: 10.1001/jamaneurol.2016.2742

Rabchevsky, A. G., Patel, S. P., and Sullivan, P. G. (2017). Targeting mitoNEET with pioglitazone for therapeutic neuroprotection after spinal cord injury. *Neural Regen. Res.* 12, 1807–1808. doi: 10.4103/1673-5374.219040

Ramalingam, M., and Kim, S. J. (2014). The role of insulin against hydrogen peroxide-induced oxidative damages in differentiated SH-SY5Y cells. *J. Recept. Signal. Transduct. Res.* 34, 212–220. doi: 10.3109/10799893.2013.876043

Ramalingam, M., and Kim, S. J. (2016a). Insulin on activation of autophagy with integrins and syndecans against MPP(+)-induced alpha-synuclein neurotoxicity. *Neurosci. Lett.* 633, 94–100. doi: 10.1016/j.neulet.2016.09.023

Ramalingam, M., and Kim, S. J. (2016b). The neuroprotective role of insulin against MPP(+) -induced parkinson's disease in differentiated SH-SY5Y Cells. *J. Cell Biochem.* 117, 917–926. doi: 10.1002/jcb.25376

Ramalingam, M., and Kim, S. J. (2017). Protective effects of activated signaling pathways by insulin on C6 glial cell model of MPP(+)-induced Parkinson's disease. *J. Recept. Signal. Transduct. Res.* 37, 100–107. doi: 10.3109/10799893.2016.1171342

Reale, M., Greig, N. H., and Kamal, M. A. (2009a). Peripheral chemo-cytokine profiles in Alzheimer's and Parkinson's diseases. *Mini Rev. Med. Chem.* 9, 1229–1241. doi: 10.2174/138955709789055199

Reale, M., Iarlori, C., Thomas, A., Gambi, D., Perfetti, B., Di Nicola, M., et al. (2009b). Peripheral cytokines profile in Parkinson's disease. *Brain Behav. Immun.* 23, 55–63. doi: 10.1016/j.bbi.2008.07.003

Rena, G., Hardie, D. G., and Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia* 60, 1577–1585. doi: 10.1007/s00125-017-4342-z

Rinne, U. K., and Molsa, P. (1979). Levodopa with benserazide or carbidopa in Parkinson disease. *Neurology* 29, 1584–1589. doi: 10.1212/wnl.29.12.1584

Rodriguez-Araujo, G., Nakagami, H., Takami, Y., Katsuya, T., Akasaka, H., Saitoh, S., et al. (2015). Low alpha-synuclein levels in the blood are associated with insulin resistance. *Sci. Rep.* 5:12081. doi: 10.1038/srep12081

Rom, S., Zuluaga-Ramirez, V., Gajghate, S., Seliga, A., Winfield, M., Heldt, N. A., et al. (2018). Hyperglycemia-Driven neuroinflammation compromises BBB leading to memory loss in both diabetes mellitus (DM) Type 1 and Type 2 mouse models. *Mol. Neurobiol.* 56, 1883–1896. doi: 10.1007/s12035-018-1195-5

Rusnak, M., Jelokova, J., Vietor, I., Sabban, E. L., and Kvetnansky, R. (1998). Different effects of insulin and 2-deoxy-D-glucose administration on tyrosine hydroxylase gene expression in the locus coeruleus and the adrenal medulla in rats. *Brain Res. Bull.* 46, 447–452. doi: 10.1016/s0361-9230(98)00033-1

Sandyk, R. (1993). The relationship between diabetes mellitus and Parkinson's disease. *Int. J. Neurosci.* 69, 125–130.

Sarkar, S., Davies, J. E., Huang, Z., Tunnacliffe, A., and Rubinsztein, D. C. (2007). Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J. Biol. Chem.* 282, 5641–5652. doi: 10.1074/jbc.M609532200

Schapira, A. H. V., Chaudhuri, K. R., and Jenner, P. (2017). Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.* 18, 435–450. doi: 10.1038/nrn.2017.62

Schernhammer, E., Hansen, J., Rugbjerg, K., Wermuth, L., and Ritz, B. (2011). Diabetes and the risk of developing Parkinson's disease in Denmark. *Diabetes Care* 34, 1102–1108. doi: 10.2337/dc10-1333

Schubert, M., Gautam, D., Surjo, D., Ueki, K., Baudler, S., Schubert, D., et al. (2004). Role for neuronal insulin resistance in neurodegenerative diseases. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3100–3105. doi: 10.1073/pnas.0308724101

Sekar, S., and Taghibiglou, C. (2018). Elevated nuclear phosphatase and tensin homolog (PTEN) and altered insulin signaling in substantia nigral region of patients with Parkinson's disease. *Neurosci. Lett.* 666, 139–143. doi: 10.1016/j.neulet.2017.12.049

Sharma, A. N., Elased, K. M., and Lucot, J. B. (2012). Rosiglitazone treatment reversed depression- but not psychosis-like behavior of db/db diabetic mice. *J. Psychopharmacol.* 26, 724–732. doi: 10.1177/0269881111434620

Sharma, S., and Taliyan, R. (2018). High fat diet feeding induced insulin resistance exacerbates 6-OHDA mediated neurotoxicity and behavioral abnormalities in rats. *Behav. Brain Res.* 351, 17–23. doi: 10.1016/j.bbr.2018.05.025

Sharma, S. K., Chorell, E., Steneberg, P., Vernersson-Lindahl, E., Edlund, H., and Wittung-Stafshede, P. (2015). Insulin-degrading enzyme prevents alpha-synuclein fibril formation in a nonproteolytical manner. *Sci. Rep.* 5:12531. doi: 10.1038/srep12531

Shokrzadeh, M., Mirshafa, A., Yekta Moghaddam, N., Birjandian, B., and Shaki, F. (2018). Mitochondrial dysfunction contribute to diabetic neurotoxicity induced by streptozocin in mice: protective effect of Urtica dioica and pioglitazone. *Toxicol. Mech. Methods* 28, 499–506. doi: 10.1080/15376516.2018.1459993

Simo, R., Ciudin, A., Simo-Servat, O., and Hernandez, C. (2017). Cognitive impairment and dementia: a new emerging complication of type 2 diabetes-The diabetologist's perspective. *Acta Diabetol.* 54, 417–424. doi: 10.1007/s00592-017-0970-975

Singh, B. S., Rajakumar, P. A., Eves, E. M., Rosner, M. R., Wainer, B. H., and Devaskar, S. U. (1997). Insulin gene expression in immortalized rat hippocampal and pheochromocytoma-12 cell lines. *Regul. Pept.* 69, 7–14. doi: 10.1016/s0167-0115(96)02120-9

Speed, N., Saunders, C., Davis, A. R., Owens, W. A., Matthies, H. J., Saadat, S., et al. (2011). Impaired striatal Akt signaling disrupts dopamine homeostasis and increases feeding. *PLoS One* 6:e25169. doi: 10.1371/journal.pone.0025169

Speed, N. K., Matthies, H. J., Kennedy, J. P., Vaughan, R. A., Javitch, J. A., Russo, S. J., et al. (2010). Akt-dependent and isoform-specific regulation of dopamine transporter cell surface expression. *ACS Chem. Neurosci.* 1, 476–481. doi: 10.1021/cn100031t

Sreenan, S., Sturis, J., Pugh, W., Burant, C. F., and Polonsky, K. S. (1996). Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. *Am. J. Physiol.* 271(4 Pt 1), E742–E747. doi: 10.1152/ajpendo.1996.271.4.E742

Sun, Y., Chang, Y. H., Chen, H. F., Su, Y. H., Su, H. F., and Li, C. Y. (2012). Risk of Parkinson disease onset in patients with diabetes: a 9-year population-based cohort study with age and sex stratifications. *Diabetes Care* 35, 1047–1049. doi: 10.2337/dc11-1511

Svenningsson, P., Wirdefeldt, K., Yin, L., Fang, F., Markaki, I., Efendic, S., et al. (2016). Reduced incidence of Parkinson's disease after dipeptidyl peptidase-4 inhibitors-A nationwide case-control study. *Mov. Disord.* 31, 1422–1423. doi: 10.1002/mds.26734

Swanson, C., and Emborg, M. (2014). Expression of peroxisome proliferator-activated receptor-gamma in the substantia nigra of hemiparkinsonian nonhuman primates. *Neurol. Res.* 36, 634–646. doi: 10.1179/1743132813Y.0000000305

Tain, L. S., Mortiboys, H., Tao, R. N., Ziviani, E., Bandmann, O., and Whitworth, A. J. (2009). Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. *Nat. Neurosci.* 12, 1129–1135. doi: 10.1038/nn.2372

Takahashi, M., Yamada, T., Tooyama, I., Moroo, I., Kimura, H., Yamamoto, T., et al. (1996). Insulin receptor mRNA in the substantia nigra in Parkinson's disease. *Neurosci. Lett.* 204, 201–204. doi: 10.1016/0304-3940(96)12357-0

Timmons, S., Coakley, M. F., Moloney, A. M., and O' Neill, C. (2009). Akt signal transduction dysfunction in Parkinson's disease. *Neurosci. Lett.* 467, 30–35. doi: 10.1016/j.neulet.2009.09.055

Tokutake, T., Kasuga, K., Yajima, R., Sekine, Y., Tezuka, T., Nishizawa, M., et al. (2012). Hyperphosphorylation of Tau induced by naturally secreted amyloid-beta at nanomolar concentrations is modulated by insulin-dependent Akt-GSK3beta signaling pathway. *J. Biol. Chem.* 287, 35222–35233. doi: 10.1074/jbc.M112.348300

Vanhanen, M., Kuusisto, J., Koivisto, K., Mykkanen, L., Helkala, E. L., Hanninen, T., et al. (1999). Type-2 diabetes and cognitive function in a non-demented population. *Acta Neurol Scand.* 100, 97–101. doi: 10.1111/j.1600-0404.1999.tb01045.x

Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M., and Andreelli, F. (2012). Cellular and molecular mechanisms of metformin: an overview. *Clin. Sci.* 122, 253–270. doi: 10.1042/CS20110386

Wahlqvist, M. L., Lee, M. S., Hsu, C. C., Chuang, S. Y., Lee, J. T., and Tsai, H. N. (2012). Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with Type 2 diabetes in a Taiwanese population cohort. *Parkinsonism Relat. Disord.* 18, 753–758. doi: 10.1016/j.parkreldis.2012.03.010

Wang, L., Zhai, Y. Q., Xu, L. L., Qiao, C., Sun, X. L., Ding, J. H., et al. (2014). Metabolic inflammation exacerbates dopaminergic neuronal degeneration in response to acute MPTP challenge in type 2 diabetes mice. *Exp. Neurol.* 251, 22–29. doi: 10.1016/j.expneurol.2013.11.001

Wang, S., Zhang, C., Sheng, X., Zhang, X., Wang, B., and Zhang, G. (2014). Peripheral expression of MAPK pathways in Alzheimer's and Parkinson's diseases. *J. Clin. Neurosci.* 21, 810–814. doi: 10.1016/j.jocn.2013.08.017

Wang, X., Yu, S., Hu, J. P., Wang, C. Y., Wang, Y., Liu, H. X., et al. (2014). Streptozotocin-induced diabetes increases amyloid plaque deposition in AD transgenic mice through modulating AGEs/RAGE/NF-kappaB pathway. *Int. J. Neurosci.* 124, 601–608. doi: 10.3109/00207454.2013.866110

Wilhelm, K. R., Yanamandra, K., Gruden, M. A., Zamotin, V., Malisauskas, M., Casaite, V., et al. (2007). Immune reactivity towards insulin, its amyloid and protein S100B in blood sera of Parkinson's disease patients. *Eur. J. Neurol.* 14, 327–334. doi: 10.1111/j.1468-1331.2006.01667.x

Williams-Gray, C. H., Evans, J. R., Goris, A., Foltynie, T., Ban, M., Robbins, T. W., et al. (2009). The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort. *Brain* 132(Pt 11), 2958–2969. doi: 10.1093/brain/awp245

Xu, L., Chen, X., Sun, B., Sterling, C., and Tank, A. W. (2007). Evidence for regulation of tyrosine hydroxylase mRNA translation by stress in rat adrenal medulla. *Brain Res.* 1158, 1–10. doi: 10.1016/j.brainres.2007.04.080

Yamada, N., Katsuura, G., Ochi, Y., Ebihara, K., Kusakabe, T., Hosoda, K., et al. (2011). Impaired CNS leptin action is implicated in depression associated with obesity. *Endocrinology* 152, 2634–2643. doi: 10.1210/en.2011-2014

Yang, L., Chen, Z., Li, B., Wang, M., Yu, L., Wan, Y., et al. (2017). Multiple evidences for association between cognitive impairment and dysglycemia in Parkinson's disease: implications for clinical practice. *Front. Aging Neurosci.* 9:355. doi: 10.3389/fnagi.2017.00355

Yang, L., Wang, H., Liu, L., and Xie, A. (2018). The role of insulin/IGF-1/PI3K/Akt/GSK3beta signaling in parkinson's disease dementia. *Front. Neurosci.* 12:73. doi: 10.3389/fnins.2018.00073

Yazbeck, R., Howarth, G. S., and Abbott, C. A. (2009). Dipeptidyl peptidase inhibitors, an emerging drug class for inflammatory disease? *Trends Pharmacol. Sci.* 30, 600–607. doi: 10.1016/j.tips.2009.08.003

Zhao, W. Q., Chen, H., Quon, M. J., and Alkon, D. L. (2004). Insulin and the insulin receptor in experimental models of learning and memory. *Eur. J. Pharmacol.* 490, 71–81. doi: 10.1016/j.ejphar.2004.02.045

Zhao, W. Q., and Townsend, M. (2009). Insulin resistance and amyloidogenesis as common molecular foundation for type 2 diabetes and Alzheimer's disease. *Biochim. Biophys. Acta* 1792, 482–496. doi: 10.1016/j.bbadis.2008.10.014

Zochodne, D., and Malik, R. A. (2014). "Diabetes and the nervous system," in *Handbook of Clinical Neurology*, Vol. 126, eds D. Zochodne and R. Malik (Amsterdam: Elsevier), 1–640.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 28 August 2019
doi: 10.3389/fnins.2019.00843

Type-2-Diabetes Alters CSF but Not Plasma Metabolomic and AD Risk Profiles in Vervet Monkeys

Kylie Kavanagh[1,2†], Stephen M. Day[3†], Morgan C. Pait[3], William R. Mortiz[4], Christopher B. Newgard[5], Olga Ilkayeva[5], Donald A. Mcclain[6] and Shannon L. Macauley[3]*

[1] Department of Pathology, Wake Forest School of Medicine, Winston-Salem, NC, United States, [2] College of Health and Medicine, University of Tasmania, Hobart, TAS, Australia, [3] Section of Gerontology and Geriatric Medicine, Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC, United States, [4] Department of Neurology, Washington University School of Medicine, St. Louis, MO, United States, [5] Sarah W. Stedman Nutrition and Metabolism Center, Duke Molecular Physiology Institute, Duke University Medical Center, Durham, NC, United States, [6] Section of Endocrinology and Metabolism, Wake Forest School of Medicine, Winston-Salem, NC, United States

Epidemiological studies suggest that individuals with type 2 diabetes (T2D) have a twofold to fourfold increased risk for developing Alzheimer's disease (AD), however, the exact mechanisms linking the two diseases are unknown. In both conditions, the majority of pathophysiological changes, including glucose and insulin dysregulation, insulin resistance, and AD-related changes in Aβ and tau, occur decades before the onset of clinical symptoms and diagnosis. In this study, we investigated the relationship between metabolic biomarkers associated with T2D and amyloid pathology including Aβ levels, from cerebrospinal fluid (CSF) and fasting plasma of healthy, pre-diabetic (PreD), and T2D vervet monkeys (*Chlorocebus aethiops sabaeus*). Consistent with the human disease, T2D monkeys have increased plasma and CSF glucose levels as they transition from normoglycemia to PreD and diabetic states. Although plasma levels of acylcarnitines and amino acids remained largely unchanged, peripheral hyperglycemia correlated with decreased CSF acylcarnitines and CSF amino acids, including branched chain amino acid (BCAA) concentrations, suggesting profound changes in cerebral metabolism coincident with systemic glucose dysregulation. Moreover, CSF $A\beta_{40}$ and CSF $A\beta_{42}$ levels decreased in T2D monkeys, a phenomenon observed in the human course of AD which coincides with increased amyloid deposition within the brain. In agreement with previous studies in mice, CSF $A\beta_{40}$ and CSF $A\beta_{42}$ were highly correlated with CSF glucose levels, suggesting that glucose levels in the brain are associated with changes in Aβ metabolism. Interestingly, CSF $A\beta_{40}$ and CSF $A\beta_{42}$ levels were also highly correlated with plasma but not CSF lactate levels, suggesting that plasma lactate might serve as a potential biomarker of disease progression in AD. Moreover, CSF glucose and plasma lactate levels were correlated with CSF amino acid and acylcarnitine levels, demonstrating alterations in cerebral metabolism occurring with the onset of T2D. Together, these data suggest that peripheral metabolic changes associated with the development of T2D produce alterations in brain metabolism that lead to early changes in the amyloid cascade, similar to those observed in pre-symptomatic AD.

