CHAPTER 3

Results

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

3.1.1 The effects of estrogen deprivation on metabolic parameters in obese-insulin resistant condition

After HF consumption for 13 weeks, the rats developed obese-insulin resistant and dyslipidemia as indicated by increased body weight, visceral fat weight, plasma insulin level, HOMA index, plasma cholesterol and LDL level when compared with the ND rats ($p<0.05$; Table 3.1).

After ovariectomy for 7 weeks, rats were determined the uterus weight and serum estradiol levels to confirm the effect of estrogen deprivation. NDO and HFO rats resulted in decreased uterus weight and serum estradiol levels when compared with NDS and HFS rats. In addition, the NDO, HFS and HFO rats suffered the metabolic disorders and oxidative stress with increased body weight, visceral fat weight, plasma insulin level, HOMA index, the area under the glucose curve (AUCg) of OGTT, plasma cholesterol, LDL level and serum MDA when compared with the NDS rats. Interestingly, the HFO rats had the most severity of metabolic disorders and oxidative stress, as indicated by the highest level of body weight, hyperglycemia, insulin resistance, dyslipidemia and serum MDA ($p<0.05$; Table 3.2). This results suggested that estrogen deprivation aggravated the severity of metabolic disorders under the obese-insulin resistant condition.

Table 3.1 Effect of high-fat diet consumption on metabolic parameters.

Parameters	Groups	
	ND	**HF**
Body weight (g)	272.08±2.69	318.20±5.48*
Plasma glucose (mg/dl)	144.92±4.27	143.54±3.47
Plasma insulin (ng/ml)	1.09±0.12	1.66±0.18*
HOMA index	7.56±1.36	14.28±2.09*
Cholesterol (mg/dl)	95.35±3.54	107.23±4.34*
HDL (mg/dl)	9.08±0.30	9.71±0.28
LDL (mg/dl)	71.82±2.26	83.96±3.17*
Triglyceride (mg/dl)	47.60±3.98	54.12±3.96
Calorie intake (kcal/day)	35.82±4.21	54.35±5.78*

Data was presented as mean ± SEM. *, $p < 0.05$ compared with ND; n = 16/group; ND = normal-diet fed rats, HF = high-fat-diet fed rats; HOMA = Homeostasis Model Assessment; HDL= high-density lipoprotein; LDL = low-density lipoprotein

Table 3.2 Effect of estrogen deprivation on metabolic changes in obese-insulin resistant condition.

Parameters	Groups			
	NDS	**NDO**	**HFS**	**HFO**
Body weight (g)	279.55±3.90	304.21±4.92*	325.56±5.33*,†	391.19±10.79*,†,‡
Visceral fat (g)	6.82±1.42	12.29±1.53*	25.98±1.30*,†	33.71±2.25*,†,‡
Uterus weight (g)	0.45±0.06	0.15±0.04*	0.38±0.03	0.13±0.02‡
Plasma glucose (mg/dl)	133.48±2.66	136.59±4.75	141.87±4.12	159.58±6.84*,†,‡
Plasma insulin (ng/ml)	0.99± 0.12	2.38±0.31*	2.44±0.38*	3.46±0.32*,†,‡
HOMA index	8.73±1.02	19.13±1.80*	17.47±3.69*	29.49±3.77*,†,‡
Plasma glucose AUC (AUCg)(mg/dl×min×10^4)	1.85±0.08	2.16±0.07*	2.26±0.08*,†	2.53±0.10*,†,‡
Cholesterol (mg/dl)	77.33±4.64	95.59±7.23	106.53±6.80*	117.57±10.50*
HDL (mg/dl)	7.72±0.21	8.34±0.25	8.12±0.39	8.56±0.31
LDL (mg/dl)	67.80± 4.82	76.36± 3.62	84.63± 8.31*	85.74± 5.82*
Triglyceride (mg/dl)	70.99±5.31	72.41±4.64	72.84±6.62	91.07±3.92*,†,‡
Calorie intake (kcal/day)	54.04± 2.06	60.83± 0.66*	60.35± 1.46*	66.71± 1.57*,†,‡
Estradiol level (pg/ml)	112.37±17.19	31.55±3.92*	48.49±2.98*,†	23.97±2.05*,‡
Serum MDA (µM)	3.51±0.25	4.26±0.20*	4.58±0.22*	5.22±0.24*,†,‡

Data was presented as mean ± SEM. *, $p < 0.05$ compared with NDS, †, $p < 0.05$ compared with NDO, ‡, $p < 0.05$ compared with HFS; n = 8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet; HOMA = Homeostasis Model Assessment; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MDA = Malondialdehyde

3.2 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

3.2.1 Effect of estrogen deprivation on hippocampal ROS production and cortical ROS production in the obese-insulin resistant condition.

