CHAPTER 3

RESULTS

3.1 The peripheral insulin resistance was developed by long-term HFD consumption for 12 weeks

At the baseline (week 0 before starting dietary feeding), the initial body weight (BW) of the normal diet (ND)-fed and high-fat diet (HFD)-fed groups was not significantly different (187.22 ± 2.06 and 192.22 ± 3.74 g; respectively; $p>0.05$). Furthermore, the initial plasma glucose levels, plasma insulin levels and HOMA index of HFD-fed group (151.67 ± 8.41 mg/dl, 2.63 ± 1.19 ng/ml and 22.35 ± 3.95, respectively) were not significantly different when compared with those of ND-fed group (147.74 ± 8.92 mg/dl, 2.35 ± 0.25 ng/ml and 18.56 ± 7.12, respectively) ($p>0.05$) (Table 3-1). These findings indicate that the animals in each dietary group did not have insulin resistance at baseline.

After dietary consumption for 12 weeks, the HFD-fed rats had significantly greater body weight than the ND-fed rats (567.50 ± 15.04 and 456.43 ± 20.43 g; respectively; $p<0.01$). Visceral fat (VF) in the 12-week HFD-fed group was also significantly elevated compared to the 12-week ND-fed group (45.36 ± 2.47 and 24.67 ± 3.13 g, respectively; $p<0.01$). Furthermore, 12-week HFD-fed group had significantly increased energy intake compared to 12-week ND-fed group (133.75 ± 8.26 and 94.76 ± 7.23 kcal, respectively; $p<0.05$). In the 12-week HFD-fed group, the plasma free fatty acid (FFA) levels and plasma triglyceride (TG) levels (0.77 ± 0.08

mM and 86.74 ± 10.00 mg%, respectively) were not significantly different when compared with those of 12-week ND-fed group (0.85 ± 0.14 mM and 75.91 ± 12.43 mg%, respectively) (p>0.05). We also found that 12-week HFD-fed group had significantly elevated plasma insulin levels compared to 12-week ND-fed group (4.23 ± 0.58 and 1.77 ± 0.45 ng/ml, respectively, p<0.01), while plasma glucose levels were not different between both 12-week HFD-fed and 12-week ND-fed groups (158.11 ± 1.72 and 146.99 ± 8.14 mg/dl, respectively; p>0.05). However, HOMA index of 12-week HFD-fed group was significantly greater than that of the 12-week ND-fed group (39.69 ± 5.69 and 15.95 ± 4.33, respectively; p<0.01) (Table 3-2). These results suggest that the peripheral insulin resistance developed after 12 weeks of HFD feeding.

Table 3-1 The metabolic parameters between dietary groups at baseline.

Metabolic parameter	**Baseline (week 0 before starting dietary feeding)**	
	ND group	**HFD group**
Body weight (g)	187.22 ± 2.06	192.22 ± 3.74
Plasma glucose (mg/dl)	147.74 ± 8.92	151.67 ± 8.41
Plasma insulin (ng/ml)	2.35 ± 0.25	2.63 ± 1.19
HOMA index	18.56 ± 7.12	22.35 ± 3.95

Each value was presented as mean ± SEM.

Table 3-2 The metabolic parameters between dietary groups at week 12.

Metabolic parameter	12-week dietary feeding	
	ND group	HFD group
Body weight (g)	456.43 ± 20.43	567.50 ± 15.04 **
Visceral fat (g)	24.67 ± 3.13	45.36 ± 2.47 **
Energy intake (kcal)	94.76 ± 7.23	133.75 ± 8.26 *
Plasma free fatty acid (mM)	0.85 ± 0.14	0.77 ± 0.08
Plasma triglyceride (mg%)	75.91 ± 12.43	86.74 ± 10.00
Plasma glucose (mg/dl)	146.99 ± 8.14	158.11 ± 1.72
Plasma insulin (ng/ml)	1.77 ± 0.45	4.23 ± 0.58 **
HOMA index	15.95 ± 4.33	39.69 ± 5.69 **

Each value was presented as mean ± SEM. $^{*}p < 0.05$; significant difference from ND-fed group. $^{**}p < 0.01$; significant difference from ND-fed group.