Keywords: metabolomics, type 2 diabetes, Alzheimer's disease, amyloid-beta, CSF, amino acids, acylcarnitine, hyperglycemia

OPEN ACCESS

Edited by:
Eugenio Barone, Sapienza University of Rome, Italy

Reviewed by:
Erik Steven Musiek, Washington University School of Medicine, United States
Lori Beason-Held, National Institute on Aging (NIA), United States

****Correspondence:***
Shannon L. Macauley
smacaule@wakehealth.edu

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *07 June 2019*
Accepted: *26 July 2019*
Published: *28 August 2019*

Citation:
Kavanagh K, Day SM, Pait MC, Mortiz WR, Newgard CB, Ilkayeva O, Mcclain DA and Macauley SL (2019) Type-2-Diabetes Alters CSF but Not Plasma Metabolomic and AD Risk Profiles in Vervet Monkeys. Front. Neurosci. 13:843. doi: 10.3389/fnins.2019.00843

INTRODUCTION

Rates of type 2 diabetes (T2D) and Alzheimer's disease (AD) are reaching epidemic proportions and are expected to continue to rise over the next several decades (Holtzman et al., 2011). T2D is a metabolic disorder characterized by elevated fasting plasma glucose levels, increased insulin levels, insulin resistance, and beta cell dysfunction with the majority of changes occurring 5–10 years before clinical diagnosis (American Diabetes Association, 2010). Similarly, pathological hallmarks of AD, including the extracellular aggregation of amyloid β (Aβ) into amyloid plaques and the intracellular accumulation of the tau protein into neurofibrillary tangles (NFTs), begin decades before cognitive decline and clinical diagnosis (Hardy and Selkoe, 2002; Bateman et al., 2012; Musiek and Holtzman, 2015). While both are considered diseases of aging and mechanisms linking the two conditions remain elusive, epidemiological and cross-sectional studies suggest that individuals with T2D have a twofold to fourfold increased risk for developing AD and dementia and show increased AD pathology (Ott et al., 1999; Crane et al., 2013; Huang et al., 2014). Preclinical studies in mouse models of cerebral amyloidosis suggest that systemic hyperglycemia increases Aβ levels within the hippocampal interstitial fluid (ISF) by 25%; an effect that is amplified when plaques are already present in the brain during the hyperglycemia challenge (Macauley et al., 2015; Stanley et al., 2016). Moreover, mouse plasma glucose, ISF glucose, and ISF Aβ are highly correlated, and elevated glucose levels drive Aβ production in the hippocampus in an activity dependent manner (Macauley et al., 2015). Conversely, systemic hyperinsulinemia at post-prandial or supra-physiological levels, only modestly increase ISF Aβ levels. This suggests that changes in glucose, rather than insulin, correlate more closely with brain Aβ levels (Stanley et al., 2016). Although these studies suggest a mechanistic link between T2D and AD, rodent models of AD do not fully recapitulate the human course of disease, and it is important to translate these observations to primates. Similar to humans, many non-human primate species develop T2D and amyloid pathology with age (Wagner et al., 2006; Latimer et al., 2019), and thus represent an important translational tool for examining the metabolic relationship between the two conditions.

Branched chain amino acids (BCAAs), including leucine, isoleucine, and valine, are essential amino acids necessary for protein synthesis, but when found in excess, impact energy homeostasis (Shimomura and Kitaura, 2018; Hudd et al., 2019; White and Newgard, 2019). Recent work demonstrated that elevated dietary BCAA intake is associated with obesity and insulin resistance in both humans and rodents (Newgard et al., 2009; Solon-Biet et al., 2019), and plasma BCAA levels are highly predictive of T2D development in normoglycemic individuals (Wang et al., 2011). Elevated levels of circulating BCAAs are associated with suppressed mitochondrial β-oxidation, reduced glucose tolerance, increased insulin resistance, and increased *de novo* lipogenesis, making BCAAs a potential biomarker of metabolic disease (Newgard et al., 2009; Weiss and Lustig, 2014). BCAAs are also integral to healthy brain function, due to their roles in neurotransmitter biosynthesis, protein synthesis, and energy production. Alterations in BCAA levels in plasma and CSF have been implicated in AD pathology, with conflicting evidence on whether they are helpful or harmful to disease progression (Griffin and Bradshaw, 2017). Nevertheless, alterations in energy homeostasis and BCAA catabolism represent one potential link between T2D and AD.

Acylcarnitines are byproducts of mitochondrial fatty acid, amino acid and glucose catabolism that serve as useful biomarkers of metabolic changes (Jones et al., 2010). Changes in the plasma acylcarnitine profile have been observed in obesity, T2D, and insulin resistance, representing alterations in several metabolic pathways (Jones et al., 2010; Schooneman et al., 2013). Moreover, acylcarnitines are key energy substrates in the brain, especially during fasting conditions when glucose levels are low (Jones et al., 2010). In AD patients, plasma levels of acylcarnitines are decreased, suggesting perturbations in energy metabolism that may be central to AD pathogenesis (Cristofano et al., 2016).

Here, we applied comprehensive metabolic profiling tools to healthy control (Ctrl), pre-diabetic (PreD), and diabetic (T2D) monkeys to explore the relationship between T2D and amyloid pathology. We utilized a cohort of aging vervet monkeys (*Chlorocebus aethiops sabaeus)*, which develop neuropathological changes consistent with human AD pathology including increased amyloid plaque burden, elevated cortical tau levels and paired helical filament tau immunoreactivity, Aβ-related vascular impairment, reduced cerebral metabolism, regional atrophy, decreased CSF $A\beta_{42}$ and increased CSF tau levels (Kalinin et al., 2013; Chen et al., 2018; Latimer et al., 2019), to ensure translational relevance of our findings. We analyzed plasma and CSF amino acids and acylcarnitine concentrations and explored how these changes related to CSF $A\beta_{40}$ and $A\beta_{42}$ levels, which are established biomarkers of disease in AD.

MATERIALS AND METHODS

Animals

The monkeys used in this study were sourced from a multigenerational pedigreed colony of vervet monkeys (*Chlorocebus aethiops sabaeus*; age = 16.5–23.5 years old). Veterinary and research staff categorized the monkeys as either healthy (Ctrl; $n = 4$), pre-diabetic (PreD; $n = 4$), or type-2 diabetic (T2D; $n = 5$) according to repeated fasting glucose measurements and American Diabetes Association criteria (American Diabetes Association, 2010) and were selected to be matched by age, bodyweight, and adiposity as measured by waist circumference. PreD and T2D categorization was only made after ≥2 consecutive fasting glucose values were ≥100 mg/dL or ≥126 mg/dL, respectively. T2D monkeys were maintained with insulin therapy, and all T2D monkeys in study had been diagnosed and treated for a minimum of 2 years. Monkeys were fed a commercial laboratory primate chow diet (Laboratory Diet 5038; LabDiet., St. Louis, MO, United States), with daily supplemental fresh fruits and vegetables. This standard laboratory diet is comprised of 13% calories from fat; 69% calories from carbohydrates; and 18% of calories from protein. All samples were collected

after 16 h fasting and withdrawal from all exogenous insulin. Cerebrospinal fluid (CSF) was collected via puncture of the atlanto-occipital space, and plasma samples were collected from the femoral vein.

All animal procedures were performed on a protocol approved by the Wake Forest University Institutional Animal Care and Use Committee according to recommendations in the Guide for Care and Use of Laboratory Animals (Institute for Laboratory Animal Research) and in compliance with the USDA animal Welfare Act and Animal Welfare Regulations (Animal Welfare Act as Amended; Animal Welfare Regulations).

AD Biomarkers

$A\beta_{40}$ and $A\beta_{42}$ levels from CSF samples were assayed using sandwich ELISAs as previously described (Bero et al., 2011; Roh et al., 2012). Briefly, $A\beta_{40}$ and $A\beta_{42}$ were quantified using monoclonal capture antibodies (a generous gift from David Holtzman) targeted against amino acids 45–50 (HJ2) or 37–42 (HJ7.4), respectively. For detection, both $A\beta_{40}$ and $A\beta_{42}$ used a biotinylated monoclonal antibody against the central domain (HJ5.1B), followed by incubation with streptavidin-poly-HRP-40. Assays were developed using Super Slow TMB (Sigma) and the plates read on a Bio-Tek Synergy 2 plate reader at 650 nm.

Metabolomics, Lipids, Glucose, and Lactate Measures

Glucose and lactate measurements within the plasma and CSF were quantified using a YSI 2900 analyzer as previously described (Macauley et al., 2015). A detailed description of blood and CSF sample preparation and coefficients of variation for these assays has been published (Haqq et al., 2005; Solon-Biet et al., 2019). Insulin was measured by ELISA (Mercodia, Uppsala, Sweden) in plasma and CSF samples. Total cholesterol, high density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglycerides were measured with kits from Roche Diagnostics (Indianapolis, IN, United States) and free fatty acids (total) and ketones (total and 3-hydroxybutyrate) with kits from Wako (Richmond, VA, United States). ApoB associated cholesterol was calculated as the total cholesterol minus HDLC. Plasma and CSF acylcarnitines and amino acids were analyzed by MS/MS as described previously (Millington et al., 1990; Chace et al., 1995; An et al., 2004; Wu et al., 2004; Ferrara et al., 2008).

Data Analysis

Data were analyzed using one-way ANOVA and correlations were determined by Pearson's correlation coefficient, *r*. To determine the relative relationship between CSF Glucose, CSF $A\beta_{42}$, and plasma lactate and CSF analytes, we transformed each data point to represent its value relative to the control group mean [% control mean value = $100^*(x/\text{control mean})$, where x = any given data point]. Data are represented as means ± SEM. Tukey's *post hoc* tests were used when appropriate.

RESULTS

Metabolic Profile of Normoglycemic, Pre-diabetic, and T2D Monkeys

Monkeys were older, ranging from 16 to 23 years (**Table 1**), which represents the last 30% of lifespan for this species and is a typical age range for the onset of metabolic diseases and neuropathological changes associated with AD (Kalinin et al., 2013; Chen et al., 2018; Latimer et al., 2019). There were no differences in body weight or waist circumference between groups (**Table 1**). PreD and T2D monkeys had elevated fasting blood glucose levels compared to normoglycemic controls [**Table 1**; $p < 0.0001$, $F(2,9) = 39.17$], but there were no differences in fasting insulin levels (**Table 1**). While HOMA scores were elevated in PreD and T2D monkeys, the differences were not significant (**Table 1**). Additionally, lipid measures illustrated higher triglycerides in T2D monkeys compared to PreD or Ctrl. Together, elevated fasting blood glucose was the most notable finding delineating the Ctrl, PreD, and T2D cohorts.

Increased CSF Glucose Levels Correlated With Decreased CSF $A\beta_{40}$ and $A\beta_{42}$ Concentrations in T2D Monkeys

T2D monkeys have elevated plasma glucose [**Figure 1A**; $p = 0.0036$, $F(2,10) = 10.39$] and CSF glucose concentrations [**Figure 1B**; $p < 0.0001$, $F(2,9) = 32.31$] compared to normoglycemic controls, while PreD had intermediate values. Data analysis revealed that plasma and CSF glucose levels had a strong positive correlation (**Figure 1C**; $p < 0.0002$, $r = 0.8739$, $R^2 = 0.7637$), which is consistent with observations from preclinical rodent models (Macauley et al., 2015). We also observed that plasma lactate levels were lower in T2D monkeys [**Figure 1D**; $p = 0.0395$, $F(2,10) = 4.544$], however, no differences in CSF lactate concentrations were observed (**Figure 1E**). We previously demonstrated that hyperglycemic APP/PS1 mice, a model of cerebral amyloidosis, have elevated Aβ within the brain's ISF (Macauley et al., 2015; Stanley et al., 2016), and non-human primates with T2D have increased Aβ deposition in several brain regions (Okabayashi et al., 2015). T2D monkeys have decreased CSF $A\beta_{40}$ [**Figure 1F**; $p = 0.0280$, $F(2,9) = 5.030$], and CSF $A\beta_{42}$ concentrations [**Figure 1J**; $p = 0.0342$, $F(2,9) = 5.463$]. Interestingly, both CSF $A\beta_{40}$ and CSF $A\beta_{42}$ were highly correlated with CSF glucose (**Figure 1G**; $p = 0.0400$, $r = -0.5979$, $R^2 = 0.3575$ and **Figure 1K**; $p = 0.0489$, $r = -0.5782$, $R^2 = 0.3343$, respectively) and plasma lactate (**Figure 1H**; $p = 0.0027$, $r = 0.7820$, $R^2 = 0.6115$ and **Figure 1L** $p = 0.0013$, $r = 0.8133$, $R^2 = 0.6614$; respectively) but not with plasma insulin (**Figures 1I,M**). CSF insulin levels were undetectable. Given that decreased CSF Aβ is indicative of increased plaque formation within the brain (Tapiola et al., 2009), these data indicate that T2D monkeys display biomarkers of early amyloid deposition and pre-symptomatic AD (Kalinin et al., 2013; Chen et al., 2018; Latimer et al., 2019) triggered by a state of energy dysregulation, which is consistent with previous work in rodent models (Macauley et al., 2015; Stanley et al., 2016).

TABLE 1 | Demographic and metabolic characteristics of monkeys included in study.

