Neuroscience Letters 571 (2014) 11–16

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Immunohistochemical analysis of tau phosphorylation and astroglial activation with enhanced leptin receptor expression in diet-induced obesity mouse hippocampus

Shunsuke Koga[c], Ayako Kojima[a], Satoshi Kuwabara[c], Yasumasa Yoshiyama[a,b,c,*]

[a] Laboratory for Neurodegenerative Disorder Research, Clinical Research Center, Chiba East National Hospital, Japan
[b] Department of Neurology, Chiba-East National Hospital, Japan
[c] Department of Neurology, Chiba University Graduate School of Medicine, Japan

HIGHLIGHTS

- Diet-induced obesity (DIO) causes tau phosphorylation in wild-type mouse hippocampus.
- DIO enhances astrogliosis, astroglial leptin receptor expression, and mild microgliosis.
- Astroglial leptin receptor may play a role in these pathological processes.
- Voluntary exercise can prevent these DIO-induced pathological changes.

ARTICLE INFO

Article history:
Received 7 January 2014
Received in revised form 21 March 2014
Accepted 18 April 2014
Available online 28 April 2014

Keywords:
Alzheimer disease
Tau phosphorylation
Wild-type mouse
Diet-induced obesity
Astrocyte
Voluntary exercise

ABSTRACT

Accumulating evidence indicates that obesity is an independent risk factor for developing Alzheimer disease (AD). Recent studies have shown that diet-induced obesity (DIO) enhances AD-related pathologies in transgenic mouse models of the disease. DIO increases amyloid β (Aβ) deposition in amyloidogenic transgenic mice and enhances tau phosphorylation in tau transgenic mice. However, it remains unclear whether DIO also enhances AD-related pathological processes in wild-type (WT) mice. In this study, we examined the effects of DIO on Aβ and tau pathology in WT mice using immunohistochemistry. In addition, we evaluated the protective effect of voluntary exercise on the DIO-induced pathological changes. DIO caused tau phosphorylation and astroglial activation in the hippocampus in WT mice. Interestingly, these changes were associated with enhanced astrocytic leptin receptor (LepR) expression and mild microgliosis, but not Aβ accumulation. Although phosphorylated tau staining was only observed in the hippocampus, astrogliosis and microgliosis were present in both the amygdala and hippocampus. However, no apparent neuronal loss was observed. Voluntary exercise prevented these DIO-induced pathological changes. Our results demonstrate for the first time that DIO causes tau phosphorylation and that astrocytic LepR might be involved in the pathological process in WT mouse hippocampus. Our findings also suggest that physical exercise is a promising strategy for the prevention of AD in patients with obesity.

1. Introduction

Alzheimer disease (AD) is the most common cause of dementia, with the number of patients increasing steadily worldwide [5]. Accumulating evidence indicates that obesity raises the risk of developing AD, independent of other factors [6,17,20]. Consequently, the risk of developing AD may be decreased by reducing obesity, a preventable disease. However, little is known about the causative link between obesity and AD. AD is pathologically characterized by the extracellular deposition of amyloid β (Aβ) as senile plaques, as well as the intracellular accumulation of

Abbreviations: AD, Alzheimer disease; DIO, diet-induced obesity; Aβ, amyloid β; WT, wild-type; LepR, leptin receptor; GFAP, glial fibrillary acidic protein; LepRs, short form of leptin receptor; LepRl, long form of leptin receptor.

* Corresponding author at: 673 Nitona, Chuo-ku, Chiba 260-8712, Japan. Tel.: +81 43 261 5171; fax: +81 43 268 2613.
E-mail addresses: neuroyy@cehpnet.com, yoshiyama@fastmail.fm (Y. Yoshiyama).

http://dx.doi.org/10.1016/j.neulet.2014.04.028

hyperphosphorylated aggregated tau in the form of neurofibrillary tangles [8]. Recent studies have shown that diet-induced obesity (DIO) enhances AD-related pathology in transgenic mouse models of AD. DIO increases Aβ deposition and memory deficits in APP_{SWE} transgenic mice expressing human APP695 containing the Swedish double mutation (KM670/671NL) [14,15]. DIO also exacerbates tau phosphorylation in the hippocampus and impairs learning ability in tau transgenic mice [12]. These studies clearly show that DIO accelerates AD-associated pathological processes and diminishes cognitive function. Furthermore, APP_{SWE} transgenic mice fed a high-fat diet and given physical exercise show less Aβ deposition than their counterparts fed a high-fat diet without physical exercise [14,15]. These findings indicate that physical exercise can prevent obesity-induced pathological changes in AD mouse models. Additionally, in WT rats, DIO increases glial fibrillary acidic protein (GFAP) expression in the hippocampus [7] and impairs cognitive function [11]. However, it remains unclear whether DIO enhances AD-related pathological processes in WT mice.

