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Energy restriction combined with dipeptidyl peptidase-4 inhibitor exerts neuroprotection in obese male rats

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Abstract

Dipeptidyl peptidase-4 (DDP-4) inhibitors and energy restriction (ER) are widely used to treat insulin resistance and type 2 diabetes mellitus. However, the effects of ER or the combination with vildagliptin on brain insulin sensitivity, brain mitochondrial function, hippocampal synaptic plasticity and cognitive function in obese insulin-resistant rats have never been investigated. We hypothesised that ER with DDP-4 inhibitor exerts better efficacy than ER alone in improving cognition in obese insulin-resistant male rats by restoring brain insulin sensitivity, brain mitochondrial function and hippocampal synaptic plasticity. A total of twenty-four male Wistar rats were divided into two groups and fed either a normal diet or a high-fat diet (HFD) for 12 weeks. At week 13, the HFD rats were divided into three subgroups (*n* 6/subgroup) to receive one of the following treatments: vehicle, ER (60 % of energy received during the previous 12 weeks) or ER plus vildagliptin (3 mg/kg per d, p.o.) for 4 weeks. At the end of the treatment, cognitive function, metabolic parameters, brain insulin sensitivity, hippocampal synaptic plasticity and brain mitochondrial function were determined. We found that HFD-fed rats demonstrated weight gain with peripheral insulin resistance, dyslipidaemia, oxidative stress, brain insulin resistance, impaired brain mitochondrial function and cognitive dysfunction. Although HFD-fed rats treated with ER and ER plus vildagliptin showed restored peripheral insulin sensitivity and improved lipid profiles, only ER plus vildagliptin rats had restored brain insulin sensitivity, brain mitochondrial function, hippocampal synaptic plasticity and cognitive function. These findings suggest that only a combination of ER with DPP-4 inhibitor provides neuroprotective effects in obese insulin-resistant male rats.

Key words: Energy restriction: Vildagliptin: Obese insulin-resistant rats: Brain mitochondrial functions: Hippocampal synaptic plasticity: Cognitive functions

Obesity is a hallmark of insulin resistance and the metabolic syndrome, which has significantly increased worldwide[1]. Previous studies demonstrated that high-energy-diet-induced obesity led to the development of insulin resistance and cognitive decline in animal models[2,3]. In addition, our previous studies showed that the development of peripheral insulin resistance, brain insulin resistance, brain mitochondrial dysfunction, as well as cognitive decline, occurred in high-fat-diet (HFD)-induced obese male rats[4,5]. The dipeptidyl peptidase-4 inhibitor, vildagliptin, is widely used in the treatment of type 2 diabetes mellitus (T2DM) because of its beneficial effects on the regulation of the blood glucose level without causing any hypoglycaemic effects[6]. In addition, our previous studies reported that vildagliptin improved not only peripheral insulin resistance but also brain insulin resistance, as indicated by improved brain insulin receptor function in obese insulin-resistant rats[4]. Furthermore, the enhancement of hippocampal neurogenesis and improvement in cognition were found in HFD-fed rodents treated with vildagliptin[4,7,8]. All of those findings suggest that vildagliptin exerts beneficial effects on metabolic control and cognitive function.

Although several types of medication have been prescribed for the treatment of T2DM or the obese insulin-resistant condition, several side effects of medication have been observed. Therefore, a lifestyle modification, including energy restriction

Abbreviations: aCSF, artificial cerebrospinal fluid; Akt/PKB, serine/threonine-specific protein kinase B; ER, energy restriction; HFD, high-fat diet; HFRV, HFD-fed rats on a restricted diet and vehicle; HFRVil, HFD-fed rats on a restricted diet and vildaglptin; HFV, HFD-fed rats treated with the vehicle; IR, insulin receptor; LTD, long-term depression; LTP, long-term potentiation; MDA, malondialdehyde; MWM, Morris Water Maze; ND, normal diet; NDV, ND-fed rats treated with vehicle; ROS, reactive oxygen species.

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(ER), has been used as an alternative therapy in the obese insulin-resistant condition and T2DM. A previous study showed that ER in older animals led to improved metabolic parameters and also an extended lifespan[9]. Although adverse effects of ER such as promoting bone loss[10] and decreasing wound healing rate[11], in rats have been reported, several studies have shown that ER mimetics conferred the benefits of ER without its side effects[12,13]. Moderate ER had a beneficial impact on metabolic parameters, and improved insulin sensitivity in *in vitro* and *in vivo* studies[14–17]. In addition, a previous study reported that ER not only exerts a positive effect on metabolic control but also leads to improved brain function[18,19]. Several studies demonstrated that ER caused an increase in an endogenous apoptosis inhibitor in the neuronal cells[20], induced neuroprotection[21], decreased mitochondrial reactive oxygen species (ROS) production in aged rat brains[22] and preserved cognitive function in aged male rats[23]. All of those findings suggest that ER has beneficial effects in neuroprotection and the preservation of cognitive function.

However, the effects of either ER or the combination of ER plus vildagliptin on brain function, including brain insulin sensitivity, brain mitochondrial function, hippocampal synaptic plasticity and cognitive function in obese insulin-resistant rats have never been investigated. This study tests the hypothesis that the combination of ER plus vildagliptin has greater beneficial effects on the brain function of obese insulin-resistant rats than ER alone.

