

ผลของการให้โกรทฮอร์โมนระยะสั้นต่อระดับเลปตินในพลาสมา ในหนูแรทปกติ และในหนูแรทที่ถูก
เหนี่ยวนำให้อ้วนจากอาหาร



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บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

สาขาวิชาสารวิทยาการสัตว์ ภาควิชาสารวิทยา

คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2560

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECTS OF SHORT-TERM GROWTH HORMONE ADMINISTRATION ON PLASMA LEPTIN
LEVELS IN NORMAL AND DIET-INDUCED OBESITY RATS



A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Animal Physiology

Department of Veterinary Physiology

Faculty of Veterinary Science

Chulalongkorn University

Academic Year 2017

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Thesis Title EFFECTS OF SHORT-TERM GROWTH HORMONE
ADMINISTRATION ON PLASMA LEPTIN LEVELS IN
NORMAL AND DIET-INDUCED OBESITY RATS

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สุธาริณี ลิขิตนุกูล : ผลของการให้โกรทฮอร์โมนระยะสั้นต่อระดับเลปตินในพลาสมา ในหนูแรพปกติ และในหนูแรพที่ถูกเหนี่ยวนำให้อ้วนจากอาหาร (EFFECTS OF SHORT-TERM GROWTH HORMONE ADMINISTRATION ON PLASMA LEPTIN LEVELS IN NORMAL AND DIET-INDUCED OBESITY RATS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. น.สพ. ดร.สัมพันธ์ ธรรมเจริญ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. สพ.ญ. ดร.สฤณี กลั่นทกานนท์ ทองทรง, หน้า.

ภาวะอ้วนมีความสัมพันธ์กับการเปลี่ยนแปลงของระดับฮอร์โมนในร่างกาย เช่น โกรทฮอร์โมน ฮอรโมนอินซูลิน และฮอรโมนเลปติน เนื่องจากโกรทฮอร์โมนมีกลไกการออกฤทธิ์ได้หลายทาง และมีความเกี่ยวข้องกับภาวะอ้วนทั้งในด้านพยาธิสรีรวิทยาและการให้โกรทฮอร์โมนเพื่อรักษาภาวะอ้วน ซึ่งอาจส่งผลต่อระดับของฮอรโมนเลปติน การศึกษาในดุษฎีนิพนธ์นี้จึงมีวัตถุประสงค์ประการแรกเพื่อแสดงผลของการให้โกรทฮอร์โมนระยะสั้นต่อระดับเลปตินในพลาสมาใน 3 รูปแบบ ของการหลังเลปติน ได้แก่ ระดับพื้นฐาน ระดับถูกกระตุ้นด้วยการกินอาหาร และระดับอดอาหาร ในหนูที่มีปริมาณเนื้อเยื่อไขมันที่ต่างกัน และวัตถุประสงค์ประการที่สอง คือ ต้องการศึกษาผลของการให้โกรทฮอร์โมนระยะสั้นต่อความไวของอินซูลิน และระดับของเนื้อเยื่อไขมันในร่างกายในหนูกลุ่มควบคุม กลุ่มที่ต้านทานต่อการเหนี่ยวนำให้อ้วนจากอาหารพลังงานสูง และกลุ่มที่ถูกเหนี่ยวนำให้อ้วนจากอาหารที่มีพลังงานสูง ในการนี้จึงได้แบ่งหนูออกเป็น 2 กลุ่ม คือกลุ่มควบคุม และกลุ่มที่ให้อาหารพลังงานสูง เป็นระยะเวลา 6 สัปดาห์ หลังจากนั้น จึงทำการคัดเลือกหนูที่ต้านทานต่อการเหนี่ยวนำให้อ้วน และหนูที่ถูกเหนี่ยวนำให้อ้วนจากอาหารที่มีพลังงานสูง เพื่อทำการศึกษาผลของการให้โกรทฮอร์โมน ขนาด 1 มก./กก. วันละ 2 ครั้ง เปรียบเทียบกับกลุ่มที่ให้เฉพาะสารละลายน้ำเกลือ พบว่า ภายหลังจากการให้โกรทฮอร์โมน เป็นเวลา 32 ชั่วโมง ระดับเลปตินในพลาสมาในหนูแรพที่ถูกเหนี่ยวนำให้อ้วนจากอาหารพลังงานสูงมีค่าลดลง สำหรับสภาวะที่ถูกกระตุ้นด้วยการกินอาหาร พบว่าโกรทฮอร์โมนไม่มีผลต่อระดับเลปตินในพลาสมาในหนูทุกกลุ่ม เมื่อเปรียบเทียบกับกลุ่มที่ได้รับสารละลายน้ำเกลือ และในสภาวะอดอาหาร การให้โกรทฮอร์โมนระยะสั้น ส่งผลต่อระดับเลปติน โดยโกรทฮอร์โมนยับยั้งผลของการอดอาหารต่อระดับเลปตินในพลาสมา ในหนูแรพทั้งกลุ่มควบคุมและกลุ่มที่มีความต้านทานต่อการเหนี่ยวนำให้อ้วนจากอาหารพลังงานสูง ภาวะดื้อต่ออินซูลินเกิดภายหลังจากการได้รับโกรทฮอร์โมนในหนูกลุ่มที่มีความต้านทานต่อการเหนี่ยวนำให้อ้วนจากอาหารพลังงานสูง โดยมีระดับอินซูลินในพลาสมาที่สูงขึ้นร่วมกับดัชนีชี้วัดความดื้อต่ออินซูลินที่มากขึ้น ดังนั้นสรุปได้ว่า การให้โกรทฮอร์โมนระยะสั้น ส่งผลต่อระดับเลปตินในพลาสมาโดยขึ้นกับปริมาณเนื้อเยื่อไขมันในร่างกายและระดับพลังงานของร่างกาย ผลการศึกษาในครั้งนี้แสดงให้เห็นถึงการออกฤทธิ์ของโกรทฮอร์โมนต่อระดับเลปตินในพลาสมา รวมทั้งอาจเสนอให้มีการใช้โกรทฮอร์โมนระยะสั้นเพื่อเป็นการรักษาร่วมในภาวะอ้วน

