CHAPTER 1

Introduction

1.1 Principle, Theory and Rational/Hypothesis

Currently, obesity has become a public health priority around the world because it could lead to a major health problem, including insulin resistance, diabetes, hyperlipidemia, hypertension and cardiovascular disease (1). The prevalence of obesity is directly related to high-caloric diet consumption and the increase of sedentary lifestyle (2-4). In previous studies, insulin takes part in an essential role in the central nervous system (CNS), particularly in cognitive function (5-7). Therefore, the effect of obese-induced insulin resistant condition on the CNS become a very interesting topic. Our previous studies found that a 12-week high-fat diet (HF) consumption in both male rats and female rats could result in not only obesity and peripheral insulin resistance, but also brain insulin resistance, indicated by the impairment of brain insulin receptor function (insulin-induced long term depression; LTD) and brain insulin signaling (8, 9). Furthermore, previous studies found that a 12-week of HF consumption led to brain mitochondrial dysfunction (induced mitochondrial reactive oxygen species (ROS) production, mitochondrial membrane potential change and mitochondrial swelling), increased brain oxidative stress (MDA levels) and cognitive decline indicated by Morris water maze (MWM) test (7, 9).

Estrogen not only takes part in a crucial role in the reproductive system, but also in the metabolic control and cognitive function (10). Loss of estrogen either from an ovariectomy (OVX) or menopause leads to a significant increase in visceral fat deposition and insulin resistance (2-4, 11-13). Moreover, OVX causes impaired hippocampal synaptic function, indicated by decreased long-term potentiation (LTP) amplitude, dendritic spine density, as well as cognitive impairment (14-17). Our recent study showed that obesity aggravated peripheral insulin resistance, brain insulin resistance, brain mitochondrial dysfunction, cognitive impairment, brain oxidative stress and

hippocampal synaptic dysfunction in ovariectomized rats (18). However, the effect of obesity followed by estrogen deprivation has not much been thoroughly investigated. Only one study by Balzetic and colleagues used the model of HF feeding prior to ovariectomy (19). They found that ovariectomized-obese rats significantly increased in body weight, body fat mass, plasma insulin and plasma glucose levels than ovariectomized rats alone (19). Nevertheless, the combined effect of obesity and estrogen deprivation on hippocampal and cortical ROS production, hippocampal and cortical apoptosis, hippocampal and cortical estradiol levels and hippocampal synaptic function, by using the model of HFD feeding prior to ovariectomy has not been investigated.

Furthermore, it is well known that learning and memory can be divided into explicit (declarative) memory and implicit (nondeclarative) memory, which are hippocampal-dependent and hippocampal-independent learning and memory, respectively (20, 21). In animal study, hippocampal-dependent memory was determined by the MWM test (22), and hippocampal-independent memory by the novel objective recognition (NOR) test (23). In MWM test, previous found that obesity or ovariectomy caused the impairment of hippocampal-dependent learning and memory, which determined by the increased time to reach the platform and decreased time spent in target quadrant (18, 24). For the NOR test, obesity or ovariectomy caused the impairment of hippocampal-independent memory, which indicated by decreased time exploring a novel object (25, 26). However, the combined effect of obesity and estrogen deprivation on hippocampal-dependent and hippocampal-independent memory, by using the model of HF feeding prior to ovariectomy has not been examined. Therefore, the present study hypothesizes that estrogen deprivation aggravates the severity of metabolic impairment, hippocampal and cortical ROS production, hippocampal and cortical apoptosis, hippocampal synaptic dysfunction and cognitive impairment (both hippocampal-dependent and hippocampal-independent learning and memory) via decreases hippocampal and cortical estradiol levels in obese female rats.

1.2 Literature Review

1.2.1 Obesity and insulin resistance

Obesity is a major public health issue, it has more widespread obesity populations since 1980 (WHO, 2016). In 2014, WHO reported that over 600 million people around

the world were become obese (WHO, 2016). Diet is the most important causative factor of obesity (27, 28). The excessive high energy diet intake especially HF consumption gives more energy than other food types, resulting to over energy accumulation in the body (29). The HF is the main cause of obesity due to adipocyte hypertrophy, which is related to risk of metabolic disorders such as diabetes, hyperlipidemia, hypertension and cardiovascular diseases (1). Obesity also accelerates the risk of insulin resistance, which is the most important underlying mechanism of these health issues (30). The body weight gain from HF consumption could activate the insulin resistance condition (31). Insulin resistance is a pathological condition in which target cells become resistance of the biological activities of insulin on glucose and lipid metabolism (32). At normal physiological function, insulin binds to the extracellular α-subunit of the insulin receptor lead to conformational changes, which are autophosphorylation and tyrosine phosphorylation of the intracellular β-subunit (33). Resulting in the activation of several pathways including the phosphorylation of intracellular insulin receptor substrate (IRS) protein. IRS phosphorylation stimulates phosphoinositide 3-kinase (PI3K) activation, and this leads to the generation of phosphatidylinositol-3,4,5-triphosphate (PIP3) by phosphorylating phosphatidylinositol-4,5-bisphosphate (PIP2), 3-phosphoinositide-dependent protein kinase 1 (PDK1) and serine/threonine-specific protein kinase (Akt) (34). Akt can activate the translocation of glucose transporter type 4 (Glut4) from intracellular stores to the plasma membrane, which is crucial for insulin regulated glucose uptake (34). In addition, the activation of Akt requires a second phosphorylation of a serine residue by the mammalian target of rapamycin complex 1 (mTORC1), mammalian target of rapamycin complex 2 (mTORC2) complex and GSK3β, which mechanism can moderate protein synthesis, cell growth, cell survival and glycogen synthesis (34). Moreover, phosphorylated of SH2-containing collagen-related proteins (Shc) subunit can stimulate growth factor receptor-bound protein 2/son of sevenless guanine nucleotide exchange factor/mitogen-activated protein kinase (Grb2/SOS/MAPK) signaling pathway, which controls gene expression, cell proliferation, cell differentiation and development (34). Insulin signaling pathways are shown in Figure 1.1. Insulin resistance reduces the responsiveness of target tissues to normal circulating levels of insulin (35), it is a pathological condition of the metabolic syndrome, known as 'the pre-diabetes mellitus type 2 (T2DM) condition (35). The crucial factor that role in insulin resistance