Previous studies found that the increased of brain ROS production was the underlying mechanism of learning and memory impairment (161, 162). Therefore, this study determined both hippocampal ROS production and cortical ROS production. The result showed that the NDO, HFS and HFO rats had the result of increased hippocampal ROS production only when compared with the NDS rats ($p<0.05$, Figure 3.1A). However, cortical ROS production was not significantly different among groups (Fig 3.1B). These data suggested that estrogen deprivation and obese-insulin resistant condition increased hippocampal reactive oxygen species production. Moreover, estrogen deprivation did not aggravate these impairments under obese conditions.

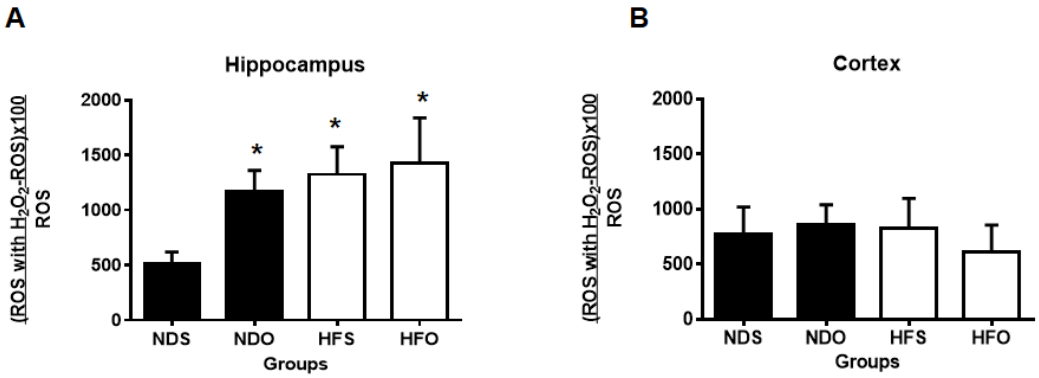

Fig 3.1 The effect of estrogen deprivation on the hippocampal ROS production and cortical ROS production in obese-insulin resistant condition. Hippocampal ROS productions of the NDS, NDO, HFS and HFO rats (**A**) and cortical ROS production of the NDS, NDO, HFS and HFO rats (**B**); *, $p<0.05$ compared with NDS; n=8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet; ROS = reactive oxygen species

3.2.2 Effect of estrogen deprivation on hippocampal apoptosis and cortical apoptosis in obese-insulin resistant condition.

Several studies demonstrated that the underlying mechanism of learning and memory impairment was brain apoptosis (163, 164). Therefore, this study determined the pro-apoptotic protein expression (Bax), anti-apoptotic protein expression (Bcl-2) and Bax/Bcl-2 ratio in both hippocampus and cortex. The result showed that Bax protein expression in both hippocampus and cortex were not significantly different among groups (Fig. 3.2A and Figure 3.2D). Interestingly, the NDO, HFS, and HFO rats had decreased hippocampal Bcl-2 protein expression and increased Bax/Bcl-2 ratio compared with the NDS rats ($p<0.05$, Figure 3.2B and Figure3.2C). However, the cortical Bcl-2 protein expression and Bax/Bcl-2 ratio were not significantly different among groups (Fig. 3.2E and Figure 3.2F). It can be concluded that the estrogen deprivation and obese-insulin resistant condition caused the apoptosis in the hippocampus. Moreover, estrogen deprivation did not aggravate these impairments under the obese conditions.