	Ctrl Mean (SEM)	PreD Mean (SEM)	T2D Mean (SEM)	One-Way ANOVA
Age (years)	20.25 (±1.109)	18.75 (±0.6292)	18.00 (±1.304)	$p = 0.3800$
Body weight (kg)	5.553 (±0.5525)	6.618 (±0.9210)	5.414 (±0.4937)	$p = 0.4100$
Waist circumference (cm)	36.98 (±1.719)	41.94 (±3.861)	38.08 (±3.441)	$p = 0.5283$
Fasting glucose (mg/dL)	67.84 (±5.097)	113.80 (±4.308)	161.10 (±11.05)	$p < 0.0001$
Fasting insulin (μIU/mL)	20.83 (±4.806)	37.43 (±8.854)	26.63 (±8.191)	$p = 0.3284$
HOMA Score (AU)	3.613 (±1.048)	10.61 (±2.721)	10.64 (±3.525)	$p = 0.1512$
TPC (mg/dL)	171.3 (±10.06)	198.9 (±33.75)	164.3 (±9.067)	$p = 0.4945$
Triglyceride (mg/dL)	74.33 (±2.541)	59.75 (±7.417)	103.80 (±16.19)	$p = 0.0407$
HDLC (mg/dL)	60.59 (±4.389)	65.50 (±5.939)	59.83 (±3.657)	$p = 0.6702$
ApoB-associated cholesterol (mg/dL)	110.70 (±6.232)	95.22 (±8.152)	104.40 (±6.809)	$p = 0.3633$
TPC/HDLC	2.845 (±0.093)	2.360 (±0.130)	2.785 (±0.148)	$p = 0.0646$

Ctrl = Control; PreD = Pre-Diabetic; T2D = Type-2 Diabetic; HOMA = Homeostatic model assessment. TPC = Total plasma cholesterol; HDLC = High-density lipoprotein cholesterol.

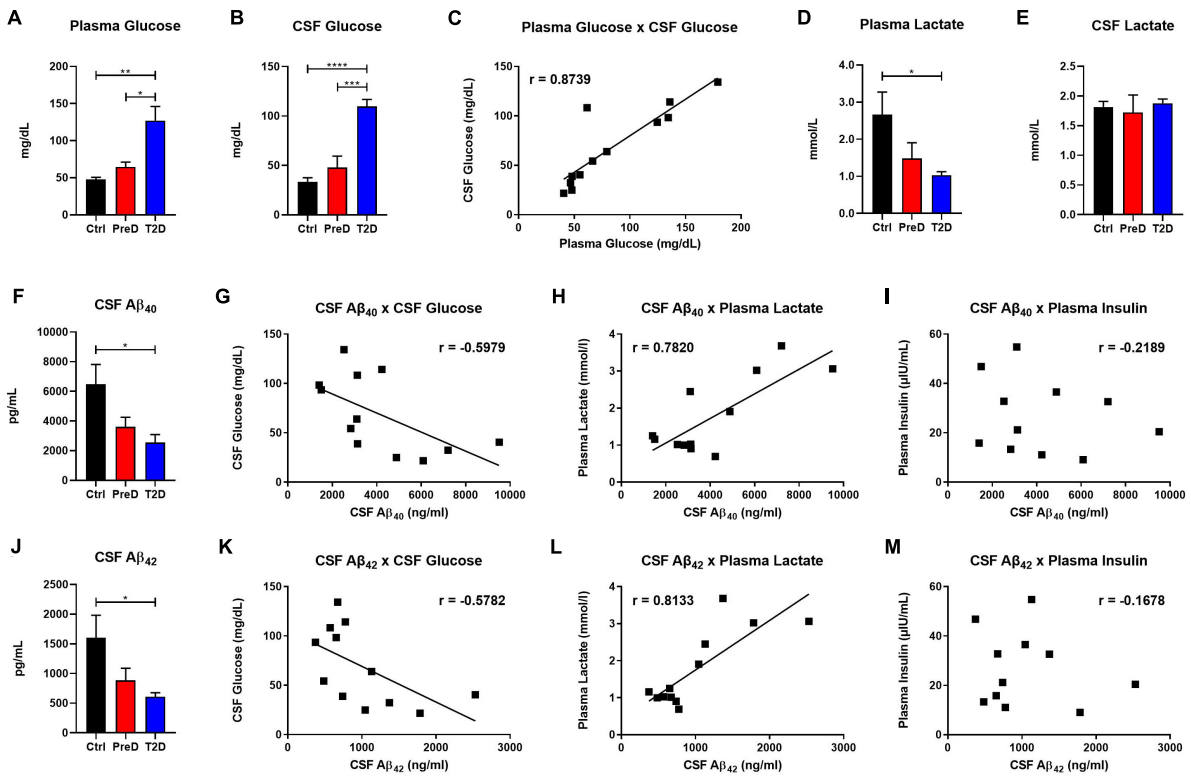

FIGURE 1 | Type 2 diabetes (T2D) monkeys have significantly decreased cerebrospinal fluid (CSF) $A\beta_{40}$ and CSF $A\beta_{42}$ concentrations, which is correlated with CSF glucose and plasma lactate concentrations. **(A)** plasma glucose and **(B)** CSF glucose concentrations were significantly elevated in T2D monkeys. **(C)** Plasma and CSF glucose concentrations were significantly correlated. **(D)** T2D monkeys had significantly decreased plasma lactate, but not **(E)** CSF lactate. **(F)** T2D monkeys had significantly decreased $A\beta_{40}$, which was correlated with **(G)** CSF glucose and **(H)** plasma lactate, but not **(I)** plasma insulin concentrations. **(J)** Similarly, T2D monkeys had significantly decreased $A\beta_{42}$ concentrations, which was correlated with **(K)** CSF glucose and **(L)** plasma lactate, but not **(M)** plasma insulin concentrations.*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$; one-way ANOVA with Tukey's *post hoc* test. Values represent mean ± SEM; solid lines represent statistically significant correlations ($p < 0.05$).

T2D and PreD Monkey Show Decreased Amino Acid and Acylcarnitines Levels in the CSF but Not the Plasma

Because plasma amino acid and acylcarnitine levels are linked to metabolic dysfunction in T2D (Schooneman et al., 2013; Shin et al., 2014), the levels of amino acids (AA) in both the plasma and CSF were quantified to further explore the energy imbalance associated with T2D. In examining the AA concentrations by their functional groups, PreD and T2D monkeys had lower concentrations of amino acids in the CSF. Amino acids were further stratified into branched-chain AA [BCAAs, **Figure 2A**; $p = 0.0038$, $F(2,9) = 11.04$], total AA [**Figure 2B**; $p = 0.0081$, $F(2,9) = 8.638$], essential AA

[**Figure 2C**; $p = 0.0065$, $F(2,9) = 9.280$], aromatic AA [**Figure 2D**; $p = 0.0046$, $F(2,9) = 10.36$], and basic AA [**Figure 2E**; $p = 0.0087$, $F(2,9) = 8.421$] (**Supplementary Table S1**). In the CSF, all AA groups were lower in T2D, with the exception of acidic AA where no changes were detected (**Figure 2F**). No differences in plasma AA concentrations were detected in any of the AA categories (**Figures 2G–L**). Interestingly, when individual AAs were measured in plasma (**Supplementary Table S3** and **Supplementary Figure S2**), there was a trend toward an increase in levels of the BCAAs valine and leucine/isoleucine (**Supplementary Figure S2F**; $p = 0.0977$ and **Supplementary Figure S2M**; $p = 0.1675$, respectively), suggesting peripheral metabolic perturbations were present, although the differences in CSF amino acids (**Supplementary Figures S1A–O** and **Supplementary Table S4**), valine and leucine/isoleucine in particular, were more striking (**Supplementary Figure S1F**; $p = 0.0219$ and **Supplementary Figure S1M**; $p = 0.0524$).

Acylcarnitines are derived from the mitochondrial oxidation of fatty acids, carbohydrates, and amino acids (Schooneman et al., 2013). Several studies have shown that T2D patients have elevated plasma acylcarnitine concentrations compared to healthy controls (Muoio, 2014). Here, T2D monkeys had lower total acylcarnitine concentrations in the CSF [**Figure 3A**; $p = 0.0208$, $F(2,9) = 6.139$], but no differences in plasma total acylcarnitine levels (**Figure 3C**), a pattern consistent with the AA data (**Supplementary Table S2**). In the CSF, T2D monkeys also had lower short-chain acylcarnitine concentrations [**Figure 3B**; $p = 0.0245$, $F(2,9) = 5.764$]. However, due to variability in the control monkeys, medium- and long-chain acylcarnitine concentrations were consistently lower in T2D monkeys, but the difference did not reach significance (Muoio, 2014; **Figures 3C,D**). Again, plasma concentrations remained comparable between groups (**Figures 3F–H**). Together, this data suggests that fuel metabolism is altered in the brains of T2D monkeys compared to normoglycemic controls.

Metabolic Dysregulation in the Brain Is Associated With Changes in CSF Glucose, Plasma Glucose, CSF Aβ_{42}, and Plasma Lactate

Lastly, we investigated the relationship between differences in CSF amino acids and CSF acylcarnitines as a function of CSF glucose, CSF Aβ_{42}, and plasma lactate concentrations (**Figure 4**) to further elucidate the interaction between early metabolic changes in T2D with early biomarker alterations in AD. There was an overall negative relationship between CSF glucose and Aβ_{40}, Aβ_{42}, total AA, essential AA, BCAA, aromatic AA, basic AA, short-chain acylcarnitine, and total acylcarnitine (**Figure 4A**). Thus, as CSF glucose increases as observed in PreD and T2D, concentrations of amino acids, acylcarnitines, and Aβ all decrease in the CSF. Plasma glucose was correlated with CSF BCAA, total AA, essential AA, aromatic AA, and basic AA, but not CSF Aβ_{40}, CSF Aβ_{42}, or CSF acylcarnitines (**Figure 4B** and **Supplementary Table S6**). Next, CSF Aβ_{42} was correlated with CSF Aβ_{40} and CSF BCAA (**Figure 4C**), reinforcing the relationship between BCAAs and amyloid pathology. Lastly, plasma lactate concentrations correlated with CSF Aβ_{40} and Aβ_{42}, CSF essential AA and BCAA, and CSF short-chain and total acylcarnitine concentrations (**Figure 4D**), demonstrating plasma lactate might be a potential biomarker for early changes in T2D and pre-symptomatic AD. Taken together, alterations in cerebral metabolism co-vary with changes in plasma glucose, plasma lactate, and CSF Aβ_{42} which highlight the importance of metabolic changes in the pathogenesis of T2D and AD.

DISCUSSION

In this study, elevated fasting blood glucose levels associated with the onset of T2D elicit changes in brain metabolism and correlate with changes in the amyloid cascade, an early indicator of presymptomatic AD (Crane et al., 2013; Macauley et al., 2015). Aged monkeys (>19 years) can develop pathology consistent with human AD including increased amyloid plaque burden, elevated cortical tau levels and paired helical filament tau immunoreactivity, Aβ-related vascular damage, reduced cerebral metabolism, regional atrophy, and alterations in CSF biomarkers including both Aβ and tau (Kalinin et al., 2013; Chen et al., 2018; Latimer et al., 2019). Moreover, reductions in CSF Aβ levels correlate with increased amyloid plaque burden and insoluble Aβ levels in the cortex, similar to findings from human studies. In this study, T2D monkeys had lower CSF Aβ_{40} and Aβ_{42} levels, which is indicative of increased amyloid deposition within the brain (Tapiola et al., 2009). In agreement with previous rodent studies (Macauley et al., 2015; Stanley et al., 2016), CSF Aβ_{40} and Aβ_{42} were highly correlated with CSF glucose levels, which suggests that increased glucose may be driving Aβ production and aggregation in these animals. Interestingly, CSF Aβ_{40} and Aβ_{42} levels also highly correlated with plasma lactate levels, which is consistent with published studies that show decreased plasma lactate correlates with AD severity (Lu et al., 2015; Verri et al., 2018). Moreover, T2D vervet monkeys had lower CSF acylcarnitine and CSF amino acids, while plasma levels were largely unchanged, suggesting either early changes in cerebral metabolism with the onset of T2D or changes in the transport of certain metabolic fuels to the brain. Reduced CSF Aβ_{40} and Aβ_{42} levels in T2D monkeys correlated with higher plasma and CSF glucose concentrations, suggesting increased amyloid plaques are related to glucose dysregulation. Lastly, we showed that CSF amino acids and acylcarnitines were negatively correlated with CSF glucose and positively correlated with CSF Aβ_{40}, CSF Aβ_{42}, and plasma lactate. Together, these data suggest that peripheral metabolic changes associated with diabetogenesis co-occur with alterations in brain metabolism. Moreover, these metabolic changes are associated with activation of the amyloid cascade typically observed in humans with pre-symptomatic AD. Future postmortem studies in T2D monkeys should examine tau hyperphosphorylation, NFTs, and amyloid plaques in addition to changes in CSF Aβ levels to further strengthen the connection between T2D and AD pathology.

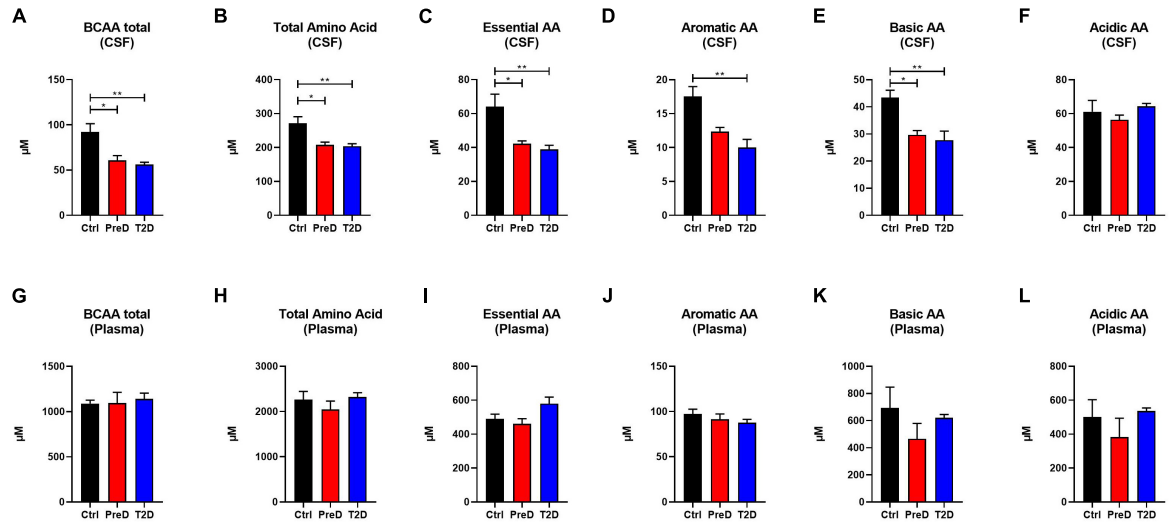

FIGURE 2 | Amino acid(AA) concentrations are decreased in the cerebrospinal fluid (CSF) but not plasma of type 2 diabetes (T2D) monkeys. **(A)** T2D monkeys had significantly decreased branch chain amino acids (BCAA), **(B)** total amino acid concentrations, **(C)** essential AAs, **(D)** aromatic AAs, and **(E)** basic AAs, but not **(F)** acidic AAs. **(G–L)** Conversely, plasma amino acid concentrations were not different between groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$; one-way ANOVA with Tukey's *post hoc* test. Values represent mean ± SEM.