3.2 The 12-week HFD consumption did not change structure and number of hippocampal CA1 neuron

Under light microscope, the hippocampal CA1 neurons of 12-weeks ND-fed and 12-week HFD-fed groups were highly ordered and dense. In addition, the hippocampal sections of 12-week ND-fed and 12-week HFD-fed groups presented morphologically intact and regular CA1 neurons. The hippocampal CA1 neurons of both dietary groups at week 12 had abundant cytoplasm and large clear nuclei. Furthermore, there was no space between the hippocampal CA1 neurons (Figure 3-

1A). These findings indicate that the neuronal damage was not detectable in both dietary groups.

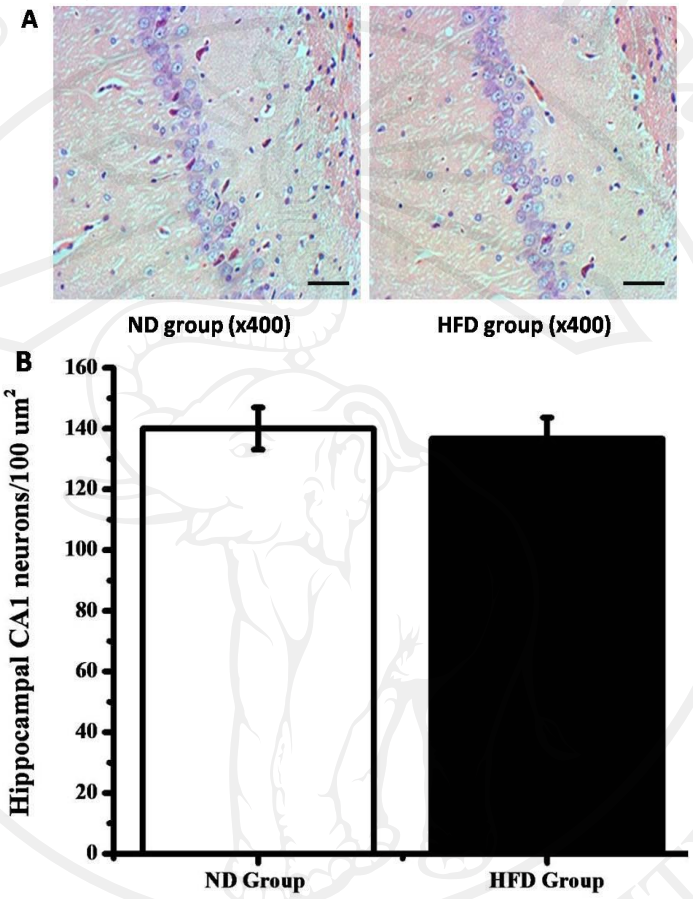

Figure 3-1 The effect of high-fat diet feeding for 12 weeks on structure and number of the hippocampal neuron. (A) Representative histological analysis of neurons in the hippocampal CA1 region of 12-week normal diet (ND)-fed group and 12-week high-fat diet (HFD)-fed group which was photographed at ×400 magnification. Scale bars in images represent 25 μm. (B) Quantitative analysis of the number of hippocampal CA1 neurons per 100 μm^2 area was determined in hippocampal CA1 regions of 12-week ND-fed and 12-week HFD-fed groups. Bar was presented as mean ± SEM. The structure and the number of hippocampal CA1 neuron between 12-week ND-fed group and 12-week HFD-fed group did not differ.

In the present study, we also found that the number of hippocampal CA1 neurons of 12-week HFD-fed group was not significantly different when compared with that of 12-week ND-fed group (136.67 ± 6.94 and 140.00 ± 6.94 hippocampal CA1 neurons/100 μm^2, respectively; p>0.05) (Figure 3-1B). These results suggest that the morphological feature of hippocampal CA1 neurons between both dietary groups were not different as shown by no change in structure and number of hippocampal CA1 neurons.