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ลายมือชื่อ อ.ที่ปรึกษาร่วม

5575407031 : MAJOR ANIMAL PHYSIOLOGY

KEYWORDS: ADIPOSE TISSUE, GROWTH HORMONE, LEPTIN, OBESITY

SUTHARINEE LIKITNUKUL: EFFECTS OF SHORT-TERM GROWTH HORMONE ADMINISTRATION ON PLASMA LEPTIN LEVELS IN NORMAL AND DIET-INDUCED OBESITY RATS. ADVISOR: ASSOC. PROF. SUMPUN THAMMACHAROEN, D.V.M.,M.Sc.,Ph.D., CO-ADVISOR: ASSOC. PROF. SARINEE KALANDAKANOND THONGSONG, D.V.M.,Ph.D., pp.

Obesity has related to the alteration of hormonal profiles, i.e. growth hormone (GH), insulin and leptin. Because of the pleiotropic mechanisms of GH, it plays important roles both in the pathophysiological mechanisms and the therapeutic hormonal supplementation, which probably affected plasma leptin. The present research aimed firstly to demonstrate the effect of short-term GH administration on plasma leptin in 3 conditions; basal, meal-induced and fasting condition, from the rats with different adipose tissue mass. Secondly, to investigate short-term effect of GH on insulin sensitivity and body adiposity in control, diet resistant (DR) and diet-induced obesity (DIO) rats. In this regard, the rats were divided to control and hypercaloric (HC) diet-feeding rats. After 6 weeks of feeding period, HC diet-feeding rats were selected for DR and DIO rats. Exogenous GH (1 mg/kg, twice daily) was injected and compared with saline-treated rats. Short-term GH treatment decreased plasma leptin only in DIO rats that significantly occurred at 32 h after the first GH injection. The leptin response probably depended on the higher body adiposity in DIO rats than that of control and DR rats. For meal-induced plasma leptin, short-term GH treatment had no effect in all rats. For fasting condition, GH treatment attenuated the effect of fasting on plasma leptin in control and DR rats, which had the lower adiposity than that of DIO rats. Insulin resistance (IR) was induced by short-term GH treatment, which demonstrated by the higher fasting insulin and increased surrogate indexes of insulin resistance in DR rats, including the homeostasis model IR and adipose tissue IR. Therefore, we conclude that the effect of short-term GH treatment on plasma leptin might depend on body adiposity and energy status. These findings reveal the evidence of short-term GH treatment on plasma leptin and may suggest the short-term GH treatment as adjunctive therapy in obese stage.