The purpose of this study was to examine the effects of DIO on tau pathology using immunohistochemistry in WT mice. We also assessed the protective effect of voluntary exercise on the pathological changes induced by DIO.

2. Material and methods

WT female mice (B6C3H/F1) were divided into high calorie diet (Fat = 15.3%; 4.15 Cal/g, Quick Fat (Clea Japan Inc., Tokyo, Japan))-fed ($n = 21$) and standard diet (Fat = 4.5%; 3.3 Cal/g)-fed (SD, $n = 6$) groups. The high calorie diet group was subdivided into no-exercise (HCD, $n = 17$) and exercise (HCD + Ex, $n = 4$) groups. Mice in the HCD and HCD + Ex groups were fed a high calorie diet, starting at the age of 12 weeks. HCD + Ex group mice were housed in cages equipped with a running wheel (Clea Japan Inc., Japan) for voluntary exercise from the age of 8 weeks. The average amount of exercise was $1.1 \pm 0.4 \times 10^4$ rounds/day ($\approx 7.4 \pm 2.5$ km/day, mean ± SD). Body weight was measured every 4 weeks. Animals were anesthetized by intraperitoneal injection of 1% xylocaine at the age of 40 weeks and decapitated. The body temperature was controlled during the anesthesia using a heating pad. We used PS19 tauopathy model mice, which carry the human tau gene with a P301S mutation [21], as positive controls for evaluating tau pathology. In all experiments, we used only female mice because there was a large difference in body weight between male and female, and the body weight of male mice varied much more widely than that of female mice in a preliminary experiment.

Intraperitoneal glucose tolerance tests (IGTTs) were performed at 40 weeks of age. On the night before the IGTTs, food was removed at 6:00 PM, and the following morning, mice were given a single dose, by intraperitoneal injection, of glucose (2 g/kg body weight). Blood samples were collected from the tail vein immediately before glucose administration and at 15, 30, 60 and 120 min after gavage. Blood glycemic content was assessed using Glutest Ace R (Sanwa Kagaku Kenkyusho Co., Ltd, Nagoya, Japan), following the manufacturer's instructions. For analyses, glucose responses over time were integrated to determine the area-under-the-curve.

Tissue preparation and immunohistochemical analysis were performed as previously described [21]. Briefly, mice were deeply anesthetized and transcardially perfused with 15 ml phosphate-buffered saline (PBS). The brains were removed, immersion-fixed for 24 h in 4% paraformaldehyde/PBS, and stored in 15% sucrose/PBS at 4 °C until use. The brains were cut into 20-μm-thick sections for immunohistochemical analysis. Free-floating sections were immunostained using streptavidin-biotin peroxidase.

The following antibodies were used in this study: mAb AT8 (1:100; Innogenetics, Gent, Belgium), mAb AT180 (1:100; Innogenetics), mAb S422 (1:100; Invitrogen, CA, USA), anti-glial fibrillary acidic protein (1:1000; Dako, CA, USA), anti-Iba-1 (1:1000; Wako, Osaka, Japan), anti-leptin receptor M18 (1:100; Santa Cruz Biotechnology, CA, USA), and anti-leptin receptor C14104 (1:100; Neuromics, MN, USA).

Images were captured with a Nikon eclipse 80i microscope and a Nikon DXM 1200C digital camera. To measure the area occupied by S422-, GFAP-, Iba-1- and M18-positive cells in the hippocampus and amygdala we obtained three images each of the CA1, CA3 and dentate gyrus regions of the hippocampus and five images each of the amygdala for each animal. The quantification of the positively labeled areas was performed using Photoshop (Adobe Systems, CA, USA). The number of neurons and the number of GFAP- and Iba-1 positive cells were measured manually using digital photomicrographs. Quantification results were analyzed using one-way ANOVA, followed by Tukey's post hoc test or Scheffe's post hoc test. Results were expressed as mean ± SD, and differences with $P < 0.05$ were considered significant.

3. Results

To evaluate the effect of diet and exercise, we measured body weight gain every 4 weeks. After 20 weeks of age, the body weight of HCD mice was significantly increased compared with SD mice or HCD + Ex mice (Fig. 1A). To estimate glucose tolerance, we performed IGTTs on the three groups. Although serum glucose at 60 min was slightly elevated in the HCD group compared with the SD or HCD + Ex group, the area-under-the-curve was not significantly different among the three groups (Fig. 1B). These findings suggest that glucose metabolism is not significantly perturbed in HCD mice.

