ORIGINAL ARTICLE

Lower insulin secretion is associated with hippocampal and parahippocampal gyrus atrophy in elderly patients with type 2 diabetes mellitus

Yuko Adachi[1], Kazuki Ota[1]*, Isao Minami[1], Tetsuya Yamada[2], Takayuki Watanabe[1]

[1]Department of Diabetes and Endocrinology, Yokohama City Minato Red Cross Hospital, Yokohama, Japan, and [2]Department of Molecular Endocrinology and Metabolism, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

ABSTRACT

Aims/Introduction: We aimed to examine the association between diabetes-related parameters and hippocampal and parahippocampal gyrus atrophy (HPGA) in patients with type 2 diabetes mellitus to elucidate the risk factors for HPGA, which is often accompanied by Alzheimer's disease.

Materials and methods: A total of 137 patients aged ≥50 years with type 2 diabetes mellitus (mean age 67.8 ± 9.8 years) underwent brain magnetic resonance imaging scans and comprehensive health examinations. We measured the volume of interest – a portion of the inner temporal lobe that includes the hippocampus, amygdala and entorhinal cortex (frontal part of the parahippocampal gyrus) – using the voxel-based specific regional analysis system for Alzheimer's disease in each patient. The diabetes-related parameters included glycated hemoglobin, fasting plasma glucose, C-peptide (CPR) index (serum CPR / fasting plasma glucose × 100) and duration of diabetes.

Results: The mean glycated hemoglobin was 9.3 ± 2.2%, the median CPR index was 1.29 (interquartile range 0.85–1.74) and the median duration of diabetes was 10 years (interquartile range 3–20 years). The severity score of volume of interest atrophy was >1.0 in 36 patients. Using multivariate logistic regression analysis, we found that age (odds ratio 1.09, 95% confidence interval 1.02–1.15) and CPR index (odds ratio 0.451, 95% confidence interval 0.216–0.940) were significantly associated with HPGA.

Conclusions: Lower insulin secretion was significantly associated with HPGA in patients with type 2 diabetes mellitus. The results of this study support the hypothesis that insulin-signaling abnormalities are involved in the pathophysiology of Alzheimer's disease.

INTRODUCTION

The increase in the number of patients with dementia is a serious social and medical problem in Japan. In 2017, the Hisayama Study reported that the age-standardized prevalence of all-cause dementia and Alzheimer's disease (AD) has increased with time, whereas no secular change was observed in vascular dementia (VaD), and that the age- and sex-adjusted incidence of all-cause dementia and AD, but not vascular dementia, also showed a time-dependent increase[1]. Therefore, it is necessary to identify the risk factors for AD to prevent a general increase in the number of patients with dementia. Recently, several studies have reported that diabetes was associated with an increased risk of dementia. A worldwide meta-analysis showed that diabetes patients had a 1.6-fold AD and 2.2-fold vascular dementia risk as compared with patients without diabetes[2].

There were several reports on the relationship between diabetes and brain atrophy. A meta-analysis showed that in patients with type 2 diabetes mellitus, the total volume of the hippocampus, basal ganglia, orbital frontal cortex and frontal lobe was reduced in addition to a reduction in the total brain volume[3]. Furthermore, in 2016, the Hisayama Study reported

atrophy of the whole brain and hippocampus in diabetes patients[4].

However, the mechanisms that cause brain atrophy in patients with diabetes have not yet been clarified. Therefore, in the present study, we aimed to examine the risk factors for hippocampal and parahippocampal gyrus atrophy (HPGA), which is often accompanied by AD, using the voxel-based specific regional analysis system for AD (VSRAD) on each of our elderly patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Participants, standard protocol approvals, registrations and patient consent

From November 2016 to November 2018, we carried out a cross-sectional study. During this period, a total of 137 participants aged ≥50 years with type 2 diabetes mellitus underwent brain magnetic resonance imaging (MRI) scans and comprehensive health examinations while they were being treated for diabetes at Yokohama City Minato Red Cross Hospital, Yokohama, Japan. The treatments of participants were as follows: no glucose-lowering medication ($n = 22$), sulfonylurea or glinide ($n = 34$), metformin ($n = 35$), sodium–glucose cotransporter 2 inhibitor ($n = 10$), dipeptidyl peptidase-4 inhibitor ($n = 73$), alpha-glucosidase inhibitor ($n = 18$), pioglitazone ($n = 8$), glucagon-like peptide-1 receptor agonists ($n = 5$) and insulin ($n = 49$). Many patients took multiple medications. We excluded individuals with obvious neurological disorders that had been diagnosed before the initiation of this study.