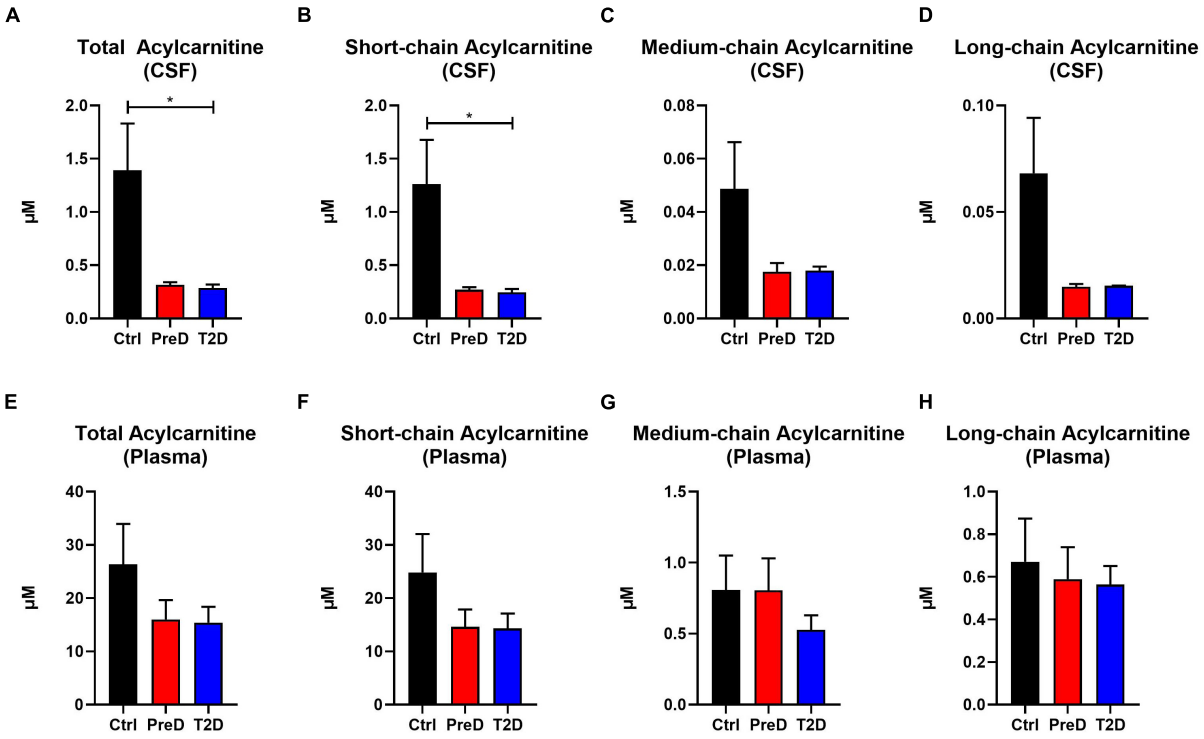

FIGURE 3 | Acylcarnitine concentrations are decreased in the cerebrospinal fluid (CSF) but not plasma of type 2 diabetes (T2D) monkeys. **(A,B)** Total and short-chain acylcarnitine concentrations were significantly decreased in the plasma of T2D and IR monkeys, **(C,D)**, however, medium- and long-chain acylcarnitines were not significantly different. **(E–H)** There were no differences in plasma acylcarnitine concentrations between groups.*$p < 0.05$; one-way ANOVA with Tukey's *post hoc* test. Values represent mean ± SEM.

Our data further supports existing evidence that chronic hyperglycemia and metabolic dysfunction are a pathological link between T2D and AD. In humans, hyperglycemia increases dementia risk in both patients with and without diabetes, causes rapid progression from mild cognitive impairment (MCI) to symptomatic AD, and increases the rate of amyloid accumulation in the brain (Crane et al., 2013; Morris et al., 2014). Moreover, hyperglycemia and increased HbA1c levels correlate with

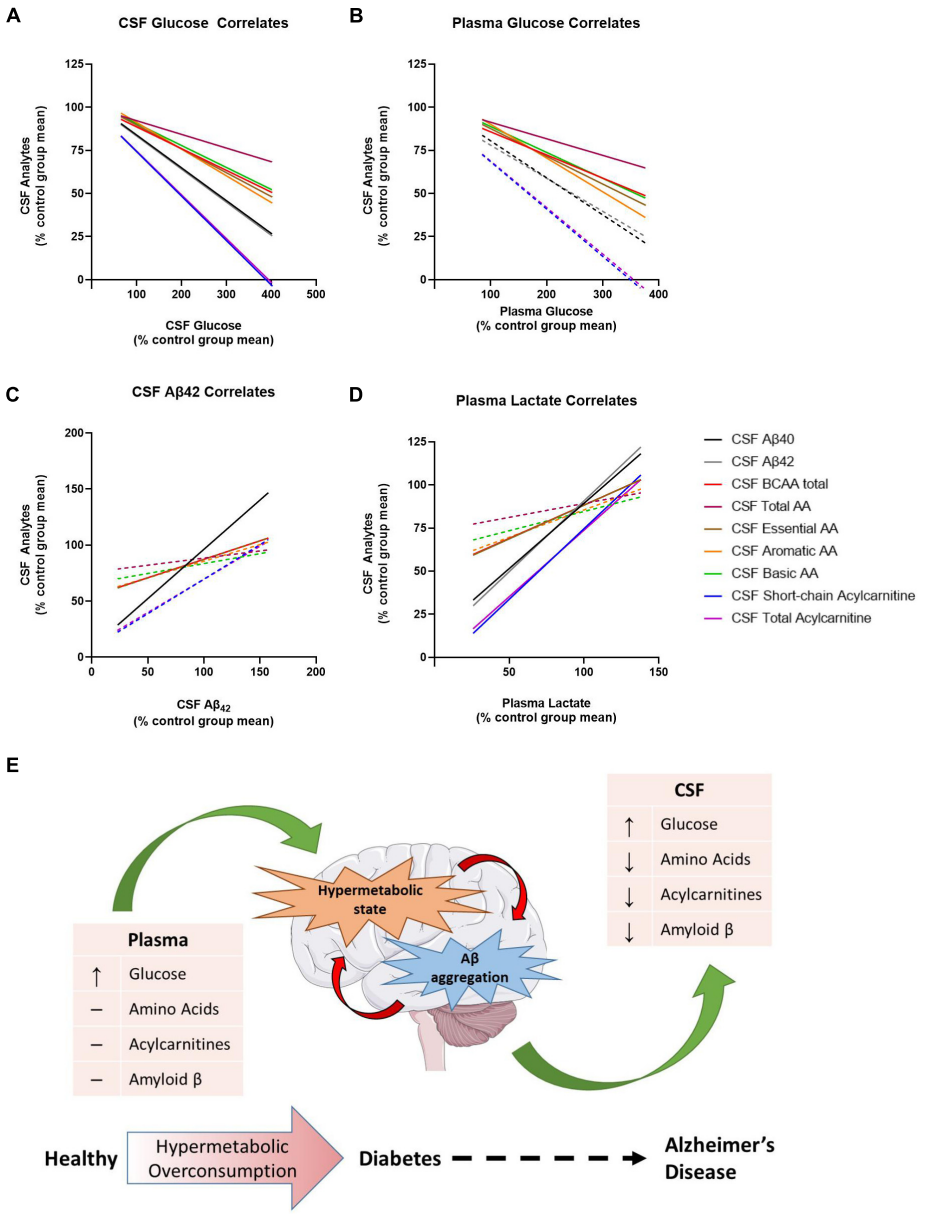

FIGURE 4 | Metabolic dysregulation is associated with changes in plasma lactate, cerebrospinal fluid (CSF) glucose, and CSF $A\beta_{40}$ and $A\beta_{42}$. Here, each data point has been converted into a value that represents the % of the control group's mean [value = 100*(*x*/control group mean)]. **(A)** Normalized CSF glucose values were negatively correlated with several normalized CSF analytes. **(B)** Normalized plasma glucose were negatively correlated with normalized CSF amino acids, but not normalized CSF $A\beta_{40}$, $A\beta_{42}$, or acylcarnitines. **(C)** Normalized CSF $A\beta_{42}$ was correlated with normalized CSF analytes. **(D)** Normalized plasma lactate values were positively correlated with several normalized CSF analytes. **(E)** Thus, the model we propose here is that type 2 diabetes (T2D) moves the brain into a state of hypermetabolic overconsumption wherein the brain consumes increased energy, leading to lower concentrations of $A\beta_{42}$ in the CSF, which is indicative of increased Aβ aggregation. Lines represent non-linear regression best fit curve; solid lines indicate statistical significance.

memory impairment, decreased functional connectivity, and increased neuronal loss, independent of T2D or AD diagnosis (Zheng et al., 2018). Data from T2D monkeys illustrates the same phenomenon; elevated blood glucose levels increase CSF glucose which correlates with changes in CSF Aβ levels, presumably due to the sequestration of Aβ into amyloid plaques in the brain (Bateman et al., 2006). Our findings in the T2D monkeys also uncovered an interesting relationship between glucose, lactate, and Aβ which supports previous findings from rodent studies. Preclinical studies in mouse models of cerebral amyloidosis demonstrate that synaptic release of Aβ occurs in an activity dependent manner, where high levels of synaptic activity increase Aβ secretion (Cirrito et al., 2003; Cirrito et al., 2005; Bateman et al., 2006; Cirrito et al., 2008). Increased synaptic activity not only drives ISF Aβ release but also the release of lactate into the extracellular space (Bero et al., 2011). According to the astrocyte neuron lactate shuttle, lactate is a preferred energy source for neurons to sustain excitatory neurotransmission and

levels of ISF lactate correlate with neuronal activity. Our previous work demonstrated that hyperglycemia not only increases ISF glucose and ISF Aβ but also ISF lactate, illustrating that increased metabolic activity is linked with increased synaptic activity and Aβ release. Since a direct measure of lactate production in the brain of T2D monkeys was unattainable in this study, we explored how plasma and CSF levels changed with peripheral hyperglycemia. Interestingly, plasma lactate, but not CSF lactate, correlated with changes in CSF glucose and Aβ. In accordance with our previous work, we hypothesize that increased glucose metabolism is increasing neuronal activity within the brain and driving both the production of Aβ and the consumption of pyruvate and lactate as fuel. Although the changes in plasma lactate levels could be due to alterations in peripheral metabolism in the T2D monkeys, we propose a different mechanism where increased lactate consumption in the brain signifies a hyperactive and hypermetabolic brain state present in both T2D and AD (**Figure 4E**). Since the concentration gradient for lactate favors transport from brain to blood (Raichle et al., 1970), decreased plasma lactate levels could reflect increased neuronal activity, lactate consumption, and Aβ production in the brain, which also makes plasma lactate levels a potential serum biomarker for AD, T2D, or both. In humans, a small cohort study established that serum lactate levels decreased in symptomatic AD, yet the authors attributed this finding to alterations in muscle metabolism, not brain (Verri et al., 2018). Because the majority of the changes in lactate were found in the plasma and not the CSF, an alternative hypothesis is that T2D suppresses neuronal activity leading to the decreased CSF $A\beta_{40}$ and $A\beta_{42}$ concentrations. However, this is unlikely given that CSF Aβ was significantly decreased in T2D monkeys relative to healthy controls in spite of the unaltered CSF lactate concentrations. Thus, additional studies are needed in order to elucidate the role of plasma lactate in T2D and AD.

In the current study, we demonstrated that T2D monkeys have lower CSF acylcarnitine and amino acid concentrations (**Figures 2**, **3**). Several studies demonstrated that circulating levels of amino acids are positively correlated with obesity, insulin resistance, metabolic dysfunction, and T2D in humans and rodents (Newgard et al., 2009; Wang et al., 2011; Solon-Biet et al., 2019). Although our data demonstrates a trend toward an increase in the BCAAs valine and isoleucine/leucine, no differences in plasma amino acid concentrations were detected in PreD or T2D monkeys. This may be explained by the fact that the T2D monkeys in this study were fed a well-controlled, balanced diet that did not replicate the traditional nutritional overconsumption seen humans with metabolic syndrome and T2D. Although the T2D monkeys were hyperglycemic, their insulin levels were unchanged, suggesting that the hyperglycemia may arise via a mechanism independent of insulin resistance. Studies reporting elevated plasma BCAAs in humans have involved obese and insulin resistant subjects (Newgard et al., 2009; Solon-Biet et al., 2019). Therefore, future studies should explore the relationship between plasma and CSF amino acid levels in a non-human primate model of dietary induced metabolic syndrome and T2D.

Another explanation for the difference in CSF AA levels could be that the T2D brain is overconsuming amino acids as fuel or rapidly increasing protein synthesis. While glucose is the primary source of energy for the brain, the brain can readily use fatty-acids as energy substrates; however, this typically occurs with decreased glucose availability, such as fasting or starvation (Costa et al., 1999). This could lead the brain to a state of hypermetabolic overconsumption if both glucose and fatty acid metabolism were upregulated. Furthermore, because many of the amino acids consumed by the brain are necessary for neurotransmitter biosynthesis or neurotransmission itself (Sperringer et al., 2017), we propose that increased amino acid consumption increases both synaptic activity and metabolic activity, leading to elevated Aβ production, oxidative stress, and Aβ aggregation. Our current data cannot discern if the decrease in amino acids and acylcarnitines in the CSF is due to increased oxidation, or by another means, such as altered amino acid transport (**Supplementary Figure S3** and **Supplementary Table S5**), therefore additional studies will need to further elucidate mechanisms underlying the changes in CSF metabolites.

CONCLUSION

The data presented here show that in the progression from healthy to PreD to T2D, the brain moves into a state of altered metabolism that results in an increase in glucose and lowering of amino acids and acylcarnitines in the CNS. Increased cerebral metabolism drives Aβ production and accelerates Aβ aggregation, which reciprocally escalates the disease cascades in T2D and AD. These findings shed further light on the metabolic link between T2D and amyloid pathology and how T2D progression could lead to AD-related pathology and cognitive decline.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or **Supplementary Files**.

ETHICS STATEMENT

Animal subjects: The animal study was reviewed and approved by Wake Forest University Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

KK, DM, SM, CN, and OI conceived on the experimental design. KK, MP, WM, CN, OI, and SM performed the experiments. SD, KK, CN, OI, DM, and SM analyzed the data and wrote the manuscript.

FUNDING

We would like to acknowledge the following grants: 1K01AG050719 (SM), R01AG061805 (SM), NCDRC Pilot Award (SM), and this work was supported by the Wake Forest University Alzheimer's Disease Research Center (P30 AG049638), funded by the National Institute on Aging (SM). We also gratefully acknowledge use of the Wake Forest Nonhuman Primate Program (KK), funded by the National Center for Advancing Translational Sciences and Office of the Director: UL1TR001420 and P40OD010965.

ACKNOWLEDGMENTS

We would like to thank Dr. David Holtzman for the ELISA antibodies.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00843/full#supplementary-material

FIGURE S1 | T2D monkeys had significantly decreased levels of several essential, branched chain, and aromatic amino acids in the CSF. **(A–E)** T2D and PreD monkeys had significantly decreased glycine, proline, methionine, aspartate/asparagine, and tyrosine. **(F–J)** T2D monkeys had significantly decreased valine, phenylalanine, ornithine, citrulline, and arginine. **(K–O)** There were no differences in alanine, serine, leucine/isoleucine, histidine, or glutamine/glutamic acid between groups.