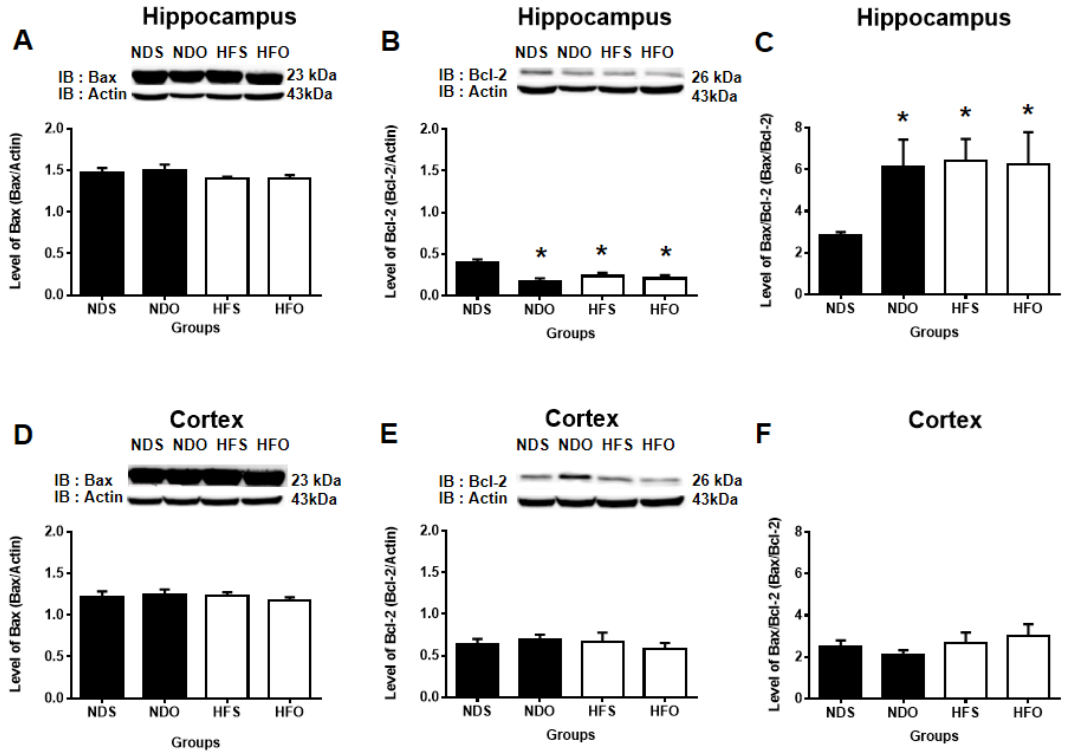

Fig 3.2 The effect of estrogen deprivation on hippocampal and cortical apoptosis in obese-insulin resistant condition. Representative blots of protein expression of Bax in hippocampus from the NDS, NDO, HFS and HFO rats (**A**), representative blots of protein expression of Bcl-2 in hippocampus from the NDS, NDO, HFS and HFO rats (**B**), Bax/Bcl-2 ratio in hippocampus from the NDS, NDO, HFS and HFO rats (**C**), representative blots of protein expression of Bax in cortex from the NDS, NDO, HFS and HFO rats (**D**), representative blots of protein expression of Bcl-2 in cortex from the NDS, NDO, HFS and HFO rats (**E**) and Bax/Bcl-2 ratio in cortex from the NDS, NDO, HFS and HFO rats (**F**); *, $p<0.05$ compared with NDS; n=8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet

3.2.3 Effect of estrogen deprivation on hippocampal estradiol level and cortical estradiol level in obese-insulin resistant condition.

Previous studies demonstrated that estradiol had the effect on learning and memory (140, 165). Therefore, our study measured estradiol level in both hippocampus and cortex. The result showed that the hippocampal estradiol levels in the NDO, HFS and HFO rats were significantly decreased when compared with NDS rats ($p<0.05$, Fig 3.3A). However, the cortical estradiol levels were not significantly different among groups (Fig 3.3B). The result suggested that the estrogen deprivation and obese-insulin resistant condition decreased hippocampal estradiol level. Moreover, estrogen deprivation did not alter cortical estradiol level under obese conditions.