The medical ethics committee at Yokohama City Minato Red Cross Hospital approved the study (date of approval: 8 November 2016; approval no. 2016-62), and we obtained written informed consent from all participants.

MRI analysis, diagnosis of type 2 diabetes and measurements of other risk factors

We used an MRI device with magnetic field strength of 1.5 Tesla (Achieva 1.5T A-series; Philips, Amsterdam, The Netherlands). We evaluated the atrophy of the volume of interest (VOI) – a portion of the inner temporal lobe that includes the hippocampus, amygdala and entorhinal cortex (frontal part of the parahippocampal gyrus) – using a free software program, VSRAD (VSRAD Advance, Eisai, Japan). VSRAD maintains a database of 80 healthy Japanese male and female volunteers aged 54–86 years (mean age 70.2 ± 7.3 years). Its validity has been previously reported[5,6]. After MRI, the VSRAD software automatically extracted the gray matter and standardized each participant's brain size and shape based on statistical parametric mapping 8 (SPM8) and Diffeomorphic Anatomical Registration Through Exponentiated Lie (DARTEL) algebra. We determined the severity of brain atrophy using the Z-score; that is, ([normal control average of voxel-level − patient's voxel-level] / [normal control standard deviation]) in each voxel. We calculated the severity score of VOI atrophy using the following equation: (total amount of positive Z-scores in VOI) / (number of voxels of positive Z-scores in VOI). The severity score of VOI atrophy was interpreted as follows: ≤1: VOI atrophy was rare and >1: VOI atrophy ranged from mild to severe[7]. Therefore, we set the threshold of the severity score of VOI atrophy as 1. The extent of gray matter (GM) atrophy indicates the percentage of areas with a Z score >2 in GM. The extent of VOI atrophy refers to the percentage of areas with a Z score >2 in the VOI. The ratio of VOI/GM atrophy is the ratio of the percentage of the VOI atrophic area to the percentage of the GM atrophic area.

We also evaluated brain lesions on MRI using the following definitions: cerebral infarctions were defined by the presence of areas with high signal intensity on T2-weighted images, and low signal intensity on T1-weighted images in the subcortical gray and white matter, respectively; white matter lesions were defined as discrete areas with high signal intensity on fluid-attenuated inversion recovery images in periventricular white matter and deep subcortical white matter; cerebral microbleeds were defined by the presence of areas with low signal intensity on T2-weighted images in the basal ganglia, corona radiata, thalamus, brain stem and cerebellum.

The participants were diagnosed with type 2 diabetes mellitus before they visited our hospital for diabetes treatment. For the purpose of our research, we considered that individuals who had a self-reported doctor's diagnosis of type 2 diabetes mellitus and those who used oral antidiabetic medication or insulin had type 2 diabetes mellitus. In addition, we checked whether the laboratory data of these type 2 diabetes mellitus patients met the diagnostic criteria of the Japan Diabetes Society[8]. We differentiated type 2 diabetes mellitus from other types of diabetes based on the patients' titer of anti-glutamic acid decarboxylase antibody, insulin secretion ability and clinical course of the disease.

