APPENDIX

APPENDIX A

Reagent for determination of brain reactive oxygen species

1. Solution for isolated brain tissue prepatrion

MSE

D-mannitol	225 mM
Sucrose	75 mM
EGTA	1 mM
HEPES	5 mM
BSA	1 mg/ml

Adjusted to 7.4 with NaOH (strong base) or HCl (strong acid)

2. Solution for brain mitochondrial respiratory buffer

KCl	150 mM
HEPES	5 mM
$K_2HPO_4.3H2O$	2 mM
$C_5H_8NNaO_4.xH_2O$	5 mM
$CH_3COCOONa$	5 mM

Adjusted to 7.2 with NaOH (strong base) or HCl (strong acid)

3. DCFH-DA preparation (2 µM DCF-DA)

DCFH-DA (stock) 1 mM

Soluble in brain mitochondrial respiratory buffer

APPENDIX B

Reagent for western blotting

1. Reagents for western blotting

Slice lysis buffer

EDTA	1 mM
EGTA	1 mM
NP-40	1% v/v
Triton-X	1% v/v
1X Protease inhibitor	

Non-ionizing lysis buffer

NaCl	100 mM
EDTA	25 mM
Tris	10 mM
Triton X-100	1% v/v
NP-40	1% v/v
1X Protease inhibitor	

Loading buffer

Tris	0.3 M
SDS	0.5 M
Glycerol	5%
Bromophenol blue	250 µl
2-mercaptoethanol	5 µ

TBS (10X)

Tris	200 mM
NaCl	1.37 M
ddH2O	
Adjusted pH to 7.4	

TBST (1X)

10XTBS	100 ml
ddH_2O	900 ml
Tween-20	1 ml

10xRunning buffer

Tris, pH 8.3	250 mM
Glycine	2 M
SDS	35mM
ddH_2O	

1X Running buffer

10X Running buffer	100 ml
ddH_2O	900 ml

10xTransfer buffer

Tris, pH 8.3	250 M
Glycine	2 M

1X Transfer buffer

10X Transfer buffer	100 ml
ddH_2O	700 ml
Methanol	200 ml

Antibody

Primary antibodies

Anti-actin

Anti- Bax

Anti-Bcl-2

Secondary antibodies

Anti-rabbit and anti-mouse IgG conjugated with horseradish peroxidase

Blueeye Prestained Protein Ladder

ECL

2. Preparation of gel

1.5M Tris pH 8.8 (200 ml)

Tris	1.5 M
Deionized water	160 ml
pH 8.8	
Add deionized water to	200 ml
Filter through membrane 0.2 um	
Store at 4^{o}C (light sensitive)	

0.5 M Tris pH 6.8 (500 ml)

Tris	30.25 g
SDS	2.0 g
ddH_2O	300 ml
Adjusted to 6.8	

0.5M Tris pH 6.8 (200 ml)

Tris	12.1 g
Deionized water	160 ml
pH 6.8	
Add deionized water to	200 ml
Filter through membrane 0.2 um	
Store at 4^{o}C (light sensitive)	

10% Acrylamide/Bisacrylamide

30% acrylamide/0.8%	3.5 ml
1.5M Tris pH 8.8	2.5 ml
Deionized water	2.85 ml
10% APS*	100 ul
TEMED*	10 ul

4% Stacking gel

Deionized water	3.05 ml
0.5M Tris pH 6.8	1.25 ml
30% acrylamide/0.8% Bis	650 ul
10% SDS	50 ul

10% APS*	25 ul
TEMED*	5 ul

*should be fresh prepared

3. Gel preparation process

1. Clean glasses for loading gel with 70%Ethanol
2. Load the 10% Acrylamide
3. Fill the space above the gel with propanol, then leave it for 30 minutes
4. Rinsed the gel with distill water and replaced with 4% Stacking gel, leave it for 30 min

4. Electrophoresis and immunoblotting process

1. Move gels in to electrophoresis chamber
2. Loading samples
   a. 7 µl for molecular weight marker
   b. 20 µl for protein sample
3. Run gel at 120 Volts, 1.20 hours until the protein arrived the end of the gel
4. Transfer gel to nitrocellulose membrane at 70 Volts, 2 hours
5. Wash the membrane with deionized water for 5 minutes
6. Block membrane with 5%Milk or 5%BSA in TBST 1 hour on an orbital shaker (room temperature)
7. Add primary antibodies in TBST incubate overnight at 4^{o}C. Protein levels: Bax (1:200), Bcl-2 (1:1000)
8. Wash the membrane with 10 ml TBST 5 minutes, 5 times
9. Add secondary antibodies 1:2000 in TBST; Anti-rabbit conjugated with horseradish peroxidase for 1 hour on an orbital shaker
10. Wash the membrane 5 minutes 5 times

