CHAPTER 4

DISCUSSION AND CONCLUSIONS

The major findings of this study are as follows 1) 12-week high-fat diet (HFD) consumption caused the peripheral insulin resistance as shown by increasing of plasma insulin levels and HOMA index, 2) 12-week HFD consumption also caused an elevation of brain oxidative stress, 3) Neuronal nitric oxide synthase (nNOS) expression in hippocampal CA1 regions in those insulin resistant rats induced by HFD consumption was significantly attenuated without any change in neuronal density, and 4) The increment of brain oxidative stress was also correlated well with the decline of nNOS expression in hippocampal CA1 regions.

The elevation in prevalence and severity of insulin resistance is quickly becoming a main public health problem, in which is extending at epidemic proportions worldwide (Matsuzaki et al., 2010). Furthermore, chronic insulin resistance are leading causes of morbidity and mortality in the developed and developing countries. Earlier works revealed that HFD feeding causes insulin resistance characterized by hyperinsulinemia, elevated corticosteroids, and reduced insulin sensitivity (Pratchayasakul et al., 2011; Riccardi et al., 2004). Both preclinical and clinical studies have shown that the development of insulin resistance is associated with HFD feeding and also linked to cognitive deficits (Greenwood and Winocur, 2005; Winocur and Greenwood, 2005). While considerable research has examined both the consequences and mechanisms of a diminished insulin response in

various peripheral tissues, only a few studies have investigated the effects of this metabolic disruption within the central nervous system (CNS), particularly the metabolic disturbance following the HFD feeding. Our recent study demonstrated that 12-week HFD feeding caused not only peripheral insulin resistance, but also neuronal insulin resistance (Pratchayasakul et al., 2011).

In the present study, peripheral insulin resistance characterized by weight gain, increased visceral fat, euglycemia, hyperinsulinemia, and increased HOMA index were observed following the 12-week HFD feeding. Our data also demonstrated that the incidence of peripheral insulin resistance following 12-week HFD feeding. However, we found that the fasted plasma triglyceride and free fatty acid levels were not changed. This finding is similar to that reported previously, in which HFD-induced obesity in rats elevated liver triglyceride content without any change in plasma triglyceride level (Buettner et al., 2006) and the recent study showed that mice fed with HFD (45% kcal from fat) for 9-12 months did not develop hypertriglyceridemia (Hwang et al., 2010). A possible explanation could be that increased ingestion of fats preferentially contributed towards the cytosolic pool by elevating liver triglyceride concentration as well as a much longer time is required for the upregulation and secretion of plasma triglyceride. Furthermore, excessive intake of fat leads to an accumulation of triglyceride in many tissues, particularly in the adipose tissue (Manco et al., 2004). Supporting our findings, the other finding also showed that rats fed with HFD for 4, 8 or 12 weeks have dramatically elevated in visceral fat without hypertriglyceridemia (Pratchayasakul et al., 2011).

Both insulin and nNOS have been shown to play the multiple functions in neurons and neuroglia in the central nervous system (Bishop et al., 2010; Zhou and

Zhu, 2009). It has been proposed that insulin serves as a key modulator in regulation of nNOS activity (Canabal et al., 2007a; Canabal et al., 2007b). For example; 1) the function and activity of nNOS in the brain contributes to insulin-mediated PI3K/Akt signaling pathway (Canabal et al., 2007a), 2) the unilateral microinjection of insulin into the nucleus tractus solitaries (NTS) of Wistar-Kyoto (WKY) rats potentiates PI3K/Akt cascade to phosphorylate nNOS at serine residue (Ser^{1416}) in a time-dependent manner (Chiang et al., 2009), 3) the study in cell culture of astrocytes and neurons of Sprague-Dawley rats showed that insulin treatment can also significantly upregulate the expression of both nNOS mRNA and protein in a dose-dependent manner (Yuan et al., 2004), 4) the injection of streptozotocin (STZ) in the rodents triggers insulin-dependent diabetes mellitus (IDDM), in which attenuated the expression of nNOS mRNA and protein in the cerebrocortex (Yu et al., 1999) and hippocampus (Reagan and McEwen, 2002), and 5) *In vivo* study, the intravenous injection of insulin in STZ-diabetic rats can reverse the attenuated expression of mRNA and protein of nNOS level in the cerebellum (Yu et al., 2000). All those findings have exhibited the role of insulin in the nNOS expression in the central nervous system. In the present study, we also found that the peripheral insulin resistance caused the decline of nNOS expression. Therefore, those previous studies and the present studies suggest that nNOS expression in the brain could be modulated by insulin action.