We asked the participants about the duration of their disease and the presence of diabetic retinopathy. We carried out a comprehensive health examination comprising: body mass index (kg/m^2), ankle-brachial index (Vasera VS-1500; FUKUDA DENSHI, Tokyo, Japan), cardio-ankle vascular index (CAVI; Vasera VS-1500; FUKUDA DENSHI), carotid intima-media thickness (Aplio i800, Aplio 500, Aplio 400, Canon, HI VISION Ascendus; HITACHI, Tokyo, Japan), nerve conduction velocity (Neuropack, MEB-2200, MBE-2312; NIHON KOHDEN, Tokyo, Japan) and coefficient of variation of R-R interval (CVRR, ECG-1450, ECG-1560, ECG2450; NIHON KOHDEN). Ankle-brachial index, CAVI and carotid intima-media thickness indicated diabetic macroangiopathy, whereas nerve conduction velocity and CVRR indicated diabetic neuropathy. After the patients fasted overnight, we measured the glycated hemoglobin (HbA1c), fasting plasma glucose and serum C-peptide immunoreactivity (CPR). We also measured albumin and C-peptide levels from the patients' 24-h urine sample. We assessed insulin secretion from urine C-peptide and CPR index (serum CPR / fasting plasma glucose × 100). The CPR index is useful in clinical decision-making and in determining

whether insulin is required to treat type 2 diabetes mellitus patients[9]. We evaluated the participants' cognitive function using the Hasegawa Dementia Scale-Revised (HDS-R) score, which comprises nine simple questions with a maximum score of 30[10].

Statistical analysis

We carried out all the statistical analyses using EZR on the R commander, version 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan). We calculated the mean or median of the diabetes-related factors among two groups, severity score of VOI atrophy ≤1 or severity score of VOI atrophy >1, and compared them using the Student's *t*-test, Mann–Whitney *U*-test or Fisher's exact test, as appropriate. We used a multivariate logistic regression analysis to determine the relationship between the diabetes-related parameters and the severity score of VOI atrophy. We expressed our calculations as odds ratios (ORs) and 95% confidence intervals (CIs), and considered *P*-values of <0.05 as statistically significant.

RESULTS

The demographic and clinical characteristics of the participants (*n* = 137) are shown in Table 1. The mean ± standard deviation age at which they underwent brain MRI was 67.8 ± 9.8 years, 67.9% of them (*n* = 93) were men, and the median duration of diabetes was 10 years (interquartile range 3–20 years). The mean HbA1c was 9.3 ± 2.2%. The median HDS-R score was 28 (interquartile range 26–29), and the median CPR index was 1.29 (interquartile range 0.85–1.74). In 26.3% of the participants (*n* = 36), the severity score of VOI atrophy was >1.0.

As shown in Table 2, we compared the mean or median values of diabetes-related parameters among groups with severity scores of VOI atrophy ≤1 or >1. The participants with a severity score of VOI atrophy >1 were older and showed significantly lower levels of body mass index, CPR index and urine

Table 1 | Demographics and clinical characteristics of the patients

Total (*n*)	137
Male sex, *n* (%)	93 (67.9)
Age (years)	67.8 ± 9.8
BMI (kg/m^2)	25.4 ± 4.1
HbA1c (%)	9.3 ± 2.2
FPG (mg/dL)	151 (124–182)
CPR index	1.29 (0.85–1.74)
Duration of diabetes (years)	10 (3–20)
HDS-R score	28 (26–29)
Severity score of VOI atrophy >1, *n* (%)	36 (26.3)

Data are shown as the mean ± standard deviation or median (interquartile range) or *n* (%). BMI, body mass index; CPR, serum C-peptide immunoreactivity; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDS-R, Hasegawa Dementia Scale-Revised; VOI, volume of interest.

Table 2 | Differences in diabetes-related parameters among groups with severity score of volume of interest atrophy ≤1 or >1

	Severity score of VOI atrophy ≤1 (*n* = 101)	Severity score of VOI atrophy >1 (*n* = 36)	*P*-value
Age (years)	66.0 ± 9.2	72.8 ± 9.9	<0.01*
Male sex, *n* (%)	70 (69.3)	23 (63.8)	0.541
BMI (kg/m^2)	26.0 ± 4.2	23.7 ± 3.6	<0.01*
Duration of diabetes (years)	10 (3–20)	12 (7–20)	0.156
HbA1c (%)	8.7 (7.8–10.5)	9.2 (8.2–11.2)	0.250
FPG (mg/dL)	151 (124–179)	159 (124–206)	0.596
CPR index	1.37 (0.95–1.83)	0.96 (0.52–1.36)	<0.01*
Urine C-peptide (μg/day)	59.4 (32.2–91.2)	34.2 (22.2–60.5)	<0.01*
ABI	1.12 (1.05–1.16)	1.07 (0.94–1.17)	0.152
CAVI	9.1 ± 1.1	9.5 ± 1.2	0.034*
IMT (mm)	0.85 (0.75–1.00)	0.80 (0.70–1.00)	0.699
NCV decline, *n* (%)	78 (79.6)	28 (80.0)	1.000
CVRR (%)	2.10 (1.59–2.89)	1.62 (1.29–2.94)	0.140
Presence of diabetic retinopathy, *n* (%)	30 (36.6)	8 (26.7)	0.374
Urine albumin (mg/day)	9.2 (4.1–40.7)	15.5 (4.2–64.0)	0.617
CCr (mL/min)	87.3 ± 31.7	77.2 ± 34.0	0.126
HDS-R score	28 (26–30)	26 (22–29)	<0.01*