condition is the free fatty acid (FFAs), which release from adipocytes (36). After long term high-fat diet consumption, the visceral adipose tissue which is a metabolically active endocrine organ, becomes dysfunction and leads to increase FFAs (36). On the other hand, insulin resistance increases plasma FFAs to activate more serine phosphorylation of IRS protein but less of tyrosine phosphorylation, leads to increase glucose production in liver and decreases glucose uptake in muscle (37). FFAs modulate insulin receptor via the activation of diacylglycerol (DAG) and protein kinase C (PKC) in liver and muscle (38), immediately become dysfunction of other insulin signals such as inhibitor of nuclear factor-kB kinase-β (IKK-β), JUN N-terminal kinase1 (JNK) and MAPK (37). IKK-β can activate nuclear factor-kB (NF-kB), a transcription factor that activates the inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) (39), resulting to trigger inflammatory response to insulin signaling (37). In addition, adipocytes can secret adipokines and inflammatory factors that inhibits insulin signaling including, increased resistin production, plasminogen activator inhibitor-1 (PAI-1), retinol-binding protein 4 (RBP4), IL-6 (40) and TNFα (37). Moreover, obesity has an increase in adipose tissue macrophages (ATMS) accumulation, which can activate adipocyte to produce inflammatory cytokines and lead to inhibit insulin signaling (41). On the other hand, level of adiponectin is decreased in obese condition (37). All of these disturb insulin signaling and many metabolic tissues including adipose tissue, liver and muscle, resulting in an increased level of insulin in bloodstream which decreased insulin sensitivity in many organs, increased free fatty acid oxidation (42) and increased ROS production (43). The relationship of endocrine, inflammatory and obesity to insulin resistance shown in Figure 1.2.

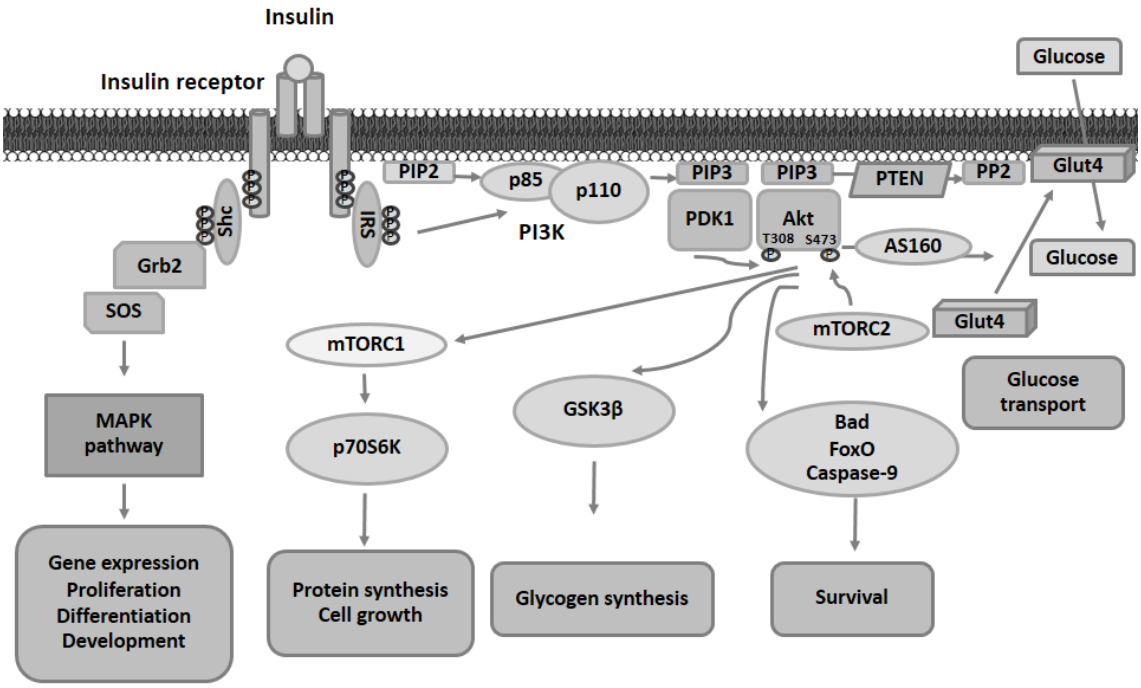

Figure 1.1 Insulin signaling pathways modified from Kim and colleagues (34)