To assess the effect of DIO on the tau phosphorylation, the neuropathological hallmarks of AD, we performed immunohistochemistry using several phosphorylation-specific tau antibodies; S422 (phospho-Ser422), AT-8 (phospho-Ser202 and phospho-Thr205) and AT-180 (phospho-Thr231). Sections from the three different groups in WT mice were not labeled with AT-8 (Fig. 1C) or AT-180 (Fig. 1D). Interestingly, dendrites in the CA1 region and mossy fiber in the CA3 region were slightly stained with S422 in HCD mice (Fig. 1G and H), but not in SD (Fig. 1E and F) or HCD + Ex (Fig. 1I and J) mice. As positive controls, perikarya and neurites in the CA1 of the hippocampus from the 24-week-old PS19 mice were strongly stained with AT-8 (Fig. 1C, inset), AT-180 (Fig. 1D, inset), or S422 (Fig. 1E, inset), respectively. Quantitative analysis revealed that the percentage of the area occupied by S422-positive cells is higher in HCD mice than in SD or HCD + Ex mice (Fig. 1K). We also examined the amygdala and other brain regions, but they were not stained with S422, AT-8 or AT-180. It is known that tau phosphorylation occurs in neurites in early phase [18]. From these results, we conjectured that DIO can at least partially induce tau phosphorylation in the hippocampus in WT mice. Next, to assess neurodegeneration, we counted the number of neurons in the hippocampus. The number of neurons in the hippocampus was not significantly different among the three different groups (data not shown), suggesting that neurodegeneration was not present.

Because astrogliosis parallels the distribution of tau phosphorylation in neurodegenerative diseases [2,19], we performed immunohistochemistry with an antibody to GFAP. The number of GFAP-immunoreactive astrocytes in the CA1 and CA3 regions of the hippocampus was significantly increased (Fig. 2B and E) and some astrocytes displayed hypertrophic changes (Fig. 2B, E, inset) in HCD mice compared with SD (Fig. 2A and D) or HCD + Ex mice (Fig. 2C and F). Similar changes were observed in the amygdala (Fig. 2G–I). Quantitative analysis revealed that the percentage of the area occupied by GFAP-positive cells (Fig. 2P) and the number of astrocytes