Methods

Animal models and experimental protocols

In total, twenty-four male Wistar rats weighing 180–200 g (approximately aged 5–6 weeks old), obtained from the National Animal Center, Salaya Campus, Mahidol University, Bangkok, Thailand, were used. All experiments were conducted in accordance with the approved protocol from the Faculty of Medicine, Chiang Mai University's Institutional Animal Care and Use Committee, in compliance with NIH guidelines (protocol number: 31/2557). All animals were housed in environmentally controlled conditions (25 ± 0·5°C and a 12 h light–12 h dark cycle) and were allowed to acclimate for 1 week. Rats were randomly divided into two groups and fed on either a normal diet (ND: 19·77 % energy (%E) from fat, *n* 6) or a HFD (59·28 %E from fat, *n* 18) for 12 weeks[24]. At the end of the 12th week, rats in the ND group received normal saline solution (NSS) via intra-gastric gavage, as indicated, and were designated the control group (ND-fed rats treated with the vehicle (NDV)), and rats in the HFD group were subdivided into three subgroups: (1) 12-week HFD-fed rats continued with HFD and in addition were given NSS via intra-gastric gavage for 4 weeks (HFD-fed rats treated with the vehicle (HFV)); (2) 12-week HFD-fed rats were switched to a restricted diet (ND, containing only 60 % energy intake compared with the energy intake of HFD), as described in a previous study[17], and were also given NSS via intra-gastric gavage for 4 weeks (HFD-fed rats on a restricted diet and vehicle (HFRV)); and (3) 12-week HFD-fed rats were switched to a restricted diet and in addition were given 3 mg/kg per d vildagliptin (Novartis, Thailand) via intra-gastric gavage for 4 weeks[6,8]. The rats' cognitive function was subsequently determined by the Morris Water Maze (MWM) test. Blood samples were collected from a tail vein at weeks 0, 12 and 16 for further plasma analysis. At the end of the experimental period, rats were deeply anesthetised with 2–3 % isoflurane and killed by decapitation. The brain of each rat was rapidly removed after death and carefully sliced in preparation for extracellular recording (insulin-induced long-term depression (LTD) and hippocampal synaptic long-term potentiation (LTP)), immunoblot and brain mitochondrial functions. The experimental protocol is shown in Fig. 1.

Blood sample assays

Plasma glucose and cholesterol levels were determined via colorimetric assay (Biotech). Plasma HDL and LDL levels were determined using a commercial colorimetric assay kit (Biovision). Plasma insulin levels were determined using the Sandwich ELISA kit (Millipore). Peripheral insulin resistance was assessed using the homoeostasis model assessment (HOMA), as previously described[5].

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed as described previously[4,8]. In brief, rats were starved overnight before the test and received 2 g/kg glucose solution via oral gavage, and then blood samples were collected at different time points. AUC were calculated to evaluate glucose tolerance.

Brain-slice preparation

The brain-slice preparation was performed as described previously[4,5,24]. In brief, the whole brain was rapidly removed after decapitation and then immersed in ice-cold high-sucrose artificial cerebrospinal fluid (aCSF). The hippocampal slices were cut using a vibratome (Vibratome Company). Following a 30-min post-slice incubation in high-sucrose aCSF, the slices were transferred to a standard aCSF for an additional 30 min at room temperature (22–24°C).

Extracellular recordings of hippocampal slices for insulin-induced long-term depression

An extracellular recording of hippocampal slices for insulin-induced LTD was performed as described in previous studies[4,5,24]. Hippocampal slices were perfused with aCSF (as a baseline condition) for 10 min, and then perfused with aCSF plus 500 nM-insulin (as an insulin-induced LTD) for an additional 10 min. After this, the slices were perfused with aCSF for an additional 50 min and readings recorded.

Extracellular recordings of hippocampal slices for synaptic long-term potentiation

The examination of electrical-induced hippocampal synaptic LTP was performed as described in a previous study[25]. In brief, LTP was induced by delivering high-frequency

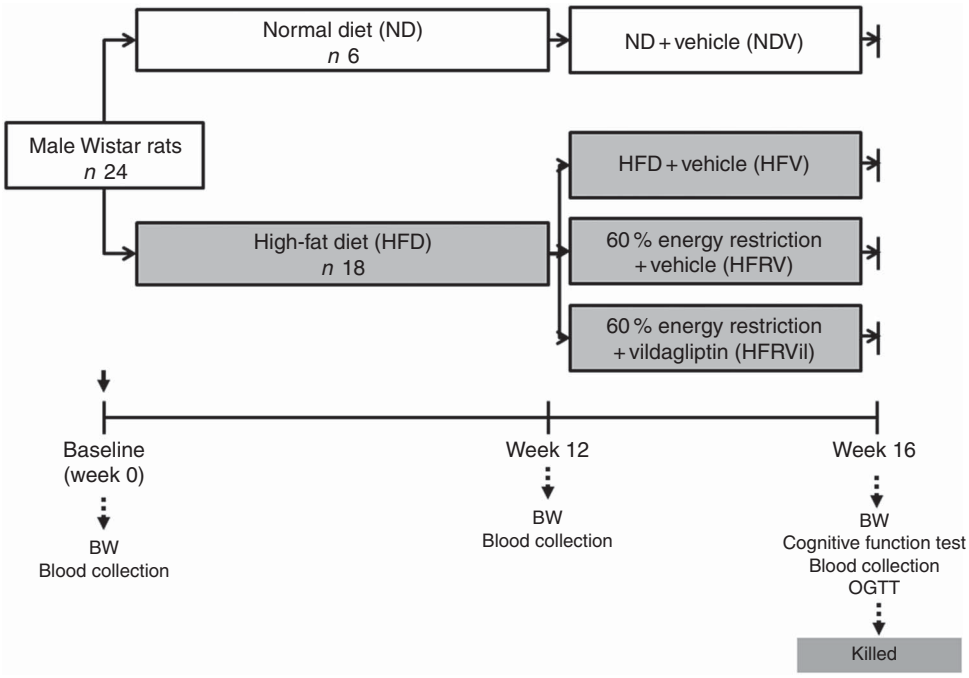

Fig. 1. The experimental protocol of the study. OGTT, oral glucose tolerance test.