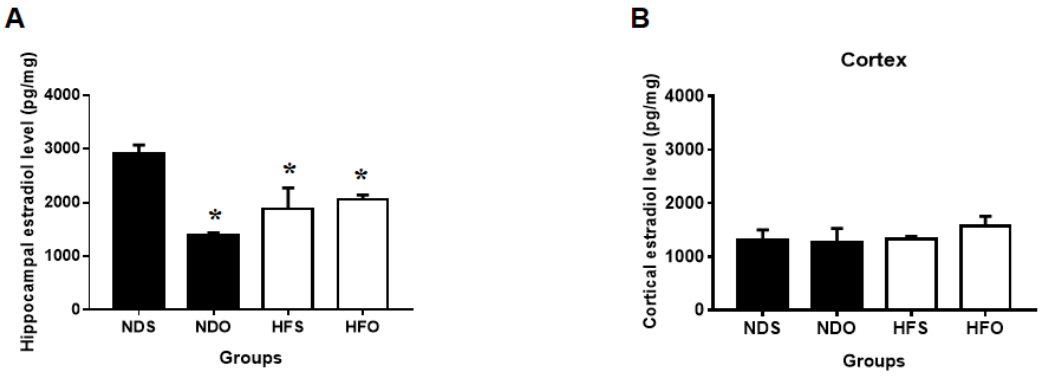

Fig 3.3 The effect of estrogen deprivation on hippocampal estradiol level and cortical estradiol level in obese-insulin resistant condition. Hippocampal estradiol level of the NDS, NDO, HFS and HFO rats (**A**) and cortical estradiol level of the NDS, NDO, HFS and HFO rats (**B)**; *, $p<0.05$ from NDS; n=8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet

3.2.4 Effect of estrogen deprivation on the hippocampal synaptic plasticity in obese-insulin resistant condition.

Several studies proved that the hippocampal synaptic plasticity had an association with learning and memory (166, 167). Therefore, the present study determined the long-term potentiation (LTP) from the hippocampal slice. The NDO, HFS and HFO rats had impaired hippocampal synaptic plasticity resulting in a significant decrease in the degree of electrical-mediated LTP and percentage increment of fEPSP slope when compared with the NDS rats, respectively (Figure 3.4A and Figure 3.4B). It can be summarized that the estrogen deprivation and obese-insulin resistant condition impaired the hippocampal synaptic plasticity. Nevertheless, the estrogen deprivation did not aggravate these impairments under obese conditions.

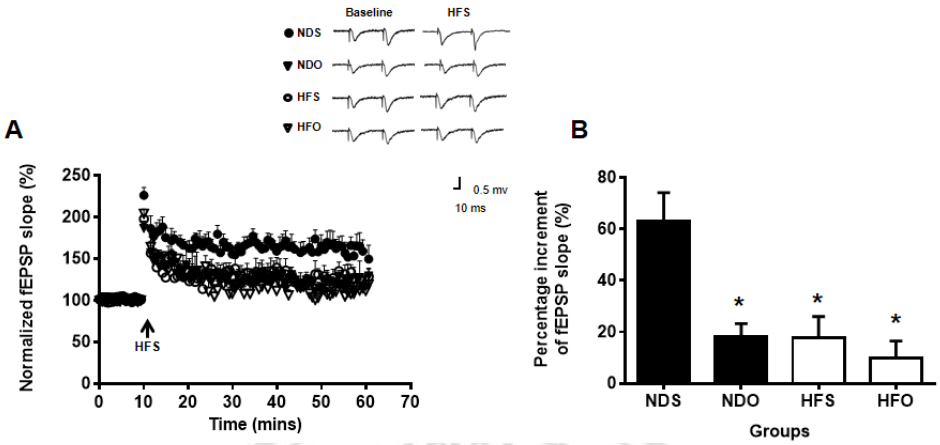

Fig 3.4 The effect of estrogen deprivation on synaptic plasticity (electrical-induced LTP) in obese-insulin resistant condition. The degree of electrical-mediated LTP observed from hippocampal slices of NDS, NDO, HFS and HFO rats (**A**) and the percentage increment of fEPSP slope from the NDS, NDO, HFS and HFO rats (**B**); *, $p<0.05$ compared with NDS, (n=7-8 independent slices, n=8 animals/group) ; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet; Hfs = high frequency stimulation; fEPSPs = field excitatory post synaptic potential

3.3 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

3.3.1 Effect of estrogen deprivation on hippocampal-dependent memory and hippocampal-independent memory in obese-insulin resistant condition.

After 13 weeks of ND or HF consumption, the locomotor activity by the open-field test was determined before the cognitive test, the speed and distance showed no significant difference between groups. These results suggested that each rat had no difference in locomotor activity (Figure 3.5A and Figure 3.5B).

The MWM test was used to determine the hippocampal-dependent learning and memory. In the acquisition test, the HF rats had significantly higher time to reach platform and distance to platform when compared with the ND rats from day 3 to day 5 (Figure 3.6A and Figure 3.6B). For probe test in day 6, the HF rats had reduced time in

target quadrant and distance in target quadrant significantly when compared with the ND rats (Figure 3.7A and Figure 3.7B). In addition, there was no difference in the swimming speed between each rat suggesting that there was no other factor affected the rat's swimming (Figure 3.6C). This result suggested that obese-insulin resistant condition impaired the hippocampal-dependent learning and memory.