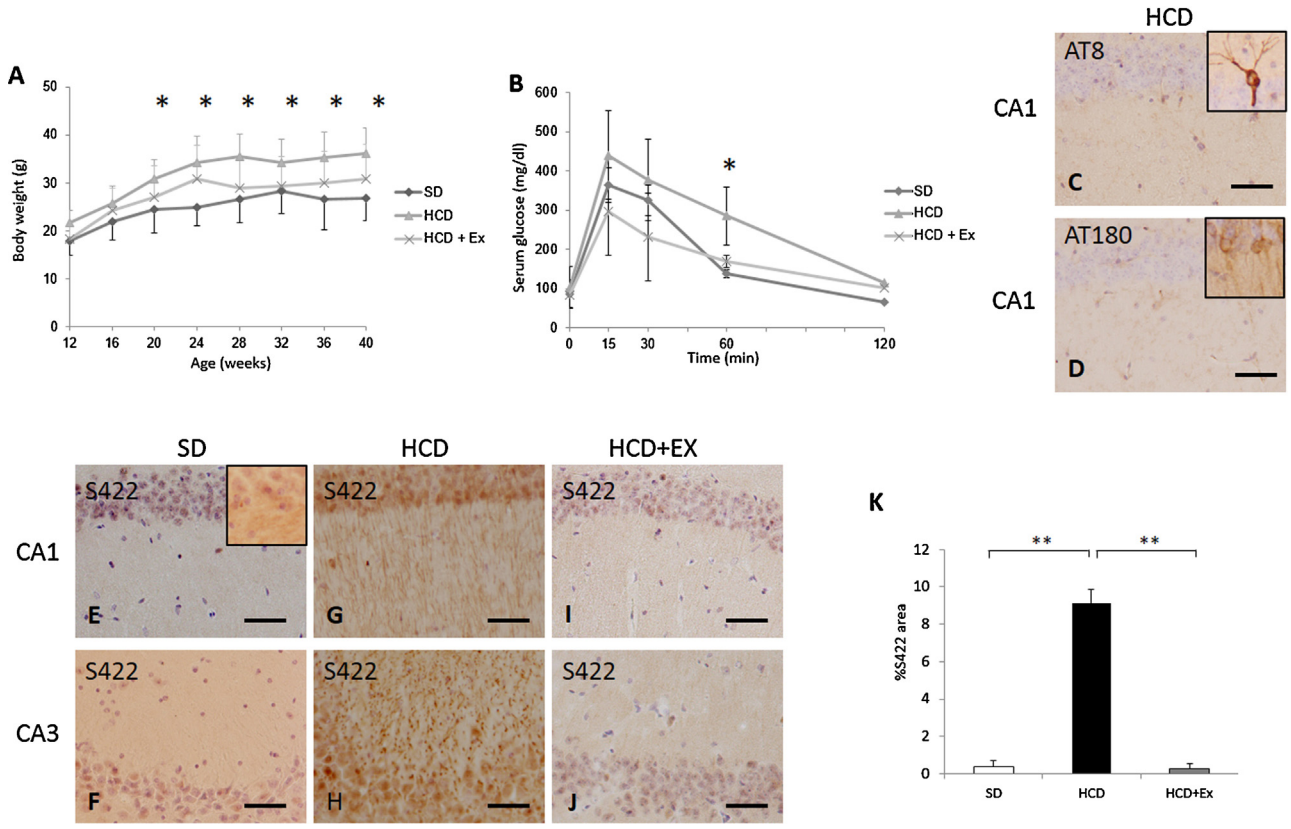

Fig. 1. Effects of a high calorie diet and voluntary exercise on body weight and glucose tolerance (A and B). Graphs show the change in body weight every 4 weeks (A) and the glucose tolerance test results at the age of 40 weeks (B). Immunohistochemistry for AT-8, AT-180-, or Ser422-phosphorylated tau in the hippocampus (C–J). Sections from CA1 region of the hippocampus in the HCD group mice are not stained with AT-8 (C) or AT-180 (against pThr231) (D) antibodies and counterstained with hematoxylin. The CA1 and CA3 regions of the hippocampus from SD (E and F), HCD (G and H) and HCD + Ex (I and J) mice are stained S422 antibody (against pSer422). Dendrites in the CA1 region (E) and mossy fiber in the CA3 region (F) are stained with S422 in HCD mice, but not in SD or HCD + Ex mice. Perikarya and neurites in the CA1 of the hippocampus from the 24-week-old PS19 mice were strongly stained with AT-8 (C, inset), AT-180 (D, inset), or S422 (E, inset), respectively. The graph shows that the area of S422 positive cells is higher in HCD mice than in SD or HCD + Ex mice (K). Scale bars: 50 μm (C–J). Data are mean ± SD. $^{*}P < 0.05$, HCD vs. SD or HCD + Ex mice. $^{**}P < 0.01$, HCD vs. SD or HCD + Ex mice.

(Fig. 2Q) were increased in the hippocampus and amygdala of HCD mice compared with SD or HCD + Ex animals. Because microglia-mediated neuroinflammation is implicated in the pathogenesis of neurodegenerative diseases [1,21–23], we assessed microglial activation using an anti-Iba-1 antibody. In HCD mice, the number of microglia was increased in the hippocampus (Fig. 2J–L) and the amygdala (Fig. 2M–O), paralleling the distribution of astrogliosis. Although significant microglial morphological differences were not seen among the three different groups (Fig. 2J–O, inset), the percentage of the area occupied by Iba-1-positive cells (Fig. 2R) and the number of microglia (Fig. 2S) were increased in the hippocampus and amygdala of HCD mice compared with SD or HCD + Ex animals. These findings of increased astrogliosis and microgliosis in the absence of neuronal loss suggest that the activation of astrocytes and microglia in HCD mice is not secondary to the neurodegenerative changes, but might be directly caused by DIO. The results also indicate that microglial activation is relatively mild compared with astroglial activation.

As described above, we observed tau phosphorylation and astrogliosis in the HCD mouse hippocampus. Our previous study using PS19 mice showed that DIO enhanced tauopathy and neuroinflammation through the activation of astrocytic leptin receptor (LepR) (Unpublished data). Therefore, focusing on the role of leptin receptor in the WT mice, we performed immunohistochemistry for LepR in the hippocampus where the overt astrogliosis occurred. LepR can be divided into two isoforms; long form (LepRl) and short form (LepRs). We used anti-M18 antibody, which recognizes the short cytoplasmic form of membrane-bound LepR [9]. M18-positive cells were seen mainly in the hippocampus (Fig. 3A–C) and the hypothalamus (data not shown). M18-positive cells were considered astrocytes on a morphological basis, and double-labeled hippocampal sections demonstrated that all M18-expressed cells were GFAP-positive astrocytes (Fig. 3D–F). To quantify the LepRs expression, we standardized the M18-positive area divided by the GFAP-positive area because LepRs was expressed in astrocytes and HCD increased the area of astrocytes. As shown in Fig. 3G, the expression of astrocytic LepRs was significantly increased in the hippocampus of HCD mice compared with SD or HCD + Ex animals. This indicated that DIO caused not only astrogliosis, but also increase expression of LepRs. We also performed immunohistochemistry with a CH14104 antibody, which recognizes the unique LepRl cytoplasmic tail sequence, but staining was barely visible in the hippocampus (data not shown).

