CHAPTER 1

INTRODUCTION

1.1 Statement and significant of the problem

An increase in rates of obesity, type 2 diabetes and cardiovascular disease and the associated metabolic abnormalities have been noticed in the world's population. Insulin resistance, defined as a sharply diminished insulin response within target tissues, has grown increasingly common and is correlated with the development of cardiovascular disease (Saely et al., 2005), type 2 diabetes (Winkler and Cseh, 2009), and the metabolic syndrome (Saely et al., 2005). Notably, *in vivo* studies reveal that the animals with high-fat diet (HFD) feeding had experience obesity (Lin et al., 2000), dyslipidemia (Yang et al., 2008), and decreased peripheral insulin sensitivity (Riccardi et al., 2004). Surprisingly, considering the dramatic rise of insulin resistance throughout the developed world (Lieberman, 2003), and the growing interest in the role of insulin within the brain (Esler et al., 2001), there have been only a few studies examining the effects of this metabolic impairment in the central nervous system (CNS). The alarming trend of an increase in high-fat diets and obesity has led to an escalating prevalence of type 2 diabetes. Although, diabetes has long been considered a peripheral disease, it is now accepted that type 2 diabetes is a risk factor for dementia (Whitmer, 2007). Furthermore, several studies suggest that a

diet rich in fat can impede cognitive performance (Greenwood and Winocur, 2005; Kalmijn et al., 2004). Our previous study has demonstrated that a significant modification of important neuronal insulin receptor signaling can be induced by a fat-enriched diet of male rats (Pratchayasakul et al., 2011b). Fed for 12 weeks, the high-fat diet clearly induced neuronal insulin resistance, which is identified as a significant reduction in the ability of insulin to induce long-term depression (LTD), and a reduction in the stimulated phosphotyrosine activity of IR, IRS-1 and Akt/PKB in brain slices (Pratchayasakul et al., 2011b).

Nitric oxide (NO) has been implicated in developmental neural plasticity as well as in hippocampal long-term potentiation (LTP), a long-lasting form of synaptic plasticity related to some types of learning and memory (Doyle et al., 1996). During LTP process, NO acts as a retrograde messenger which produces from the post-synaptic neuron, diffuses through the extracellular space for directly acting in the pre-synaptic neuron (Abbott and Nahm, 2004). In hippocampus, NO production is controlled by neuronal nitric oxide synthase (nNOS) (Vallance and Leiper, 2002). nNOS is a main enzyme isoform in the brain (Huang et al., 1993). It is widely distributed in the amygdala, cerebellum, cerebral cortex and hippocampus (Endoh et al., 1994). Studies with nNOS inhibitor suggested a role of nNOS in hippocampal synaptic plasticity. These studies showed that LTP in hippocampal brain slices were decreased by nNOS inhibitor (Zhao and Zhang, 1999). In addition, deletions of the genes that encode nNOS isoforms reduce the inducibility of LTP (Kelley et al., 2009). Furthermore, rats which received nNOS inhibitors showed the impairment of spatial learning when determined by water maze (Markvartova and Vozeh, 2008). In addition, previous study shown that nNOS expression in cerebellum significantly

decreased in aged rats (Yu et al., 2000). This finding suggested that the causes of impaired cognitive function in aging may be related to nNOS expression. However, the effects of high-fat diet consumption on nNOS expression in brain have not been investigated.

We previously showed that long-term HFD consumption not only caused peripheral insulin resistance, but also induced neuronal insulin resistance and increased neuronal stress (Pratchayasakul et al., 2011b). Moreover, previous studies demonstrated that insulin resistance condition caused by HFD consumption could reduce insulin transportation into the brain (Kaiyala et al., 2000), reduce hippocampal neurogenesis (Lindqvist et al., 2006), and increase oxidative stress in the central nervous system (Stranahan et al., 2008). However, the correlation among peripheral insulin resistance, the nNOS expression in hippocampus and the brain oxidative stress level in rat fed with 12-week HFD have not yet been investigated. Therefore, in this study we tested the hypothesis that high-fat diet consumption can cause not only the peripheral insulin resistance but also the impaired nNOS expression and increased brain oxidative stress. Aims of the present study are as following: 1) to investigate whether 12-week HFD consumption can cause peripheral insulin resistance and the reduction of nNOS expression in hippocampus, 2) to examine the brain oxidative stress levels in 12-week HFD-fed rats compared with the 12-week ND-fed rats, and 3) to determine the relationship among the peripheral insulin resistance, nNOS expression in hippocampus, and brain oxidative stress levels of 12-week HFD-fed rats.

1.2 Literature review

1.2.1 The role of insulin in the brain

Insulin is a peptide hormone composed of 51 amino acids and has a molecular weight of 5805 Da (Huang et al., 2010; Oliva et al., 2000). It is synthesized and secreted by islets of Langerhans in pancreas (Huang et al., 2010). Interestingly, insulin is also released from neurons upon depolarization (Wei et al., 1990). Insulin was first discovered in the central nervous system (CNS) of rats by immunohistochemical methods (Havrankova et al., 1978). In addition, the peripheral insulin from blood circulation can transport into CNS across the blood brain barrier (BBB) by receptor-mediated transcytosis (RMT) (Jones and Shusta, 2007; Patel et al., 2009; Wang et al., 2009). The RMT is the transport system that applies the vesicular trafficking mechanism of the endothelium to transport insulin between the blood and the brain (Wang et al., 2009). However, the central insulin cannot transport back from brain into circulation (Cashion et al., 1996).