Department: Veterinary Physiology

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Field of Study: Animal Physiology

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Academic Year: 2017

Co-Advisor's Signature

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my kind advisor, Assoc. Prof. Dr. Sumpun Thammacharoen. It is my pleasure to be his student. I am deeply thankful to him for his guidance, encouragement, support and persistent advice given to me. He also motivates and inspires me to take up the career of a researcher/instructor.

I would also like to express my grateful thanks to my thesis co-advisor, Assoc. Prof. Dr. Sarinee Kalandakanond-Thongsong for her valuable suggestions, opinions and support throughout my study.

At the same time, my special thanks are extended to the Chairman, Asst. Prof. Dr. Suwanakiet Sawangkoon, and other committee members, namely Assoc. Prof. Dr. Sutthasinee Poonyachoti, Assoc. Prof. Dr. Gunnaporn Suriyaphol and Assoc. Prof. Dr. Punnee Nusuetrong for their valuable comments and recommendations in correcting my dissertation to be of better quality and presentation.

My appreciation and recognition go to all teachers in Animal Physiology Program, Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University for their kindness, cooperation and assistance rendered to me. Additionally, I would like to thank all staffs at Department of Physiology for their very useful help in providing all facilities for my study.

This dissertation could not be successful without the financial support from The 100th Anniversary Chulalongkorn University for Doctoral Scholarship. I am very grateful for the financial support by the graduate school, Chulalongkorn University for me to attend and make a poster presentation at the 95th Annual Meeting of the Physiological Society of Japan, Takamatsu, Japan, enabling me to earn practical and precious experience.

Additionally, I would like to mention with sympathy and thanks relating to all laboratory animals in my research. They sacrificed their valuable life for my experiments.

Finally, my graduation would not be achieved without the best supports, understanding and encouragement from members of my beloved family, Mr. Chingchai Likitnukul, Mrs. Orawan Likitnukul, Mr. Sittha Likitnukul and Mr. Piyapong Thanatrakul. They always give me the greatest wishes and willpower during my entire study. I dedicate this dissertation to my beloved family. Last but not least, I would also express thanks to my classmates for helping me in my lab work, which enables me to enjoy working and studying in Animal Physiology Program of the Department.