stimulation (HFS; four trains at 100 Hz; 0·5-s duration; 20-s interval). Experiments were performed for 40 min after HFS.

Immunoblotting for brain insulin signalling

To investigate the expression of insulin receptor phosphorylation (p-IR), insulin receptors (IR), serine/threonine-specific protein kinase B (Akt/PKB) and insulin-mediated Akt Ser473 phosphorylation, homogenated brain slices from each subgroup were used as described in our previous study[4,5,24]. The p-IR, IR, Akt/PKB at serine 473 kinase phosphorylation and Akt/PKB were electrophoresed and immunoblotted with rabbit anti-IR at tyrosine phosphorylation (p-IR$^{tyr1162/1163}$) (1:1000, sc-25103-R; Santa Cruz Biotechnology), IR (1:1000, sc-711; Santa Cruz Biotechnology), Akt/PKB at serine 473 kinase phosphorylation (1:1000, no. 9271; Cell Signaling Technology) and Akt/PKB (1:1000, no. 9272; Cell Signaling Technology). For a loading control, the immunoblot for each membrane was incubated with anti-β-actin (1:4000, no. 4967; Cell Signaling Technology). All membranes enabling the visualisation of the phosphorylation and the protein levels of IR and Akt/PKB expression were incubated with secondary goat anti-rabbit antibody conjugated with horseradish peroxidase (1:2000, no. 7074; Cell Signaling Technology). Band densities of phosphorylated IR or Akt/PKB at serine 473 kinase phosphorylation were represented as a ratio of insulin stimulation (+):no insulin stimulation (–) and were normalised to total IR or Akt/PKB. Band intensities were quantified using Scion Imaging, and the results were shown as average signal intensity (arbitrary) units.

Serum and brain malondialdehyde levels

HPLC method was used to evaluate the concentrations of serum and brain malondialdehyde (MDA), as described previously[8,26–28].

Brain mitochondrial function study

Brain mitochondria were isolated using the method described previously[4]. Brain mitochondrial function including brain mitochondrial ROS, mitochondrial membrane potential change ($\Delta\Psi$m) and mitochondrial swelling were determined. Brain mitochondrial ROS were measured using dichloro-hydrofluorescein diacetate fluorescent dye. The change in mitochondrial membrane potential ($\Delta\Psi$m) was measured using the fluorescent dye 5, 52, 6, 62-tetrachloro-1, 12, 3, 32-tetraethyl benzimidazol carbocyanine iodide (JC-1) and brain mitochondrial swelling was determined by measuring the change in the absorbance of brain mitochondrial suspension at 540 nm. All were determined by following the methods described previously[4,8].

Cognitive function test

The open-field test (OFT) was used to screen locomotor activity, as described in previous studies[8,29]. The assessment of cognitive function was performed using the MWM test with two assessments including the acquisition test, which was carried out for 5 consecutive days, and the probe test, which was performed on day 6[8,30]. Data analysis of the MWM test was done manually from video tape recordings by the investigators, who were blinded to experimental groups.

Golgi staining and morphological analysis of dendritic spines

Golgi staining and morphological analysis were carried out using the method described previously[31]. After decapitation, brains were removed and rinsed with double-distilled water and were processed for Golgi staining using a commercially

available kit (FD Rapid GolgiStain™ Kit #PK401; FD Neurotechnologies, Inc.). For analysis of dendritic spine density, the secondary and tertiary dendrites of three neurons in the CA1 hippocampus area were counted. Dendritic segments were viewed through an inverted microscope (IX-81; Olympus).

Statistical analysis

Data were expressed as mean values with their standard errors. For all comparisons, the significance of the differences in peripheral biochemical parameters was calculated using the Mann–Whitney *U* test. The comparisons in the percentage of insulin-induced LTD, the percentage of LTP, brain mitochondrial function, immunoblot, the OFT tests and the MWM tests for the probe test between groups were performed using the one-way ANOVA test, followed by *post hoc* Fisher's least significant difference (LSD) analysis. As the acquisition phase of the MWM test has two independent factors (treatment and time), we used the two-way ANOVA followed by *post hoc* Fisher's LSD analysis to analyse the data. $P<0{\cdot}05$ was considered as a measure of statistical significance.

Results

Energy restriction and the combination of energy restriction plus vildagliptin in obese insulin-resistant rats equally restored peripheral insulin sensitivity

At baseline levels (week 0), the metabolic parameters were not significantly different between the ND-fed rats and the HFD-fed rats (Table 1). After 12 weeks of diet regimens, the HFD group had significantly increased body weight, plasma insulin, HOMA index, total cholesterol levels and LDL-cholesterol levels, when compared with the ND group (Table 1). However, plasma glucose levels were not significantly different between the groups (Table 1).