Insulin acts via the activation of insulin receptors. Insulin receptors are widely distributed in brain, particularly in the cerebral cortex, hypothalamus, and hippocampus (Chiu and Cline, 2010; Huang et al., 2010; Schulingkamp et al., 2000). Furthermore, insulin receptors are presented in higher concentrations in neurons and in lower concentrations in glial cells (Gerozissis, 2008). The messenger RNA (mRNA) of insulin receptor is abundantly localized in cell bodies of neuron (neuronal soma) and protein of its receptor is found in both cell bodies and synapses (Schwartz et al., 1992; Zhao et al., 2004). Insulin receptors are tyrosine kinase receptors that have a molecular weight of approximately 400 kDa (Olson et al., 1988). Moreover, insulin receptors are large heterodimeric transmembrane glycoproteins composed of

α- and β-subunit that are linked together by disulfide bond (Chiu and Cline, 2010; Olefsky, 1990; Schulingkamp et al., 2000). The α-subunits (ligand-binding domain) are located entirely on the extracellular surface with a binding site for insulin while the β-subunits (ATP-binding and kinase domain) are transmembrane proteins possessing tyrosine kinase activity (Chiu and Cline, 2010; Taylor et al., 1992). In addition, the apparent molecular weight of the α-subunits (135 kDa) is slightly higher than the β-subunits (95 kDa) (Grunfeld et al., 1985). The schematic structures of insulin receptor are shown in Figure 1-1.

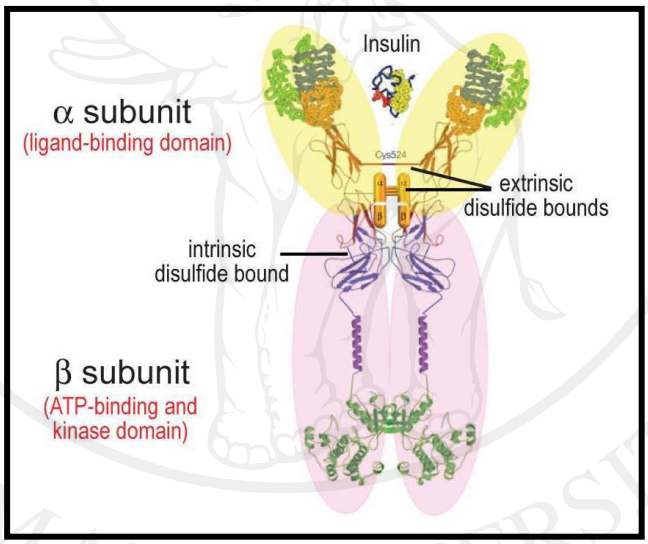

Figure 1-1 Schematic illustration of insulin receptor structure including α- and β-subunit (Chiu and Cline, 2010).

The biological effects of insulin depend on the availability of its binding to specific receptors and the activation of its intracellular signaling pathways. Insulin exerts its effect by binding to insulin receptors on the plasma membrane of neurons that initiate signal transduction complex to regulate several cellular functions (Porte et al., 2005). The specific binding of insulin triggers a rapid autophosphorylation at

tyrosine residue of β-subunits (Huang et al., 2010). Tyrosine autophosphorylation of insulin receptor promotes specific recruitment of Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domain containing protein that recognize phosphorylated tyrosine residue (Craft and Watson, 2004; van der Heide et al., 2006). The phosphorylated insulin receptors initiate its physiological effects by activating at least two major intracellular insulin signaling pathways: an insulin receptor substrate (IRS)-dependent pathway (PI3K/Akt cascade) and a SH2-domain containing protein (Shc)-dependent pathway (classical MAPK cascade) (Virkamaki et al., 1999). The activated insulin receptors phosphorylated Shc and IRS (Huang et al., 2010; Muniyappa et al., 2007). Subsequently, the Shc interact with the other adapter proteins such as growth factor receptor-bound protein 2 (Grb2)/Son of sevenless homolog (SOS) complex (Okada et al., 1998). The association of the Grb-2/SOS complex with the insulin receptor via phosphorylated Shc couples the insulin receptor to Ras/Raf activation, which in turn triggers kinase phosphorylation of mitogen-activated protein kinase (MAPK) cascades (Belfiore et al., 2009; Trakul and Rosner, 2005). Finally, the phosphorylated MAPK regulates gene transcription, protein synthesis, cell growth, and cell differentiation (Belfiore et al., 2009; Muniyappa et al., 2007). The insulin-dependent Shc cascade is shown in Figure 1-2.

Moreover, the phosphorylated IRS activates phosphatidylinositol 3-kinase (PI3K), in which convert phosphatidylinositol 4, 5-bisphosphate (PIP_2) to phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3) (Belfiore et al., 2009). However, PIP_3 can convert back to PIP_3 via phosphatase activity of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (He et al., 2010). The phosphorylated PIP_3 can stimulate 3-phosphoinositide dependent protein kinase-1 (PDK1) that

promotes Akt/protein kinase B (PKB) activity (Lustig, 2010; Muniyappa et al., 2007) and also activate MAPK activity via protein kinase C (PKC) upregulation (Le Good et al., 1998). Furthermore, Akt/PKB can be phosphorylated by PIP_3. Finally, the phosphorylated Akt/PKB regulates neuronal survival and release of neurotransmitter (Craft and Watson, 2004). The insulin-dependent IRS cascade is shown in Figure 1-2.