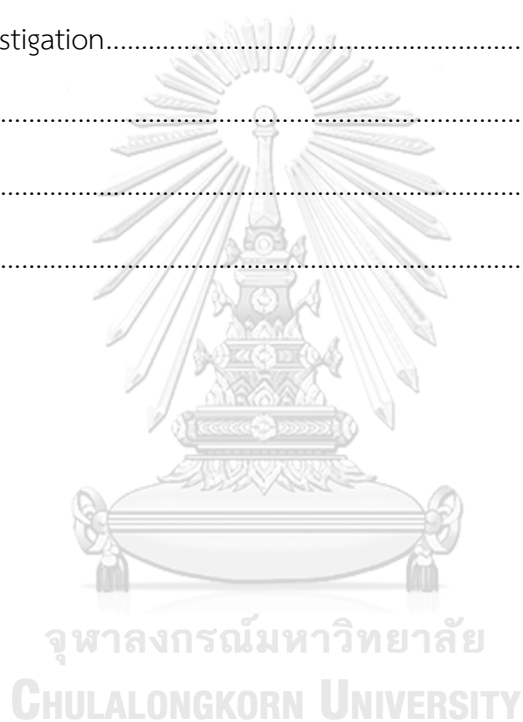
CONTENTS

	Page
THAI ABSTRACT	iv
ENGLISH ABSTRACT	v
ACKNOWLEDGEMENTS	vi
CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xiv
CHAPTER I INTRODUCTION	1
CHAPTER II LITERATURE REVIEW	5
A. Update of obesity problem and the initiation factors	5
B. Animal model of obesity research	6
C. Leptin synthesis and adipose tissue	9
D. The roles of leptin in physiology	12
E. Regulation of plasma leptin levels	14
F. Leptin in obesity	19
G. Growth hormone in obesity	22
H. Effects of growth hormone on leptin levels	25
I. Effects of growth hormone on plasma insulin and insulin sensitivity	26
CHAPTER III MATERIALS AND METHODS	29
1. Approvals:	29
2. Animals and animals procedures:	29
3. Diet preparation:	30

	Page
Study part 1: The effects of short-term GH administration on basal plasma leptin levels in control and DIO rats	31
1. Experimental procedures:	31
2. Analytical procedures for measurements of blood samples	34
Blood sample preparation	34
ELISA method	34
3. Data analysis:	36
Statistical analysis	36
Study part 2: The effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels and insulin function in control, DR and DIO rats	37
1. Experimental procedures:	37
2. Analytical procedures for measurements of blood samples	39
Blood sample preparation	39
ELISA method	40
The measurement of NEFA	40
3. Data analysis:	41
Calculation	41
Statistical analysis	42
CHAPTER IV RESULTS	44
Study part 1: The effects of short-term GH administration on plasma leptin levels in control and DIO rats	44
A. Characterization of DIO and DR rats	44

	Page
B. Effects of short-term GH administration on plasma leptin in control and DIO rats.....	46
C. Effects of short-term GH administration on plasma IGF-1 in control and DIO rats.....	48
D. Effects of short-term GH administration on food intake and energy intake in control and DIO rats.....	49
E. Body adiposity and liver of GH-treated control and DIO rats.....	51
Study part 2: The effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels and insulin function in control, DR and DIO rats	53
A. Characterization of DIO and DR rats.....	53
B. Effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels in control, DR and DIO rats	55
C. Effects of short-term GH administration on insulin function in control, DR and DIO rats.....	61
a. Effects of short-term GH administration on plasma insulin, glucose and NEFA	61
b. Effects of short-term GH administration on HOMA-IR and Adipo-IR.....	61
D. Effects of short-term GH administration on body adiposity and liver weight in control, DR and DIO rats	61
CHAPTER V DISCUSSION	65
Study part 1: The effects of short-term GH administration on plasma leptin levels in control and DIO rats	65

Study part 2: The effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels and insulin function in control, DR and DIO rats	67
CHAPTER VI SUMMARY.....	72
A. Conclusions.....	72
B. Implication.....	74
C. Further investigation.....	75
.....	76
REFERENCES	76
VITA.....	126



LIST OF TABLES

Table 1 Diet composition in this study.....	31
Table 2 The characteristic of control and DIO rats.	45
Table 3 Body adiposity and liver weight of control and DIO rats.	52
Table 4 The characteristic of control, DR and DIO rats.	54
Table 5 The effect of short-term GH treatment on basal plasma leptin at 24 h and 32 h after GH injection in control (n = 6, each), DR (n = 4 for saline and n = 5 for GH) and DIO rats (n = 6, each).....	57
Table 6 The effect of short-term GH treatment on fasting plasma leptin in control (n = 6, each), DR (n = 4 for saline and n = 5 for GH) and DIO rats (n = 6, each).....	60
Table 7 The effect of short-term GH treatment on fasting plasma insulin, glucose and NEFA in control (n = 6, each), DR (n = 4 for saline and n = 5 for GH) and DIO rats (n = 6, each).....	62
Table 8 The effect of short-term GH treatment on body adiposity and liver weight of control (n = 6, each), DR (n = 4 for saline and n = 5 for GH) and DIO rats (n = 6, each).....	64