After 4 weeks of the treatment, HFV had significantly increased body weight, visceral fat, plasma insulin levels, HOMA index, plasma glucose AUC of OGTT (AUCg), total cholesterol and plasma LDL-cholesterol levels, but had decreased plasma HDL-cholesterol levels, when compared with NDV (Table 2). These findings indicated that peripheral insulin resistance was still observed in HFV rats after 16 weeks. Interestingly, HFRV and HFD-fed rats on a restricted diet and vildaglptin (HFRVil) had significantly decreased body weight, visceral fat, plasma insulin, HOMA index, plasma glucose AUCg, total plasma cholesterol and LDL-cholesterol levels, but increased plasma HDL-cholesterol levels, when compared with HFV rats (Table 2). However, there was no significant difference in plasma glucose and plasma TAG levels between all groups (Table 2).

Obesity caused the impairment of brain insulin receptor function, which was only restored by the combination of energy restriction plus vildagliptin

After 4 weeks of the treatment, the degree of insulin-induced LTD in HFV rats was significantly reduced, when compared with that in NDV rats (*n* 2–3 independent slices/animal, *n* 6 animals/group, Fig. 2(a)). Interestingly, the degree of insulin-induced LTD in only HFRVil rats showed a significant increase, when compared with that of HFV rats and HFR rats (*n* 2–3 independent slices/animal, *n* 6 animals/group, Fig. 2(a)). Regarding brain insulin signalling, the levels of IR and Akt/PKB proteins expression showed no difference between the groups (Fig. 2(d) and (e)). The p-IR and Akt/PKB at the serine 473 site in both HFV and HFRV rats showed a significant decrease, when compared with that of NDV rats (Fig. 2(b) and (c)). However, the p-IR levels and Akt/PKB at the serine 473 site of only HFRVil rats showed a significant increase when compared with those of HFV rats (Fig. 2(b) and (c)).

Obesity led to brain mitochondrial dysfunction, which improved following treatment with the combination of energy restriction plus vildagliptin

After 4 weeks of treatment, brain mitochondrial dysfunction was observed in HFV rats as indicated by increased brain mitochondrial ROS production, brain mitochondrial membrane depolarisation and brain mitochondrial swelling (Fig. 3(a), (b) and (c)). HFRV rats also had a significant increase in brain mitochondrial ROS production, brain mitochondrial depolarisation

Table 1. Metabolic parameters at week 0 (baseline) and the end of week 12 (before treatment) (Mean values with their standard errors)

	Baseline				Week 12			
	ND		HFD		ND		HFD	
Metabolic parameters	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (g)	199·6	2·7	206·9	2·4	476·3	6·0	560·8	12·5*
Plasma glucose (mg/dl)	139·0	5·4	130·5	4·5	144·6	5·1	143·3	9·0
Plasma insulin (ng/ml)	2·4	0·6	2·4	0·5	2·9	0·4	4·7	0·6*
HOMA index	12·0	2·8	10·9	2·5	24·5	3·9	38·6	3·9*
Plasma total cholesterol (mg/dl)	68·0	3·4	67·8	3·8	66·9	3·9	81·5	4·2*
Plasma TAG (mg/dl)	51·9	3·7	48·1	4·5	60·2	7·6	62·0	8·7
HDL-cholesterol (mg/dl)	32·5	1·5	29·7	1·3	30·1	1·6	28·0	3·3
LDL-cholesterol (mg/dl)	23·6	3·5	28·9	4·0	23·7	2·7	39·9	3·0*

ND, normal-diet-fed rats; HFD, high-fat-diet-fed rats; HOMA, homoeostasis model assessment.
* Compared with ND at the same time interval.

Table 2. Metabolic parameters after energy restriction and energy restriction plus vildagliptin treatment (Mean values with their standard errors)

	Groups							
	NDV		HFV		HFRV		HFRVil	
Metabolic parameters	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (g)	490·7	9·4	597·9	15*	519·1	10·1†	511·3	10·5†
Visceral fat (g)	24·6	1·3	52·7	2·9*	30·0	1·3†	28·5	2·3†
Plasma glucose (mg/dl)	153·5	16·8	163·2	7·1	152·7	3·2	157·5	5·6
Plasma insulin (ng/ml)	4·2	0·2	6·4	0·8*	3·6	0·5†	4·0	0·6†
HOMA index	34·6	5·3	59·2	8·8*	32·7	4·4†	34·5	6·1†
Plasma glucose AUC (AUCg) (mg/dlxminx10^4)	4·8	0·2	6·0	0·3*	4·8	0·3†	4·9	0·2†
Plasma total cholesterol (mg/dl)	70·5	4·2	97·3	6·0*	76·5	2·4†	75·4	3·8†
Plasma TAG (mg/dl)	52·1	7·2	54·7	5·0	51·8	5·3	53·3	7·2
HDL-cholesterol (mg/dl)	33·9	1·9	24·5	1·9*	35·9	2·2†	34·7	2·0†
LDL-cholesterol (mg/dl)	26·9	3·1	52·8	6·0*	31·8	6·5†	28·3	4·3†
Serum MDA (μmol/ml)	1·42	0·05	5·30	1·33*	1·63	0·12†	1·54	0·11†
Brain MDA (μmol/mg protein)	0·47	0·05	0·68	0·07*	0·57	0·04	0·50	0·02†

NDV, normal-diet-fed rats treated with vehicle; HFV, high-fat-diet-fed rats treated with vehicle; HFRV, high-fat-diet-fed rats reversed to normal diet with 60 % energy restriction treated with vehicle; HFRVil, high-fat-diet-fed rats reversed to normal diet with 60 % energy restriction treated with vildagliptin; HOMA, homoeostasis model assessment; MDA, malondialdehyde.
* Compared with NDV.
† Compared with HFV.

and brain mitochondrial swelling, when compared with NDV rats (Fig. 3(a), (b) and (c)). Interestingly, only HFRVil rats showed a significant decrease in brain ROS production, and improvement in brain mitochondrial depolarisation and brain mitochondrial swelling, when compared with HFV rats (Fig. 3(a), (b) and (c)).