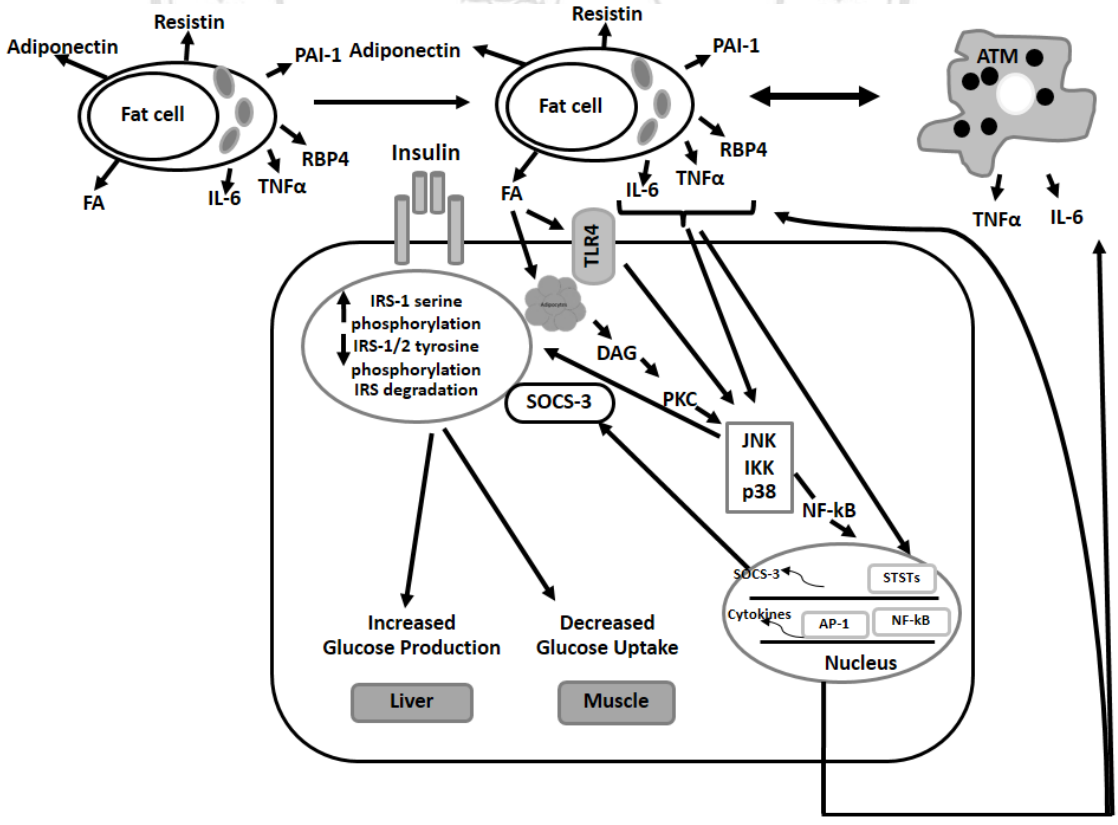

Figure 1.2 The relationship of endocrine, inflammatory and obesity to insulin resistance modified from Qatanani and colleagues (37)

1.2.2 Effect of insulin resistance on brain function

Malfunctioning of insulin signaling in the peripheral and neuronal tissues have been known to involve in Alzheimer's disease, diabetes and ageing (5, 44-48). In Alzheimer's disease, the reduction of cerebral insulin levels is thought to be the result of functional disturbances of the insulin receptors (insulin-resistant brain state) (48). Moreover, several cross-sectional epidemiological studies in elderly humans have indicated that there is an association between hyperinsulinemia or Type II diabetes and Alzheimer's dementia (49-51). In addition, the impairment of insulin or insulin resistance also have adverse effect on neuronal functions including synapse formation and synaptic plasticity (52). Synaptic plasticity is the main mechanism for learning and memory processes (53, 54). Learning and memory processes composed of two opposite forms of activity-dependent synaptic plasticity including LTP and LTD (53, 54). LTP and LTD can be initiated by many factors such as a large rise in intracellular calcium levels, a stronger depolarization and a brief high-frequency (100 Hz) stimulation which leads to a long lasting synaptic transmission of LTP (53, 54). On the other hand, a small rise of the intracellular calcium level, a decrease level of post synaptic depolarization and a prolonged low-frequency (1 Hz) stimulation lead to a persistent reduction in synaptic strength or LTD (53, 55, 56). Therefore, both LTP and LTD are the primary experimental protocols to investigate the basic of synaptic learning and memory processes (54).

Some studies has been done using animal models of insulin resistance to investigate the changes of LTP and LTD (7, 8, 57). In the insulin resistance model of fructose-fed hamsters, it found that hippocampal synaptic plasticity was impaired as indicated by diminish of LTD (58). Excessive fat consumption has known as a key role player in the development of insulin resistance and Type II diabetes (59). Previous study indicated that the high-calorie diet consumption for 32 weeks impaired synaptic plasticity by reducing the LTP in Cornu Ammonis 1 (CA1) hippocampus, and impaired cognitive function in rats (60). Furthermore, several studies suggested that consumption of a high-fat diet for 3 months can initiate peripheral insulin resistance and lead to impair cognitive function (6, 61-64). These results proved that the cause of insulin resistance induced cognitive decline which is related to HF. Our previous study found that neuronal insulin receptor function, which is determined by insulin-mediated LTD in CA1 hippocampus,

was diminished in model of 12-week HF-fed rats (9, 18). The depression of LTD might be due to the significant decrease in insulin-induced tyrosine phosphorylation of the insulin receptor signaling including insulin receptor (IR), IRS-1 and serine/threonine protein kinase/protein kinase B (Akt/PKB) in brain slices of the 12-week HF group (9).