LIST OF FIGURES

- Figure 1** The HC feeding rats were fed with HC diet for 6 weeks, only HF diet was fed during the first 2 weeks, then HF and HFHC were fed during the last 4 weeks. After 6-week period, IPGTT was performed. 30
- Figure 2** Experimental procedure of IPGTT. First, the rats were fasted for 16 h overnight and blood was collected for glucose measurement. Then, glucose solution (2g/kg) was injected intraperitoneally and blood glucose was measured at 15, 30, 60, 90 and 120 minutes following glucose injection. 33
- Figure 3** Experimental procedure, GH was injected to control and DIO rats (n = 9, each) from day 1-5. Blood was collected for measuring plasma leptin and IGF-1 at day 1 (before GH injection), day 3, 5 (during GH injection) and day 7 (after stopping GH injection)..... 34
- Figure 4** Experimental procedure, either GH or saline was injected to control, DR and DIO rats for 3 days. Blood was collected for measuring plasma hormones, glucose and NEFA:..... 39
- Figure 5** Percentage of DIO (n = 9/ 14, 64%) and DR (n = 5/ 14, 36%) rats from study part 1..... 45
- Figure 6** Glucose curve from IPGTT. From 15 minutes to 120 minutes after glucose injection, blood glucose from DIO rats was significantly higher than that of control rats. * P < 0.05, ** P < 0.01 when compared to control using unpaired t-test..... 46
- Figure 7** The correlation between plasma leptin and relative body fat mass (%) in control and DIO rats..... 47
- Figure 8** Basal plasma leptin in control and DIO rats before GH treatment (day1), during GH treatment (day 3 and 5) and after stopping GH (day 7). Short-term GH treatment decreased basal plasma leptin significantly at day 3 and day 5 in DIO rats. The black bar indicates the duration of GH injections. * P < 0.05 when

compared to day 1 within group using one-way repeated measured ANOVA followed by Bonferroni post-hoc. # $P < 0.01$ when compared to control at day 1 using unpaired t-test..... 47

Figure 9 Plasma IGF-1 was increased after GH treatment in control and DIO rats. In DIO rats, plasma IGF-1 was increased significantly at day 5. However, plasma IGF-1 was increased at day 3 and 5 in control rats. On day 7, plasma IGF-1 from both groups was decreased to the pre-treatment level of day 1. The black bar indicates the duration of GH injections. * $P < 0.05$ when compared to day 1 within group using one-way repeated measured ANOVA followed by Bonferroni post-hoc. # $P < 0.05$ when compared to control at day 3 using unpaired t-test..... 49

Figure 10 Short-term GH treatment decreased FI in both control and DIO rats. ** $P < 0.01$, *** $P < 0.001$ when compared to before GH treatment within group using two-way ANOVA followed by Bonferroni post-hoc. # $P < 0.05$ when compared to control at the same time point using two-way ANOVA followed by Bonferroni post-hoc. 50

Figure 11 Short-term GH treatment decreased EI in both control and DIO rats. Additionally, EI was significantly lower in control rats than that of DIO rats. ** $P < 0.01$ when compared to before GH treatment within group using two-way ANOVA followed by Bonferroni post-hoc. # $P < 0.001$ when compared to control at the same time point using two-way ANOVA followed by Bonferroni post-hoc. 51

Figure 12 Gross appearance of adipose tissue and liver in control (A, C) and DIO (B, D) rats. Higher amount of fat mass and enlarged liver with pale color was found in DIO rats..... 52

Figure 13 Percentage of DIO (n = 12/ 21, 57%) and DR rats (n = 9/ 21, 43%) from study part 2..... 54

Figure 14 Glucose curve from IPGTT. Blood glucose from DIO rats was significantly higher than that of control rats at 60 and 90 minutes after glucose injection. * $P < 0.05$ when compared to control rats at the same time point using one-way ANOVA followed by Bonferroni post-hoc..... 55