11. ECL exposure to ChemiDoc Touch Imaging system (Bio-Rad Laboratories, CA, USA)

APPENDIX C

Reagents for extracellular recording of hippocampal slices

1. Reagent

High sucrose aCSF containing

NaCl	85	mM
KCl	2.5	mM
$MgSO_4$	4	mM
$CaCl_2$	0.5	mM
NaH_2PO_4	1.25	mM
$NaHCO_3$	25	mM
glucose	25	mM
sucrose	75	mM
kynurenic acid	2	mM
ascorbate	0.5	mM

saturated with 95%O_2/5%CO_2 (pH 7.4)

Standard aCSF containing

NaCl	119	mM
KCl	2.5	mM
$MgSO_4$	1.3	mM
$CaCl_2$	2.5	mM
NaH_2PO_4	1.0	mM
$NaHCO_3$	26	mM
glucose	10	mM

saturated with 95%O_2/5%CO_2 (pH 7.4**)**

2. Equipment

Patch clamp setup

1. anti-vibration isolate table

2. dissecting microscope
3. Axopatch 200B amplifier (Axon Instruments)
4. manipulators (Newport)
5. computer for data acquisition (Digidata, Axon)
6. oscilloscope
7. Vibratome (Vibratome Company, St. Louis)
8. Sutter electrode puller

APPENDIX D

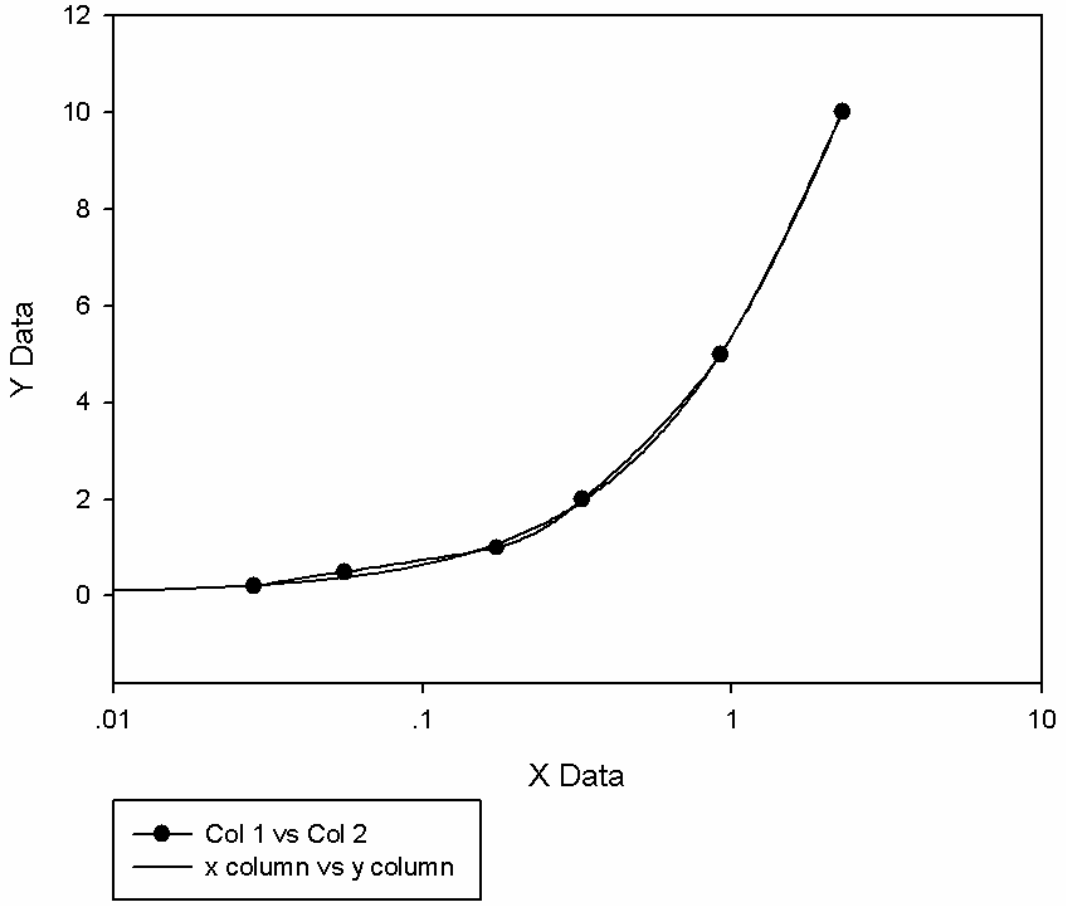

Figure D.1 Standard curve for determination of insulin concentration based on Sandwich ELISA method