In the physiological condition, mitochondria are adenosine triphosphate (ATP) producers, playing a crucial role in the regulation of body energy balance and cellular function, which produces ATP through the mitochondrial respiratory chain (65, 66). By producing ROS from the respiratory chain, it can damage cells and lead to various pathological conditions, such as neurodegenerative diseases (67, 68). Previous study in skeletal muscle of insulin resistance mice induced by HF consumption proved mitochondrial dysfunction, down-regulated genes required for the mitochondrial respiratory chain, and increased ROS production (69, 70). In addition, it is well known that brain mitochondria take part in controlling the energy demanding neurotransmission and calcium homeostasis, which are essential mechanisms for learning and memory processes (71). Our studies also found that long-term HF consumption in rats caused brain mitochondrial dysfunction, pointed out by increased in brain mitochondrial ROS production, brain mitochondrial depolarization and mitochondrial swelling (8, 72, 73). Furthermore, previous study in the hypothalamus of male Wistar rats fed on a HF diet for 8 weeks can induced apoptosis by increasing TUNEL positive cells at arcuate and lateral hypothalamic nuclei and increased Bax protein expression in hypothalamus (74). Moreover, study in adult male Sprague-Dawley rats found that diabetic rats which induced by a single intraperitoneal injection streptozotocin (STZ) after 2 weeks of a high sucrose, HF increased Bax protein expression and Bax/B-cell lymphoma 2 (Bcl-2) ratio with decreased Bcl-2 protein expression in the dentate gyrus (75). The study of male C57BL/6 mice showed that high-cholesterol diet from normal chow plus 5% cholesterol for 20 weeks led to increase the activation of caspase-3 and caspase-12 in the hippocampus (76). Another study in postnatal day 21 male Wistar rat fed a high sucrose diet for 9 weeks showed the increased of hippocampal caspase-3 and CCAAT-enhancer-binding protein homologous protein (CHOP) mRNA expression (77). The experiment in 20-week-old male Sprague-Dawley indicated that HF consumption (60 kcal% fat) for 28 weeks increased caspase-3 and Bax protein expression but decreased Bcl-2 protein expression and Bcl-2/Bax ratio in the hippocampus (78). In addition, our previous studies

in male Wistar rats found the HF diet (59.28% E) fat for 16 weeks led to increase in the expression of a pro-apoptotic marker (Bax), decrease anti-apoptotic marker (Bcl-2) and increase Bax/Bcl2 ratio in the brain when compared with normal diet (19.77% E fat) rats (57, 79). It could be summarized that insulin resistance is associated with the brain oxidative stress, brain apoptosis and hippocampal synaptic. Nevertheless, the study of combined effects of estrogen deprivation and obesity by using the model of HF consumption prior to ovariectomy on brain function have never been investigated.

1.2.3 Effect of insulin resistance on hippocampal-dependent and hippocampal-independent learning and memory

Learning is the strengthening of existing responses or formation of new responses to existing stimuli that occurs because of practice or repetition (80). Learning is acquisition of the information that makes this possible and memory is the retention and storage of that information (80). Memory is the mental function that it can acquire, retain and recall of the sensation, impression, information, and thinking (80). It can be differentiated memory into several types such as temporal categories which can be classified as short-term, intermediate long-term memory and long-term memory (81). Short-term memory is working of thinking about currently information (81). The information in short-term memory is not permanent, it is just able to keep information for second to minutes (80). For intermediate long-term memory, it is the ability to keep the information for several minutes to several weeks (82). Lastly, long-term memory is able to store information for many years or until a lifetime (81). Long-term memory can also be divided into explicit and implicit forms (80). Implicit or nondeclarative memory does not take part in awareness and its retention does not correlate with the hippocampus (83). It is important for training reflexive motor or perceptual skills (81). Implicit memory involves in priming which is related with neocortex brain area (83). Procedural also included in implicit memory, it involves in skills and habits which happen in striatum area (81). Moreover, the associative learning both in classical and operant conditioning also including in implicit memory (83). These memory compose of emotional response which is from the amydala area and skeletal musculature which is controlled by cerebellum area (80). As well as nonassociative learning, the implicit memory involves in habituation and sensitization which were controlled by reflex pathways (84). For the explicit or declarative memory, it is related to consciousness, awareness and depend on

the medial temporal lobe and hippocampus areas (81). It is associated with fact or semantic and events or episodic memory (85, 86). Forms of long-term memory as shown in Figure 1.3. Several studies used the Morris water maze (MWM) test to determine the hippocampal-dependent learning and memory and used novel object recognition (NOR) test to assess the hippocampal-independent learning and memory (81).