FIGURE S2 | There were no differences in amino acid concentrations between groups in the plasma. **(A–O)** Plasma amino acid concentrations were similar between Ctrl, PreD, and T2D monkeys.

FIGURE S3 | Amino acids carried by large neutral amino acid or L-type transporters, but not A, N, or EAAT transporters, were decreased in the CSF of T2D monkeys. **(A,B)** Amino acids (AAs) carried by large neutral amino acid (LNAA)- or L-type-transporter were decreased in the CSF of T2D monkeys. **(C,D)** There were no differences in A-, N-, and EAAT-transporter AA concentrations in the CSF of PreD and T2D monkeys. **(F–J)** There were no differences in LNAA-, L-type-, A-, N-, and EAAT-transported amino acid concentrations in plasma of PreD or T2D monkeys compared to controls.

TABLE S1 | Descriptive statistics of amino acid data (see **Figure 2**).

TABLE S2 | Descriptive statistics of acylcarnitine data (see **Figure 3**).

TABLE S3 | Descriptive statistics of CSF amino acid data (see **Supplementary Figure S1**).

TABLE S4 | Descriptive statistics of plasma amino acid data (see **Supplementary Figure S2**).

TABLE S5 | Descriptive statistics of amino acid data, grouped by AA transporter (see **Supplementary Figure S3**).

TABLE S6 | Descriptive statistics of normalized CSF glucose, plasma glucose, CSF Aβ42, and plasma lactate with various CSF analytes. See **Figure 4**.

REFERENCES

American Diabetes Association, (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care* 33(Suppl. 1), S62–S69.

An, J., Muoio, D. M., Shiota, M., Fujimoto, Y., Cline, G. W., Shulman, G. I., et al. (2004). Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. *Nat. Med.* 10, 268–274. doi: 10.1038/nm995

Bateman, R. J., Munsell, L. Y., Morris, J. C., Swarm, R., Yarasheski, K. E., Holtzman, D. M., et al. (2006). Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid in vivo. *Nat. Med.* 12, 856–861. doi: 10.1038/nm1438

Bateman, R. J., Xiong, C., Benzinger, T. L. S., Fagan, A. M., Goate, A., Fox, N. C., et al. (2012). Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med.* 367, 795–804. doi: 10.1056/NEJMoa1202753

Bero, A. W., Yan, P., Roh, J. H., Cirrito, J. R., Stewart, F. R., Raichle, M. E., et al. (2011). Neuronal activity regulates the regional vulnerability to amyloid-beta deposition. *Nat. Neurosci.* 14, 750–756. doi: 10.1038/nn.2801

Chace, D. H., Hillman, S. L., Millington, D. S., Kahler, S. G., Roe, C. R., Naylor, E. W., et al. (1995). Rapid diagnosis of maple syrup urine disease in blood spots from newborns by tandem mass spectrometry. *Clin. Chem.* 41, 62–68.

Chen, J. A., Fears, S. C., Jasinska, A. J., Huang, A., Al-Sharif, N. B., Scheibel, K. E., et al. (2018). Neurodegenerative disease biomarkers Abeta1-40, Abeta1-42, tau, and p-tau181 in the vervet monkey cerebrospinal fluid: relation to normal aging, genetic influences, and cerebral amyloid angiopathy. *Brain Behav.* 8:e00903. doi: 10.1002/brb3.903

Cirrito, J. R., Kang, J. E., Lee, J., Stewart, F. R., Verges, D. K., Silverio, L. M., et al. (2008). Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo. *Neuron* 58, 42–51. doi: 10.1016/j.neuron.2008.02.003

Cirrito, J. R., May, P. C., O'Dell, M. A., Taylor, J. W., Parsadanian, M., Cramer, J. W., et al. (2003). In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. *J. Neurosci.* 23, 8844–8853. doi: 10.1523/jneurosci.23-26-08844.2003

Cirrito, J. R., Yamada, K. A., Finn, M. B., Sloviter, R. S., Bales, K. R., May, P. C., et al. (2005). Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron* 48, 913–922. doi: 10.1016/j.neuron.2005.10.028

Costa, C. C., de, Almeida IT, Jakobs, C., Poll-The, B. T., and Duran, M. (1999). Dynamic changes of plasma acylcarnitine levels induced by fasting and sunflower oil challenge test in children. *Pediatr. Res.* 46, 440–444.

Crane, P. K., Walker, R., Hubbard, R. A., Li, G., Nathan, D. M., Zheng, H., et al. (2013). Glucose levels and risk of dementia. *N. Engl. J. Med.* 369, 540–548.

Cristofano, A., Sapere, N., La Marca, G., Angiolillo, A., Vitale, M., Corbi, G., et al. (2016). Serum levels of acyl-carnitines along the continuum from normal to Alzheimer's Dementia. *PLoS One* 11:e0155694. doi: 10.1371/journal.pone.0155694

Ferrara, C. T., Wang, P., Neto, E. C., Stevens, R. D., Bain, J. R., Wenner, B. R., et al. (2008). Genetic networks of liver metabolism revealed by integration of metabolic and transcriptional profiling. *PLoS Genet.* 4:e1000034. doi: 10.1371/journal.pgen.1000034

Griffin, J. W., and Bradshaw, P. C. (2017). Amino acid catabolism in Alzheimer's disease brain: friend or foe? *Oxid. Med. Cell Longev.* 2017:5472792. doi: 10.1155/2017/5472792

Haqq, A. M., Lien, L. F., Boan, J., Arlotto, M., Slentz, C. A., Muehlbauer, M. J., et al. (2005). The study of the effects of diet on metabolism and nutrition (STEDMAN) weight loss project: rationale and design. *Contemp. Clin. Trials* 26, 616–625. doi: 10.1016/j.cct.2005.09.003

Hardy, J., and Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353–356. doi: 10.1126/science.1072994

Holtzman, D. M., Morris, J. C., and Goate, A. M. (2011). Alzheimer's disease: the challenge of the second century. *Sci. Transl. Med.* 3:77sr1. doi: 10.1126/scitranslmed.3002369

Huang, C. C., Chung, C. M., Leu, H. B., Lin, L. Y., Chiu, C. C., Hsu, C. Y., et al. (2014). Diabetes mellitus and the risk of Alzheimer's disease: a

nationwide population-based study. *PLoS One* 9:e87095. doi: 10.1371/journal.pone.0087095

Hudd, F., Shiel, A., Harris, M., Bowdler, P., McCann, B., Tsivos, D., et al. (2019). Novel blood biomarkers that correlate with cognitive performance and hippocampal volumetry: potential for early diagnosis of Alzheimer's disease. *J. Alzheimers Dis.* 67, 931–947. doi: 10.3233/JAD-180879

Jones, L. L., McDonald, D. A., and Borum, P. R. (2010). Acylcarnitines: role in brain. *Prog. Lipid Res.* 49, 61–75. doi: 10.1016/j.plipres.2009.08.004

Kalinin, S., Willard, S. L., Shively, C. A., Kaplan, J. R., Register, T. C., Jorgensen, M. J., et al. (2013). Development of amyloid burden in African Green monkeys. *Neurobiol. Aging* 34, 2361–2369. doi: 10.1016/j.neurobiolaging.2013.03.023

Latimer, C. S., Shively, C. A., Keene, C. D., Jorgensen, M. J., Andrews, R. N., Register, T. C., et al. (2019). A nonhuman primate model of early Alzheimer's disease pathologic change: implications for disease pathogenesis. *Alzheimers Dement.* 15, 93–105. doi: 10.1016/j.jalz.2018.06.3057

Lu, W., Huang, J., Sun, S., Huang, S., Gan, S., Xu, J., et al. (2015). Changes in lactate content and monocarboxylate transporter 2 expression in Abeta(2)(5)(-)(3)(5)-treated rat model of Alzheimer's disease. *Neurol. Sci.* 36, 871–876. doi: 10.1007/s10072-015-2087-3

Macauley, S. L., Stanley, M., Caesar, E. E., Yamada, S. A., Raichle, M. E., Perez, R., et al. (2015). Hyperglycemia modulates extracellular amyloid-β concentrations and neuronal activity in vivo. *J. Clin. Invest.* 125, 2463–2467. doi: 10.1172/JCI79742

Millington, D. S., Kodo, N., Norwood, D. L., and Roe, C. R. (1990). Tandem mass spectrometry: a new method for acylcarnitine profiling with potential for neonatal screening for inborn errors of metabolism. *J. Inherit. Metab. Dis.* 13, 321–324. doi: 10.1007/bf01799385

Morris, J. K., Vidoni, E. D., Honea, R. A., Burns, J. M., Alzheimer's Disease Neuroimaging Initiative, et al. (2014). Impaired glycemia increases disease progression in mild cognitive impairment. *Neurobiol. Aging* 35, 585–589. doi: 10.1016/j.neurobiolaging.2013.09.033

Muoio, D. M. (2014). Metabolic inflexibility: when mitochondrial indecision leads to metabolic gridlock. *Cell* 159, 1253–1262. doi: 10.1016/j.cell.2014.11.034

Musiek, E. S., and Holtzman, D. M. (2015). Three dimensions of the amyloid hypothesis: time, space and 'wingmen'. *Nat. Neurosci.* 18, 800–806. doi: 10.1038/nn.4018

Newgard, C. B., An, J., Bain, J. R., Muehlbauer, M. J., Stevens, R. D., Lien, L. F., et al. (2009). A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab.* 9, 311–326. doi: 10.1016/j.cmet.2009.02.002

Okabayashi, S., Shimozawa, N., Yasutomi, Y., Yanagisawa, K., and Kimura, N. (2015). Diabetes mellitus accelerates Aβ pathology in brain accompanied by enhanced GAβ generation in nonhuman primates. *PLoS One* 10:e0117362. doi: 10.1371/journal.pone.0117362

Ott, A., Stolk, R. P., van Harskamp, F., Pols, H. A., Hofman, A., Breteler, M. M., et al. (1999). Diabetes mellitus and the risk of dementia: the rotterdam study. *Neurology* 53, 1937–1942.

Raichle, M. E., Posner, J. B., and Plum, F. (1970). Cerebral blood flow during and after hyperventilation. *Arch. Neurol.* 23, 394–403.

Roh, J. H., Huang, Y., Bero, A. W., Kasten, T., Stewart, F. R., Bateman, R. J., et al. (2012). Disruption of the sleep-wake cycle and diurnal fluctuation of beta-amyloid in mice with Alzheimer's disease pathology. *Sci. Transl. Med.* 4:150ra122. doi: 10.1126/scitranslmed.3004291

Schooneman, M. G., Vaz, F. M., Houten, S. M., and Soeters, M. R. (2013). Acylcarnitines: reflecting or inflicting insulin resistance? *Diabetes* 62, 1–8. doi: 10.2337/db12-0466

Shimomura, Y., and Kitaura, Y. (2018). Physiological and pathological roles of branched-chain amino acids in the regulation of protein and energy metabolism and neurological functions. *Pharmacol. Res.* 133, 215–217. doi: 10.1016/j.phrs.2018.05.014

Shin, A. C., Fasshauer, M., Filatova, N., Grundell, L. A., Zielinski, E., Zhou, J. Y., et al. (2014). Brain insulin lowers circulating BCAA levels by inducing hepatic BCAA catabolism. *Cell Metab.* 20, 898–909. doi: 10.1016/j.cmet.2014.09.003

Solon-Biet, S. M., Cogger, V. C., and Simpson, S. J. (2019). Branched-chain amino acids impact health and lifespan indirectly via amino acid balance and appetite control. *Nat. Metab.* 1, 532–545. doi: 10.1038/s42255-019-0059-2

Sperringer, J. E., Addington, A., and Hutson, S. M. (2017). Branched-chain amino acids and brain metabolism. *Neurochem. Res.* 42, 1697–1709. doi: 10.1007/s11064-017-2261-5

Stanley, M., Macauley, S. L., Caesar, E. E., Koscal, L. J., Moritz, W., Robinson, G. O., et al. (2016). The effects of peripheral and central high insulin on brain insulin signaling and Amyloid-β in young and old APP/PS1 mice. *J. Neurosci.* 36, 11704–11715. doi: 10.1523/jneurosci.2119-16.2016

Tapiola, T., Alafuzoff, I., Herukka, S. K., Parkkinen, L., Hartikainen, P., Soininen, H., et al. (2009). Cerebrospinal fluid β-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. *Arch. Neurol.* 66, 382–389. doi: 10.1001/archneurol.2008.596

Verri, M., Aquilani, R., Ricevuti, G., Rondanelli, M., Ghitti, M., Bongiorno, A. I., et al. (2018). Plasma energy substrates at two stages of Alzheimer's disease in humans. *Int. J. Immunopathol. Pharmacol.* 32: 2058738418817707.

Wagner, J. D., Kavanagh, K., Ward, G. M., Auerbach, B. J., Harwood, H. J. Jr., Kaplan, J. R., et al. (2006). Old world primate models of type 2 diabetes mellitus. *ILAR J.* 47, 259–271. doi: 10.1093/ilar.47.3.259

Wang, T. J., Larson, M. G., Vasan, R. S., Cheng, S., Rhee, E. P., McCabe, E., et al. (2011). Metabolite profiles and the risk of developing diabetes. *Nat. Med.* 17, 448–453. doi: 10.1038/nm.2307

Weiss, R., and Lustig, R. H. (2014). "Obesity, metabolic syndrome, and disorders of energy balance," in *Pediatric Endocrinology*, ed. M. Sperling (Philadelphia, PA: Elsevier Saunders), 956–1014.

White, P. J., and Newgard, C. B. (2019). Branched-chain amino acids in disease. *Science* 363, 582–583.

Wu, J. Y., Kao, H. J., Li, S. C., Stevens, R., Hillman, S., Millington, D., et al. (2004). ENU mutagenesis identifies mice with mitochondrial branched-chain aminotransferase deficiency resembling human maple syrup urine disease. *J. Clin. Invest.* 113, 434–440. doi: 10.1172/jci200419574

Zheng, F., Yan, L., Yang, Z., Zhong, B., and Xie, W. (2018). HbA1c, diabetes and cognitive decline: the English longitudinal study of ageing. *Diabetologia* 61, 839–848. doi: 10.1007/s00125-017-4541-7

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Neuroscience

MINI REVIEW
published: 18 October 2019
doi: 10.3389/fnins.2019.01112

Glucagon-Like Peptide-1: A Focus on Neurodegenerative Diseases

*Maddalena Grieco[1], Alessandra Giorgi[1], Maria Cristina Gentile[2], Maria d'Erme[1], Susanna Morano[2], Bruno Maras[1] and Tiziana Filardi[2]**

[1] Department of Biochemical Sciences, Faculty of Pharmacy and Medicine, Sapienza University of Rome, Rome, Italy,
[2] Department of Experimental Medicine, Faculty of Medicine and Dentistry, Sapienza University of Rome, Rome, Italy

Diabetes mellitus is one of the major risk factors for cognitive dysfunction. The pathogenesis of brain impairment caused by chronic hyperglycemia is complex and includes mitochondrial dysfunction, neuroinflammation, neurotransmitters' alteration, and vascular disease, which lead to cognitive impairment, neurodegeneration, loss of synaptic plasticity, brain aging, and dementia. Glucagon-like peptide-1 (GLP-1), a gut released hormone, is attracting attention as a possible link between metabolic and brain impairment. Several studies have shown the influence of GPL-1 on neuronal functions such as thermogenesis, blood pressure control, neurogenesis, neurodegeneration, retinal repair, and energy homeostasis. Moreover, modulation of GLP-1 activity can influence amyloid β peptide aggregation in Alzheimer's disease (AD) and dopamine (DA) levels in Parkinson's disease (PD). GLP-1 receptor agonists (GLP-1RAs) showed beneficial actions on brain ischemia in animal models, such as the reduction of cerebral infarct area and the improvement of neurological deficit, acting mainly through inhibition of oxidative stress, inflammation, and apoptosis. They might also exert a beneficial effect on the cognitive impairment induced by diabetes or obesity improving learning and memory by modulating synaptic plasticity. Moreover, GLP-1RAs reduced hippocampal neurodegeneration. Besides this, there are growing evidences on neuroprotective effects of these agonists in animal models of neurodegenerative diseases, regardless of diabetes. In PD animal models, GPL-1RAs were able to protect motor activity and dopaminergic neurons whereas in AD models, they seemed to improve nearly all neuropathological features and cognitive functions. Although further clinical studies of GPL-1RAs in humans are needed, they seem to be a promising therapy for diabetes-associated cognitive decline.