3.3 The nNOS expression in hippocampal CA1 regions was reduced following 12 weeks of HFD consumption

It has been previously demonstrated that the impairment of intracellular insulin signaling can diminish nNOS function in the nervous system (Erol, 2008). However, changes of nNOS expression in hippocampal CA1 regions during 12 weeks of HFD consumption-induced peripheral insulin resistance have not been investigated. In the present study, our results revealed that nNOS-positive neurons in all animals were observed in the interneurons of hippocampal CA1 regions (Figure 3-2A). The number of nNOS-positive neurons in CA1 regions of hippocampus of 12-week HFD-fed group was significantly attenuated compared to that of the 12-week ND-fed group (34.72 ± 3.00 and 68.89 ± 3.62 nNOS-positive neurons/100 μm^2, respectively; p<0.01) (Figure 3-2B). These data suggest that 12-week HFD consumption-induced peripheral insulin resistance may cause the reduction of nNOS expression in the hippocampal CA1 regions without neuronal loss and damage in neuronal structure.

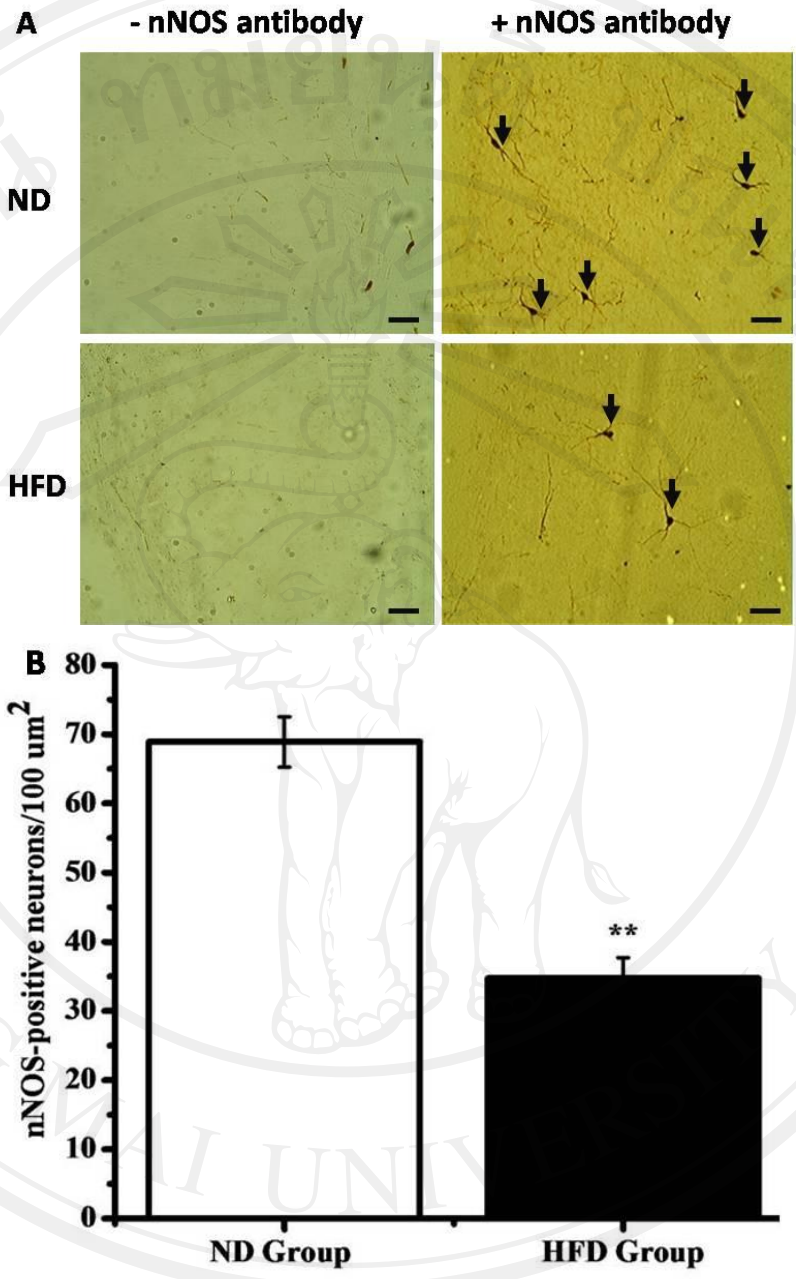

Figure 3-2 The effect of high-fat diet feeding for 12 weeks on the nNOS expression in CA1 regions of hippocampus. (A) Representative images of nNOS-positive neurons in CA1 hippocampus of normal diet (ND)-fed group and high-fat diet (HFD)-fed group which were photographed at ×100 magnification. Scale bars in images represent 100 μm. Arrow indicated nNOS-positive neurons (dark brown color). (B) Quantitative analysis of the number of nNOS-positive neurons per 100 μm^2 area was