After 13 weeks of ND or HF consumption, the NOR test was used to determine the hippocampal-independent learning and memory. It showed no significant difference in the percent exploration time as well as index preference when compared between ND and HF groups (Figure 3.8A and Figure 3.8B). This result indicated that obese-insulin resistant condition did not impair the hippocampal-independent learning and memory.

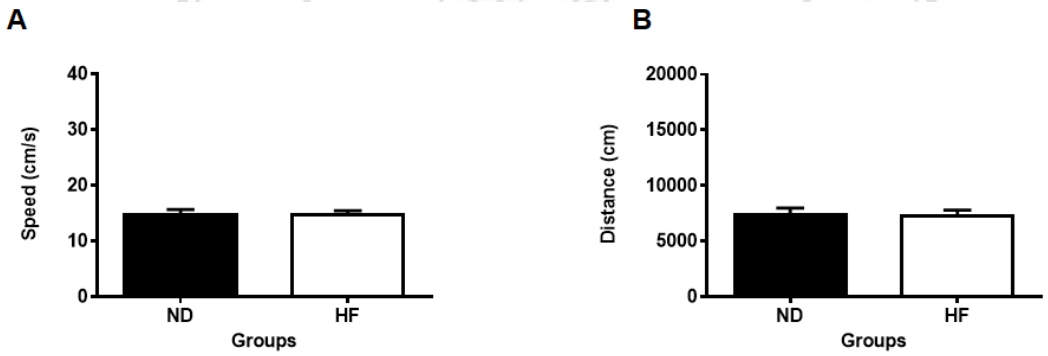

Fig 3.5 The effect of obese-insulin resistant condition on locomotor activity. Speed in open-field test of the ND and HF rats (**A**) and distance in open-field test of the ND and HF rats (**B**); n=16/group; ND = normal-diet fed rats; HF = high-fat-diet fed rats

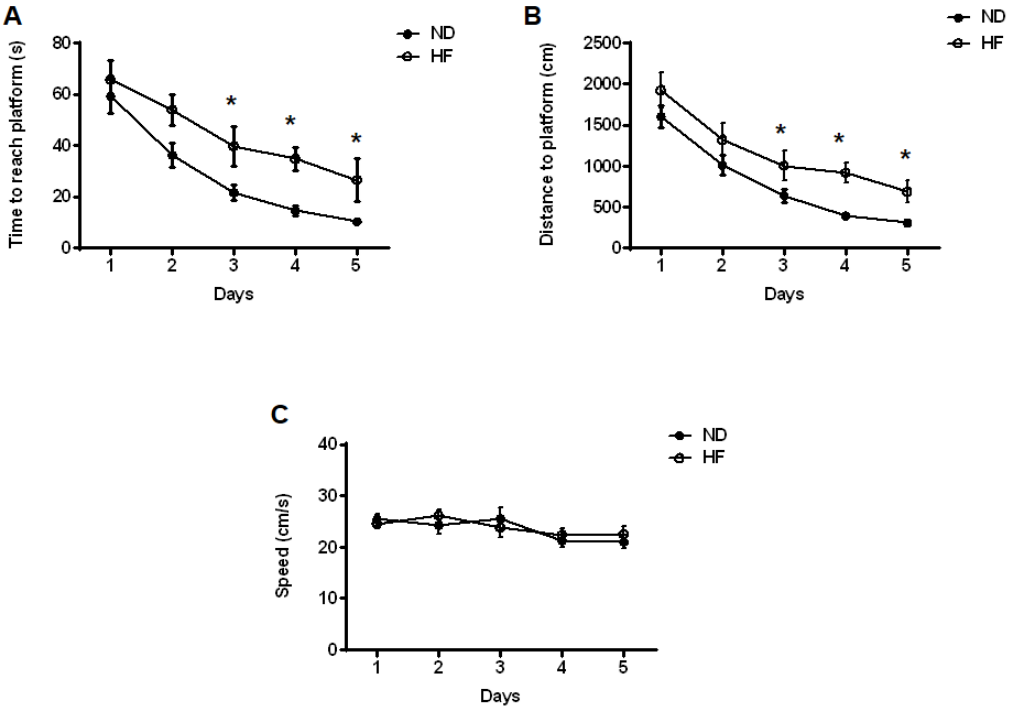

Fig 3.6 The effect of obese-insulin resistant condition on the hippocampal-dependent learning and memory (acquisition test). Time to reach platform in acquisition test from the ND and HF rats (**A**), distance to platform in acquisition test from the ND and HF rats (**B**) and speed in acquisition test of the ND and HF rats (**C**); *, $p<0.05$ compared with ND; n=16/group; ND = normal-diet fed rats; HF = high-fat-diet fed rats