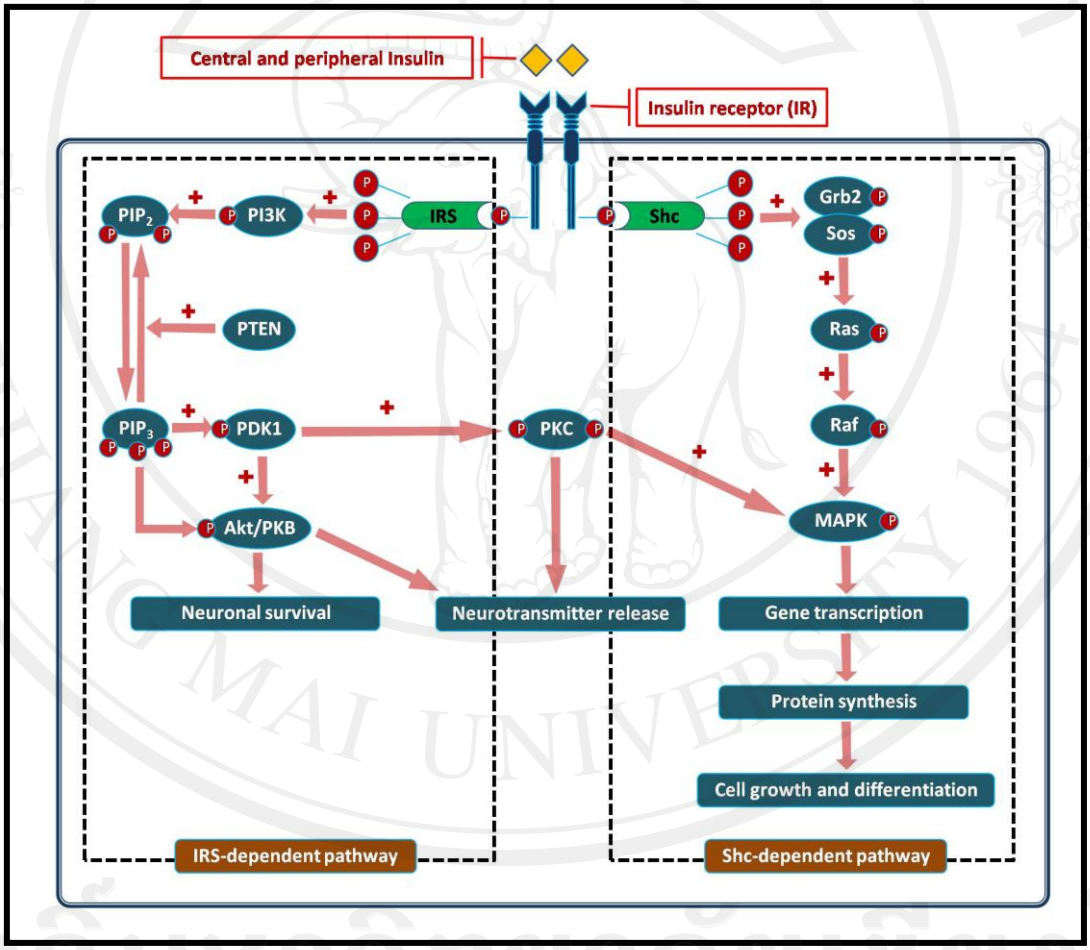

Figure 1-2 Schematic illustration of major intracellular signaling of insulin action both IRS-dependent pathway (PI3K/Akt cascade) and Shc-dependent pathway (classical MAPK cascade) (Craft and Watson, 2004; van der Heide et al., 2006).

The efficient insulin activity in the nervous system has been implicated in maintaining energy balance and glucose homeostasis (Gerozissis, 2008). The alteration in central insulin action is directly involved in metabolic dysfunction such as obesity and type 2 diabetes (Lin et al., 2004). The previous studies have shown that intact insulin in the brain control energy and glucose homeostasis via IRS/PI3K pathway (Carvalheira et al., 2001; Niswender et al., 2003; Porte et al., 2005). However, the specific neuronal deletion of insulin receptor in mice exhibits elevation of body weight and food intake (Bruning et al., 2000). In addition, insulin signaling in the CNS can also regulate synaptic plasticity (Bishop et al., 2010; Huang et al., 2010; McNay and Cotero, 2010). The two main types of synaptic plasticity either involve a long-lasting decrease (long-term depression; LTD) or increase (long-term potentiation; LTP) (Citri and Malenka, 2008). The activation of insulin receptors by insulin enhances the LTD in the hippocampus and cerebellum via α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor internalization from synaptic membrane of neuron into cytoplasm (Chiu and Cline, 2010; Huang et al., 2010; Wang and Linden, 2000). Moreover, experimental studies found that amyloid β (Aβ) protein-induced impairment of hippocampal LTP can also rescue by insulin (Lee et al., 2009; Zhao et al., 2010).