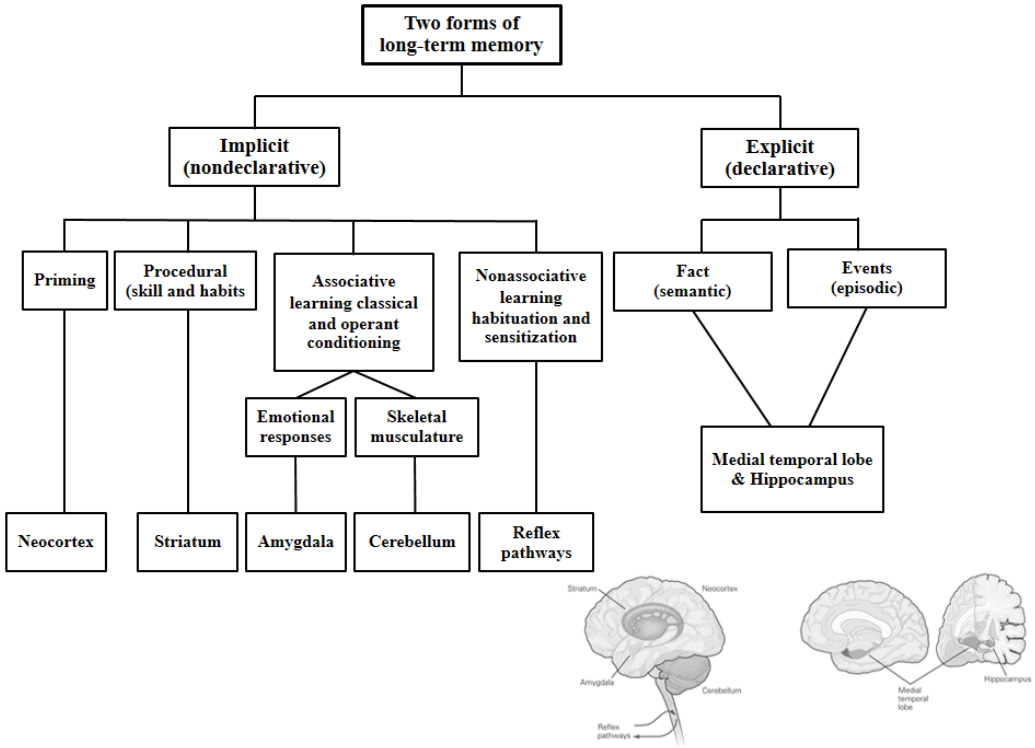

Figure 1.3 Forms of long-term memory modified from Mullally and colleagues (86)

It has been known that obesity is associated with cognitive dysfunction in both animal and human models. Several in vivo studies suggested that obese-insulin resistant is associated with the impairment of cognition (both hippocampal-dependent and hippocampal-independent memory (7, 72, 73, 87-90). In MWM test, the study in the 4-months old male Wistar rats, rats received 35% sucrose to induced the pre-diabetic state for 9 weeks found that high sucrose rats spent less time in the correct quadrant, more time in the opposite quadrant, had a higher latency to reach the platform zone and reduced number of crossings in the platform zone when compared to control rats (90). Our experiments in Wistar rats received HF consumption for 12 weeks, resulting in cognitive

impairment determined by increased time to reach the platform in acquisition phase and decreased time spent in the target quadrant in the probe test of MWM test (7, 72, 73, 88). Previous study in male Wistar rats after 4 months of HF (59% fat) showed the impairment of cognitive function as indicated by the increased of escape latency time and decreased time in the target quadrant than control (91). Another study in male Sprague-Dawley rats found that after 16 weeks of HF intake led to impair spatial memory by increased escape latency and decreased the number of platform crossings and time spent around the platform than control (92). The study in naïve male Sprague-Dawley rats received high-fat chow (60% kcal/g of fat) and high-fructose chow (55% kcal/g of fructose) for 7 weeks found that high-fructose fed rat entered the target quadrant significantly lesser time than high-fat fed rat in the reverse MWM task (93). In contrast, the experiment in male Wistar rats after 3 months of HF consumption showed no difference of the Morris water maze tests when compared between control and HF group (94). However, the impairment of hippocampal-independent memory under the HF-induced obese-insulin resistant is still unclear. The study in adult male Sprague-Dawley rats that received HF contained 45% energy from fat for 8 weeks found that obese rats had lower discrimination ratios for tests of NOR than normal diet rats (87). In the study of C57BL/6J male mice which gained 60% calories from fat after 3 and 6 weeks, had impaired discrimination index for NOR when compared to low-fat diet-fed mice that containing only 10% calories from fat (89). The study in male Sprague-Dawley rat found that after 16 weeks of HF consumption resulting to decreased discriminate index (92). However, there are the studies showed no effect of HF on hippocampal-independent learning and memory. Previous study in Male Wistar rats after 20 weeks of HF administration found no difference of exploration time when compared with standard diet group (95). In addition, in Male Sprague-Dawley rats received cafeteria diet (chow and access to one sucrose bottle at 10% solution) for 20 days showed no difference of exploration time when compared with regular diet group (96). Nevertheless, the study of combined effects of estrogen deprivation and obesity by using the model of HF consumption prior to ovariectomy on both hippocampal-dependent memory and hippocampal-independent memory have never been investigated.

1.2.4 Effect of estrogen deprivation on metabolic disturbance

In mammals, there are three major forms of estrogens including estrone (E1), estradiol (E2 or 17β-estradiol) and estriol (E3) (97). And another type of estrogen called estetrol (E4) which is secreted only during pregnancy (97). The predominant form in the human body is E2, which has the highest biopotency (97). Main source of estrogens is the ovary, specifically the growing follicles (97). Other source of estrogens is the placenta, responsible for a large extent of high levels of estrogens during pregnancy (97). Estradiol is also locally produced in several different tissues, including ovary, bone and nervous tissues (97). Moreover, the adipose tissue is also known to secrete estrogens (98). According to previous studies, estrogen plays an important role in the development of sexually dimorphic anatomical, functional and behavioral characteristics, which exists in many tissues including the brain, adipose tissue, skin and placenta (99). Moreover, several studies showed that estrogen plays a role in metabolic regulation (100, 101). Endogenous estrogen can maintain glucose homeostasis in both humans and animals (100, 101).