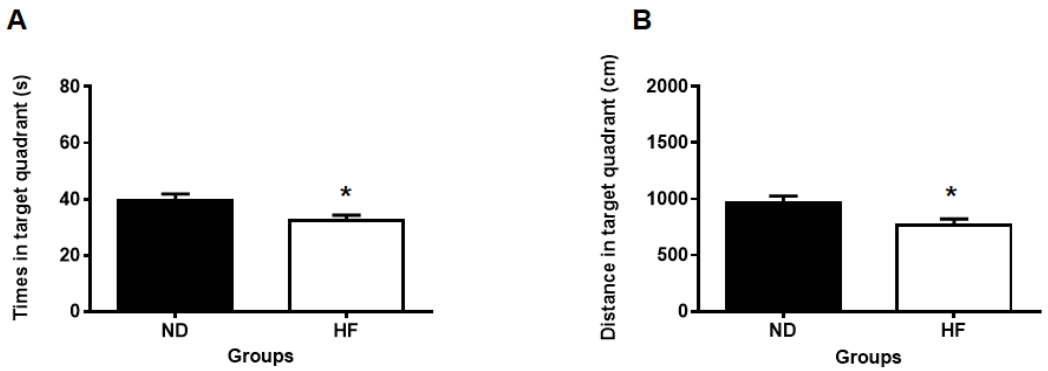

Fig 3.7 The effect of obese-insulin resistant condition on the hippocampal-dependent learning and memory (probe test). Time in target quadrant in probe test from the ND and HF rats (**A**) and distance in target quadrant in probe test from the ND and HF rats (**B**); *, $p<0.05$ compared with ND; n=16/group; ND = normal-diet fed rats; HF = high-fat-diet fed rats

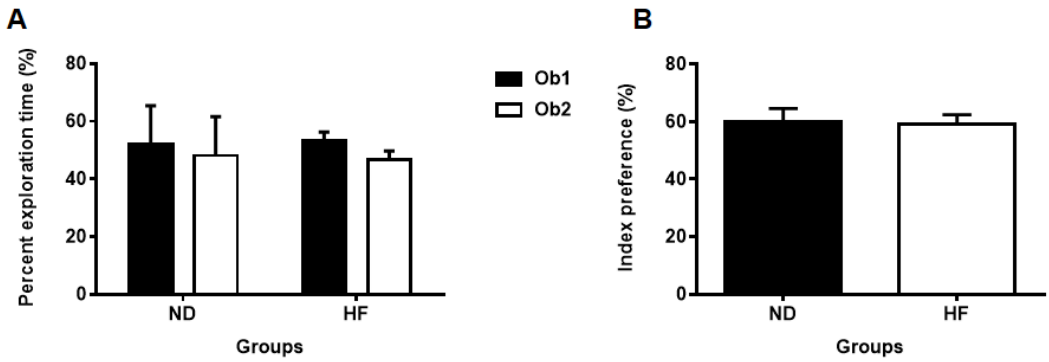

Fig 3.8 The effect of obese-insulin resistant condition on the hippocampal-independent learning and memory. Percent exploration time from the ND and HF rats (**A**) and index preference from the ND and HF rats (**B**); n=16/group; ND = normal diet; HF = normal diet; Ob1 = object 1; Ob 2 = object2

At week 20, the locomotor activity did not showed any significant difference between NDS, NDO, HFS and HFO rats, which was determined by no significant difference in speed and distance (Figure 3.9A and Figure 3.9B).

In the acquisition test of MWM, the NDO, HFS and HFO rats had significant higher time to reach platform and distance to platform when compared with the NDS rats from day 3 to day 5 (Figure 3.10A and Figure 3.10B). For the probe test of MWM in day 6, the NDO, HFS and HFO rats had significant decrease in the time and distance in target quadrant when compared with the NDS rats (Figure 3.11A and Figure 3.11B). In addition, there was no difference in the swimming speed among rats (Figure 3.10C). All of these findings indicated that both estrogen deprivation and obesity impaired hippocampal-dependent memory. Moreover, estrogen deprivation did not aggravate these cognitive impairments under obese conditions.