1.2.2 The role of high-fat diet consumption on insulin resistance

Insulin resistance is a main metabolic perturbation of type 2 diabetes characterized by high plasma insulin levels (hyperinsulinemia) with progression from normal glucose tolerance to impaired glucose tolerance (Gill et al., 2005). The peripheral insulin resistance is a pathophysiological state, in which target cells in the

peripheral tissues (such as livers, skeletal muscle cell, and adipocytes) are impaired responsiveness to the physiological action of insulin (Boden and Hoeldtke, 2003; Pawlak and Derlacz, 2011). On the other hand, the neuronal insulin resistance is a pathophysiological state, in which target cells in the nervous tissues (such as neurons and glial cells) are impaired responsiveness to the physiological action of insulin (Rasgon et al., 2011; Taghibiglou et al., 2009).

In both rodent and humans, the high-fat diet is sufficient to stimulate the perturbation of a number of aspects of metabolism in a progressive response and systematic manner (Figure 1-3) (Iqbal, 2007; Zhang et al., 2009). During the progression of high-fat diet-induced metabolic disturbance from healthy euglycemic state to unhealthy diabetic state, high-density lipoprotein (HDL) gradually decreased while insulin, fasting glucose, triglyceride, blood pressure, and body mass index (BMI) gradually increased (Zhang et al., 2009). In addition, the impairment of insulin action and the high levels of circulating insulin are also associated with increased glucose levels (Zhang et al., 2009).

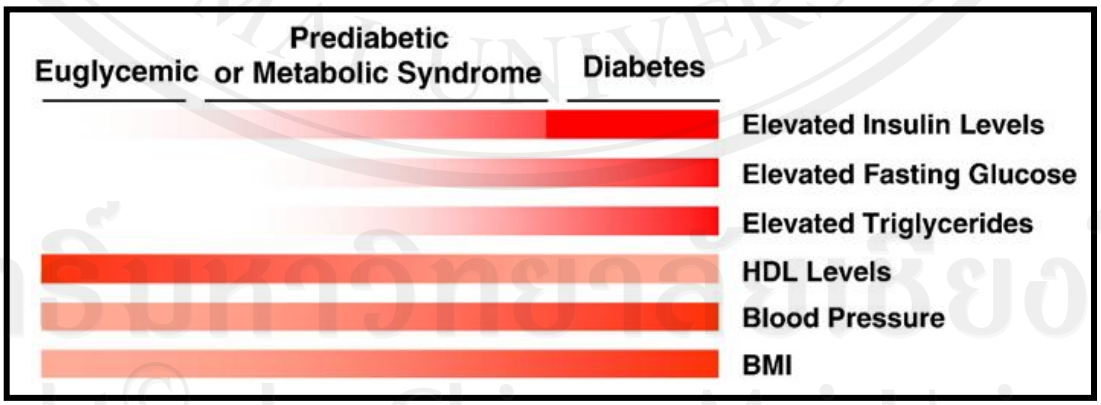

Figure 1-3 Schematic illustration of the significant role of high-fat diet consumption on progressive response from healthy euglycemic state to unhealthy diabetic state (Zhang et al., 2009)

The high-fat diet (HFD) has been implicated in the development of peripheral and neuronal insulin resistance in the animal models (Boghossian et al., 2009; Ono et al., 2008; Pratchayasakul et al., 2011a). In human clinical study, high-fat diet is the main cause of hypertrophic obesity due to adipocyte hypertrophy (Fung et al., 2001). The underlying mechanism of high-fat diet-related obesity in generating peripheral insulin resistance may affect on the adipocyte-derived cytokine and adipocyte-derived hormone. The secretion of cytokine from adipose tissue, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), can stimulate the progression of peripheral insulin resistance (Bastard et al., 2006). Other putative mechanism, adipocyte-derived hormone, especially adiponectin, can trigger insulin sensivity in peripheral tissues that is the second connection between obesity and insulin resistance (Bastard et al., 2006). In obese human, adiponectin is secreted less than in lean people (Faraj et al., 2004). However, the other adipocyte-derived hormone, especially resistin, has also been involved in the development of peripheral insulin resistance in peripheral tissues (Bastard et al., 2006).

Pratchayasakul and colleague have revealed that the level of neuronal corticosterone in male Wistar rats was elevated after 12-week HFD feeding (Pratchayasakul et al., 2011b). Corticosterone is glucocorticoid hormone in the rodent that acts in the brain to influence multiple aspects of neuronal processes (Droste et al., 2008). It has been previously observed that the basal level of corticosterone physiologically promotes neuronal cell survival while the high level of corticosterone can accelerate neuronal cell death (McEwen, 2007). The administration of exogenous corticosterone generates insulin resistance in brain of the animal models (Osmanovic et al., 2010; Piroli et al., 2007). The high level of

corticosterone abolishes phosphorylation of insulin receptor, Akt levels, and glucose transporter 4 (GLUT4) levels in the rat hippocampus (Piroli et al., 2007). Furthermore, exposure to excessive corticosterone level completely inhibits translocation of hippocampal GLUT4 to neuronal membranes (Piroli et al., 2007). These findings suggest that the stress level of corticosterone in the brain following chronic HFD feeding may be a possible mechanism for development of neuronal insulin resistance.