Menopause occurs naturally in women when there is a gradual loss of ovarian follicles and can be termed as the complete cessation of menstrual periods (101). It is predicted that 1.2 billion of women worldwide will become menopausal or postmenopausal by the year 2030 (102). The study found that menopause and ovariectomy lead to increased adipose tissue, involved with loss of estrogen signaling and caused an increase in visceral fat (11). Moreover, postmenopausal women have the alteration of lipid metabolism and body fat distribution (103). In model of female ER-α knockout (αERKO) mice also showed an increased in adipose tissue (103). Ovariectomy can stimulate hepatic fat and cholesterol accumulation in HF fed rat model (104).

Several studies have reported that estrogen replacement therapy is valuable for improving insulin sensitivity, reducing central body fatness, lowering lipid and cholesterol levels, and decreasing the risk of developing Type II diabetes (105-107). These beneficial effects of estrogen on insulin resistance depend on dosage, route of administration, and duration of treatment (108). According to a previous study, the administration of estrogen in rats can increases the pancreatic insulin content (108). Subsequently, a partial pancreaectomised rat model showed that after administration of

estrogen, it was associated with hypertrophy and the regeneration of islets (109). Furthermore, it has been demonstrated that female rodents were protected against hyperglycemia unless they were ovariectomized, while diabetes in male rodents was reversed by estradiol perfusion (110). Studies using transgenic mice and mice with genetic alterations in estrogen secretion or estrogen action have shown a little anti-diabetic properties of estrogen at the tissue-specific levels (111). These in vivo studies described above suggested that the estrogen had the beneficial effects on insulin resistance. However, some studies have shown that estrogen has no effect on the impairment of insulin sensitivity (112, 113). Previous study showed that the administration of low dose estradiol in an animal model increased the rate of IRS-1 phosphorylation, promoted the association between IRS-1 and the subunit of PI3K, p85alpha, caused a decrease in the rate of IRS-1 serine phosphorylation and increased the rate of Akt phosphorylation (10). In addition, the study showed that estrogen treatment exerted anti-diabetic and anti-obesity effects in rats that received 34.9 g% fat for 10 months (106). The study showed that estradiol treatment in the HF fed rat model reduced insulin levels by 50% during a hyperinsulinemic euglycemic clamp study (113). The treatment also improved insulin signaling (Akt phosphorylation) in insulin-stimulated skeletal muscles (113).

1.2.5 Effect of estrogen deprivation on brain function

In the central nervous system, estradiol controls synaptic plasticity, adult neurogenesis, reproductive behavior, aggressive behavior, pain processing, and cognition (114). Estrogen acts at estrogen receptor (ER-α or ER-β) binding sites associated with the plasma membrane and translocation of the estrogen receptor complexes to the nucleus, activation of the intracellular cascade to modulate the activity of multiple signal transduction (115). The effects of estrogen are on cells and tissues, including neuroprotective and neurotrophic via the activation of anti-apoptotic genes, sprouting and activation of glial response (116). In addition, estrogen activates the intracellular cascade consisting of the extracellular-signal-regulated kinases (ERK)/MAPK pathway (115). This pathway regulates both additional genomic events after translocation of activated ERK into the nucleus and also membrane receptors such as the α-amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) or N-methyl-D-aspartate (NMDA) receptors, ion channels, and intracellular enzymes (117). The tyrosine phosphorylation of NMDA

receptors leads to the increased function of the receptors and facilitation of LTP formation, as well as learning and memory (116). This pathway also could be involved in the synaptic remodeling which took place in hippocampus and other brain structures during the estrus cycle (116). The cellular effects of estrogen in the brain are as shown in Figure 1.3.

Jiang and colleagues stated that 42 days after OVX, the OVX group impaired amplitudes of field excitatory postsynaptic potentials (fEPSPs) when compared with sham group (118). Moreover, the study of female Lister Hooded rats found after 5 weeks of OVX led to impair percent fEPSP slope than sham estrous group (119).

After estradiol administration to hippocampal slices of OVX rats found that 24 and 48 hours after the last injection could increase fEPSP slope than the OVX rats (17). As well as the Schaffer collateral-CA1 of Sprague-Dawley ovariectomized rats that received series of injections of 17β-estradiol found that low-frequency stimulation led to a significant induced LTD (120). The underlying mechanisms of estradiol on improving synaptic plasticity may be involved in increasing apical dendritic spines (121-123), decreasing GABAergic inhibition (124, 125), increasing in NMDA receptor (NMDAR) expression and transmission (113, 126, 127).

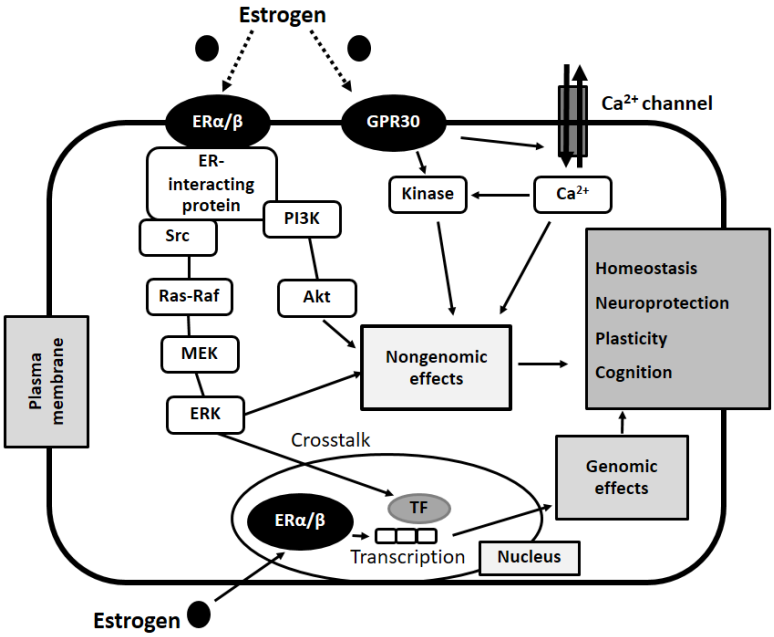

Figure 1.4 The cellular effects of estrogen in the brain modified from Bi and colleagues (116)