Data are shown as mean ± standard deviation, median (interquartile range) or *n* (%). *Statistical significance (*P* < 0.05). ABI, ankle brachial index; BMI, body mass index; CAVI, cardio-ankle vascular index; CCr, creatinine clearance; CPR, serum C-peptide immunoreactivity; CVRR, coefficient of variation of R-R interval; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDS-R, Hasegawa Dementia Scale-Revised; IMT, carotid intima media thickness; NCV, nerve conduction velocity.

C-peptide. They also showed significantly higher levels of CAVI. The severity score of VOI atrophy was negatively correlated with the HDS-R score (*R* = −0.261, *P* < 0.02). As shown in Table 3, we estimated the effect of each diabetes-related factor on the presence of VOI atrophy in multivariate logistic regression analysis. We analyzed the age, body mass index, CPR index and CAVI – all of which showed significant differences in univariate analysis – and the duration of diabetes, which was predicted as a risk factor in previous studies[4,11]. Age (OR 1.09, 95% CI 1.02–1.15) and CPR index (OR 0.451, 95% CI 0.216–0.940) were correlated with the severity score of VOI atrophy. When we used different thresholds of the severity score of VOI atrophy, such as the median, tertile and quartile values, all of these showed the same result with the threshold set at 1; that is, the CPR index and age were independent risk factors of VOI atrophy. Multiple logistic regression analysis was carried out after adding HbA1c; however, the results were the same before the addition of HbA1c; that is, only the age and CPR index were correlated with the severity score of VOI atrophy.

Table 3 | Effects of each diabetes-related factor on the presence of volume of interest atrophy in multivariate logistic regression analysis

	Odds ratios	95% confidence intervals	P-value
Age (years)	1.09	1.02–1.15	<0.01*
BMI (kg/m^2)	0.953	0.841–1.080	0.444
Duration of diabetes (years)	0.984	0.947–1.020	0.411
CPR index	0.451	0.216–0.940	0.034*
CAVI	1.04	0.652–1.640	0.884

Multivariate logistic regression analysis was carried out with age, body mass index (BMI), serum C-peptide immunoreactivity (CPR) index and cardio-ankle vascular index (CAVI; all of which showed significant differences in univariate analysis) and the duration of diabetes, which was predicted to be a risk factor based on previous studies. *Statistical significance ($P < 0.05$).

Furthermore, we investigated the relationship between diabetes-related parameters and the extent of GM atrophy, extent of VOI atrophy and ratio of VOI/GM atrophy, and only age was correlated with the extent of GM atrophy, extent of VOI atrophy and ratio of VOI/GM atrophy. The associations between several brain lesions detected on the MRI carried out in the present study and various other factors were examined. Patients with cerebral infarctions ($n = 45$), all of which were asymptomatic microinfarctions, had a significantly greater extent of GM atrophy and lower HDS-R, but showed no correlation with the severity score of VOI atrophy, extent of VOI atrophy, ratio of VOI/GM atrophy and diabetes-related parameters compared with those who had no pathological lesions (control group; $n = 37$). Patients with white matter lesions ($n = 71$) or cerebral microbleeds ($n = 26$) had no significant difference in the HDS-R, atrophy of VOI or GM, ratio of VOI/ GM atrophy, or in diabetes-related parameters compared with the control group.

The use of insulin, glucagon-like peptide-1 receptor agonist or any oral antidiabetic agents were not associated with the atrophy of VOI or GM or ratio of VOI/GM atrophy. Only the use of sulfonylureas or glinides was negatively associated with the HDS-R score ($P < 0.05$).

DISCUSSION

In the present study, we showed that older age and lower insulin secretion were risk factors for HPGA, which is characteristic of AD. A few previous studies examined the relationship between hippocampal atrophy and diabetes-related factors in Japanese patients with type 2 diabetes mellitus[4,12,13], but none of them examined the relationship between atrophy and insulin secretion.

The association between AD and insulin-signaling disorders has been shown in previous research. Amyloid β (Aβ) and neurofibrillary tangles are known to form in the brains of patients with AD[14], and Aβ overproduction has been reported in the brains of mice that were administered streptozotocin[15,16]. Excessive phosphorylation of tau proteins promotes their polymerization to form neurofibrillary tangles[17,18]. A deficiency in insulin signaling promotes tau phosphorylation mediated by the glycogen synthase kinase 3β, which is one of the tau phosphorylases[19,20]. Several studies have reported that tau phosphorylation was significantly promoted not only in type 2 diabetes mellitus models showing insulin resistance, but also in models of type 1 diabetes[21-24]. These data show that a lower insulin concentration leads to impaired insulin signaling in the brain, thereby resulting in Aβ overproduction and hyperphosphorylation of the tau protein.

Some previous reports showed that insulin resistance or hyperinsulinemia was associated with cognitive decline; however, others did not[25,26]. One of the possible mechanisms of this association is that, during hyperinsulinemia, the insulin permeability of the blood–brain barrier is reduced due to downregulation of the insulin receptors in the vascular endothelium, thereby reducing the insulin concentration in the cerebrospinal fluid[27]. A previous report showed that the ratio of the concentration of insulin in cerebrospinal fluid to serum insulin was reduced in non-diabetic AD patients with higher plasma insulin levels[28].

In contrast, the present study showed that patients with lower insulin secretion abilities tended to have VOI atrophy. A low concentration of serum insulin leads to a low concentration of insulin in the cerebrospinal fluid, which gives rise to insulin signal disorders and results in Aβ overproduction, excessive amount of hyperphosphorylated tau protein, and atrophy of hippocampal and parahippocampal regions. Although these findings disagree with the aforementioned report, other studies provided evidence that supports the present results. Indeed, a prospective study of a community-based cohort of Japanese-American elderly men showed that both low and high levels of insulin were associated with an increased risk of developing dementia[29]. In addition, in the population-based Uppsala Longitudinal Study of Adult Men, impaired acute insulin response during the intravenous glucose tolerance test in middle-aged participants was associated with an increased risk of AD up to 35 years later[30]. Furthermore, intranasal insulin administration improved cognitive performance in patients with type 2 diabetes mellitus[31]. Finally, in an experimental study, insulin infusion into the brain lateral ventricles of streptozotocin-diabetic rats for 12 weeks prevented brain atrophy[32]. Therefore, insulin deficiency could be one of the important mechanisms for cognitive decline in diabetes mellitus through hyperglycemia, high glucose variability or the defects in insulin signaling in the brain.

The Hisayama study showed that hippocampal volume and total brain volume were significantly lower in participants with diabetes than in those without diabetes, and this atrophy was associated with a longer duration of diabetes[4]. The present study, VOI atrophy was not associated with the duration of

diabetes; however, the recorded duration might have been inaccurate, as the information was based on self-reported data and was not obtained from a review of the medical records.

Hanyu *et al.*[11,33] suggested that there was a dementia subgroup among diabetes patients, characterized by high HbA1c, long duration of diabetes mellitus and high frequency of insulin therapy – they called it 'diabetes-related dementia.' MRI findings in 'diabetes-related dementia' patients showed no evidence of vascular lesions, less-severe medial temporal lobe atrophy, but diffuse cortical atrophy. We examined associations between the HDS-R score and HbA1c, duration of disease and insulin use. These were features of diabetes-related dementia, but these were not correlated with the HDS-R score. As almost all of our participants had not manifested overt dementia, we might not have been able to sow the characteristics of diabetes-related dementia in the present study.

Many reports claim that diabetes promotes AD pathology[34], whereas others suggest that it promotes brain atrophy and brain damage without increasing AD pathology[35]. However, AD is still considered to be the most common cause of dementia. This is because >40% of type 2 diabetes mellitus patients show intermediate or severe AD pathology at the time of brain autopsy[34].

We examined the associations of glucose-lowering medicines with brain atrophy or cognitive function, and patients who were treated with sulfonylureas or glinides had a significantly lower HDS-R. The possible reason for this association might be that these medicines can cause severe hypoglycemia.

There were some limitations in this study that need to be mentioned. A history of severe hypoglycemia and education could affect brain atrophy and cognitive function; however, we could not assess the influence of these factors, as this information could not be obtained from the patients. The effect of glucose-lowering medicines on cognitive function might have been difficult to determine accurately, because many patients were on multidrug regimens at various periods. The ability of the VSRAD might have been influenced by the whole-brain volume; for example, if there was atrophy of the whole brain, the value of severity score of VOI atrophy would indicate false negatives. In the present study, there were just two patients with whole-brain atrophy, but their severity scores of VOI atrophy were >1; therefore, this potential influence did not affect the present results.

Further studies are required in the future to verify the present results, and determine whether there is a direct link between lower insulin secretion and HPGA in diabetes patients.

We successfully concluded that lower levels of insulin secretion in patients with type 2 diabetes mellitus were significantly associated with the occurrence of HPGA. The results of the present study, which are supplemented by MRI findings, support the hypothesis that insulin-signaling abnormalities are involved in the pathophysiology of AD. For preventing AD in type 2 diabetes mellitus patients, early treatment intervention will be important to maintain insulin secretion ability.

REFERENCES

1. Ohara T, Hata J, Yoshida D, *et al*. Trends in dementia prevalence, incidence, and survival rate in a Japanese community. *Neurology* 2017; 88: 1925–1932.

2. Ninomiya T. Diabetes mellitus and dementia. *Curr Diab Rep* 2014; 14: 487.

3. Moulton CD, Costafreda SG, Horton P, *et al*. Meta-analyses of structural regional cerebral effects in type 1 and type 2 diabetes. *Brain Imaging Behav* 2015; 9: 651–652.

4. Hirabayashi N, Hata J, Ohara T, *et al*. Association between diabetes and hippocampal atrophy in elderly Japanese: the Hisayama study. *Diabetes Care* 2016; 39: 1543–1549.

5. Hirata Y, Matsuda H, Nemoto K, *et al*. Voxel-based morphometry to discriminate early Alzheimer's disease from controls. *Neurosci Lett* 2005; 382: 269–274.

6. Matsuda H, Mizumura S, Nemoto K, *et al*. Automatic voxel-based morphometry of structural MRI by SPM8 plus diffeomorphic anatomic registration through exponentiated lie algebra improves the diagnosis of probable Alzheimer Disease. *Am J Neuroradiol* 2012; 33: 1109–1114.

7. Matsuda H. MRI in Alzheimer's disease. *Gazoushindan* 2018; 38: 889–896 (Japanese).

8. Seino Y, Nanjo K, Tajima N, *et al*. Report of the committee on the classification and diagnostic criteria of diabetes mellitus (Revision for International Harmonization of HbA1c in Japan). *J Jpn Diab Soc* 2012; 55: 485–504 (Japanese).

9. Asano T, Kawamura M, Watanabe T, *et al*. Indices of urinary and serum C-peptide corrected with fasting plasma glucose for decision-making of insulin therapy in type 2 diabetes-validation and comparison. *J Jpn Diab Soc* 2008; 51: 759–763 (Japanese).

10. Katoh S, Simogaki H, Onodera A, *et al*. Development of the revised version of Hasegawa's dementia scale (HDS-R). *Jpn J Geriatr Psychiatry* 1991; 2: 1339–1347 (Japanese).

11. Fukazawa R, Hanyu H, Sato T, *et al*. Subgroups of Alzheimer's disease associated with diabetes mellitus based on brain imaging. *Dement Geriatr Cogn Disord* 2013; 35: 280–290.

12. Shimomura T, Anan F, Masaki T, *et al*. Homocysteine levels are associated with hippocampal volume in type 2 diabetic patients. *Eur J Clin Invest* 2011; 41: 751–758.

13. Masaki T, Anan F, Shimomura T, et al. Association between hippocampal volume and serum adiponectin in patients with type 2 diabetes mellitus. Metabolism 2012; 61: 1197–1200.

14. Selkoe DJ. The molecular pathology of Alzheimer's disease. Neuron 1991; 6: 487–498.

15. Devi L, Alldred MJ, Ginsberg SD, et al. Mechanisms underlying insulin deficiency-induced acceleration of β-amyloidosis in a mouse model of Alzheimer's disease. PLoS One 2012; 7: e32792.

16. Lannert H, Hoyer S. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. Behav Neurosci 1998; 112: 1199–1208.

17. Mandelkow EM, Biernat J, Drewes G, et al. Tau domains, phosphorylation, and interactions with microtubules. Neurobiol Aging 1995; 16: 355–363.

18. Gómez-Isla T, Hollister R, West H, et al. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol 1997; 41: 17–24.

19. Hanger DP, Hughes K, Woodgett JR, et al. Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. Neurosci Lett 1992; 147: 58–62.

20. Dozza B, Smith MA, Perry G, et al. Regulation of glycogen synthase kinase-3beta by products of lipid peroxidation in human neuroblastoma cells. J Neurochem 2004; 89: 1224–1232.

21. Salkovic-Petrisic M, Tribl F, Schmidt M, et al. Alzheimer-like changes in protein kinase B and glycogen synthase kinase-3 in rat frontal cortex and hippocampus after damage to the insulin signalling pathway. J Neurochem 2006; 96: 1005–1015.

22. Plaschke K, Kopitz J, Siegelin M, et al. Insulin-resistant brain state after intracerebroventricular streptozotocin injection exacerbates Alzheimer-like changes in Tg2576 AbetaPP-overexpressing mice. J Alzheimers Dis 2010; 19: 691–704.

23. Jolivalt CG, Hurford R, Lee CA, et al. Type 1 diabetes exaggerates features of Alzheimer's disease in APP transgenic mice. Exp Neurol 2010; 223: 422–431.

24. Ke YD, Delerue F, Gladbach A, et al. Experimental diabetes mellitus exacerbates tau pathology in a transgenic mouse model of Alzheimer's disease. PLoS One 2009; 4: e7917.

25. Feinkohl I, Price JF, Strachan MW, et al. The impact of diabetes on cognitive decline: potential vascular, metabolic, and psychosocial risk factors. Alzheimers Res Ther 2015; 7: 46.

26. Reijmer YD, van den Berg E, de Bresser J, et al. Accelerated cognitive decline in patients with type 2 diabetes: MRI correlates and risk factors. Diabetes Metab Res Rev 2011; 27: 195–202.

27. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat Rev Neurol 2018; 14: 168–181.

28. Craft S, Peskind E, Schwartz MW, et al. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. Neurology 1998; 50: 164–168.

29. Pelia R, Rodriguez BL, White LR, et al. Fasting insulin and incident dementia in an elderly population of Japanese-American men. Neurology 2004; 63: 228–233.

30. Rönnemaa E, Zethelius B, Sundelöf J, et al. Impaired insulin secretion increases the risk of Alzheimer disease. Neurology 2008; 71: 1065–1071.

31. Novak V, Milberg W, Hao Y, et al. Enhancement of vasoreactivity and cognition by intranasal insulin in type 2 diabetes. Diabetes Care 2014; 37: 751–759.

32. Serbedzija P, Madl JE, Ishii DN. Insulin and IGF-I prevent brain atrophy and DNA loss in diabetes. Brain Res 2009; 1303: 179–194.

33. Hanyu H, Hirose D, Fukasawa R, et al. Guidelines for the clinical diagnosis of diabetes mellitus-related dementia. J Am Geriatr Soc 2015; 63: 1721–1723.

34. Biessels GJ, Despa F. Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. Nat Rev Endocrinol 2018; 14: 591–604.

35. Kalaria RN. Neurodegenerative disease: diabetes, microvascular pathology and Alzheimer disease. Nat Rev Neurol 2009; 5: 305–306.