1.2.3 The relationship between high-fat diet-induced insulin resistance and oxidative stress in the brain

It has been shown that the brain is highly vulnerable to oxidative stress for several reasons (Figure 1-4): 1) it is a post-mitotic tissues with a high energy demand, 2) it requires very high amounts of oxygen consumption (about 20% of oxygen consumed by the whole body in human) for glucose utilization, 3) it has a high content of peroxidizable polyunsaturated fatty acids that are extremely sensitive to oxidation, 4) it has a high content of iron (Fe), particularly Fe plus ascorbate, that are potential constituent (powerful pro-oxidant) in causing membrane lipid peroxidation, and 5) it contains relatively poor concentrations of non-enzymatic antioxidants (such as reduced glutathione (GSH), α-tocopherol (vitamin E), and uric acid) as well as enzymatic antioxidants (such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)) that are not sufficient to prevent oxidative accumulation (Floyd and Hensley, 2002; Moreira et al., 2009).

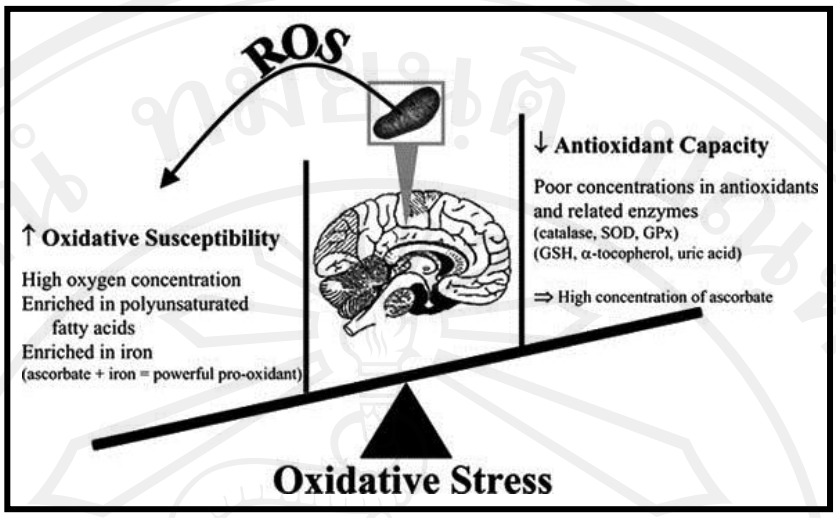

Figure 1-4 Schematic illustration of basic concepts of brain oxidative stress (Gill and Tuteja, 2010; Moreira et al., 2009; Scandalios, 2005).

Several clinical trials suggest that oxidative stress has been linked to insulin resistance and diabetes, and that treatment with antioxidant improves insulin sensitivity in insulin-resistant and diabetic patients (Ceriello, 2000; Evans et al., 2003; Evans et al., 2005; Maddux et al., 2001). It has been recently proposed that oxidative stress may be the central processes in the etiology of insulin resistance (Figure 1-5) (Styskal et al., 2012). The oxidative stress is caused by elevated mitochondrial reactive oxygen species (ROS), increased NADPH oxidase activity, and decreased antioxidant level (Evans et al., 2003; Styskal et al., 2012). Subsequently, the activation of oxidative stress promotes a pro-inflammatory state through macrophage infiltration and accumulation of inflammatory cytokine, such as nuclear factor-kappa B (NF-κB), TNF-α, and IL-6, that can also stimulate oxidative stress (Evans et al., 2003; Styskal et al., 2012). Oxidative stress may directly inhibit multiple downstream targets of insulin such as insulin receptor and PI3K to abolish intracellular insulin

signaling (Styskal et al., 2012). In addition, oxidative stress has been shown to enhance cellular stress-sensitive serine/threonine (Ser/Thr) kinase pathways, particularly c-Jun N-terminal kinase (JNK) (Styskal et al., 2012), that can disturb cellular insulin signaling through IRS-1 inhibition by phosphorylation at serine residue (Ser^{307}) (Evans et al., 2003; Rui et al., 2001). Furthermore, pro-inflammatory state can also reduce IRS-1 activity (Styskal et al., 2012). These data indicate that oxidative stress may directly or indirectly affect the severity of insulin resistance.

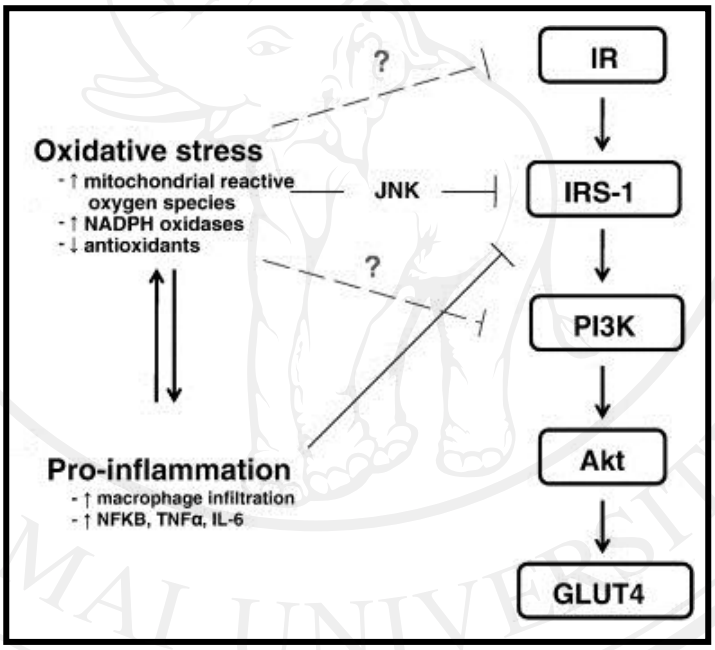

Figure 1-5 Schematic illustration of proposed mechanism of oxidative stress-related insulin resistance (Styskal et al., 2012)..