determined in hippocampal CA1 regions of ND- and HFD-fed groups. Bar was presented as mean ± SEM. The number of nNOS-positive neuron in CA1 regions of hippocampus in the 12-week HFD-fed group was significantly attenuated compared to the 12-week ND-fed group. $^{**}p < 0.01$; statistically significant difference from ND-fed group at 12 weeks.

3.4 The 12 weeks of HFD consumption increased brain oxidative stress

The previous studies have been reported that the progression of the peripheral insulin resistance exhibited a significantly higher intracellular oxidative product, such as malondialdehyde (MDA, the biomarker of lipid peroxidation) (Alsultan et al., 2010; Koca et al., 2009). Moreover, the increasing phenomenon of peripheral insulin resistance significantly decreased antioxidant such as reduced glutathione (GSH) (Ghareeb et al., 2011). In the present study, we found that the MDA levels in the brain of HFD-fed group at 12 weeks were significantly increased compared to that of ND-fed group at 12 weeks (755.57 ± 125.51 and 101.89 ± 54.18 nM/mg protein, respectively; $p<0.01$) (Figure 3-3A). The GSH levels in the brain of 12-week HFD-fed group were also significantly decreased compared to that of 12-week ND-fed group (16.62 ± 1.12 and 23.31 ± 1.18 µM/mg protein, respectively; $p<0.01$) (Figure 3-3B). These data revealed that brain oxidative stress coexisted with the development of peripheral insulin resistance.