For the NOR test, it showed no significant different in the percent exploration time as well as index preference when compared among groups (Figure 3.12A and Figure 3.12B). This result indicated that estrogen deprivation and obese-insulin resistant condition did not impair the hippocampal-independent learning and memory.

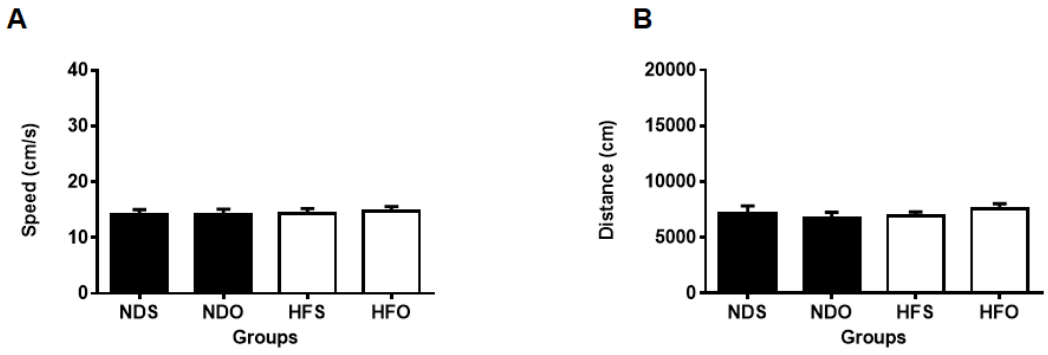

Fig 3.9 The effect of estrogen deprivation on locomotor activity in obese-insulin resistant condition. Speed in open-field test from the NDS, NDO, HFS and HFO rats (**A**) and distance in open-field test from the NDS, NDO, HFS and HFO rats (**B**); n=8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed

with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet

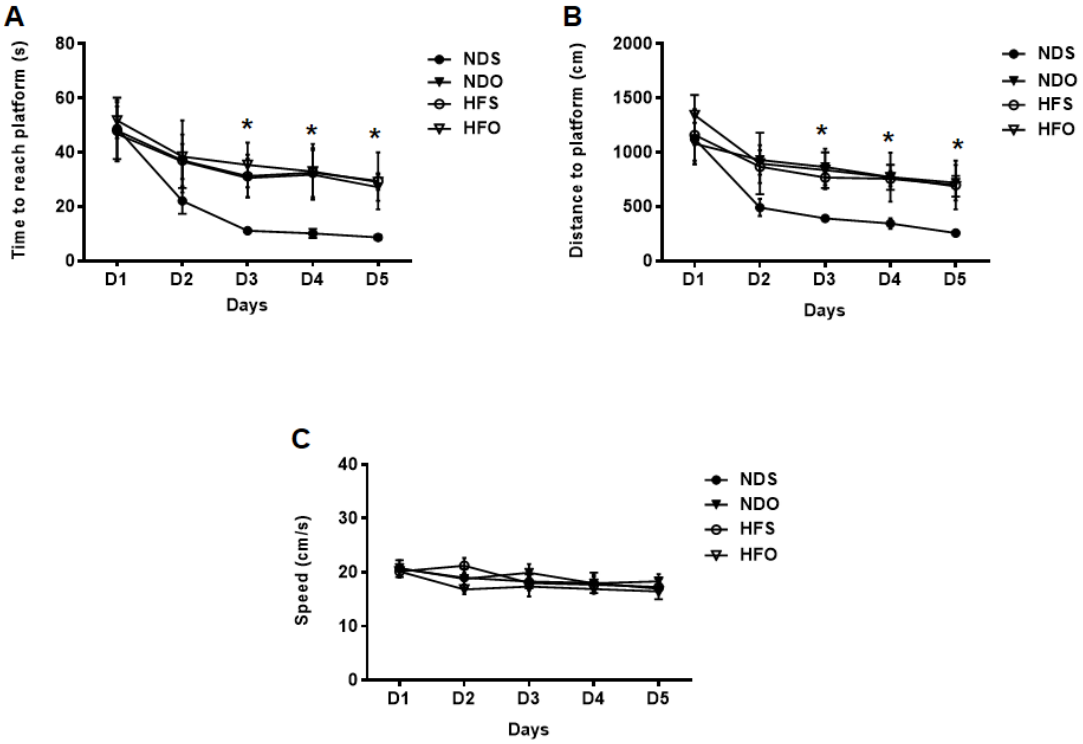

Fig 3.10 The effect of estrogen deprivation on the hippocampal-dependent learning and memory (acquisition test) in obese-insulin resistant condition. Time to reach platform in acquisition test from the NDS, NDO, HFS and HFO rats (**A**), distance to platform in acquisition test from the NDS, NDO, HFS and HFO rats (**B**) and speed in acquisition test from the NDS, NDO, HFS and HFO rats (**C**); *, $p<0.05$ compared with NDS; n=8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet

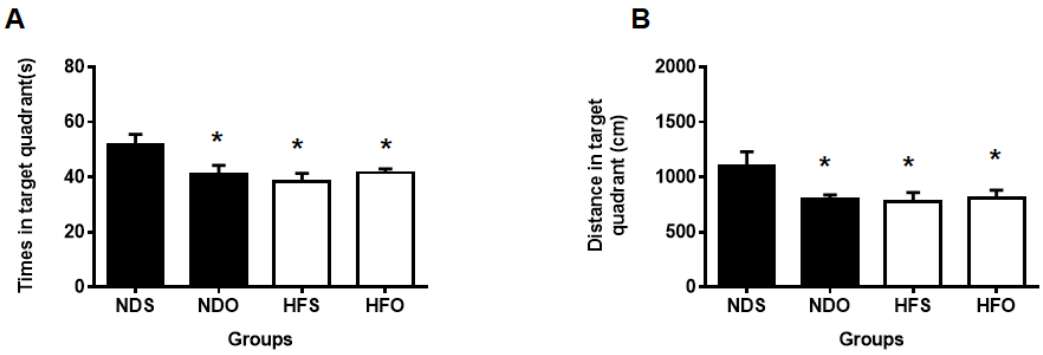

Fig 3.11 The effect of estrogen deprivation on the hippocampal-dependent learning and memory (probe test) in obese-insulin resistant condition. Time in target quadrant in probe test from the NDS, NDO, HFS and HFO rats (**A**) and distance in target quadrant in probe test from the NDS, NDO, HFS and HFO rats (**B**); *, $p<0.05$ compared with NDS; n=8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet

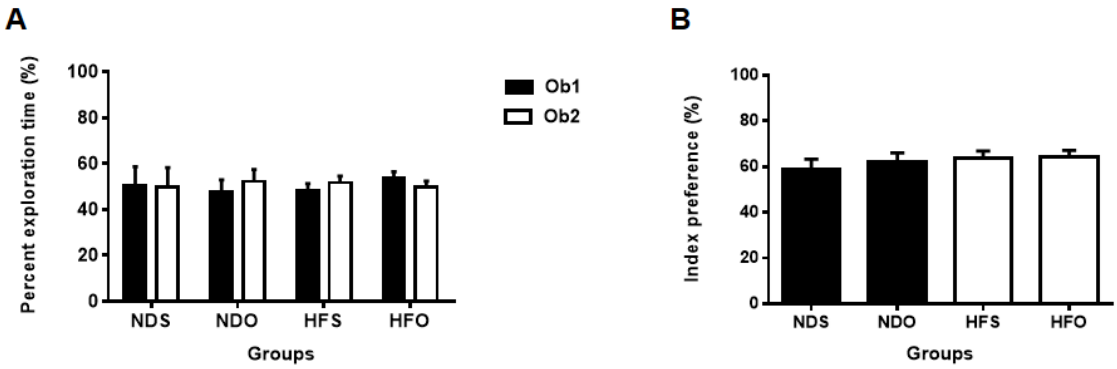

Fig 3.12 The effect of estrogen deprivation on the hippocampal-independent learning and memory in obese-insulin resistant condition. Percent exploration time from the NDS, NDO, HFS and HFO rats (**A**) and index preference from the NDS, NDO, HFS and HFO rats (**B**); n=8/group; NDS = sham-operated female rats fed with normal diet; NDO = ovariectomized female rats fed with normal diet; HFS = sham-operated females rats fed with high-fat diet; HFO = ovariectomized female rats fed with high-fat diet; Ob1 = object 1; Ob 2 = object2