Furthermore, there were morphological changes in brain mitochondria; representatives from all groups are shown in Fig. 3(d). Brain mitochondrial swelling was observed in both the HFV and HFRV rats, as indicated by markedly unfolded cristae (Fig. 3(d)). Interestingly, brain mitochondrial morphology with apparent folded cristae was observed in only HFRVil rats (Fig. 3(d)).

Obesity led to increased brain oxidative stress levels, which was attenuated by the combination of energy restriction plus vildagliptin

After 4 weeks of treatment, both circulating MDA and brain MDA levels significantly increased in HFV rats compared with NDV rats (Table 2). HFRV rats only showed a significant decrease in circulating MDA, when compared with HFV rats (Table 2). Interestingly, HFRVil rats showed a significant decrease in both circulating MDA and brain MDA levels, when compared with HFV rats (Table 2).

Obesity caused impaired hippocampal synaptic plasticity and cognitive decline, which only showed restoration with the combination of energy restriction plus vildagliptin

After 4 weeks of treatment, the results demonstrated that the degrees of electrical-induced LTP significantly decreased in both HFV and HFRV rats, when compared with NDV rats (n 2–3 independent slices/animal, n 6 animals/group, Fig. 4(a)). The reduction of electrical-induced LTP in both HFV and HFRV rats was not significantly different (n 2–3 independent slices/animal, n 6 animals/group, Fig. 4(a)). However, the degree of electrical-induced LTP of HFRVil rats significantly increased, when compared with that of HFV rats (n 2–3 independent slices/animal, n 6 animals/group, Fig. 4(a)).

The density of hippocampal dendritic spines was determined at the secondary and tertiary dendrites in the apical dendrite of the CA1 hippocampus to investigate the underlying mechanism of memory function. It was found that the dendritic spine density of HFV and HFRV rats decreased significantly, when compared with that of NDV rats (Fig. 4(b)). After treatment regimens, the dendritic spine density of both HFRV and HFRVil rats showed a significant increase, when compared with that of HFV rats (Fig. 4(b)). However, dendritic spine density of HFRVil rats was significantly greater than that of HFRV rats (Fig. 4(b)).

The OFT was used to screen locomotor activity. The results showed that there was no significant difference between all groups, indicating that the locomotor activity did not differ in all groups. In addition, the study determined cognitive function by using the MWM test after 4 weeks of the treatment regimens. For the acquisition test, it was found that both HFV and HFRV rats showed a significantly increased time to reach the platform, when compared with NDV rats (Fig. 4(c)). However, HFRVil rats showed a significantly decreased time to reach the platform, when compared with HFV rats (Fig. 4(c)). For the probe test, both HFV and HFRV rats had a significantly decreased time spent in the target quadrant, when compared with NDV rats (Fig. 4(d)). In contrast, HFRVil rats had a significantly increased time spent in the target quadrant, when compared with HFV rats (Fig. 4(d)).

Discussion

The major findings of this study are as follows: (1) obesity induced by HFD consumption leads to the development of peripheral insulin resistance, impairment of brain insulin sensitivity, brain mitochondrial dysfunction, impaired hippocampal synaptic