In vitro study in Male Wistar rats, the dose-dependent attenuation of NOS activity in the brain tissue is caused by the elevation of oxidative stress (Onufriev et al., 1999). The investigation in female Fisher rat has shown that a high-fat, high-sucrose (HFS) diet consumption for 7 months caused an increase in oxidant (such as

H_2O_2 and protein carbonyl) as well as a decrease in plasma total antioxidant capacity (Roberts et al., 2005). Subsequently, an imbalance between oxidant and antioxidant results in oxidative stress. Furthermore, nNOS protein expression in the brain of female Fisher rat consuming the HFS diet was significantly attenuated (Roberts et al., 2005). In the present study, we also found that the reduced nNOS expression in CA1 regions of hippocampus and the raised brain oxidative stress were found in 12-week HFD-fed rats. Our results revealed that the attenuation of nNOS-positive neuron in hippocampal CA1 regions was significantly correlated with the progression of brain oxidative stress. However, structure and number of hippocampal neuron of 12-week HFD-fed rats was not different. Therefore, these studies indicated that the decreased nNOS expression in hippocampal CA1 regions could be related to the increment of the brain oxidative stress without hippocampal neuron loss or damage.

Our previous study demonstrated that 12 weeks of HFD-fed male rats significantly increased the neuronal corticosterones (stress hormone) levels (Pratchayasakul et al., 2011). The experimental studies in the rodent have also been reported that exposure to stress levels of corticosterone generates impairment of insulin signaling in the brain leading to neuronal insulin resistance (Osmanovic et al., 2010; Piroli et al., 2007). In addition, the high levels of corticosterone can disturb brain mitochondrial function to produce oxidative stress (Liu and Zhou, 2012), inhibit long-term potentiation (LTP) formation in the hippocampus (Zhou et al., 2000), diminish dendritic arborization in developing hippocampal CA1 neuron (Alfarez et al., 2009), and stimulate neuronal damage or loss in the brain (Xu et al., 2011). In the present study, we found that the HFD feeding for 12 weeks increased brain oxidative stress in insulin-resistant rats. These finding suggest that obesity-induced peripheral

insulin resistance following 12-week HFD consumption could lead to an increased brain oxidative stress via the high rise in corticosterone levels.

Our findings about the correlations between peripheral insulin resistance and brain oxidative stress were supported by several previous studies. For example; 1) it has been reported that the increasing phenomenon of insulin resistance is associated with the development of oxidative stress in the brain (de la Monte et al., 2009; Evans et al., 2005) and 2) insulin resistance could accelerate the excessive production of MDA (oxidant) level in the brain (Abbas and Elsamanoudy, 2011; Pipatpiboon et al., 2012) and the low production of GSH (antioxidant) level in the brain (Reddy et al., 2009) that refers to severity of oxidative stress (Chakravarty and Rizvi, 2011).

In the present study, we measured the nNOS expression in hippocampus. However, the nNOS activity in the hippocampus should be measured to confirm the reduction of NO, in which NO has been implicated in developmental neuronal plasticity as well as in hippocampal LTP.