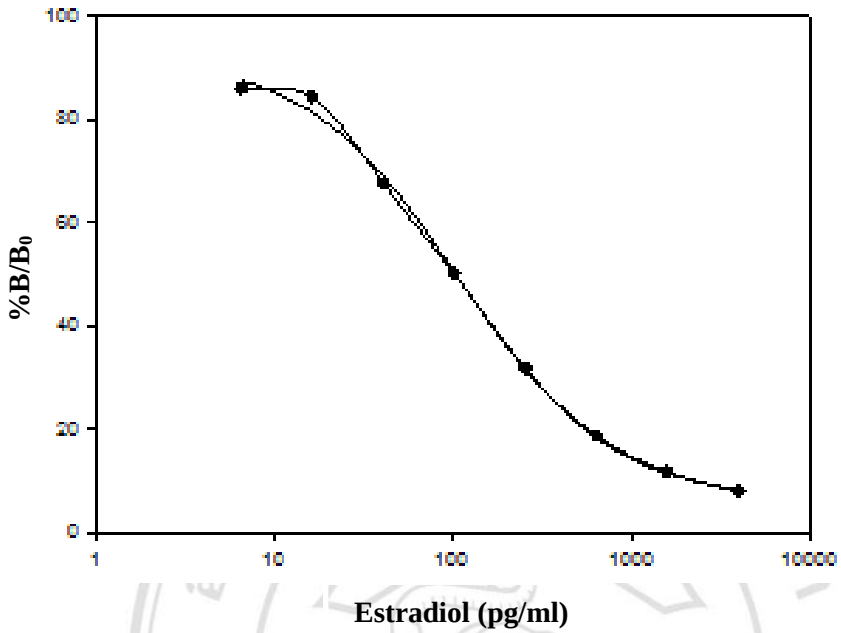

Figure D.2 Standard curve for determination of serum estrogen based on Sandwich ELISA method

APPENDIX E

Determination of SERUM MDA level

1. Reagent

Phosphate buffer

$NaH_2PO_42H_2O$	712	mg
H_2PO_4	68	l
ddH_2O	1000	ml

Phosphoric acid

H_3PO_4	30	ml
ddH_2O	970	ml

10%TCA in 50 ppm BHT

TCA	100	g
BHT	50	mg
ddH_2O	1000	ml

50 ppm BHT in methanol

BHT	50	mg
Methanol	1000	ml

0.6% TBA in ddH_2O

TBA	6	g
ddH_2O	1000	ml

MDA standard

MDA stock solution	100	ml
ddH_2O	9900	ml

2. Mobile phase for MDA determination

1 M $K_2HPO_4.3H_2O$

$K_2HPO_4.3H_2O$	780	g
ddH_2O	5000	ml

1 M KH_2PO_4

KH_2PO_4	1141.15	g
ddH_2O	5000	ml

100mM KPB

KH_2PO_4	192.5	ml
$K_2HPO_4.3H_2O$	307.5	ml
ddH_2O	4500	ml

50 mM KPB

KPB	3250	ml
Methanol	1750	ml

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Peer reviewed abstract

Palee S, Minta W, **Mantor D**, Sutham W, Pratchayasakul W, Chattipakorn SC, Chattipakorn N. Estrogen deprivation aggravates cardiometabolic dysfunction and intracellular calcium dyshomeostasis in obese-insulin eesistance rats. J Am Coll Cardio 2017;69(11, Supplement):681. (Impact Factor=17.759)

Conference abstract

Mantor D, Pratchayasakul W, Minta W, Sutham W, Palee S, Sripetchwandee J, Kredphoo S, Jaiwongkum T, Sriwichaiin S, Krintratun W, Chattipakorn N, Chattipakorn SC. The Effects of Estrogen Deprivation on Hippocampal Synaptic Plasticity and Cognitive Function in Obese-Insulin Resistant Female Rats. Abstract to the 45 th Annual Meeting of the Physiological Society of Thailand, Khon Kaen, Thailand, 2017;33.

Minta W, Palee S, **Mantor D**, Sutham W, Pratchayasakul W, Kumfu S, Chattipakorn SC, Chattipakorn N. Estrogen Deprivation Aggravates Cardiometabolic Dysfunction in Obese-Insulin Resistant Rats. Abstract to the 45 th Annual Meeting of the Physiological Society of Thailand, Khon Kaen, Thailand, 2017;32.

Sutham W, Sripetchwandee J, Minta W, **Mantor D**, Pattakuhar S, Palee S , Chattipakorn N, Chattipakorn SC. Estrogen Deprivation on Skeletal Muscle Function in Obese-Insulin Resistant Rats. Abstract to the 45 th Annual Meeting of the Physiological Society of Thailand, Khon Kaen, Thailand**,** 2017;34.

Presentations at national meeting

December 2017 45 th Annual Meeting of the Physiological Society of Thailand, Khon Kaen, Thailand