Keywords: glucagon-like peptide-1, GLP-1 receptor agonists, Parkinson's disease, Alzheimer's disease, neurodegenerative diseases, type 2 diabetes

OPEN ACCESS

Edited by:
Mara Dierssen, Centre for Genomic Regulation (CRG), Spain

Reviewed by:
Alberto Granzotto, Center for Research on Aging Sciences and Translational Medicine Center (CeSI-MeT), Italy
Christian Hölscher, Lancaster University, United Kingdom

****Correspondence:***
Tiziana Filardi
tiziana.filardi@uniroma1.it

Specialty section:
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

Received: *14 June 2019*
Accepted: *02 October 2019*
Published: *18 October 2019*

Citation:
Grieco M, Giorgi A, Gentile MC, d'Erme M, Morano S, Maras B and Filardi T (2019) Glucagon-Like Peptide-1: A Focus on Neurodegenerative Diseases. Front. Neurosci. 13:1112. doi: 10.3389/fnins.2019.01112

INTRODUCTION

The concern for neurodegeneration, a worldwide expanding set of diseases, stimulated the research on risk factors related to the lifestyle of the population, leading to interesting findings on the association between dysmetabolism and brain impairment. In this perspective, gut/brain axis and altered insulin release and response seem to be the main actors in establishing the pathological metabolic set up for the development of neurodegenerative diseases. Indeed, insulin works as pro-survival neurotrophic factor with its receptor widespread in cognitive areas as hippocampus and in dopaminergic system (Haas et al., 2016; Fiory et al., 2019).

In the last years particular attention has been devoted to unravel the role of glucagon-like peptide-1 (GLP-1), a gut released hormone that not only is one of the major components of the gut/brain axis, but it is also able to protect pancreatic beta-cells from apoptosis and to induce insulin secretion (Cabou and Burcelin, 2011).

Glucagon-like peptide-1 is an endogenous peptide hormone released by intestinal L-cells in response to meal. Gene expression generates pro-glucagon (PG), which is processed by prohormone convertases (PC1/3) to release the GLP-1 (1–37) peptide precursor (Sandoval and D'Alessio, 2015). Proteolytic cleavage and amidation of the precursor protein GLP-1(1–37) generate two GLP-1 active forms with the same biological activity namely GLP-1 (7–37) and the amidated GLP-1 (7–36). GLP-1 is degraded by a dipeptidyl-peptidase IV (DPP IV), a serine aminopeptidase expressed in the different organ, such as liver, pancreas, gut, and brain (Hopsu-Havu and Glenner, 1966; Smith et al., 2019). GLP-1 stimulates insulin secretion from the pancreatic beta-cells under hyperglycemic conditions and reduces glucagon secretion from the alfa-cells recovering insulin sensitivity and enhancing glycemic homeostasis (Meloni et al., 2013; Katsurada and Yada, 2016).

Glucagon-like peptide-1 signal transduction is mediated by GLP-1 receptor (GLP-1R), a G-protein coupled receptor, leading to cyclic adenosine monophosphate (cAMP) dependent activation of protein kinase A (PKA) and of cAMP-regulated guanine nucleotide exchange factor (Epac). The activation of Epac and PKA potentiates in a synergistic way the insulin release from the beta-pancreatic cells through phosporylation of the SNARE-associated protein Snapin and activation of L-type voltage gated calcium channels (Song et al., 2011).

It is noteworthy that GPL-1R may operate signal transduction even by activating the PI3K/AKT axis as observed in GLP-1 protection against apoptosis with the regulation of CREB and protein survival factors like Bcl-2 and Bcl-XL, through the action of β-arrestin-1 and the phosphorylation of ERK1/2. Furthermore, the activation of the PI3K/AKT axis can induce the inhibition of specific caspases and of NF-κB, with the resulting inhibition of the release of pro-inflammatory cytokines (Farilla et al., 2003; Athauda and Foltynie, 2016; Tramutola et al., 2017; Yang et al., 2018).

Plasticity of GLP-1 action at molecular level is mirrored also in different tissues like the cardiac compartment and the brain. GLP-1 plays a pivotal role preventing cardiovascular disorders, which makes GLP-1 and its analogs a great resource in the treatment of these diseases (Pozo et al., 2019). GLP-1 is also involved in the reduction of the oxidative stress, in the regulation of autophagy, and in the modulation of central nervous system (CNS) pathways with protective functions and elicitation of anti-inflammatory signaling (Li et al., 2009).

MULTIFUNCTIONAL ROLE OF GLP-1

Glucagon-like peptide-1 is produced at neuronal level of the solitary tract within the brainstem. In addition, this peptide, released from the gut, activates the GLP-1R located on vagal sensory neurons that constitute the hepato-portal glucose sensor, communicating with brainstem neurons, extending its action to different brain regions. Several studies have shown the influence of GLP-1 on neuronal function such as thermogenesis, blood pressure control, neurogenesis, neurodegeneration, retinal repair, and energy homeostasis (Katsurada and Yada, 2016). Since GLP-1Rs are expressed in different brain regions, GLP-1 behaves as a neuropeptide, involved in different peculiar effects including the control of satiety, water intake, and stress reaction (van Dijk and Thiele, 1999; Meier et al., 2002). Kinzig et al. (2003) reported that GLP-1-stimulated brain GLP-1Rs are mediator of multiple stress responses. GLP-1 administration directly into the rat brain increases anxiety level, associated with a higher production of stress-activated hormones ACTH and corticosterone, demonstrating that GLP-1 is able to stimulate at the same time a response by both amygdala and by the paraventricular nucleus of the hypothalamus (Kinzig et al., 2003). The increase of GLP-1 in the circulation could reach the brain and regulate food intake (Ruttimann et al., 2009). Recent studies showed that GLP-1 in combination with dexamethasone (GLP-1/Dexa) can decrease food intake and lower body weight in obese mice without inducing mood or memory deficits (Decarie-Spain et al., 2019). In type 2 diabetes (T2D), alteration of insulin sensitivity and disturbances of neurogenesis are correlated with a reduction in GLP-1 levels in response to food, and its signaling activity (Nauck et al., 2011). Recently, several groups reported that GLP-1 contributes to the regulation of neurologic and cognitive functions (Muscogiuri et al., 2017). Indeed, GLP-1 is also involved in the control of the synaptic plasticity and in some forms of neuroprotection and thus has a regulative role in various signaling pathways associated with learning, memory, and other synaptic function (Gault and Holscher, 2008; Yildirim Simsir et al., 2018).

GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS (GLP-1RAS) AS NEUROPROTECTIVE AGENTS IN DIABETES-ASSOCIATED COGNITIVE IMPAIRMENT

Type 2 diabetes is a chronic disease with an increasing global prevalence. Besides the well-known micro- and macro-vascular complications, cognitive decline is thought to be an emerging consequence of diabetes (Koekkoek et al., 2015).

Over the last decade GLP-1RAs have emerged as effective glucose-lowering drugs. Exenatide was the first GLP-1RA approved for the treatment of T2D. It is a synthetic form of exendin-4, a natural GLP-1-like peptide extracted from the saliva of the lizard *Heloderma suspectum*. Exenatide shares 53% homology with native GLP-1. Exenatide has a half-life of 2.4 h, whereas GLP-1 has a half-life of 2 min. Lixisenatide is based on the structure of exendin-4 and has a half-life of 3 h. Liraglutide was the first GLP-1RA deriving from native GLP-1, sharing 97% homology and with a half-life of 13 h (Aroda, 2018). Semaglutide, a modified form of liraglutide has a half-life of ∼7 days due

to a 5.6 times higher affinity with albumin than liraglutide (Gomez-Peralta and Abreu, 2019).

Several studies have investigated the neuroprotective actions of GLP-1RAs in animal models of diabetes. Many of them have focused on the effects of GLP-1RAs on cerebral ischemia/reperfusion injury. In diabetic rats with cerebral ischemia/reperfusion damage caused by middle cerebral artery occlusion, recombinant GLP-1 improved neurological deficit and reduced cerebral infarct area, mainly through the inhibition of oxidative stress and apoptosis (Fang et al., 2018). GLP-1RAs exert favorable effects, such as the reduction of cognitive impairment induced by diabetes or obesity.

Indeed, it has been observed that peripheral administration of lixisenatide for 40 days (50 nmol/kg bw, twice-daily) in high-fat fed mice with established obesity, insulin resistance, and impaired cognition resulted in marked improvement in recognition memory, which was associated with up-regulation of hippocampal expression of neurotrophic tyrosine kinase receptor type 2 and mammalian target of rapamycin (mTOR) genes involved in modulating synaptic plasticity and long-term potentiation. Lixisenatide treatment promoted also hippocampal progenitor cells proliferation and increased immature neurons in the hippocampal dentate gyrus (Lennox et al., 2014). Liraglutide showed effects against hippocampal neurodegeneration induced by streptozotocin (STZ), an animal model of diabetes and neurodegeneration associated with cognitive decline. In particular, liraglutide improved learning and memory, and reduced hippocampal neuronal death (Palleria et al., 2017). In addition, in a STZ-induced mouse model of diabetes, pre-treatment with liraglutide contrasted neuronal and synaptic damage in the hippocampal CA1 region (Kong et al., 2018).

Notably, neuroprotective activity of GLP-1RAs seem not to be entirely related to glycemia normalization. Indeed, there is growing evidence about neuroprotective effects of GLP-1RAs in animal models of neurodegenerative diseases, regardless of diabetes. Liraglutide reduced infarct size in the brain of diabetic and non-diabetic rats but decreased neurologic deficits only in non-diabetic rats, suggesting that the GLP-1 RAs effects on cognitive function are not associated with diabetes and glycemia normalization. Indeed, both liraglutide and metformin, a glucose lowering agent acting via AMP-activated protein kinase-dependent pathways, induced euglycaemia in diabetic rats, but only liraglutide treatment reduced ischemic brain damage (Filchenko et al., 2018).

GLP-1RAs AS NEUROPROTECTIVE AGENTS IN NEURODEGENERATIVE DISEASES

Considering the beneficial effects of GLP-1RAs on neuropathological features it is conceivable a link between T2D and neurodegenerative disease such as Parkinson's disease (PD) and Alzheimer's Disease (AD). Neurodegenerative diseases have a considerable physical, psychological, social, and economical impact, both on affected people and on their careers, families, and society in general (World Health Organization, 2017).

Parkinson's Disease

Parkinson's disease is a progressive nervous system disorder whose etiology remains still unclear, although genetic and environmental factors seem to be involved. PD's clinical features include resting tremor, rigid muscles, slowed movement (bradykinesia), postural instability, and loss of purposeful movement (Kalia and Lang, 2015). Pathological features are characterized by neurons impairment of substantia nigra pars compacta with concomitant formation of intracellular Lewy bodies and loss of dopaminergic neurons. Lewy bodies are abnormal aggregates of α-synuclein protein, which is involved in dopamine (DA) metabolism and function. Dopaminergic neurons dysfunction and death by apoptosis or autophagy are also associated with mitochondrial activity alteration, oxidative stress, altered protein handling, and inflammatory condition (Olanow and Tatton, 1999). Rare dominant form of PD in familial and sporadic cases is associated to point mutations, duplications, and triplications in the α-synuclein gene (Lesage and Brice, 2009).

In different preclinical models of PD, GLP-1RAs showed neuroprotective effects, influencing motor activity, dopaminergic neurons, cortical activity, and energy utilization in the brain. Harkavyi et al. tested the efficacy of exendin-4 in rat models of PD treated with 6-hydroxydopamine (6-OHDA) and lipopolysaccaride (LPS) (Harkavyi et al., 2008). They observed that in striatal tissue DA concentrations were markedly higher in 6-OHDA/LPS + exendin-4 treated rats with respect to 6-OHDA/LPS + vehicle groups. This effect was associated with an increase in the tyrosine hydroxylase enzyme involved in the production of L-dopa, a DA precursor. In the same PD rat model exendin-4 was able to promote adult neurogenesis *in vitro* e *in vivo*, normalizing DA imbalance, showing an increase in tyrosine hydroxylase- and vesicular monoamine transporter 2-positive neurons in the substantia nigra (Bertilsson et al., 2008). Other authors observed that the administration of exendin-4, liraglutide, and lixisenatide in the same mouse model prevented both motor dysfunction and tyrosine hydroxylase levels reduction in the substantia nigra and basal ganglia. Furthermore, liraglutide and lixisenatide induced a marked increase in anti-apoptotic pathways compared to exendin-4 (Liu et al., 2015).

Recently, the long-term administration of liraglutide was found to rescue dopaminergic neuronal loss and motor impairment also in diabetic db/db mice, an established model of diabetes, with a mutation in the gene encoding the leptin receptor (Ma et al., 2019), suggesting that long-term injection of liraglutide might prevent motor function impairment and PD development also in patients with T2D. In rotenone-induced PD model, liraglutide together with sitagliptin, a DPP IV inhibitor, increased striatal DA and tyrosine hydroxylase protein levels, reduced neuroinflammation, and reversed neuronal loss (Badawi et al., 2017). Liraglutide was also able to attenuate dyskinesia, a serious complication of long-term therapy with L-dopa (Badawi et al., 2019).

In the MPTP mouse model of PD, semaglutide improved most of neuropathological features of PD, reversing motor impairment, inducing the increase of tyrosine hydroxylase levels, and attenuating neuroinflammation and apoptosis in the substantia nigra and striatum (Zhang et al., 2018). A reduction in α-synuclein aggregation occurred after this treatment, not observed with other GLP-1RAs (Zhang et al., 2019), highlighting semaglutide as an effective treatment for PD.

Preliminary clinical studies were performed with subcutaneous injections of exenatide in PD patients. Athauda et al. (2017) reported the results of the first randomized, double-blind, placebo-controlled trial in 62 patients affected by moderate PD. Patients were randomly assigned to receive subcutaneous injections of exenatide 2 mg once-weekly (n.32) or placebo (n.30) for 48 weeks. Exenatide had positive and sustained effects (12 weeks after exposure) on clinically assessed motor function. A *post hoc* analysis indicated that even non-motor symptoms, such as clinically evaluated mood and emotional well-being, improved in patients treated with exenatide although these beneficial effects did not last after interruption (Athauda et al., 2018). Patients treated with exenatide had significantly higher tyrosine phosphorylation of insulin receptor (IR) substrate 1 and higher expression of total Akt and phosphorylated mTOR than placebo-treated patients providing a possible insulin-based molecular mechanism explanation for the results observed in clinical trial (Athauda et al., 2019).