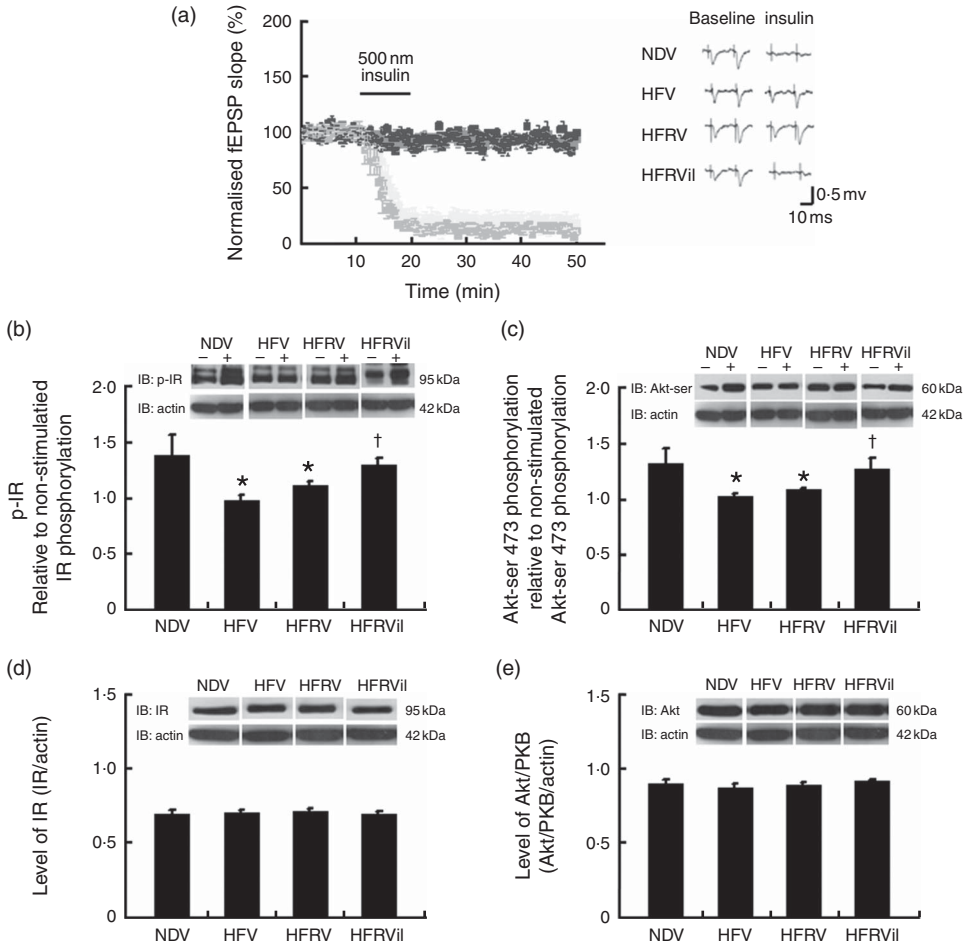

Fig. 2. The effects of energy restriction (ER) and the combination of ER plus vildagliptin on brain insulin receptor function (insulin-induced long-term depression (LTD)) and brain insulin signalling, including the phosphorylation of insulin receptors (p-IR), Akt-Ser 473, insulin receptors (IR) and serine/threonine-specific protein kinase B (Akt/PKB) protein expression in obese insulin-resistant rats. The combination of ER plus vildagliptin improves the ability of insulin-induced LTD in high-fat-diet-fed rats reversed to normal diet with 60 % ER treated with vildagliptin (HFRVil), when compared with high-fat-diet-fed rats treated with vehicle (HFV) (a). After 4 weeks of treatment, both p-IR and Akt-Ser 473 expression levels were significantly decreased in HFV and high-fat-diet-fed rats reversed to normal diet with 60 % ER treated with vehicle (HFRV) when compared with normal-diet-fed rats treated with vehicle (NDV) ((b) and (c)). Both p-IR and Akt-Ser 473 expression increased significantly after the combination of ER plus vildagliptin when compared with HFV rats ((b) and (c)). However, there was no difference in IR and Akt/PKB protein expression between all groups ((d) and (e)). *$P<0{\cdot}05$ *v.* NDV and † $P<0{\cdot}05$ *v.* HFV. □, NDV; ■, HFV; ■, HFRV; ■, HFRVil. fEPSP, field excitatory postsynaptic potential.

plasticity, decreased dendritic spine density and cognitive decline; (2) both ER and the combination of ER plus vildagliptin showed equally improved peripheral insulin sensitivity and lipid profiles; (3) ER alone showed a decrease only in circulating oxidative stress, whereas the combination of ER plus vildagliptin showed a decrease in both circulating and brain oxidative stress levels; and (4) only the combination of ER plus vildagliptin showed a correlation with restored brain insulin sensitivity, brain mitochondrial function, hippocampal synaptic plasticity and cognitive function in obese insulin-resistant rats.

This study has demonstrated that diet-induced obesity led to the development of the peripheral insulin resistance via impaired peripheral insulin sensitivity, impaired lipid profiles and an increase in both circulating and brain oxidative stress. These findings confirmed other studies and our previous studies that diet-induced obesity not only caused peripheral insulin resistance but also brain insulin resistance[24,32]. The present findings indicated that obesity increased oxidative stress and impaired brain mitochondrial function, resulting in brain insulin resistance and cognitive decline.

This study demonstrates that ER enhanced peripheral insulin sensitivity and improved lipid profiles in obese insulin-resistant rats. A possible explanation of these findings may be that ER decreased visceral fat and dyslipidaemia. Decreased fat has been shown to reduce systemic inflammation[33], as well as oxidative stress, as shown in Table 2 and in a previous study[34]. An increase in inflammation and oxidative stress led to the impairment of peripheral insulin sensitivity[35,36]. Therefore, ER in the present study led to improved peripheral insulin sensitivity, possibly via the reduction of oxidative stress.

Although ER led to the restoration of peripheral insulin sensitivity and decreased circulating oxidative stress, ER alone failed to cause improvement in brain insulin sensitivity and brain mitochondrial dysfunction, as well as failed to restore hippocampal synaptic plasticity and cognitive decline in obese insulin-resistant rats. This may be because of our finding that ER