Furthermore, several studies showed that loss of ovarian function through either the menopause or an ovariectomy resulted in increased oxidative stress, indicated by increased brain malondialdehyde (MDA) levels and brain mitochondrial ROS production (7, 128). In addition, estradiol modulates the control of apoptosis (129). Previous study in female Wistar albino rats found that after 14 days of OVX result in an increased of caspase-3 and caspase-9 expression in dorsal root ganglion and hippocampus neuron than the control group (130). In addition, 15, 21 and 36 days after OVX in female Wistar rats found the increased of Bax expression and TUNEL positive nuclei with decreased of Bcl-2 expression in the hippocampus than control rats (131). Another study in 8 week-old Sprague-Dawley rats showed that OVX for 8 weeks increased Bax and decreased Bcl-2 expression in the hippocampus (132). Estradiol reduces apoptosis and facilitates the incorporation of newly generated neurons from the subventricular zone to the ischemic regions to protect neurons in the brain ischemia model (133, 134).

1.2.6 Effect of estrogen deprivation on hippocampal-dependent and hippocampal-independent learning and memory

Previous animal and human models studies demonstrated that loss of estrogen hormone leads to a significant decrease in cognitive function, which was prevented or enhanced by estrogen replacement (101, 109, 110, 112, 135, 136).

In animal model, the ovariectomized rats had longer escape latencies and distances at day 3 to 5 of acquisition phase than the normal rats, the percentage of crossing and the time in the target quadrant of probe trial were obviously reduced in the OVX rats when compared with the normal rats (24). In the present study, Sprague-Dawley female rats 2 weeks after OVX, showed impaired object location (hippocampal-dependent) test by lesser exploration time than the control group (137). In addition, 3 months after OVX rats had impaired hippocampal-dependent cognitive function by a significant increase in escape latency of MWM test when compared with the sham group (138). In the present study, adult female Sprague-Dawley OVX group rats had prolonged latency and lesser target quadrant time than the sham group (139).

Several studies reported the beneficial effects of estrogen on improving memory function in human and animal models (121, 123, 140). Estrogen administration in ovariectomized animals leads to increase cognitive performance in a hippocampal-

dependent manner (137). The results showed that after 3 months OVX of female Sprague-Dawley rats, had a significant increase in escape latency of MWM test when compared with the sham group (138). On the other hand, 3 month of estrogen treatment could reduce escape latency of MWM test in OVX rats (138). Moreover, a study in C57BL/6J female mice found that OVX treated with estrogen for 7 or 40 days could improve the performance of T-maze test (141). One study of adult female Sprague-Dawley rats proved that estrogen replacement therapy for 20 weeks could ameliorate cognition in OVX rats by decreasing latency and increasing target quadrant time of MWM test better than the OVX rats alone (139).

Loss of estrogen hormone leads to a significant decrease in cognitive function, which was prevented or enhanced by estrogen replacement (99, 107-109). Observational studies found that postmenopausal women who has been given by estrogen treatment performed the tests of verbal fluency and verbal memory significantly better than estrogen-nonusers who were matched for relevant control variables (24, 26, 121, 138). Longitudinal studies, which carried out for several years for determining the aspects of cognitive functioning decline at different rates of increasing age, reported that estrogen-users over the age of 65 years had significantly higher scores on verbal memory, verbal fluency and visual memory when compared to age-matched nonusers (142, 143).

In hippocampal-independent memory, OVX decreased recognition index of NOR when compared with sham group (25, 26, 144). In Sprague-Dawley female rats showed 2 weeks after OVX impaired object recognition test by decreased exploration time than control group (137). After 5 weeks of estrogen replacement therapy, it can increase recognition index than the OVX untreated group (26). In the study of females C57BL/6 mice found that 1 and 6 weeks after OVX did not change time with novel object while 12 weeks after OVX led to reduced time with novel object than sham group (25). The experiment in C57BL/6J female mice reported that after OVX for 1 week, could decrease recognition index lesser than the sham group (144).

The NOR test, studies of ovariectomized Sprague-Dawley rats found that a single subcutaneous injection of estrogen at dose 5μg/kg enhanced memory in NOR by increasing exploration ratio (142). In addition, the percent time spent with the novel object of E2-treated after 24 and 48 hours was significantly greater in E2-treated

compared to vehicle-treated rats (143). After 5 weeks of estrogen replacement therapy, it can increase recognition index than the OVX untreated group (26). However, the studies in middle-aged female C57BL/6 mice were ovariectomized that received estrogen ingested doses about 70, 110, and 180 μg/kg per day for 5 weeks before NOR testing (145). The results found that there were no differences in the number of visits to novel object for any group when compared with control group (145). In the study of aging ovariectomy female mice treated with continuous 0.2 mg/kg of 17β-estradiol and intermittent group (twice/week of E2) for 3 months did not find the beneficial effect of estrogen treatment on the NOR (146). From all results, there are still have the controversy in the effect of estrogen on hippocampal-independent learning and memory. These different results maybe from the different study design, protocol, timing, animal species and age of the model.