- Figure 15** The effect of short-term GH treatment on plasma leptin per fat mass. There was the significant effect of GH on leptin per fat mass in DIO rats. * $P < 0.05$ when compared to saline treatment using two-way ANOVA followed by Bonferroni post-hoc. 58
- Figure 16** Meal-induced plasma leptin in control, DR and DIO rats. There was the significantly effect of meal on plasma leptin only in DIO rats. * $P < 0.05$ when compared to saline treatment at the same time using two-way ANOVA followed by Bonferroni post-hoc. # $P < 0.05$ when compared to plasma leptin at pre meal using two-way ANOVA followed by Bonferroni post-hoc..... 58
- Figure 17** The effect of short-term GH treatment on meal-induced plasma leptin in control, DR and DIO rats was shown as the normalized leptin of post- and pre-meal (%). There was no effect of GH on meal-induced plasma leptin in all rats..... 59
- Figure 18** The energy intake from meal-induced plasma leptin study revealed that DIO rats had higher energy intake over both control and DR rats. * $P < 0.05$ when compared between groups using two-way ANOVA followed by Bonferroni post-hoc..... 59
- Figure 19** The effect of short-term GH treatment on HOMA-IR. It was noted that GH effect induced insulin resistance in DR rats. * $P < 0.05$ when compared to saline treatment using two-way ANOVA followed by Bonferroni post-hoc. 63
- Figure 20** The effect of short-term GH treatment on Adipo-IR. The result revealed that the effect of GH can induce insulin resistance in DR rats. * $P < 0.05$ when compared to saline treatment using two-way ANOVA followed by Bonferroni post-hoc..... 63
- Figure 21** Diagrams represent the pathways in which short-term GH administration altered plasma leptin in basal, meal-induced and fasting condition in control (A), DR (B) and DIO rats (C) (+ : increase, - : decrease, O : unchanged, dashed line indicated that insulin could alter plasma leptin in fasting condition). 74

LIST OF ABBREVIATIONS

Ab	Antibody
ACOD	Acyl-CoA oxidase
ACS	Acyl-CoA synthetase
<i>ad/ad</i>	Adipose mouse
Adipo-IR	Adipose tissue insulin resistance
AGRP	Agouti-related protein
AUC	Area under the curve
BMI	Body mass index
BSA	Bovine serum albumin
BW	Body weight
CART	Cocaine- and amphetamine- regulated transcript
CoA	Coenzyme A
CSF	Cerebrospinal fluid
<i>db/db</i>	Diabetes mouse
DIO	Diet-induced obesity
DR	Diet resistant
EI	Energy intake
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum

<i>fa/fa</i>	Fatty Zucker rat
FFA	Free fatty acids
FI	Food intake
GH	Growth hormone
GHBP	Growth hormone binding protein
GHD	Growth hormone deficiency
GHRH	Growth hormone releasing hormone
GHS	Growth hormone secretagogue
GSIS	Glucose-stimulated insulin secretion
GTG	Gold thioglucose
HC	Hypercaloric diet
HF	High-fat diet
HFHC	High-fat high-carbohydrate diet
HOMA-IR	Homeostasis model of insulin resistance
HRP	Horseradish peroxidase
IGF-1	Insulin-like growth factor 1
IGFBP	Insulin-like growth factor 1 binding protein
IPGTT	Intraperitoneal glucose tolerance test
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1
JAK2	Janus kinase 2

LepRb	Leptin receptor long isoform
MEHA	3-Methyl-N-ethyl-N-(β -hydroxyethyl)-aniline
MSG	Monosodium glutamate
NEFA	Non-esterified fatty acid
NPY	Neuropeptide Y
NZO	New Zealand obese mouse
<i>Ob</i>	Obese gene
<i>ob/ob</i>	Obese mouse
PBS	Phosphate buffered saline
PI3	Phosphatidylinositol
POD	Peroxidase
POMC	Pro-opiomelanocortin
PTP1B	Phosphotyrosine phosphatase-1B
SGLT	Sodium glucose cotransporter
SOCS3	Suppressor-of-cytokine-signaling 3
STAT5	Signal-transducer-and-activator-of-transcription 5
T3	Triiodothyronine
TMB	Tetramethylbenzidine
TNF- α	Tumor necrosis factor- α
VLDL	Very low-density lipoprotein