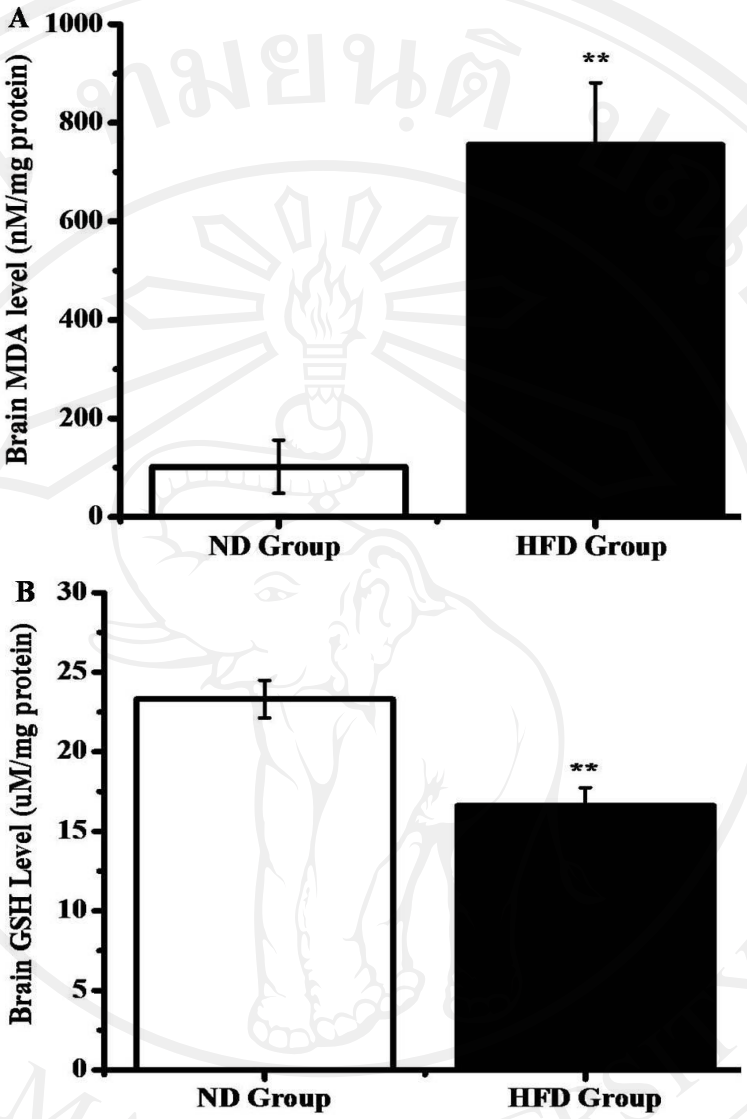

Figure 3-3 The effect of high-fat diet feeding for 12 weeks on the oxidative stress in the brain. (A) The malondialdehyde (MDA) levels in the brain of 12-week high-fat diet (HFD)-fed group were significantly elevated compared to that of 12-week normal diet (ND)-fed group. (B) The reduced glutathione (GSH) levels in the brain of 12-week HFD-fed group were significantly attenuated compared to that of 12-week ND-fed group. Bar was presented as mean ± SEM. $^{**}p < 0.01$; statistically significant difference from ND-fed group at 12 weeks.

3.5 The relationship among the peripheral insulin resistance, the hippocampal nNOS expression, and the brain oxidative stress

The correlation among the peripheral insulin resistance, the nNOS expression in CA1 regions of hippocampus, and the brain oxidative stress in rats that received normal diet or high-fat diet for 12 weeks were examined (Table 3-3). We found that the brain MDA levels were positively correlated with visceral fat ($r = 0.769$, $p<0.05$) and plasma insulin levels ($r = 0.641$, $p<0.05$). However, the brain GSH levels were negatively correlated with visceral fat ($r = -0.817$, $p<0.01$), and plasma insulin levels ($r = -0.607$, $p<0.05$). Our finding showed that the brain MDA levels were negatively correlated with the brain GSH levels ($r = -0.954$, $p<0.01$). These findings indicate that 12-week HFD consumption-induced peripheral insulin resistance caused an increase in the brain oxidative stress. Interestingly, we also found that the number of nNOS-positive neurons in hippocampal CA1 regions were negatively correlated with the brain MDA levels ($r = -0.895$, $p<0.01$) and the brain GSH levels ($r = 0.912$, $p<0.01$). These results suggest that the brain oxidative stress, possibly following peripheral insulin resistance, may be one of the important factors related to the reduction of nNOS expression in CA1 regions of hippocampus.

Table 3-3 Correlation among peripheral insulin resistance, hippocampal nNOS expression, and brain oxidative stress in rats that received normal diet or high-fat diet for 12 weeks.

Parameters	BW (g)	VF (g)	Plasma glucose (mg/dl)	Plasma TG (mg%)	Plasma FFA (mM)	Plasma insulin (ng/ml)	HOMA index	nNOS-positive neuron (neurons/100 μm^2)	Brain MDA (nM/mg)	Brain GSH (μM/mg)
BW (g)	1	0.729*	0.445	0.281	-0.006	0.747**	0.740**	-0.500	0.588	-0.530
VF (g)	0.729*	1	0.009	0.256	-0.101	0.712*	0.694*	-0.595	0.769*	-0.817**
Plasma glucose (mg/dl)	0.445	0.009	1	0.350	0.228	0.492	0.560*	-0.261	0.126	-0.178
Plasma TG (mg%)	0.281	0.256	0.350	1	0.766**	0.495	0.477	-0.567	0.438	-0.520
Plasma FFA (mM)	-0.006	-0.101	0.228	0.766**	1	0.234	0.205	-0.315	-0.075	-0.072
Plasma insulin (ng/ml)	0.747**	0.712*	0.492	0.495	0.234	1	0.994**	-0.545	0.641*	-0.607*
HOMA index	0.740**	0.694*	0.560*	0.477	0.205	0.994**	1	-0.528	0.621	-0.582
nNOS-positive neuron (neurons/100 μm^2)	-0.500	-0.595	-0.261	-0.567	-0.315	-0.545	-0.528	1	-0.895**	0.912**
Brain MDA (nM/mg)	0.588	0.769*	0.126	0.438	-0.075	0.641*	0.621	-0.895**	1	-0.954**
Brain GSH (μM/mg)	-0.530	-0.817**	-0.178	-0.520	-0.072	-0.607*	-0.582	0.912**	-0.954**	1

*; Correlation was significant at the 0.05 level (2-tailed). **; Correlation was significant at the 0.01 level (2-tailed).