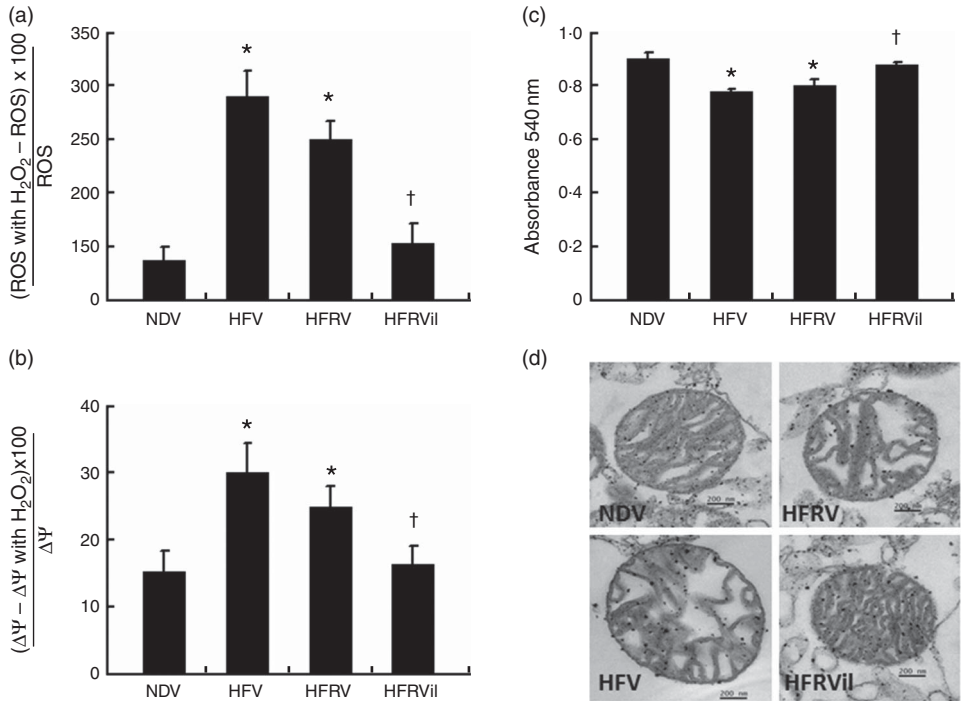

Fig. 3. The effects of energy restriction (ER) and the combination of ER plus vildagliptin on brain mitochondrial function and representative images of brain mitochondrial morphology by transmission electron microscopy (JEM-2200FS field emission electron microscope, original magnification 20 000 ×) in obese insulin-resistant rats. High-fat-diet-fed rats treated with vehicle (HFV) and high-fat-diet-fed rats reversed to normal diet with 60 % ER treated with vehicle (HFRV) demonstrated brain mitochondrial dysfunction when compared with normal-diet-fed rats treated with vehicle (NDV), as indicated by increased brain mitochondrial reactive oxygen species (ROS) production following H_2O_2 application (a), increased brain mitochondrial membrane potential change following H_2O_2 application (b) and decreased absorbance values, indicating brain mitochondrial swelling (c). The combination of ER plus vildagliptin in high-fat-diet-fed rats reversed to normal diet with 60 % ER treated with vildagliptin (HFRVil) showed improved brain mitochondrial function when compared with HFV rats, as indicated by significantly decreased brain mitochondrial ROS production (a), decreased brain mitochondrial membrane potential change (b) and increased absorbance values (c). Furthermore, normal folding of cristae in brain mitochondrial morphology was shown in both NDV and HFRVil rats (d). However, brain mitochondrial swelling, as indicated by unfolded cristae in both HFV and HFRV rats, was observed (d). *$P < 0{\cdot}05$ *v.* NDV and † $P < 0{\cdot}05$ *v.* HFV.

alone could neither decrease brain oxidative stress levels nor brain mitochondrial dysfunction, whereas treatment with combined ER and vildagliptin effectively improved both parameters in obese insulin-resistant rats (Table 2).

Unlike this study, previous studies showed that ER had beneficial effects on neuroprotection and preservation of cognition. For example, (1) a previous study demonstrated that 40 % of ER for 24 months reduced the rate of mitochondrial H_2O_2 production via reduced brain ROS production in aged rats[22]. (2) The study involving 40 % ER for 22–29 months also reported that ER prevented age-related deficit synaptic LTP and the preservation of the NR1-subunit of *N*-methyl-D-aspartate (NMDA) receptors in aged Fisher 344 rats[37]. (3) The study of 60 % ER for 4 weeks in male rats demonstrated that ER stabilised synaptophysin expression and preserved cognitive function[23]. (4) A recent study demonstrated that 70 % ER for 28 d improved metabolic effects and cognitive decline via up-regulation of brain-derived neurotrophic factor and decreased hippocampal oxidative stress in the obese-metabolic syndrome male rats induced by a moderated HFD (35 %E from fat)[19]. The different findings between previous studies and the present study could be dependent on the percentage of HFD feeding and the duration of ER. Future studies are needed to verify this issue.

One important finding from this study is that ER combined with vildagliptin restored peripheral insulin sensitivity, brain insulin sensitivity, brain mitochondrial function, synaptic plasticity and cognitive function in obese insulin-resistant rats. This means of intervention provided better efficacy than ER alone in this model. These findings suggest that the beneficial effects on brain function in this group could be because of the action of vildagliptin. This is consistent with our previous studies, which showed that vildagliptin led to the restoration of cognitive function in the obese model, via improved brain insulin sensitivity and reduced brain oxidative stress[4,8]. As demonstrated in this study, the combined ER with vildagliptin improved/restored both brain insulin resistance and brain oxidative stress better than ER alone. These findings indicated that ER in combination with the vildagliptin treatment provided more beneficial effects than ER alone in our study model. However, other ER feeding regimens and delivery could have given similar or different results as observed in the present study and require further investigation.

Limitations of the study

The two-factorial design study was not conducted in the present study; therefore, the effects of ER or its combination with vildagliptin in ND-fed rats (lean rats) were not known. However, our previous studies showed that the administration of vildagliptin in ND-fed rats has no effect on the metabolic parameters or brain function, when compared with vehicle-treated ND-fed rats[4,8].