Previous study has been exhibited insulin resistance condition can also promote oxidative stress (de la Monte et al., 2009). In human and rodent, compensatory hyperinsulinemia (an indicator for insulin resistance) is positively correlated with oxidative stress during prolonged periods of peripheral insulin

resistance (Habib et al., 1994; Krieger-Brauer and Kather, 1992). Numerous scientific studies have revealed that insulin resistance triggers formation of advanced glycation end-products (AGEs) through alteration of glucose and lipid metabolism, which contribute to the progression of oxidative stress (Figure 1-6) (Miyata and van Ypersele de Strihou, 2009; Vasdev et al., 2006). The elevation of insulin resistance can also accelerate altered glucose or lipid metabolism, in which is able to stimulate the formation of highly reactive aldehyde (Vasdev et al., 2006). These excess reactive aldehydes react with sulfhydryl (SH) group and amino group (such as cysteine, arginine, and lysine) of protein to form AGEs that lead to oxidative stress (Zeng and Davies, 2005). Furthermore, AGEs can also trigger insulin resistance via oxidative stress generation (Tomino et al., 2011).

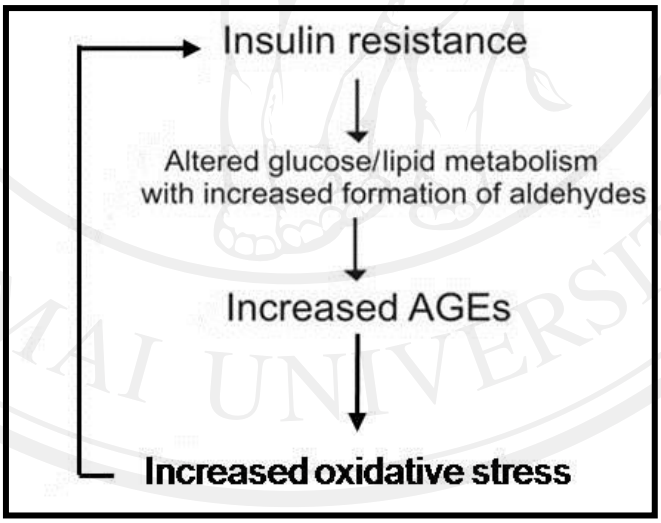

Figure 1-6 Schematic illustration of proposed mechanism of insulin resistance-mediated oxidative stress via AGEs action (Vasdev et al., 2006).

1.2 4 The role of eNOS in the brain

Endothelial nitric oxide synthase or eNOS (also known as NOS-3) is catalytic enzyme mainly expressed in vascular endothelial cells (Shaul et al., 1994). In addition, it is also expressed in human platelets (Sase and Michel, 1995), cardiac myocytes (Feron et al., 1996), gastrointestinal smooth muscles (Teng et al., 1998), and various neurons, especially pyramidal neurons of hippocampus, where it is coexpressed with nNOS (Dinerman et al., 1994). It consists of 1153 amino acids and has a molecular weight of 131 kDa (Marsden et al., 1992). As the eNOS function is regulated by calcium (Ca^{2+})/calmodulin (CaM), this enzyme is therefore termed Ca^{2+}/CaM-dependent eNOS (Spratt et al., 2007).

The eNOS is a prominent enzyme that is crucial elements of cerebral vasoregulatory mechanisms in hypertension (Jones et al., 2003) and ischemia (Endres et al., 2004). The phosphorylation of eNOS by Akt at serine residue (Ser^{1179}) in rodents (Atochin et al., 2007) and Ser^{1177} in human (Yoshitomi et al., 2011) is associated with elevation of NO generation during hypertension (Iwakiri et al., 2002). In eNOS knockout mice, it is shown that the reduction of blood flow in the ischemic border zone (Chen et al., 2005) and progression of cerebral infarctions (Huang et al., 1996). In addition, repression of eNOS action by N^{ω}-nitro-L-arginine methyl ester (L-NAME) attenuates cerebral blood flow and elevates the cerebral infarct size (Endres et al., 2004). However, intravenous administration of eNOS substrate (L-arginine) enhances NO production to confer regional cerebral blood flow-dependent functional recovery in an experimental stroke model (Dalkara et al., 1994). These data indicate that eNOS-driven NO activity prevents the cerebral ischemia and sufficiently sustaining high cerebral blood flow.

1.2.5 The role of iNOS in the brain

Inducible nitric oxide synthase or iNOS (also known as NOS-2) is catalytic enzyme mainly expressed in macrophages (Xie et al., 1992). In addition, it is also expressed in hepatocytes (Geller et al., 1993), glial cells (microglia and astrocytes) (Alderton et al., 2001), as well as vascular smooth muscle cells (Nunokawa et al., 1993). It consists of 1203 amino acids and has a molecular weight of 133 kDa (Geller et al., 1993). As the iNOS function is strongly regulated by cytokine and partially regulated by Ca^{2+}/CaM, this enzyme is therefore termed Ca^{2+}/CaM-independent iNOS (Spratt et al., 2007).