In the conclusion, 12-week HFD consumption clearly decreased nNOS expression in hippocampus, which is significantly correlated to a significant increment in brain oxidative stress. In addition, it has been shown that the defective nNOS expression has been implicated in cognitive impairment (Kirchner et al., 2004). Therefore, the reduction in nNOS expression found in 12-week HFD consumption, may lead to the impairment of cognition in the animal models. In the present study, we suggested the reduction of nNOS expression in hippocampus following 12-week HFD consumption could be due to 1) the elevation of brain oxidative stress or 2) the impairment of neuronal insulin signaling induced by peripheral insulin resistance. The summarized of proposed mechanism in the present study is shown in Figure 4-1.

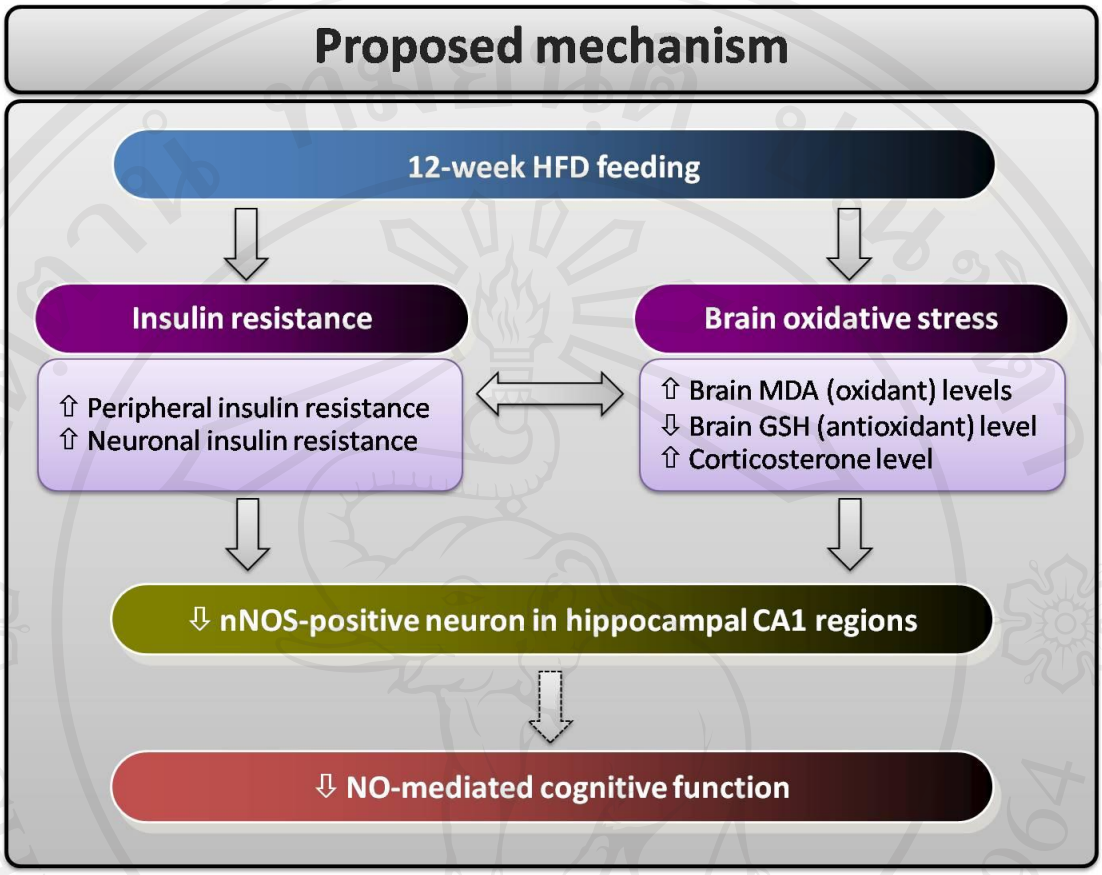

Figure 4-1 The proposed mechanism of high-fat diet (HFD) feeding for 12 weeks on the reduction of nNOS expression in the hippocampal CA1 regions through peripheral insulin resistance or brain oxidative stress.