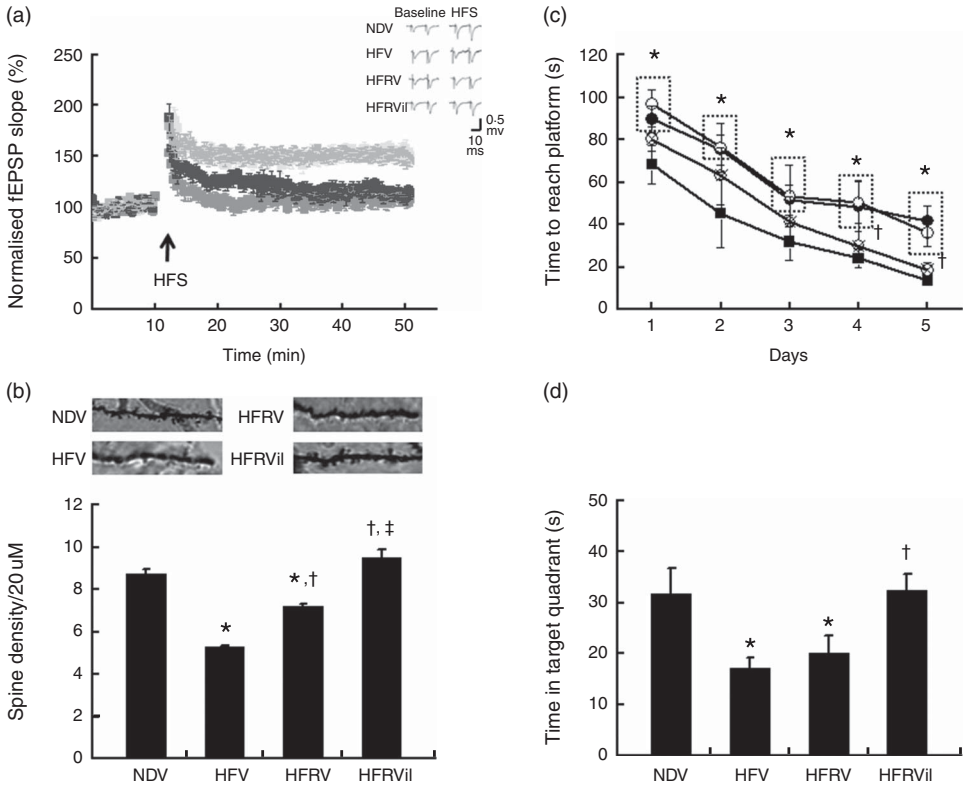

Fig. 4. The effects of energy restriction (ER) and the combination of ER plus vildagliptin on hippocampal synaptic long-term potentiation (LTP) and the number of dendritic spines on tertiary dendrites on apical dendrites, as well as cognitive function, in obese insulin-resistant rats. Both high-fat-diet-fed rats treated with vehicle (HFV) and high-fat-diet-fed rats reversed to normal diet with 60 % ER treated with vehicle (HFRV) showed a significantly reduced degree of LTP, when compared with normal-diet-fed rats treated with vehicle (NDV) (a). The degree of LTP showed improvement after a combination of ER plus vildagliptin when compared with HFV rats (a). The number of dendritic spines on secondary or tertiary dendrites in hippocampal apical dendrites was decreased in HFV rats (b). Both HFRV and high-fat-diet-fed rats reversed to normal diet with 60 % ER treated with vildagliptin (HFRVil) showed significantly increased numbers of dendritic spines after the treatment regimen (b). HFV and HFRV rats showed a significantly increased time to reach the platform in the acquisition test, as well as having a decreased time spent in the target quadrant in the probe test, when compared with NDV rats ((c) and (d)). HFRVil rats showed a significantly decreased time to reach the platform in the acquisition test, as well as an increased time spent in the target quadrant in the probe test, when compared with HFV rats ((c) and (d)). HFS, high-frequency stimulation. *$P<0{\cdot}05$ *v.* NDV, † $P<0{\cdot}05$ *v.* HFV and ‡ $P<0{\cdot}05$ *v.* HFRV. (a) □, NDV; ■, HFV; ■, HFRV; ■, HFRVil; (c) ■, NDV; ●, HFV; ○, HFRV; ⊗, HFRVil. fEPSP, field excitatory postsynaptic potential.

In addition, ER had been shown to exert benefits in attenuating cognitive decline via an up-regulation of the brain-derived neurotrophic factor and tropomyosin-related kinase B in obese, hypertensive rats[19]; these markers were not investigated in this study.

Conclusion

This study indicates that although ER restored peripheral insulin sensitivity and decreased circulating oxidative stress levels regardless of the addition of vildagliptin, only ER plus vildagliptin exerted the best efficacy in effectively decreasing brain oxidative stress levels, as well as the restoration of brain insulin sensitivity, brain mitochondrial function, hippocampal synaptic plasticity and cognition. All of these findings suggest that the addition of vildagliptin to ER provides greater therapeutic benefits on brain function than ER alone in obesity.

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H. P., N. C., S. C. C. performed the experiments, analysed the data and wrote the manuscript. P. T., W. P., P. Sa-nguanmoo, T. C. performed the experiments and analysed the data. P. Satjaritanun, L. L. analysed the data.

The authors have no conflicts of interest to disclose.

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