The previous review has been reported that iNOS function in the brain is detectable in unhealthy nerve tissue (Amitai, 2010). The activity of iNOS in microglia and astrocytes is associated with neuronal diseases (Garden and Moller, 2006; Wong et al., 2001). Moreover, the high NO level in microglia is produced by iNOS that can react with $O_2^{\bullet-}$ to forms peroxynitrite ($ONOO^{\bullet-}$) (Dasgupta et al., 2005). The peroxynitrite is capable of nitrosating tyrosine residues of protein to form nitrotyrosine that leads to neuronal cell death (Dasgupta et al., 2005).

1.2.6 The role of nNOS in the brain

Neuronal nitric oxide synthase or nNOS (also known as NOS-1) is catalytic enzyme mainly expressed in neurons and glial cells (Chen et al., 2004; Jiang et al., 2004). In addition, it is also expressed in cardiac, smooth, and skeletal muscles (Rothe et al., 2005; Schwarz et al., 1999). The nNOS is one of the three known NOS isoforms in mammalian that synthesizes nitric oxide (NO) and citrulline as a byproduct from L-arginine as substrate, as well as O_2 and NADPH as co-substrate

(Figure 1-7) (Vallance and Leiper, 2002). In the nervous system, it is mainly located at the post-synaptic terminal where nNOS-produced NO acts as retrograde messenger to deliver signal from post-synaptic neuron to pre-synaptic nerve ending of pre-synaptic neuron (Abbott and Nahm, 2004). Moreover, nNOS-driven NO also acts as second messenger in post-synaptic neuron (Jesko et al., 2003). It consists of 1433 amino acids and has a molecular weight of 161 kDa (Boissel et al., 1998). As the nNOS function is regulated by Ca^{2+}/CaM, this enzyme is therefore termed Ca^{2+}/CaM-dependent nNOS (Spratt et al., 2007).

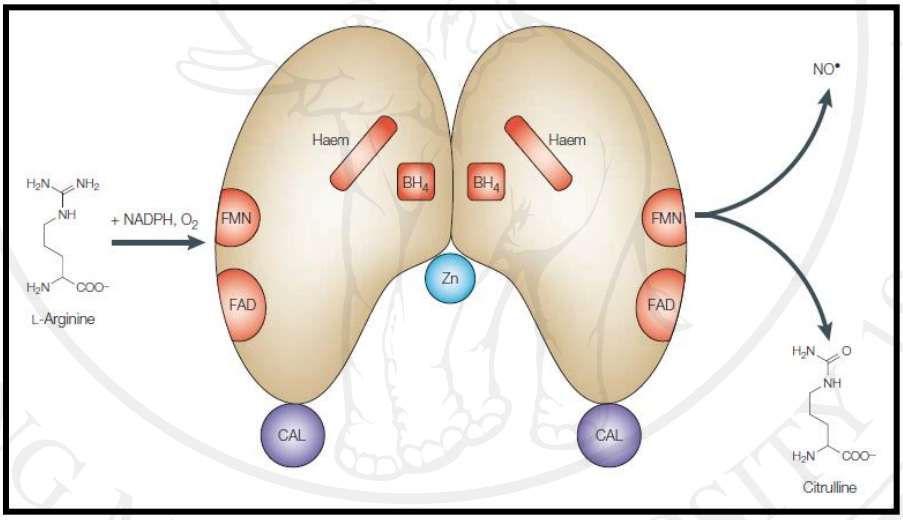

Figure 1-7 The schematic illustration of the metabolic pathway of NO formation through nNOS activity (Vallance and Leiper, 2002).

The nNOS activity in the brain has a prominent role in both physiological and pathophysiological states (Figure 1-8), in which depending upon its concentration of nNOS-derived NO (Zhou and Zhu, 2009). The low nNOS-driven NO levels are a neuromodulator while the high nNOS-driven NO levels are a neurotoxic factor (Zhou and Zhu, 2009). Under normal physiological conditions, the nNOS is the most predominant NO regulator in the neuron of both the central and peripheral nervous

system (PNS) which controls multiple neuronal functions both inhibitory regulation (neurogenesis and sympathetic outflow) as well as stimulatory regulation (neuronal survival, neurotransmitter release, and synaptic plasticity-related cognition) (Nishida et al., 2001; Zhou and Zhu, 2009). In the pathophysiological conditions, the excessive levels of nNOS-derived NO is an important factor in the development of depression, Parkinson's disease (PD), Alzheimer's disease (AD), and neuronal excitotoxicity (Toda et al., 2009; Zhou and Zhu, 2009).