1.2.7 The combined effects of estrogen deprivation and obesity on metabolic disturbance

Although previous studies showed that either diet-induced obesity or menopause had worse effects on metabolic function (19, 147, 148), few studies demonstrated the effect of the interaction of these two conditions on metabolic changes. Prior studies showed that HF-induced obesity and menopause by ovariectomy interacted with the increased body weight and increased fat mass (149, 150). In addition, the analysis of fat mass by MRI imaging has shown that the size of adipocytes in visceral, subcutaneous and perigonadal fat pads were enlarged in ovariectomized obese mice (150). Furthermore, triacylglycerol (TG) and DAG contents in skeletal muscle were also increased in ovariectomized obese mice (149). Ovariectomized obese mice also increased levels of two adipogenic enzymes including lipoprotein lipase (LPL) and fatty acid synthase (FAS) in perigonadal adipose tissue (150). The biochemical analysis showed that the combination of ovariectomy and obesity had higher levels of serum total cholesterol, serum TG, plasma leptin and lower serum adiponectin level compared to ovariectomized mice alone (151). In addition, the combination of ovariectomy and obesity also reduced whole-body energy expenditure and decreased energy expenditure with downregulation of uncoupling proteins in the brown adipose tissue (149, 150). In inflammatory processes, the combination of ovariectomy and obesity showed the increase of inflammation, indicated by increased number of CD11c-positive macrophages, the expression of TNF

and IL-6 levels in white adipose tissue (149, 151). Moreover, the combination of ovariectomy and obesity can aggravate more impairment of insulin sensitivity, as indicated by increased plasma insulin level, increased Homeostasis model assessment (HOMA) index level, impaired glucose tolerance and decreased Akt phosphorylation, compared with HF-fed rats or ovariectomized rats alone (148, 149, 151-153). Furthermore, our recent study found that obesity on top of estrogen deprivation may generate the development of Type II diabetes, indicated by increased plasma glucose levels in ovariectomized obese rats (19). To investigate the combined effects of obesity and estrogen deprivation on metabolic changes, several prior studies used ovariectomized model following by HF feeding in their studies. However, there was only one study that used the model of HF feeding prior to ovariectomy which is the most likely situation in menopause women who suffer from obesity. Blazetic and colleagues found that ovariectomized obese rats significantly increased body weight, body weight gain, body fat mass, plasma insulin and glucose concentration than ovariectomized rats alone (19). Nevertheless, further studies are required to provide more evidence to support the effects of this model on metabolic changes.

1.2.8 The combined effects of estrogen deprivation and obesity on brain function and cognitive function

Interestingly, the combined effects of estrogen deprivation and obesity on brain functions such as neuroinflammation, food intake regulation, insulin sensitivity and cognition were also investigated by using ovariectomized model following by HF feeding. In neuroinflammation, the protein and mRNA expression of brain-derived neurotrophic factor (BDNF) levels, IL-6 and TNF-α levels were decreased in the hippocampal tissues of ovariectomized obese rats (154). The study in female Wistar rats (4 weeks old) which received 8 weeks of HF follow by OVX for 10 weeks feeding found that HF or ovariectomy increased leptin receptor (Ob-R) distribution in lateral hypothalamic nuclei, but there was no effect on arcuatus nuclei (19). Our recent studies showed that obesity accelerated brain oxidative stress, hippocampal synaptic dysfunction, brain mitochondrial dysfunction, brain insulin resistance and cognitive impairment (only studied in hippocampal-dependent learning and memory) of estrogen-deprived rats (18, 128). In the previous study, 3 months old female Wistar rats were divided into ND fed sham-operated group, HF fed sham-operated group (60% fat) and HF fed OVX group for

12 weeks (154). The results showed that the HF fed sham-operated group had increased TNF-α mRNA, IL6- mRNA, TNF-α protein and IL-6 protein but decreased BDNF mRNA and protein in the hippocampus when compared with the ND fed sham-operated group (154). Moreover, the HF fed OVX group aggravated the severity of all parameters more than the ND fed sham-operated group and the HF fed sham-operated group (154). In addition, our study in the model of OVX and HF consumption for 16 weeks found that obesity aggravated the decreased of dendritic spine density and increased brain oxidative stress levels in estrogen-deprived rats (8). Although several studies found worse effects of combined estrogen deprivation and obesity on brain function, the study of brain function by using the model of HF feeding prior to ovariectomy on brain pathology, hippocampal-dependent and hippocampal-independent learning and memory have not been investigated. Therefore, further studies are required to provide more evidence to clarify.

1.3 Purposes and hypotheses of the study

Aim 1: To investigate the effects of estrogen deprivation on metabolic parameters in obese-insulin resistant condition

Hypothesis 1: Estrogen deprivation aggravates the severity of metabolic impairment in the obese-insulin resistant condition.

Aim 2: To investigate the effects of estrogen deprivation on hippocampal and cortical reactive oxygen species and hippocampal synaptic plasticity in the obese-insulin resistant condition

Hypothesis 2: Estrogen deprivation aggravates the severity of hippocampal and cortical reactive oxygen species and the impairment of hippocampal synaptic plasticity in the obese-insulin resistant condition.

Aim 3: To investigate the effects of estrogen deprivation on cognitive function (both hippocampal-dependent and hippocampal-independent memory) in the obese-insulin resistant condition

Hypothesis 3: Estrogen deprivation aggravates the impairment of cognitive function (both hippocampal-dependent and hippocampal-independent memory) in the obese-insulin resistant condition.