Alzheimer's Disease

Dementia is a chronic disease, which affects memory, other cognitive abilities and behavior. It is estimated that approximately 50 million people worldwide have dementia. Currently, it is the 7th leading cause of death and it is one of the major causes of disability worldwide. Pre-diabetic risk factors, obesity, and metabolic syndrome can promote cognitive dysfunction. AD is the most common form of dementia, contributing to 60–70% of cases. The main neuropathological features of AD are neurofibrillary tangles, formed by hyperphosphorylated tau proteins, which aggregate into oligomers, and the amyloid plaques, formed by aggregated β-amyloid peptides (Aβ) (Calsolaro and Edison, 2015).

Increasing evidence suggests a link between T2D and AD. In particular, these conditions might share defects in insulin signaling. Interestingly, in a mouse model of genetically induced AD-like neuropathology (3xTg-AD mice) peripheral glucose intolerance was observed. Treatment with pioglitazone, a glucose lowering drug, greatly improved cognitive impairment of these mice confirming the neurotrophic role of insulin (Masciopinto et al., 2012). In the same model, high-fat diet further potentiated glucose intolerance and enhanced neuropathological features of AD and memory deficits. Insulin adoption reversed the negative effect of high-fat diet, interrupting the vicious cycle between diabetes and AD (Vandal et al., 2014). Both studies highlighted the neurotrophic role of insulin in brain.

Conversely, hyperinsulinemia induced by peripheral administration of insulin increased tau phosphorylation by in C57BL/6 mice (Freude et al., 2005).

Aggregated β-amyloid peptide oligomers induced reduction of IRs activity due to the phosphorylation of IRS-1 at serine residues (IRS-1pSer), with a consequent loss of substrate affinity as observed in T2D. As reported by Bomfim et al. (2012), in the mouse hippocampal neurons Aβ oligomers are also thought to activate the TNF-α/JNK signaling, inducing insulin resistance (De Felice, 2013). The GLP-1RAs not only prevent JNK/IKK activation, but promote insulin activation by PI3K/AKT axis, with the subsequent activation of mTOR and the block of GSK-3β, an essential kinase also involved for the phosphorylation of tau protein (Moloney et al., 2010). Ma et al. (2015) reported that liraglutide administration prevented tau hyperphosphorylation associated with aging in diabetic db/db mouse.

The role of vascular dysfunction has recently emerged as significant contributor in the pathophysiology of AD. Blood–brain barrier and cerebral blood flow reduction might precede Aβ oligomers and tau deposition and it is associated to cognitive decline (Hachinski et al., 2019; Nation et al., 2019). In APP/PS1 transgenic mice liraglutide reduced the incidence of cerebral microanuerysms and leakage (Kelly et al., 2015).

Glucagon-like peptide-1 receptor agonists have shown neuroprotective effects in several preclinical studies in AD. Notably, they seem to improve nearly all neuropathological features in AD and cognitive functions as well. In 12-month-old female APP/PS1/tau AD mouse model, neurofibrillary tangles, amyloid plaques, and neuroinflammation in the hippocampi have been reduced by lixisenatide (Cai et al., 2018). In the rat model, lixisenatide also prevented synaptic damage induced by Aβ accumulation and strengthened spatial memory by affecting the PI3K-Akt-GSK3β (Cai et al., 2014). The GLP-1RA exenatide (20 μgr/kg/day, intraperitoneally for 2 weeks) reduced neuroinflammation by suppressing the TNF-α levels in rats. Furthermore, it improved memory and prevented the loss of hippocampal neurons (Solmaz et al., 2015).

Recently, it has been observed that 4-week-treatment with exendin-4 reversed memory impairment in APP/PS1 mice, downregulating the aberrant *N*-acetylglucosaminyltransferase III expression through the Akt/GSK-3β/β-catenin signaling pathway in neurons. *N*-Acetylglucosamine levels seem to be increased in the cerebrospinal fluid of most AD patients, and the levels of *N*-acetylglucosaminyltransferase III, a glycosyltransferase responsible for synthesizing a bisecting GlcNAc residue, were found to be highly expressed in the brains of AD patients as well (Wang et al., 2018).

Liraglutide (25 nmol/kg, intraperitoneally, for 2 months) improved spatial memory in 14-month-old APP/PS1 mouse model, compared to saline-treated mice. It also reduced inflammation and plaque load, while neuronal progenitor cell in the dentate gyrus increased. Long-term potentiation was significantly enhanced as well and synapse numbers increased in the hippocampus and cortex (McClean and Holscher, 2014). In another study, the same authors observed that liraglutide might also protect from progressive neurodegeneration that develops in AD: in 2-month old mice, liraglutide (once-daily intraperitoneally for 8 months) contrasted synaptic damage and improved memory. In addition, amyloid plaque load was reduced, inflammation was reduced in the cortex, and

neurogenesis was enhanced in the dentate gyrus (McClean et al., 2015). On the contrary, other authors did not report beneficial effects of liraglutide on cerebral plaque load, in APP/PS1 transgenic mouse models of AD with two different clinical APP/PS1 mutations (Hansen et al., 2016). In the mouse model, memory deficit was improved by subcutaneous administration of liraglutide (25 nmol/day once daily for 8 week), decreasing the phosphorylation of tau (Qi et al., 2016).

Furthermore, in APP/PS-1 mice at different ages, chronic administration of liraglutide promoted neural progenitor cells proliferation. Both acute and chronic treatment increased the number of immature neurons in animals at all ages, and the differentiation into mature neurons was observed for most immature cells (Parthsarathy and Holscher, 2013).

Even in a mouse model of pathological aging, which shares neurobehavioral and neuropathological dysfunction with sporadic AD at an early phase, liraglutide increased the number of CA1 pyramidal neuron in hippocampus and improved memory (Hansen et al., 2015).

The effects of GLP-1RAs on synaptic protection might involve the modulation of the brain-derived neurotrophic factor (BDNF), a trophic factor which promotes neural progenitor cell differentiation and survival. Indeed, exenatide activates the transcription factor CREB with an increase of BDNF protein expression promoting the activation of neurotrophic pathway and inhibiting apoptosis in a mouse model of age-dependent cognitive dysfunction, potentiating long-term memory (Bomba et al., 2018). Even in a mouse model of AD (the 3xTg-AD undergoing high fat diet), exenatide reverted the impairment of BDNF signaling and neuroinflammation (Bomba et al., 2019).

In the last few years, even dual and triple receptor agonists have been developed, with remarkable results in animal models. Indeed, GLP-1/gastric inhibitory polypeptide (GIP) dual agonist DA5-CH strengthened working memory and long-term spatial memory in APP/PS1 transgenic AD mouse model (9-month-old). It also led to a reduction in hippocampal amyloid senile plaques and in phosphorylated tau protein. The deficits in hippocampal late-phase long-term potentiation were reversed and p-PI3K and p-AKT growth factor kinases were up regulated. The excessive activation of p-GSKβ was prevented in the hippocampus (Cai et al., 2018). Promising results have been observed with the dual GLP-1/GIP receptor agonist DA-JC4 as well, which decreased phosphorylated tau levels in the rat cerebral cortex and hippocampus, prevented spatial learning dysfunction, attenuated chronic inflammation response in the brain, reduced apoptosis, and reactivated insulin signaling pathways in STZ-induced AD rat model (Shi et al., 2017). Recently, a triple receptor agonist, activating GLP-1, GIP, and glucagon receptors, rescued memory dysfunction, showed anti-apoptotic effects, enhanced synaptophysin, protected from synaptic loss, reduced the total amount of Aβ, and reduced neuroinflammation (activated microglia and astrocytes) and oxidative stress in the cortex and hippocampus (Tai et al., 2018).

Despite the large amount of evidence about the neuroprotective effects of GLP-1RAs in animal models of AD, human studies are still scant. In a randomized, controlled, double-blind intervention study in AD patients, no effect on the deposition of Aβ was observed in patients treated for 6 months with liraglutide, compared to placebo (Egefjord et al., 2012). In a more recent 26-week, double-blind RCT, although glucose metabolism increased in multiple regions in patients with AD treated with liraglutide compared to placebo, the statistical power of the study was insufficient to reach a conclusion about Aβ load and cognition measures (Gejl et al., 2016).

CONCLUSION

Overall, the results on the effects of GLP-1RAs in animal models of neurodegenerative diseases are encouraging. However, further clinical research is needed to clarify whether they might be potential agents for the treatment of PD and AD and other forms of cognitive impairment.

AUTHOR CONTRIBUTIONS

MD'E and SM conceptualized and critically revised the manuscript. TF, MG, and MCG performed the PubMed search and wrote the mini-review. BM and AG critically revised the article for intellectual content.

REFERENCES

Aroda, V. R. (2018). A review of GLP-1 receptor agonists: evolution and advancement, through the lens of randomised controlled trials. *Diabetes Obes. Metab.* 20(Suppl. 1), 22–33. doi: 10.1111/dom.13162

Athauda, D., and Foltynie, T. (2016). The glucagon-like peptide 1 (GLP) receptor as a therapeutic target in Parkinson's disease: mechanisms of action. *Drug Discov. Today* 21, 802–818. doi: 10.1016/j.drudis.2016.01.013

Athauda, D., Gulyani, S., Karnati, H. K., Li, Y., Tweedie, D., Mustapic, M., et al. (2019). Utility of neuronal-derived exosomes to examine molecular mechanisms that affect motor function in patients with parkinson disease: a secondary analysis of the exenatide-PD trial. *JAMA Neurol.* 76, 420–429. doi: 10.1001/jamaneurol.2018.4304

Athauda, D., Maclagan, K., Budnik, N., Zampedri, L., Hibbert, S., Skene, S. S., et al. (2018). What effects might exenatide have on non-motor symptoms in parkinson's disease: a post hoc analysis. *J. Parkinsons Dis.* 8, 247–258. doi: 10.3233/JPD-181329

Athauda, D., Maclagan, K., Skene, S. S., Bajwa-Joseph, M., Letchford, D., Chowdhury, K., et al. (2017). Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. *Lancet* 390, 1664–1675. doi: 10.1016/S0140-6736(17)31585-4

Badawi, G. A., Abd El Fattah, M. A., Zaki, H. F., and El Sayed, M. I. (2017). Sitagliptin and liraglutide reversed nigrostriatal degeneration of rodent brain in rotenone-induced Parkinson's disease. *Inflammopharmacology* 25, 369–382. doi: 10.1007/s10787-017-0331-6

Badawi, G. A., Abd El Fattah, M. A., Zaki, H. F., and El Sayed, M. I. (2019). Sitagliptin and liraglutide modulate L-dopa effect and attenuate dyskinetic movements in rotenone-lesioned rats. *Neurotox. Res.* 35, 635–653. doi: 10.1007/s12640-019-9998-3

Bertilsson, G., Patrone, C., Zachrisson, O., Andersson, A., Dannaeus, K., Heidrich, J., et al. (2008). Peptide hormone exendin-4 stimulates subventricular zone

neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. *J. Neurosci. Res.* 86, 326–338. doi: 10.1002/jnr.21483

Bomba, M., Granzotto, A., Castelli, V., Massetti, N., Silvestri, E., Canzoniero, L. M. T., et al. (2018). Exenatide exerts cognitive effects by modulating the BDNF-TrkB neurotrophic axis in adult mice. *Neurobiol. Aging* 64, 33–43. doi: 10.1016/j.neurobiolaging.2017.12.009

Bomba, M., Granzotto, A., Castelli, V., Onofrj, M., Lattanzio, R., Cimini, A., et al. (2019). Exenatide reverts the high-fat-diet-induced impairment of BDNF signaling and inflammatory response in an animal model of Alzheimer's disease. *J. Alzheimers Dis.* 70, 793–810. doi: 10.3233/JAD-190237

Bomfim, T. R., Forny-Germano, L., Sathler, L. B., Brito-Moreira, J., Houzel, J. C., Decker, H., et al. (2012). An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Abeta oligomers. *J. Clin. Invest.* 122, 1339–1353. doi: 10.1172/JCI57256

Cabou, C., and Burcelin, R. (2011). GLP-1, the gut-brain, and brain-periphery axes. *Rev. Diabet. Stud.* 8, 418–431. doi: 10.1900/RDS.2011.8.418

Cai, H. Y., Holscher, C., Yue, X. H., Zhang, S. X., Wang, X. H., Qiao, F., et al. (2014). Lixisenatide rescues spatial memory and synaptic plasticity from amyloid beta protein-induced impairments in rats. *Neuroscience* 277, 6–13. doi: 10.1016/j.neuroscience.2014.02.022

Cai, H. Y., Yang, J. T., Wang, Z. J., Zhang, J., Yang, W., Wu, M. N., et al. (2018). Lixisenatide reduces amyloid plaques, neurofibrillary tangles and neuroinflammation in an APP/PS1/tau mouse model of Alzheimer's disease. *Biochem. Biophys. Res. Commun.* 495, 1034–1040. doi: 10.1016/j.bbrc.2017.11.114

Calsolaro, V., and Edison, P. (2015). Novel GLP-1 (Glucagon-Like Peptide-1) analogues and insulin in the treatment for Alzheimer's disease and other neurodegenerative diseases. *CNS Drugs* 29, 1023–1039. doi: 10.1007/s40263-015-0301-8

De Felice, F. G. (2013). Alzheimer's disease and insulin resistance: translating basic science into clinical applications. *J. Clin. Invest.* 123, 531–539. doi: 10.1172/JCI64595

Decarie-Spain, L., Fisette, A., Zhu, Z., Yang, B., DiMarchi, R. D., Tschop, M. H., et al. (2019). GLP-1/dexamethasone inhibits food reward without inducing mood and memory deficits in mice. *Neuropharmacology* 151, 55–63. doi: 10.1016/j.neuropharm.2019.03.035

Egefjord, L., Gejl, M., Moller, A., Braendgaard, H., Gottrup, H., Antropova, O., et al. (2012). Effects of liraglutide on neurodegeneration, blood flow and cognition in Alzheimer s disease - protocol for a controlled, randomized double-blinded trial. *Dan. Med. J.* 59:A4519.