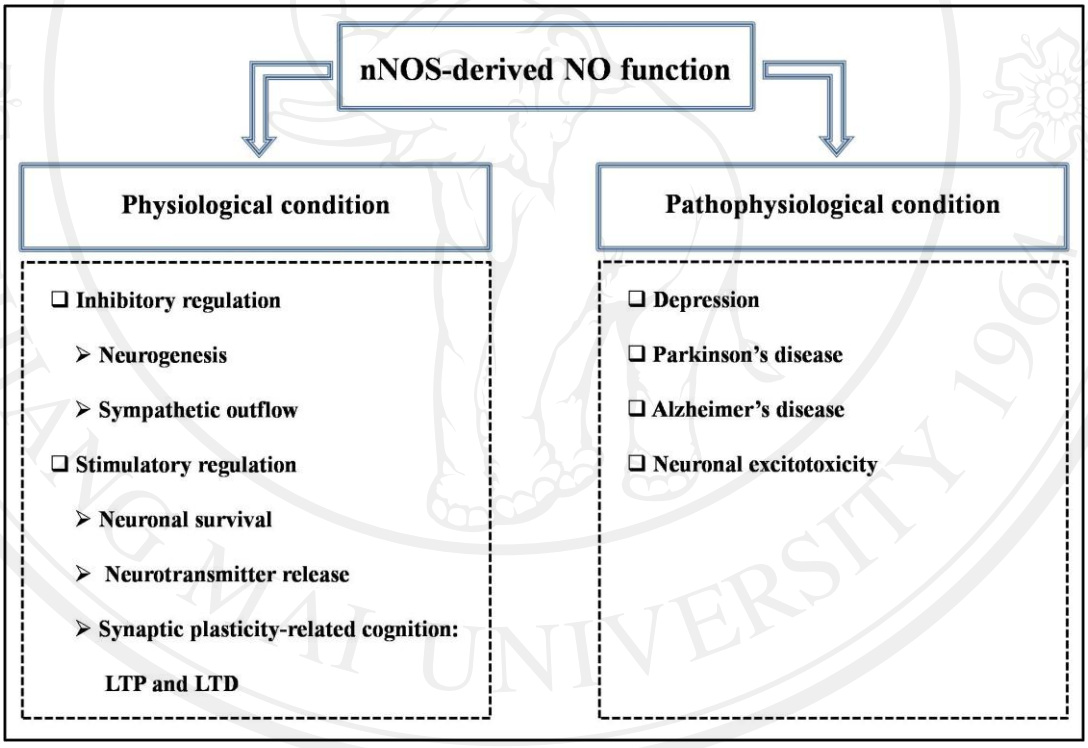

Figure 1-8 The schematic illustration of the significant role of nNOS-derived NO function both physiological and pathophysiological condition (Nishida et al., 2001; Zhou and Zhu, 2009).

1.2.7 The relationship between nNOS function and insulin activity in the brain

Experimental investigations in Sprague-Dawley rats have been reported that insulin can control nNOS-driven NO generation in glucose-inhibited (GI) neuron of ventromedial hypothalamus (VMH) via PI3K/Akt signaling pathway (Figure 1-9) (Canabal et al., 2007a; Canabal et al., 2007b). In addition, insulin phosphorylates IRS to activate PI3K/Akt cascade that can subsequently trigger nNOS-derived NO production (Canabal et al., 2007a). Afterward, NO is released from GI neurons to diffuse into adjacent cells containing soluble guanylate cyclase (sGC), including glucose-excited (GE) neuron, non-glucose-sensing neuron (N-GSN), as well as glial cell (GLIA) (Canabal et al., 2007b). However, suppression of PI3K actions with wortmanin (PI3K inhibitor) attenuate nNOS-derived NO level in the neuron (Canabal et al., 2007a).

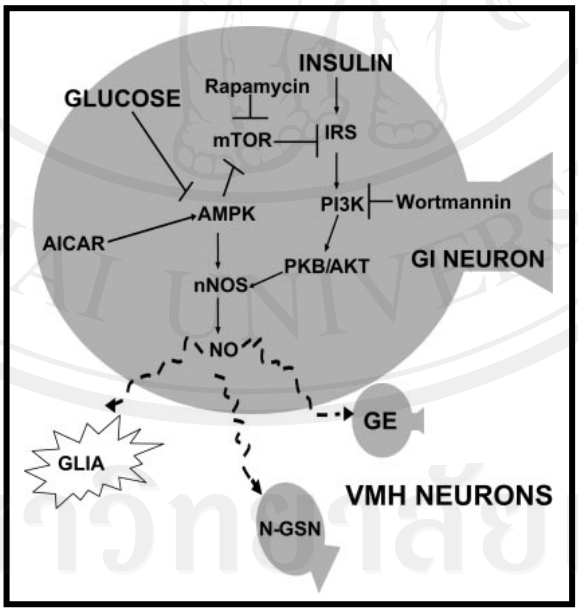

Figure 1-9 The schematic illustration of the significant role of insulin-mediated PI3K/Akt cascade in glucose-inhibited (GI) neuron of ventromedial hypothalamus (VMH) on nNOS-derived NO activity (Canabal et al., 2007a; Canabal et al., 2007b).

1.3 Objectives of this study

1.3.1 To investigate whether 12-week HFD consumption can cause peripheral insulin resistance and the reduction of nNOS expression in hippocampus.

1.3.2 To examine the brain oxidative stress levels in 12-week HFD-fed rats compared with the 12-week ND-fed rats.

1.3.3 To determine the relationship among the peripheral insulin resistance, nNOS expression in hippocampus, and brain oxidative stress levels of 12-week HFD-fed rats.

1.4 Hypothesis of this study

1.4.1 Rats fed with 12-week HFD have peripheral insulin resistance and the reduction of nNOS expression in hippocampal CA1 regions.

1.4.2 Rats fed with 12-week HFD increase the oxidative stress in brain.

1.4.3 The attenuation of nNOS expression in hippocampus correlates with the elevation of brain oxidative stress in 12-week HFD-induced insulin resistance model.