4. Discussion

Our results demonstrate that persistent obesity from early life induces tau phosphorylation in the hippocampus accompanied by enhanced astroglial LepRs expression, which might be directly related to obesity. Although phosphorylated tau staining was only seen in the hippocampus, astroglial activation and microgliosis were seen in the amygdala and hippocampus, both regions in which tau pathology is preferentially seen in AD. The difference in distribution suggests that astroglial activation and microgliosis might

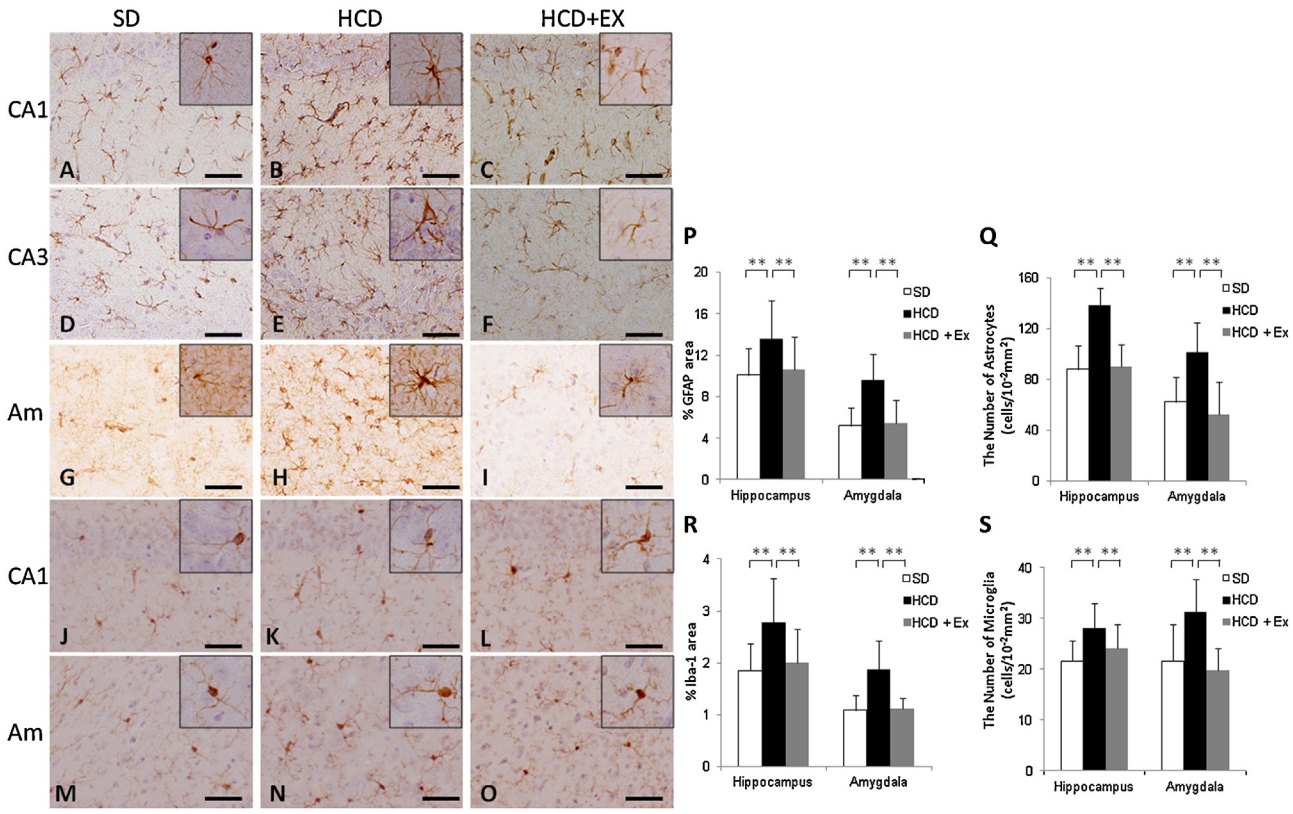

Fig. 2. Immunohistochemistry for GFAP and Iba-1 in the hippocampus and amygdala. The CA1 (A–C and J–L) and CA3 (D–F) regions of the hippocampus and amygdala (G–I and M–O) from SD (A, D, G, J and M), HCD (B, E, H, K and N) and HCD + Ex (C, F, I, L and O) mice were stained for GFAP (A–I) or Iba-1 (J–O). HCD mice show marked expression of GFAP in the hippocampus (B and E) and amygdala (H). Some astrocytes in HCD animals show hypertrophic changes (B, E, H, inset). HCD mice have an increased number of microglia in the hippocampus (K) and the amygdala (N); however, morphological differences between the three different groups were not observed (J–O, inset). The graphs show that the area of GFAP- (P) or Iba-1-positive cells (R) and the number of astrocytes (Q) or microglia (S) in the hippocampus and amygdala are greater in HCD mice than in SD or HCD + Ex mice. Am, Amygdala; Scale bars: 50 μm (A–O). Data are mean ± SD. $^{**}P < 0.01$, HCD vs. SD or HCD + Ex mice.