Fang, Y., Jiang, D., Wang, Y., Wang, Q., Lv, D., Liu, J., et al. (2018). Neuroprotection of rhGLP-1 in diabetic rats with cerebral ischemia/reperfusion injury via regulation of oxidative stress, EAAT2, and apoptosis. *Drug Dev. Res.* 79, 249–259. doi: 10.1002/ddr.21439

Farilla, L., Bulotta, A., Hirshberg, B., Li Calzi, S., Khoury, N., Noushmehr, H., et al. (2003). Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 144, 5149–5158. doi: 10.1210/en.2003-323

Filchenko, I., Simanenkova, A., Chefu, S., Kolpakova, M., and Vlasov, T. (2018). Neuroprotective effect of glucagon-like peptide-1 receptor agonist is independent of glycaemia normalization in type two diabetic rats. *Diab. Vasc. Dis. Res.* 15, 567–570. doi: 10.1177/1479164118788079

Fiory, F., Perruolo, G., Cimmino, I., Cabaro, S., Pignalosa, F. C., Miele, C., et al. (2019). The relevance of insulin action in the dopaminergic system. *Front. Neurosci.* 13:868. doi: 10.3389/fnins.2019.00868

Freude, S., Plum, L., Schnitker, J., Leeser, U., Udelhoven, M., Krone, W., et al. (2005). Peripheral hyperinsulinemia promotes tau phosphorylation in vivo. *Diabetes* 54, 3343–3348. doi: 10.2337/diabetes.54.12.3343

Gault, V. A., and Holscher, C. (2008). GLP-1 agonists facilitate hippocampal LTP and reverse the impairment of LTP induced by beta-amyloid. *Eur. J. Pharmacol.* 587, 112–117. doi: 10.1016/j.ejphar.2008.03.025

Gejl, M., Gjedde, A., Egefjord, L., Moller, A., Hansen, S. B., Vang, K., et al. (2016). In Alzheimer's disease, 6-month treatment with glp-1 analog prevents decline of brain glucose metabolism: randomized, placebo-controlled, double-blind clinical trial. *Front. Aging Neurosci.* 8:108. doi: 10.3389/fnagi.2016.00108

Gomez-Peralta, F., and Abreu, C. (2019). Profile of semaglutide in the management of type 2 diabetes: design, development, and place in therapy. *Drug Des. Dev. Ther.* 13, 731–738. doi: 10.2147/DDDT.S165372

Haas, C. B., Kalinine, E., Zimmer, E. R., Hansel, G., Brochier, A. W., Oses, J. P., et al. (2016). Brain insulin administration triggers distinct cognitive and neurotrophic responses in young and aged rats. *Mol. Neurobiol.* 53, 5807–5817. doi: 10.1007/s12035-015-9494-6

Hachinski, V., Einhaupl, K., Ganten, D., Alladi, S., Brayne, C., Stephan, B. C. M., et al. (2019). Preventing dementia by preventing stroke: the Berlin Manifesto. *Alzheimers Dement.* 15, 961–984. doi: 10.1016/j.jalz.2019.06.001

Hansen, H. H., Fabricius, K., Barkholt, P., Kongsbak-Wismann, P., Schlumberger, C., Jelsing, J., et al. (2016). Long-term treatment with liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, has no effect on beta-amyloid plaque load in two transgenic APP/PS1 mouse models of Alzheimer's disease. *PLoS One* 11:e0158205. doi: 10.1371/journal.pone.0158205

Hansen, H. H., Fabricius, K., Barkholt, P., Niehoff, M. L., Morley, J. E., Jelsing, J., et al. (2015). The GLP-1 receptor agonist liraglutide improves memory function and increases hippocampal CA1 neuronal numbers in a senescence-accelerated mouse model of Alzheimer's disease. *J. Alzheimers Dis.* 46, 877–888. doi: 10.3233/JAD-143090

Harkavyi, A., Abuirmeileh, A., Lever, R., Kingsbury, A. E., Biggs, C. S., and Whitton, P. S. (2008). Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. *J. Neuroinflammation* 5:19. doi: 10.1186/1742-2094-5-19

Hopsu-Havu, V. K., and Glenner, G. G. (1966). A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-beta-naphthylamide. *Histochemie* 7, 197–201. doi: 10.1007/bf00577838

Kalia, L. V., and Lang, A. E. (2015). Parkinson's disease. *Lancet* 386, 896–912. doi: 10.1016/S0140-6736(14)61393-3

Katsurada, K., and Yada, T. (2016). Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist. *J. Diabetes Investig.* 7(Suppl. 1), 64–69. doi: 10.1111/jdi.12464

Kelly, P., McClean, P. L., Ackermann, M., Konerding, M. A., Holscher, C., and Mitchell, C. A. (2015). Restoration of cerebral and systemic microvascular architecture in APP/PS1 transgenic mice following treatment with Liraglutide. *Microcirculation* 22, 133–145. doi: 10.1111/micc.12186

Kinzig, K. P., D'Alessio, D. A., Herman, J. P., Sakai, R. R., Vahl, T. P., Figueiredo, H. F., et al. (2003). CNS glucagon-like peptide-1 receptors mediate endocrine and anxiety responses to interoceptive and psychogenic stressors. *J. Neurosci.* 23, 6163–6170. doi: 10.1523/jneurosci.23-15-06163.2003

Koekkoek, P. S., Kappelle, L. J., van den Berg, E., Rutten, G. E., and Biessels, G. J. (2015). Cognitive function in patients with diabetes mellitus: guidance for daily care. *Lancet Neurol.* 14, 329–340. doi: 10.1016/S1474-4422(14)70249-2

Kong, F. J., Wu, J. H., Sun, S. Y., Ma, L. L., and Zhou, J. Q. (2018). Liraglutide ameliorates cognitive decline by promoting autophagy via the AMP-activated protein kinase/mammalian target of rapamycin pathway in a streptozotocin-induced mouse model of diabetes. *Neuropharmacology* 131, 316–325. doi: 10.1016/j.neuropharm.2018.01.001

Lennox, R., Flatt, P. R., and Gault, V. A. (2014). Lixisenatide improves recognition memory and exerts neuroprotective actions in high-fat fed mice. *Peptides* 61, 38–47. doi: 10.1016/j.peptides.2014.08.014

Lesage, S., and Brice, A. (2009). Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum. Mol. Genet.* 18, R48–R59. doi: 10.1093/hmg/ddp012

Li, Y., Perry, T., Kindy, M. S., Harvey, B. K., Tweedie, D., Holloway, H. W., et al. (2009). GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1285–1290. doi: 10.1073/pnas.0806720106

Liu, W., Jalewa, J., Sharma, M., Li, G., Li, L., and Holscher, C. (2015). Neuroprotective effects of lixisenatide and liraglutide in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Neuroscience* 303, 42–50. doi: 10.1016/j.neuroscience.2015.06.054

Ma, D., Liu, X., Liu, J., Li, M., Chen, L., Gao, M., et al. (2019). Long-term liraglutide ameliorates nigrostriatal impairment via regulating AMPK/PGC-1a signaling in diabetic mice. *Brain Res.* 1714, 126–132. doi: 10.1016/j.brainres.2019.02.030

Ma, D. L., Chen, F. Q., Xu, W. J., Yue, W. Z., Yuan, G., and Yang, Y. (2015). Early intervention with glucagon-like peptide 1 analog liraglutide prevents tau hyperphosphorylation in diabetic db/db mice. *J. Neurochem.* 135, 301–308. doi: 10.1111/jnc.13248

Masciopinto, F., Di Pietro, N., Corona, C., Bomba, M., Pipino, C., Curcio, M., et al. (2012). Effects of long-term treatment with pioglitazone on cognition and glucose metabolism of PS1-KI, 3xTg-AD, and wild-type mice. *Cell Death Dis.* 3:e448. doi: 10.1038/cddis.2012.189

McClean, P. L., and Holscher, C. (2014). Liraglutide can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a model of Alzheimer's disease. *Neuropharmacology* 76(Pt A), 57–67. doi: 10.1016/j.neuropharm.2013.08.005

McClean, P. L., Jalewa, J., and Holscher, C. (2015). Prophylactic liraglutide treatment prevents amyloid plaque deposition, chronic inflammation and memory impairment in APP/PS1 mice. *Behav. Brain Res.* 293, 96–106. doi: 10.1016/j.bbr.2015.07.024

Meier, J. J., Gallwitz, B., Schmidt, W. E., and Nauck, M. A. (2002). Glucagon-like peptide 1 as a regulator of food intake and body weight: therapeutic perspectives. *Eur. J. Pharmacol.* 440, 269–279. doi: 10.1016/s0014-2999(02)01434-6

Meloni, A. R., DeYoung, M. B., Lowe, C., and Parkes, D. G. (2013). GLP-1 receptor activated insulin secretion from pancreatic beta-cells: mechanism and glucose dependence. *Diabetes Obes. Metab.* 15, 15–27. doi: 10.1111/j.1463-1326.2012.01663.x

Moloney, A. M., Griffin, R. J., Timmons, S., O'Connor, R., Ravid, R., and O'Neill, C. (2010). Defects in IGF-1 receptor, insulin receptor and IRS-1/2 in Alzheimer's disease indicate possible resistance to IGF-1 and insulin signalling. *Neurobiol. Aging* 31, 224–243. doi: 10.1016/j.neurobiolaging.2008.04.002

Muscogiuri, G., DeFronzo, R. A., Gastaldelli, A., and Holst, J. J. (2017). Glucagon-like peptide-1 and the central/peripheral nervous system: crosstalk in diabetes. *Trends Endocrinol. Metab.* 28, 88–103. doi: 10.1016/j.tem.2016.10.001

Nation, D. A., Sweeney, M. D., Montagne, A., Sagare, A. P., D'Orazio, L. M., Pachicano, M., et al. (2019). Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. *Nat. Med.* 25, 270–276. doi: 10.1038/s41591-018-0297-y

Nauck, M. A., Vardarli, I., Deacon, C. F., Holst, J. J., and Meier, J. J. (2011). Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 54, 10–18. doi: 10.1007/s00125-010-1896-4

Olanow, C. W., and Tatton, W. G. (1999). Etiology and pathogenesis of Parkinson's disease. *Annu. Rev. Neurosci.* 22, 123–144. doi: 10.1146/annurev.neuro.22.1.123

Palleria, C., Leo, A., Andreozzi, F., Citraro, R., Iannone, M., Spiga, R., et al. (2017). Liraglutide prevents cognitive decline in a rat model of streptozotocin-induced diabetes independently from its peripheral metabolic effects. *Behav. Brain Res.* 321, 157–169. doi: 10.1016/j.bbr.2017.01.004

Parthsarathy, V., and Holscher, C. (2013). Chronic treatment with the GLP1 analogue liraglutide increases cell proliferation and differentiation into neurons in an AD mouse model. *PLoS One* 8:e58784. doi: 10.1371/journal.pone.0058784

Pozo, L., Bello, F., Suarez, A., Ochoa-Martinez, F. E., Mendez, Y., Chang, C. H., et al. (2019). Novel pharmacological therapy in type 2 diabetes mellitus with established cardiovascular disease: current evidence. *World J. Diabetes* 10, 291–303. doi: 10.4239/wjd.v10.i5.291

Qi, L., Ke, L., Liu, X., Liao, L., Ke, S., Liu, X., et al. (2016). Subcutaneous administration of liraglutide ameliorates learning and memory impairment by modulating tau hyperphosphorylation via the glycogen synthase kinase-3beta pathway in an amyloid beta protein induced alzheimer disease mouse model. *Eur. J. Pharmacol.* 783, 23–32. doi: 10.1016/j.ejphar.2016.04.052

Ruttimann, E. B., Arnold, M., Hillebrand, J. J., Geary, N., and Langhans, W. (2009). Intrameal hepatic portal and intraperitoneal infusions of glucagon-like peptide-1 reduce spontaneous meal size in the rat via different mechanisms. *Endocrinology* 150, 1174–1181. doi: 10.1210/en.2008-1221

Sandoval, D. A., and D'Alessio, D. A. (2015). Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. *Physiol. Rev.* 95, 513–548. doi: 10.1152/physrev.00013.2014

Shi, L., Zhang, Z., Li, L., and Holscher, C. (2017). A novel dual GLP-1/GIP receptor agonist alleviates cognitive decline by re-sensitizing insulin signaling in the Alzheimer icv. STZ rat model. *Behav. Brain Res.* 327, 65–74. doi: 10.1016/j.bbr.2017.03.032

Smith, N. K., Hackett, T. A., Galli, A., and Flynn, C. R. (2019). GLP-1: molecular mechanisms and outcomes of a complex signaling system. *Neurochem. Int.* 128, 94–105. doi: 10.1016/j.neuint.2019.04.010

Solmaz, V., Cinar, B. P., Yigitturk, G., Cavusoglu, T., Taskiran, D., and Erbas, O. (2015). Exenatide reduces TNF-alpha expression and improves hippocampal neuron numbers and memory in streptozotocin treated rats. *Eur. J. Pharmacol.* 765, 482–487. doi: 10.1016/j.ejphar.2015.09.024

Song, W. J., Seshadri, M., Ashraf, U., Mdluli, T., Mondal, P., Keil, M., et al. (2011). Snapin mediates incretin action and augments glucose-dependent insulin secretion. *Cell Metab.* 13, 308–319. doi: 10.1016/j.cmet.2011.02.002

Tai, J., Liu, W., Li, Y., Li, L., and Holscher, C. (2018). Neuroprotective effects of a triple GLP-1/GIP/glucagon receptor agonist in the APP/PS1 transgenic mouse model of Alzheimer's disease. *Brain Res.* 1678, 64–74. doi: 10.1016/j.brainres.2017.10.012

Tramutola, A., Arena, A., Cini, C., Butterfield, D. A., and Barone, E. (2017). Modulation of GLP-1 signaling as a novel therapeutic approach in the treatment of Alzheimer's disease pathology. *Expert Rev. Neurother.* 17, 59–75. doi: 10.1080/14737175.2017.1246183

van Dijk, G., and Thiele, T. E. (1999). Glucagon-like peptide-1 (7-36) amide: a central regulator of satiety and interoceptive stress. *Neuropeptides* 33, 406–414. doi: 10.1054/npep.1999.0053

Vandal, M., White, P. J., Tremblay, C., St-Amour, I., Chevrier, G., Emond, V., et al. (2014). Insulin reverses the high-fat diet-induced increase in brain Abeta and improves memory in an animal model of Alzheimer disease. *Diabetes* 63, 4291–4301. doi: 10.2337/db14-0375

Wang, Y., Chen, S., Xu, Z., Chen, S., Yao, W., and Gao, X. (2018). GLP-1 receptor agonists downregulate aberrant GnT-III expression in Alzheimer's disease models through the Akt/GSK-3beta/beta-catenin signaling. *Neuropharmacology* 131, 190–199. doi: 10.1016/j.neuropharm.2017.11.048

World Health Organization (2017). *Dementia Fact Sheets* [Online]. Available at: https://www.who.int/en/news-room/fact-sheets/detail/dementia (accessed June 11, 2019).

Yang, Y., Fang, H., Xu, G., Zhen, Y., Zhang, Y., Tian, J., et al. (2018). Liraglutide improves cognitive impairment via the AMPK and PI3K/Akt signaling pathways in type 2 diabetic rats. *Mol. Med. Rep.* 18, 2449–2457. doi: 10.3892/mmr.2018.9180

Yildirim Simsir, I., Soyaltin, U. E., and Cetinkalp, S. (2018). Glucagon like peptide-1 (GLP-1) likes Alzheimer's disease. *Diabetes Metab. Syndr.* 12, 469–475. doi: 10.1016/j.dsx.2018.03.002

Zhang, L., Zhang, L., Li, L., and Holscher, C. (2018). Neuroprotective effects of the novel GLP-1 long acting analogue semaglutide in the MPTP Parkinson's disease mouse model. *Neuropeptides* 71, 70–80. doi: 10.1016/j.npep.2018.07.003

Zhang, L., Zhang, L., Li, L., and Holscher, C. (2019). Semaglutide is neuroprotective and reduces alpha-synuclein levels in the chronic MPTP mouse model of Parkinson's disease. *J. Parkinsons Dis.* 9, 157–171. doi: 10.3233/JPD-181503

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership