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Original Article

Focus on orexin-A in obese diabetes rats: upregulation of orexin-A receptor in the diabetic brain

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ABSTRACT — Diabetes mellitus and brain toxicity are closely linked, and oxidative stress, obesity, insulin resistance, and glucose toxicity can affect the brain. Orexin-A, also known as hypocretin-1, through its activated receptor, participates in many physiological processes. Orexin-A has been associated with feeding behavior, obesity, and pathogenesis of Alzheimer's disease. We have recently established that high-dose thiamine in obese diabetic Otsuka Long–Evans Tokushima Fatty (OLETF) rats leads to reduced obesity and metabolic disorders. Additionally, we found that plasma orexin-A levels in OLETF rats can be modulated by thiamine supplementation under conditions of oxidative stress. Here, we focused on orexin-A in obese diabetic OLETF rats, which at 58 weeks of age and as expected, showed an increase in body weight and blood glucose levels. Plasma orexin-A was measured by ELISA and tended to be higher in obese diabetic OLETF rats than in non-obese diabetic control rats. We evaluated hypocretin receptor 1 (Hcrtr1, also orexin-A receptor) gene expression in the brain of diabetic OLETF rats by reverse transcription (RT)- polymerase chain reaction (PCR) and show that, compared to controls, diabetic OLETF rats exhibited greater orexin-A receptor gene expression in the brain. The results presented here are expected to provide a better understanding of the role of orexin-A and its contribution to brain toxicity in obese diabetic rats.

Key words: Diabetic brain, Orexin-A receptor expression, Plasma orexin-A level, Obese diabetic OLETF rat, RT-PCR products

INTRODUCTION

Diabetes is the most common metabolic disease, and its prevalence is increasing (Ng *et al*., 2014; WHO, 2016, 2017; Afshin *et al*., 2017; Kohda, 2018). It is associated with an increased risk of mild cognitive impairment, dementia, and stroke (Pasquier *et al*., 2006; Roriz-Filho *et al*., 2009; VanElderen *et al*., 2010; Bornstein *et al*., 2014; Gaspar *et al*., 2016; Bogush *et al*., 2017). Diabetes mellitus and brain toxicity are closely linked because insulin resistance, glucose toxicity, and oxidative stress can affect the brain (Pasquier *et al*., 2006; Roriz-Filho *et al*., 2009; VanElderen *et al*., 2010; Bornstein *et al*., 2014; Gaspar *et al*., 2016; Bogush *et al*., 2017). Although the exact mechanisms by which diabetes affects the brain remain unclear, changes to brain vasculature, disturbances in cerebral insulin signaling, and alterations in amyloid metabolism are all thought to be involved (Pasquier *et al*., 2006; Roriz-Filho *et al*., 2009; VanElderen *et al*., 2010; Bornstein *et al*., 2014; Gaspar *et al*., 2016; Bogush *et al*., 2017).

Orexin, also known as hypocretin, is produced in the hypothalamus, and orexinergic neurons project into several brain areas (DeLecea *et al*., 1998; Sakurai *et al*.,

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1998). There are two known isoforms, namely, orexin-A and orexin-B (DeLecea *et al*., 1998; Sakurai *et al*., 1998). By activating its receptor, orexin-A participates in many physiological processes, such as sleep/wake regulation, modulation of energy metabolism, feeding, learning, and memory (Sakurai *et al*., 1998, 2010; DeLecea, 2012). Diabetes and obesity are among the modifiable risk factors for Alzheimer's disease, and research has indicated that, compared to non-diabetics, people with either type 1 or type 2 diabetes may be at greater risk of developing Alzheimer's disease (Boles *et al*., 2017; Jiang *et al*., 2017; Pugazhenthi *et al*., 2017; Arnold *et al*., 2018; Tumminia *et al*., 2018; Ebrahimpour *et al*., 2020). However, the mechanisms underlying diabetes-related brain toxicity remain elusive.

We have recently established that continuous thiamine consumption by obese diabetic Otsuka Long–Evans Tokushima Fatty (OLETF) rats leads to a reduction in obesity and metabolic disorders (Tanaka *et al*., 2010; Kohda *et al*., 2012, 2017; Kohda, 2020). Thiamine plays an important role in the Wernicke-Korsakoff syndrome, which is a form of amnesia caused by brain damage that occurs in long-term alcoholics who predominantly rely on alcohol for nutrition (Day *et al*., 2013; Latt and Dore, 2014; Chandrakumar *et al*., 2018). The acute syndrome is normally reversible as its progress can be arrested by a timely injection of high-dose thiamine; however, it may develop into profound dementia (Day *et al*., 2013; Latt and Dore, 2014; Gibson *et al*., 2016; Chandrakumar *et al*., 2018). We have demonstrated that thiamine modifies cerebral oxidative stress and that thiamine supplementation can modulate plasma orexin-A levels in OLETF rats under oxidative stress conditions (Kohda and Matsumura, 2019). Orexin-A has been linked to feeding behavior, obesity, and pathogenesis of Alzheimer's disease, and it has been suggested that thiamine may have a beneficial effect on cerebral oxidative stress and Alzheimer's disease (Karuppagounder *et al*., 2009; Lu'o'ng and Nguyen, 2011; Gibson *et al*., 2013, 2020; Liu *et al*., 2017; Wang *et al*., 2017). Aging is also a risk factor for obesity, diabetes, insulin resistance, glucose toxicity, and oxidative stress, and can lead to brain toxicity, especially in the obese diabetic brain (Salmon, 2016; Boles *et al*., 2017). Hence, we focused on orexin-A levels in old OLETF rats (58 weeks old) and evaluated hypocretin receptor 1 (Hcrtr1, also known as orexin-A receptor) gene expression in the rat brain using reverse transcription (RT)- polymerase chain reaction (PCR). The results of this study are expected to provide a better understanding of the role of orexin-A and its contribution to brain toxicity in obese diabetic animals.

MATERIALS AND METHODS

Chemicals

Orexin-A ELISA kit was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Glucose pilot meter and blood glucose test strips (Aventir Biotech, Carlsbad, CA, USA) were used for blood glucose testing. RNAlater solution was purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of the highest purity available from Nacalai Tesque (Kyoto, Japan).

Animals and experimental design

Animals were handled as per the institutional guidelines for animal research, and the experimental work was approved by the Experimental Animal Research Committee of the Osaka Medical and Pharmaceutical University. We chose OLETF rats that exhibit progressive obesity and metabolic disorders due to similarities with the human metabolic syndrome. Male OLETF rats (n = 4; Japan SLC, Inc., Shizuoka, Japan) aged 5 weeks, were used initially, along with Long–Evans Tokushima Otsuka (LETO, n = 4) rats, non-obese/lean counterparts of OLETF rats, also aged 5 weeks. The rats were housed in the animal facility in cages, were provided a standard diet, and had access to water ad libitum. They were housed under controlled temperature and humidity conditions with 12-hr light-dark cycle.

Obesity was assessed based on body weight and diabetes was assessed based on glucose levels in blood collected from the tail vein of the rats. Glucose levels were determined using a blood glucose test strip and random blood glucose levels were measured in obese diabetic OLETF rats and non-obese diabetic LETO rats.

Both obese diabetic and normal rats were sacrificed at 58 weeks of age as follows. Animals were anesthetized with 50 mg/kg pentobarbital and blood samples were collected from the ventral aorta. Whole brain tissue was immediately harvested after the rats were exsanguinated and stored at −80°C in RNAlater solution (Sigma) until RNA preparation. Isolated RNA was converted to complementary DNA (cDNA) by RT, and the cDNA subjected to amplification by PCR.

Plasma orexin-A level measurement

Blood samples were collected from the ventral aorta and into heparin tubes. Plasma was separated from whole blood by centrifugation at 3000 rpm for 10 min in a refrigerated bench-top centrifuge (Kubota Corp., Tokyo, Japan), and plasma aliquots were stored at −80°C until analysis. Plasma orexin-A content was measured by

enzyme-linked immunosorbent assay (ELISA) and absorbance was read in a microplate reader (FUJIFILM Wako Pure Chemical Corp.). The kit did not cross-react with orexin-B. Plasma orexin-A assays were performed in duplicate. We also evaluated spike and recovery for the ELISA using plasma samples from each group.

RNA preparation and PCR amplification of the hypocretin receptor 1(Hcrtr1) targeted region

Diabetic brain tissue was stored at −80°C in RNAlater solution (Sigma) until RNA preparation. Total RNA was extracted using NucleoSpinRNA Plus (Takara Bio Inc., Shiga, Japan). RNA purity and concentration were determined by measuring optical density at 260 nm and 280 nm before use. The optical density ratio at 260/280 ranged from 1.8 to 2.1. For reverse transcription, 1 μg of total RNA was reverse transcribed into cDNA using PrimeScript RT Reagent Kit (Takara Bio Inc.).

The PCR reaction used primer sets RA062454-F with the sequence 5'-TGCGGCCAACCCTATCATCTA-3' and RA062454-R with the sequence 5'-ACCGGCTCTGCAAGGACAA-3', which encodes hypocretin receptor 1 gene fragment (Hcrtr1; syn. orexin-A receptor), and RA015375-F with the sequence 5'-GGAGATTACTGCCCTGGCTCCTA-3' and RA015375-R with the sequence 5'-GACTCATCGTACTCCTGCTTGCTG-3', which encodes β-actin gene fragment (Actb). For PCR, 10 μL of Premix Ex Tag (Takara Bio Inc.) and 0.4 μL of each of the forward and reverse primers (50 μM stock; Takara Bio Inc.) were added per PCR tube (0.2 mL; Thermo Fisher Scientific K.K., Tokyo, Japan). Finally, 2 μL of cDNA template and 7.2 μL of nuclease-free water were added to each tube for a final reaction volume of 20 μL.

PCR was performed using a standard thermocycler (SimpliAmp Thermal Cycler, Thermo Fisher Scientific K.K.) and PCR conditions were as follows: Initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation (95°C for 30 sec), annealing (60°C for 30 sec) and extension (72°C for 30 sec), and a final extension at 72°C for 4 min. PCR products were removed from the thermocycler and maintained at room temperature for a few minutes before separation on an agarose gel.

Preparation of agarose gels and electrophoresis of RT-PCR products

Ready-to-use precast 4% agarose gels with 12 wells (Funakoshi Co., Ltd., Tokyo, Japan) were used for electrophoresis. The gel tray was placed in a submerge-mini electrophoresis system (ATTO Corp., Tokyo, Japan) and the electrophoresis chamber was filled with 1X Tris-borate EDTA buffer until approximately 1 cm above the gel. Subsequently, 10 μL of each RT-PCR product was loaded into each well and electrophoresis was performed for 50 min. The power supply was set to 100 V when 4% agarose gels were run at room temperature. DNA size markers comprised 100 bp–200 bp DNA ladder marker (Takara Bio Inc.). After separation, electrophoresis gels were stained for 30 min with GelGreen nucleic acid gel stain (FUJIFILM Wako Pure Chemical Corp.) and RT-PCR products were visualized and photographed in an Ez-Capture MG machine (ATTO Corp.).

Statistical analyses

Data are expressed as mean ± S.E. Group comparisons used the two-tailed Student's *t*-test. All statistical analyses were performed on the Pharmaco Basic software (Scientist Press Co., Ltd., Tokyo, Japan). A p-value < 0.05 indicated statistical significance.

RESULTS

Metabolic parameters in obese diabetic OLETF rats

At 5 weeks of age, both the OLETF and LETO rats had similar body weights and blood glucose levels. At 58 weeks of age, body weight and blood glucose levels increased as expected and were higher in obese diabetic OLETF rats than non-obese diabetic normal LETO rats (Fig. 1A and 1B).

Plasma orexin-A levels in obese diabetic rats

The levels of plasma orexin-A in obese diabetic rats that were overweight (Fig. 1A) and had high blood glucose levels were investigated (Fig. 1B). Plasma orexin-A levels tended to be higher in obese diabetic OLETF rats than in non-obese diabetic LETO rats (Fig. 2).

Orexin-A receptor expression in obese diabetic OLETF rat brain

Expression of hypocretin receptor 1 (orexin-A receptor) was evident in the brain of obese diabetic OLETF rats (Fig. 3A). The obtained RT-PCR product of 137 bp corresponded to the predicted size of the orexin-A receptor and obese diabetes OLETF rats exhibited greater orexin-A receptor gene expression in the brain compared to non-obese diabetic LETO rat brain (Fig. 3A). The gene expression of β-actin in the brain was shown as an internal standard (Fig. 3B).

DISCUSSION

Here, we have evaluated plasma orexin-A levels in OLETF rats that were old, overweight, and had high

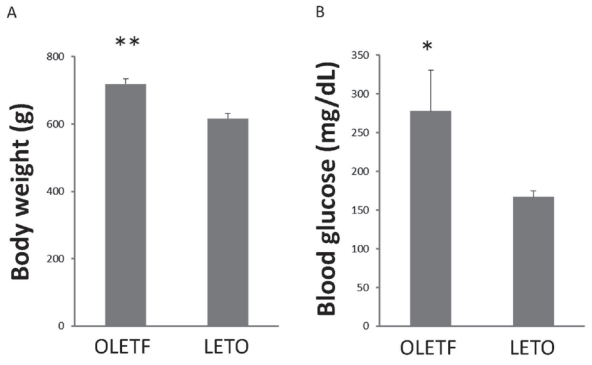

Fig. 1. Body weight (A) and blood glucose (B) levels at 58 weeks of age in Otsuka Long–Evans Tokushima Fatty (OLETF) and Long–Evans Tokushima Otsuka (LETO) rats. Significant differences were observed between OLETF (obese diabetic rat, n = 4) and LETO (non-obese, non-diabetes normal rat, n = 4) animals at 58 weeks of age. Each bar represents mean ± S.E.; **p < 0.01 and *p < 0.05 compared to the LETO rat.

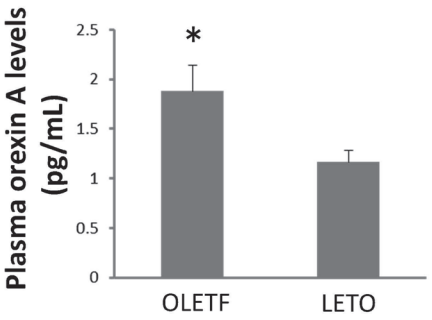

Fig. 2. Level of plasma orexin-A in 58-week-old Otsuka Long–Evans Tokushima Fatty (OLETF) and Long–Evans Tokushima Otsuka (LETO) rats. Significant differences were observed between OLETF (obese diabetic rat, n = 4) and LETO (non-obese diabetes normal rat, n = 4) animals at 58 weeks of age. Each bar represents the mean ± S.E.; *p < 0.01 compared to the LETO rat.

blood glucose levels, and show that obese diabetic rats exhibited higher plasma orexin-A levels compared to non-obese diabetic LETO rats. Orexin-A was originally identified as a factor that enhanced feeding behavior and available research suggests that orexin can elicit polyphagia and obesity (Sakurai *et al*., 1998, 2010; DeLecea, 2012). We evaluated OLETF rats developed by Kawano *et al*. (1991, 1992)—these polyphagia-induced OLETF rats lack functional receptors for cholecystokinin-A, which is associated with satiety control.

Orexin receptors are expressed throughout the brain, and we focused on hypocretin-1 receptor (syn. orexin-A receptor) gene expression. We assessed cerebral orexin-A receptor gene expression by RT-PCR and the PCR product obtained from OLETF and LETO rat whole brain tissue was of expected size. As enhanced orexin-A receptor expression was seen in the brain of obese OLETF rats, our results suggest that orexin-A receptor is susceptible to upregulation in the diabetic OLETF rat brain.

Orexin-A has been suggested to control obesity and be involved in the pathogenesis of Alzheimer's disease (Karuppagounder *et al*., 2009; Lu'o'ng *et al*., 2011; Gibson *et al*., 2013, 2020; Liu *et al*., 2017; Wang *et al*., 2017). Moreover, orexin is reported to be involved in the oxidative stress response (Li *et al*., 2020), and higher plasma orexin-A levels and orexin-A receptor expression in the rat brain might play important roles in oxidative stress-induced injury in OLETF rats. The effect of cerebral oxidative stress and associated upregulation of orexin-A receptor expression in the obese diabetic brain require

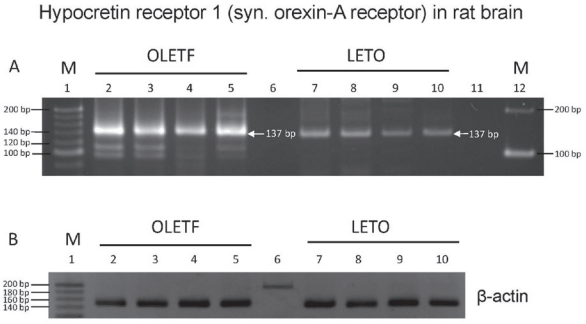

Fig. 3. Agarose gel electrophoresis of RT-PCR amplified products of the hypocretin receptor 1 (Hcrtr1; syn. orexin-A receptor) (A) gene fragment using the primer pair RA062454-F and RA062454-R, and the β-actin (Actb) (B) gene fragment using the primer pair RA015375-F and RA015375-R. RT-PCR products were separated on a 4% agarose gel by electrophoresis and stained with nucleic acid gel stain solution. Lane 1: 100 bp–200 bp DNA ladder; lane 2-5: diabetic Otsuka Long–Evans Tokushima Fatty (OLETF) rat brain; lane 7-10: non-diabetic Long–Evans Tokushima Otsuka (LETO) rat brain; lane 12: 100 bp–200 bp DNA ladder. RT-PCR product of expected size was obtained in all samples from OLETF and LETO rat whole brain tissue. Product size, 137 bp. Bp; base pairs, RT-PCR; reverse transcription-polymerase chain reaction.

further investigation.

It has been demonstrated that metabolic diseases like obesity, diabetes, and insulin resistance are associated with alterations in the central nervous system, that they

are linked with the development of cognitive and memory impairment, and that they present higher risk of developing dementia and Alzheimer's disease (Boles *et al*., 2017; Jiang *et al*., 2017; Pugazhenthi *et al*., 2017; Arnold *et al*., 2018; Tumminia *et al*., 2018; Ebrahimpour *et al*., 2020). In fact, type 3 diabetes is the new label given to Alzheimer's disease (Kandimalla *et al*., 2017; Nguyen *et al*., 2020). Thus, the threat of premature aging represents another reason to effectively control insulin resistance. Further, aging itself is associated with progressive worsening of glucose tolerance and a reduction in the ability of pancreatic β-cells to secrete insulin in response to a glucose challenge (Kulstad *et al*., 2006; Chiu *et al*., 2008). Obesity is related to a decline in the activities of daily living in the elderly and is related to a high prevalence of disorders, such as diabetes, hyperlipidaemia, and hypertension (Ogden *et al*., 2003; DeWinter *et al*., 2012; Bozkurt *et al*., 2016). It has been demonstrated that older adults with metabolic syndrome were 20% more likely to suffer from cognitive decline on a memory test than those who did not suffer from the metabolic syndrome (Raffaitin *et al*., 2011).

The rising prevalence of diabetes, together with its increasing earlier onset, suggests that diabetes-related cognitive dysfunction will increase in the near future. A reduction in insulin secretion or action, dysregulation of glucose homeostasis, impairment of the hypothalamic–pituitary–adrenal axis, and obesity, may act to disrupt neuronal homeostasis and cause diabetes-associated cognitive decline (Stranahan *et al*., 2008; Gaspar *et al*., 2016). Multiple studies have shown that orexin-A is involved in the pathogenesis of Alzheimer's disease and available evidence indicates that a gene linked to the development of type 2 diabetes may also be a factor in the pathogenesis of Alzheimer's disease (Karuppagounder *et al*., 2009; Lu'o'ng *et al*., 2011; Gibson *et al*., 2013, 2020; Liu *et al*., 2017; Wang *et al*., 2017). In the past few years, key strategies involving neuroprotective and antioxidant drugs have emerged as promising therapies for brain disease, which remains preventable or treatable (Kikuchi *et al*., 2014; Feng *et al*., 2019; Singh *et al*., 2019).

We hope that fundamental studies in the OLETF rat, such as focus on orexin-A, will lead to a treatment breakthrough and the development of preventive measures against obesity and brain toxicity in obese diabetics.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Afshin, A., Forouzanfar, M.H., Reitsma, M.B., Sur, P., Estep, K., *et al*. and GBD 2015 Obesity Collaborators. (2017): Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N. Engl. J. Med., **377**, 13-27.

Arnold, S.E., Arvanitakis, Z., Macauley-Rambach, S.L., Koenig, A.M., Wang, H.Y., Ahima, R.S., Craft, S., Gandy, S., Buettner, C., Stoeckel, L.E., Holtzman, D.M. and Nathan, D.M. (2018): Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat. Rev. Neurol., **14**, 168-181.

Bogush, M., Heldt, N.A. and Persidsky, Y. (2017): Blood Brain Barrier Injury in Diabetes: Unrecognized Effects on Brain and Cognition. J. Neuroimmune Pharmacol., **12**, 593-601.

Boles, A., Kandimalla, R. and Reddy, P.H. (2017): Dynamics of diabetes and obesity: epidemiological perspective. Biochim. Biophys. Acta Mol. Basis Dis., **1863**, 1026-1036.

Bornstein, N.M., Brainin, M., Guekht, A., Skoog, I. and Korczyn, A.D. (2014): Diabetes and the brain: issues and unmet needs. Neurol. Sci., **35**, 995-1001.

Bozkurt, B., Aguilar, D., Deswal, A., Dunbar, S.B., Francis, G.S., Horwich, T., Jessup, M., Kosiborod, M., Pritchett, A.M., Ramasubbu, K., Rosendorff, C. and Yancy, C. (2016): Contributory risk and management of comorbidities of hypertension, obesity, diabetes mellitus, hyperlipidemia, and metabolic syndrome in chronic heart failure: A scientific statement from the American Heart Association. Circulation, **134**, e535-e578.

Chandrakumar, A., Bhardwaj, A. and 't Jong, G.W. (2018): Review of thiamine deficiency disorders: Wernicke encephalopathy and Korsakoff psychosis. J. Basic Clin. Physio.l Pharmacol., **30**, 153-162.

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.

Day, E., Bentham, P.W., Callaghan, R., Kuruvilla, T. and George, S. (2013): Thiamine for prevention and treatment of Wernicke-Korsakoff Syndrome in people who abuse alcohol. Cochrane Database Syst. Rev., **2013**, CD004033.

De Lecea, L. (2012): Hypocretins and the neurobiology of sleep-wake mechanisms. Prog. Brain Res., **198**, 15-24.

De Lecea, L., Kilduff, T.S., Peyron, C., Gao, X., Foye, P.E., Danielson, P.E., Fukuhara, C., Battenberg, E.L., Gautvik, V.T., Bartlett, F.S., Frankel, W.N., Van Den Pol, A.N., Bloom, F.E., Gautvik, K.M. and Sutcliffe, J.G. (1998): The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc. Natl. Acad. Sci. USA, **95**, 322-327.

De Winter, C.F., Bastiaanse, L.P., Hilgenkamp, T.I., Evenhuis, H.M. and Echteld, M.A. (2012): Cardiovascular risk factors (diabetes, hypertension, hypercholesterolemia and metabolic syndrome) in older people with intellectual disability: results of the HA-ID study. Res. Dev. Disabil., **33**, 1722-1731.

Ebrahimpour, S., Zakeri, M. and Esmaeili, A. (2020): Crosstalk between obesity, diabetes, and alzheimer's disease: introducing quercetin as an effective triple herbal medicine. Ageing Res. Rev., **62**, 101095.

Feng, T., Yamashita, T., Shang, J., Shi, X., Nakano, Y., Morihara, R., Tsunoda, K., Nomura, E., Sasaki, R., Tadokoro, K., Matsumoto, N., Hishikawa, N., Ohta, Y. and Abe, K. (2019): Clinical and

pathological benefits of edaravone for Alzheimer's disease with chronic cerebral hypoperfusion in a novel mouse model. J. Alzheimers Dis., **71**, 327-339.

Gaspar, J.M., Baptista, F.I., Macedo, M.P. and Ambrósio, A.F. (2016): Inside the Diabetic Brain: Role of Different Players Involved in Cognitive Decline. ACS Chem. Neurosci., **7**, 131-142.

Gibson, G.E., Hirsch, J.A., Cirio, R.T., Jordan, B.D., Fonzetti, P. and Elder, J. (2013): Abnormal thiamine-dependent processes in Alzheimer's Disease. Lessons from diabetes. Mol. Cell. Neurosci., **55**, 17-25.

Gibson, G.E., Hirsch, J.A., Fonzetti, P., Jordan, B.D., Cirio, R.T. and Elder, J. (2016): Vitamin B1 (thiamine) and dementia. Ann. N. Y. Acad. Sci., **1367**, 21-30.

Gibson, G.E., Luchsinger, J.A., Cirio, R., Chen, H., Franchino-Elder, J., Hirsch, J.A., Bettendorff, L., Chen, Z., Flowers, S.A., Gerber, L.M., Grandville, T., Schupf, N., Xu, H., Stern, Y., Habeck, C., Jordan, B. and Fonzetti, P. (2020): Benfotiamine and Cognitive Decline in Alzheimer's Disease: Results of a Randomized Placebo-Controlled Phase IIa Clinical Trial. J. Alzheimers Dis., **78**, 989-1010.

Jiang, C., Li, G., Huang, P., Liu, Z. and Zhao, B. (2017): The Gut Microbiota and Alzheimer's Disease. J. Alzheimers Dis., **58**, 1-15.

Kandimalla, R., Thirumala, V. and Reddy, P.H. (2017): Is Alzheimer's disease a Type 3 Diabetes? A critical appraisal. Biochim. Biophys. Acta Mol. Basis Dis., **1863**, 1078-1089.

Karuppagounder, S.S., Xu, H., Shi, Q., Chen, L.H., Pedrini, S., Pechman, D., Baker, H., Beal, M.F., Gandy, S.E. and Gibson, G.E. (2009): Thiamine deficiency induces oxidative stress and exacerbates the plaque pathology in Alzheimer's mouse model. Neurobiol. Aging, **30**, 1587-1600.

Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M. and Natori, T. (1991): New inbred strain of Long-Evans Tokushima lean rats with IDDM without lymphopenia. Diabetes, **40**, 1375-1381.

Kawano, K., Hirashima, T., Mori, S., Saitoh, Y., Kurosumi, M. and Natori, T. (1992): Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes, **41**, 1422-1428.

Kikuchi, K., Tanaka, E., Murai, Y. and Tancharoen, S. (2014): Clinical trials in acute ischemic stroke. CNS Drugs, **28**, 929-938.

Kohda, Y. (2020): Age-related hepatic glucose-dependent insulinotropic polypeptide expression is modified by ongoing thiamine supplementation in obese diabetic rats. Fundam. Toxicol. Sci., **7**, 291-299.

Kohda, Y. (2018): Paradigm change to future health enhancement through comprehending the concept of obesity disease in Japan. J. Clin. Toxicol., **8**, 389.

Kohda, Y., Maekita, A., Tanaka, T. and Matsumura, H. (2017): Hepatic glucose-dependent insulinotropic polypeptide expression is modified by supplementing high-dose thiamine in obese diabetic rats. Fundam. Toxicol. Sci., **4**, 279-284.

Kohda, Y. and Matsumura, H. (2019): Obesity-related hypertension and enhanced plasma orexin-A level are attenuated by the consumption of thiamine water in diabetic rats under cerebral oxidative stress conditions. Fundam. Toxicol. Sci., **6**, 383-390.

Kohda, Y., Tanaka, T. and Matsumura, H. (2012): Role of thiamine in obesity-related diabetes: Modification of the gene expression. Food and Nutritional Components in Focus No.4, B Vitamins and Folate: Chemistry, Analysis, Function and Effects. The Royal Society of Chemistry, 580-591.

Kulstad, J.J., Green, P.S., Cook, D.G., Watson, G.S., Reger, M.A., Baker, L.D., Plymate, S.R., Asthana, S., Rhoads, K., Mehta, P.D. and Craft, S. (2006): Differential modulation of plasma beta-amyloid by insulin in patients with Alzheimer disease. Neurology, **66**, 1506-1510.

Latt, N. and Dore, G. (2014): Thiamine in the treatment of Wernicke encephalopathy in patients with alcohol use disorders. Intern. Med. J., **44**, 911-915.

Li, M., Meng, Y., Chu, B., Shen, Y., Xue, X., Song, C., Liu, X., Ding, M., Cao, X., Wang, P., Xu, S., Bi, J. and Xie, Z. (2020): Orexin-A exacerbates Alzheimer's disease by inducing mitochondrial impairment. Neurosci. Lett., **718**, 134741.

Liu, D., Ke, Z. and Luo, J. (2017): Thiamine Deficiency and Neurodegeneration: the Interplay Among Oxidative Stress, Endoplasmic Reticulum Stress, and Autophagy. Mol. Neurobiol., **54**, 5440-5448.

Lu'o'ng, K.V. and Nguyen, L.T. (2011): Role of thiamine in Alzheimer's disease. Am. J. Alzheimers Dis. Other Demen., **26**, 588-598.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., *et al*. (2014): Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet, **384**, 766-781.

Nguyen, T.T., Ta, Q.T., Nguyen, T.K., Nguyen, T.T. and Giau, V.V. (2020): Type 3 Diabetes and Its Role Implications in Alzheimer's Disease. Int. J. Mol. Sci., **21**, 3165.

Ogden, C.L., Carroll, M.D. and Flegal, K.M. (2003): Epidemiologic trends in overweight and obesity. Endocrinol. Metab. Clin. North Am., **32**, 741-760.

Pasquier, F., Boulogne, A., Leys, D. and Fontaine, P. (2006): Diabetes mellitus and dementia. Diabetes Metab., **32**, 403-414.

Pugazhenthi, S., Qin, L. and Reddy, P.H. (2017): Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. Biochim. Biophys. Acta Mol. Basis Dis., **1863**, 1037-1045.

Raffaitin, C., Féart, C., Goff, M.L., Amieva, H., Helmer, C., Akbaraly, T.N., Tzourio, C., Gin, H. and Barberger-Gateau, P. (2011): Metabolic syndrome and cognitive decline in French elders: the Three-City Study. Comparative Study Neurology, **76**, 518-525.

Roriz-Filho, S.J., Sá-Roriz, T.M., Rosset, I., Camozzato, A.L., Santos, A.C., Chaves, M.L., Moriguti, J.C. and Roriz-Cruz, M. (2009): (Pre)diabetes, brain aging, and cognition. Biochim. Biophys. Acta, **1792**, 432-443.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozlowski, G.P., Wilson, S., Arch, J.R., Buckingham, R.E., Haynes, A.C., Carr, S.A., Annan, R.S., McNulty, D.E., Liu, W.S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J. and Yanagisawa, M. (1998): Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell, **92**, 573-585.

Sakurai, T., Mieda, M. and Tsujino, N. (2010): The orexin system: roles in sleep/wake regulation. Ann. N. Y. Acad. Sci., **1200**, 149-161.

Salmon, A.B. (2016): Beyond Diabetes: Does Obesity-Induced Oxidative Stress Drive the Aging Process? Antioxidants, **5**, 24.

Singh, S.K., Srivastav, S., Castellani, R.J., Plascencia-Villa, G. and Perry, G. (2019): Neuroprotective and antioxidant effect of Ginkgo biloba extract against AD and other neurological disorders. Neurotherapeutics, **16**, 666-674.

Stranahan, A.M., Arumugam, T.V., Cutler, R.G., Lee, K., Egan, J.M. and Mattson, M.P. (2008): Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. Nat. Neurosci., **11**, 309-317.

Tanaka, T., Kono, T., Terasaki, F., Yasui, K., Soyama, A., Otsuka, K., Fujita, S., Yamane, K., Manabe, M., Usui, K. and Kohda, Y. (2010): Thiamine prevents obesity and obesity-associated meta-

bolic disorders in OLETF rats. J. Nutr. Sci. Vitaminol. (Tokyo), **56**, 335-346.

Tumminia, A., Vinciguerra, F., Parisi, M. and Frittitta, L. (2018): Type 2 Diabetes Mellitus and Alzheimer's Disease: Role of Insulin Signalling and Therapeutic Implications. Int. J. Mol. Sci., **19**, 3306.

Van Elderen, S.G., DeRoos, A., De Craen, A.J., Westendorp, R.G., Blauw, G.J., Jukema, J.W., Bollen, E.L., Middelkoop, H.A., Van Buchem, M.A. and Van Der Grond, J. (2010): Progression of brain atrophy and cognitive decline in diabetes mellitus: a 3-year follow-up. Neurology, **75**, 997-1002.

Wang, X., Xu, M., Frank, J.A., Ke, Z.J. and Luo, J. (2017): Thiamine deficiency induces endoplasmic reticulum stress and oxidative stress in human neurons derived from induced pluripotent stem cells. Toxicol. Appl. Pharmacol., **320**, 26-31.

WHO. (2016): World health statistics 2016: Monitoring health for the SDGs. Geneva: World Health Organization

WHO. (2017): 10 facts on obesity, updated February 2017. Geneva: World Health Organization