precede tau phosphorylation. Because there was no evidence of neurodegeneration in the hippocampus even though tau phosphorylation was observed in this region, obesity itself may not induce the entire pathological process of tau-phosphorylation-related neurodegeneration or might require a much longer period of time to induce neurodegeneration. In this study, tau phosphorylation was observed only at Ser422, but not at Ser202/Thr205. There is a consistent pattern of tau phosphorylation that corresponds to the degree of neuronal cytopathology during neurofibrillary tangle formation [3]. Ser422 is phosphorylated earlier than Ser202/Thr205, which is recognized by AT-8 [3]. In addition, tau phosphorylation is detected in neurites earlier than in cell body in early phase [18]. Therefore, we presumably observed one of the earliest tau pathological changes.

Recently, Jayaram et al. that reported that DIO did not increase GFAP expression in astrocyte-specific LepR knockout mice; rather, it reduced [10]. Although they did not mention the isoform of astrocytic LepR, our study showed that LepRs, not LepRl, was expressed in astrocytes. Therefore, astrocytic LepRs is thought to be involved in the process of DIO-induced astrogliosis. Furthermore, a recent study showed that the LepRs functions as a proinflammatory factor during leptin resistance [13]. Thus, our results suggest that DIO might enhance astroglial leptin sensitivity and activation by up-regulating the LepRs, which in turn might promote tau phosphorylation by enhanced inflammatory responses. However, the link between astroglial activation, enhanced LepRs expression and inflammation remains unclear and requires further investigation.

Recent studies have shown differing results on whether DIO enhances tau phosphorylation in WT mice [4,12,16]. Both Moroz's study (60%-fat chow, fed for 4 months from 1 month of age) and Leboucher's study (59%-fat chow, fed for 5 months from 2 months of age) reported that DIO increased the level of tau but did not induce tau phosphorylation [12,16]. On the other hand, Bhat's study (21%-fat chow, fed for 2 months from 4 months of age) and our present study (15.3%-fat chow, fed for 7 months from 3 months of age) showed that DIO increased tau phosphorylation in WT mice [4]. The discrepancy in results probably arises from food nutrition, feeding duration, or both. Interestingly, studies feeding mice a mildly high-fat diet demonstrated tau phosphorylation. We therefore conjecture that DIO itself can promote a subset of tau phosphorylation but depends on conditions which remain unclear.

Some studies have shown that physical exercise alleviates pathological changes elicited by DIO in the AD model without reduction in body weight [14,15]. Our results in WT mice are in line with the findings in these reports; voluntary exercise inhibits DIO-induced tau phosphorylation, astroglial activation and microgliosis in the hippocampus. However, it remains unclear whether voluntary exercise directly alleviates these pathological changes in this study because the body weight of mice in the HCD + Ex group was significantly lower than that of mice in the HCD group. Moreover, HCD mice showed slight glucose intolerance and voluntary exercise mitigated the change. It is possible that improvement of obesity and/or glucose intolerance ameliorated these pathological changes.

In conclusion, the present study demonstrates that DIO at least partially causes tau phosphorylation and that astrocytic LepRs may play a role in the pathogenetic process in WT mouse hippocampus. While obesity itself does not appear to directly cause

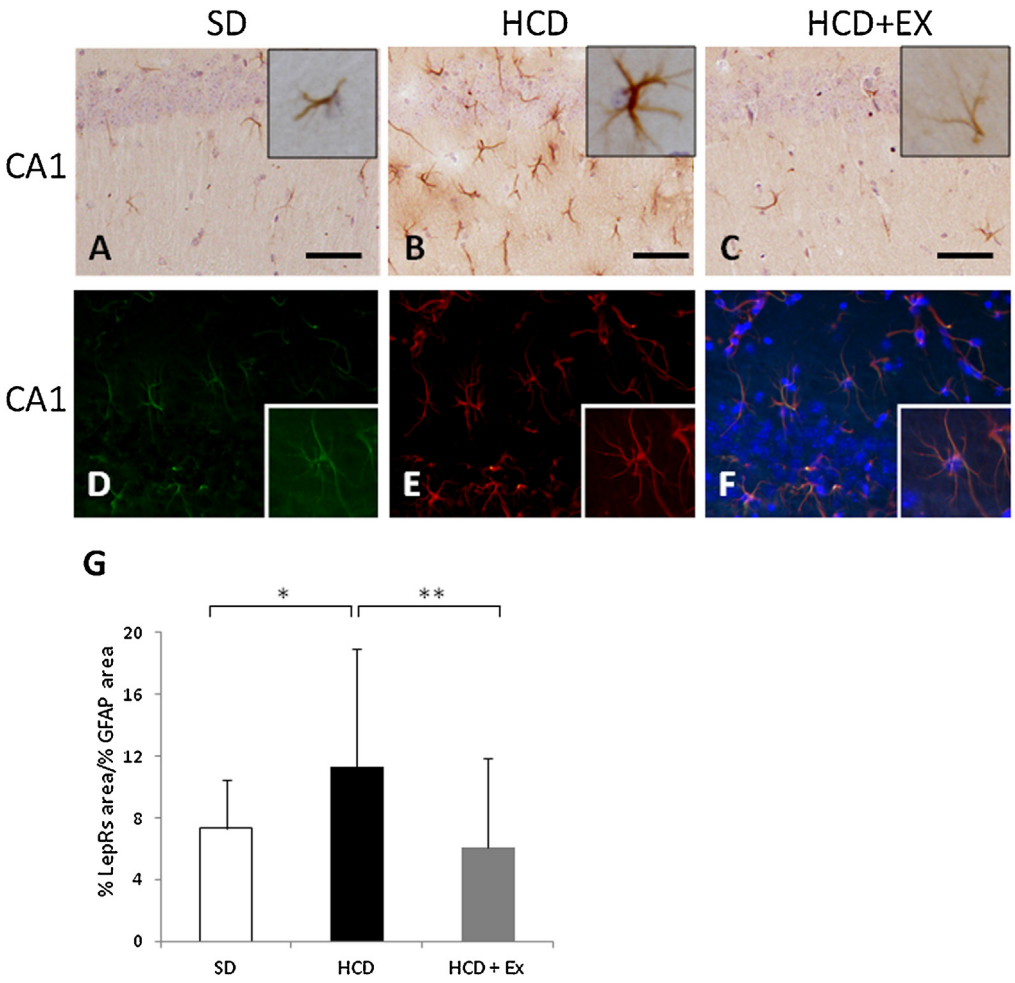

Fig. 3. Immunohistochemistry for M18 in the hippocampus. The CA1 regions of the hippocampus from SD (A), HCD (B) and HCD + Ex (C) mice were stained for M18 which recognizes the short form of the leptin receptor. HCD mice also show marked expression of M18 compared with SD or HCD + Ex mice (A–C). The merged image of immunofluorescent staining (F) shows colocalization of GFAP (D) and M18 (E). The graph shows that the ratio of M18-positive/GFAP positive area is higher in the HCD group than in the SD or HCD + Ex groups (G). Scale bars: 50 μm (A–F). Data are mean ± SD. $^{*}P < 0.05$, HCD vs. SD or HCD + Ex mice. $^{**}P < 0.01$, HCD vs. SD or HCD + Ex mice.

neurodegeneration, it might accelerate pathological processes in neurodegenerative disorders. Our findings also indicate that physical exercise might be a promising strategy for the prevention of AD in patients with obesity.

References

[1] H. Akiyama, T. Arai, H. Kondo, E. Tanno, C. Haga, K. Ikeda, Cell mediators of inflammation in the Alzheimer disease brain, Alzheimer Dis. Assoc. Disord. 14 (2000) 47–53.
[2] P.V. Arriagada, J.H. Growdon, E.T. Hedley-Whyte, B.T. Hyman, Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease, Neurology 42 (1992) 631–639.
[3] J.C. Augustinack, A. Schneider, E.M. Mandelkow, B.T. Hyman, Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease, Acta Neuropathol. 103 (2002) 26–35.
[4] N.R. Bhat, L. Thirumangalakudi, Increased tau phosphorylation and impaired brain insulin/IGF signaling in mice fed a high fat/high cholesterol diet, J. Alzheimers Dis. 36 (2013) 781–789.
[5] C.P. Ferri, M. Prince, C. Brayne, H. Brodaty, L. Fratiglioni, M. Ganguli, K. Hall, K. Hasegawa, H. Hendrie, Y. Huang, A. Jorm, C. Mathers, P.R. Menezes, E. Rimmer, M. Scazufca, Alzheimer's Disease International, Global prevalence of dementia: a Delphi consensus study, Lancet 366 (2005) 2112–2117.
[6] A.L. Fitzpatrick, L.H. Kuller, O.L. Lopez, P. Diehr, E.S. O'Meara, W.T. Longstreth Jr., J.A. Luchsinger, Midlife and late-life obesity and the risk of dementia: cardiovascular health study, Arch. Neurol. 66 (2009) 336–342.
[7] E. Fuente-Martín, C. García-Cáceres, M. Granado, M.L. de Ceballos, M.Á. Sánchez-Garrido, B. Sarman, Z.W. Liu, M.O. Dietrich, M. Tena-Sempere, P. Argente-Arizón, F. Díaz, J. Argente, T.L. Horvath, J.A. Chowen, Leptin regulates glutamate and glucose transporters in hypothalamic astrocytes, J. Clin. Invest. 122 (2012) 3900–3913.
[8] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[9] H. Hsuchou, Y. He, A.J. Kastin, H. Tu, E.N. Markadakis, R.C. Rogers, P.B. Fossier, W. Pan, Obesity induces functional astrocytic leptin receptors in hypothalamus, Brain 132 (2009) 889–902.
[10] B. Jayaram, W. Pan, Y. Wang, H. Hsuchou, A. Mace, C.G. Cornelissen-Guillaume, P.K. Mishra, R.A. Koza, A.J. Kastin, Astrocytic leptin-receptor knockout mice show partial rescue of leptin resistance in diet-induced obesity, J. Appl. Physiol. 114 (2013) 734–741.
[11] B.T. Jeon, E.A. Jeong, H.J. Shin, Y. Lee, D.H. Lee, H.J. Kim, S.S. Kang, G.J. Cho, W.S. Choi, G.S. Roh, Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet, Diabetes 61 (2012) 1444–1454.
[12] A. Leboucher, C. Laurent, F.J. Fernandez-Gomez, S. Burnouf, L. Troquier, S. Eddarkaoui, D. Demeyer, R. Caillierez, N. Zommer, E. Vallez, K. Bantubungi, C. Breton, P. Pigny, V. Buée-Scherrer, B. Staels, M. Hamdane, A. Tailleux, L. Buée, D. Blum, Detrimental effects of diet-induced obesity on τ pathology are independent of insulin resistance in τ transgenic mice, Diabetes 62 (2013) 1681–1688.
[13] J.C. Leung, L.Y. Chan, M.F. Lam, S.C. Tang, C.W. Chow, A.I. Lim, K.N. Lai, The role of leptin and its short-form receptor in inflammation in db/db mice infused with peritoneal dialysis fluid, Nephrol. Dial. Transplant. 27 (2012) 3119–3129.
[14] M. Maesako, K. Uemura, M. Kubota, A. Kuzuya, K. Sasaki, M. Asada, K. Watanabe, N. Hayashida, M. Ihara, H. Ito, S. Shimohama, T. Kihara, A. Kinoshita, Environmental enrichment ameliorated high-fat diet-induced Aβ deposition and memory deficit in APP transgenic mice, Neurobiol. Aging 33 (2012), 1011.e11-23.
[15] M. Maesako, K. Uemura, M. Kubota, A. Kuzuya, K. Sasaki, N. Hayashida, M. Asada-Utsugi, K. Watanabe, M. Uemura, T. Kihara, R. Takahashi, S. Shimohama, A. Kinoshita, Exercise is more effective than diet control in preventing high fat diet-induced β-amyloid deposition and memory deficit in amyloid precursor protein transgenic mice, J. Biol. Chem. 287 (2012) 23024–23033.

[16] N. Moroz, M. Tong, L. Longato, H. Xu, S.M. de la, Monte; Limited Alzheimer-type neurodegeneration in experimental obesity and type 2 diabetes mellitus, J. Alzheimers Dis. 15 (2008) 29–44.
[17] L.A. Profenno, A.P. Porsteinsson, S.V. Faraone, Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders, Biol. Psychiatry 67 (2010) 505–512.
[18] J.H. Su, B.J. Cummings, C.W. Cotman, Early phosphorylation of tau in Alzheimer's disease occurs at Ser-202 and is preferentially located within neuritis, Neuroreport 17 (1994) 2358–2362.
[19] T. Togo, D.W. Dickson, Tau accumulation in astrocytes in progressive supranuclear palsy is a degenerative rather than a reactive process, Acta Neuropathol. 104 (2002) 398–402.
[20] R.A. Whitmer, The epidemiology of adiposity and dementia, Curr. Alzheimer Res. 4 (2007) 117–122.
[21] Y. Yoshiyama, M. Higuchi, B. Zhang, S.M. Huang, N. Iwata, T.C. Saido, J. Maeda, T. Suhara, J.Q. Trojanowski, V.M. Lee, Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model, Neuron 53 (2007) 337–351.
[22] Y. Yoshiyama, A. Kojima, C. Ishikawa, K. Arai, Anti-inflammatory action of donepezil ameliorates tau pathology, synaptic loss, and neurodegeneration in a tauopathy mouse model, J. Alzheimers Dis. 22 (2010) 295–306.
[23] Y. Yoshiyama, A. Kojima, K. Itoh, T. Uchiyama, K. Arai, Anticholinergics boost the pathological process of neurodegeneration with increased inflammation in a tauopathy mouse model, Neurobiol. Dis. 45 (2012) 329–336.