WHO

World health organization

α -MSH

α -Melanocyte stimulating hormone



CHAPTER I

INTRODUCTION

The prevalence of obese population has increased worldwide, which acts as predisposing factor to life-threatening diseases; i.e. cardiovascular disease, hypertension, diabetes mellitus. Moreover, metabolic and hormonal disturbances have been linked to pathophysiology of obesity. For instance, obese people shows lower circulating growth hormone (GH) and higher leptin and insulin. It should be noted that GH has metabolic effect which related to plasma insulin and leptin (Moller and Jorgensen, 2009).

Leptin is mainly produced by white adipose tissue. The pathways of leptin synthesis and secretion are associated with many factors. It was demonstrated that circulating level of leptin is correlated with body adiposity (Ahren et al., 1997; Friedman and Halaas, 1998). The pattern of circulating leptin in humans and rodents has been reported in many studies (Sinha et al., 1996; Ahren et al., 2000; Bodosi et al., 2004). The circulating level of leptin, including basal and meal-induced leptin secretion, was affected by multiple factors, such as energy and nutritional status, hormonal status and substrate levels (Langendonk et al., 1998; Coleman and Herrmann, 1999; Ahren et al., 2000; Cammisotto et al., 2003; Bodosi et al., 2004). GH administration has been also demonstrated to affect plasma leptin (Boni-Schnetzler et al., 1999; Lissett et al., 2001; Malmlof et al., 2002; Leury et al., 2003; Thammacharoen et al., 2014). The long-term GH administration led to decrease body adiposity in accordance with lowering plasma leptin (Florkowski et al., 1996; Malmlof et al., 2002; Malmlof and Johansen, 2003; Johansen et al., 2005; List et al., 2009; Chaiyabutr et al., 2015). However, the short-term effect of GH treatment on plasma leptin in which body fat mass does not alter

is uncertain. For instance, short-term GH led to increase plasma leptin at 24 h and decrease its level at 72 h after GH treatment in healthy humans (Lissett et al., 2001), however GH treatment increased plasma leptin after 4 days in goats (Thammacharoen et al., 2014). The present study aimed to investigate the short-term effect of GH on plasma leptin, which brought the interesting reasons for us. Firstly, plasma GH in obese subjects was shown negative correlation with body adiposity and exogenous GH has been used for obesity treatment in some studies (Campfield et al., 1998; Bray and Tartaglia, 2000; Berryman et al., 2013). Secondly, short-term GH treatment led to decrease food intake (FI), which was reported in rodents and goats (Malmlof et al., 2002; Thammacharoen et al., 2014). This point remained to clarify whether the decreased FI was resulted from the direct effect of GH or indirect effect via modification of circulating leptin. Lastly, the metabolic effects of GH was related to plasma insulin and leptin as aforementioned (Moller and Jorgensen, 2009). To study the effect of GH on plasma leptin, this study focused on the short-term effect of GH and investigated the optimal duration of GH treatment in diet-induced obesity (DIO) rats. Furthermore, this study clearly defined plasma leptin in 3 conditions, consisting of basal, meal-induced and fasting condition in control, diet-resistant (DR) and DIO rats. The information from this study help us to clarify the relationship between GH and leptin, thus provide the benefit of obesity research.

Therefore, the objectives of this study were:

1. To acquire the optimal duration of GH treatment on basal plasma leptin in control and DIO rats.

2. To investigate the short-term effect of GH on basal, meal-induced and fasting plasma leptin in control, DR and DIO rats.
3. To investigate the short-term effect of GH on insulin and insulin sensitivity in control, DR and DIO rats.
4. To investigate the short-term effect of GH on body adiposity in control, DR and DIO rats.

The research questions of this study were:

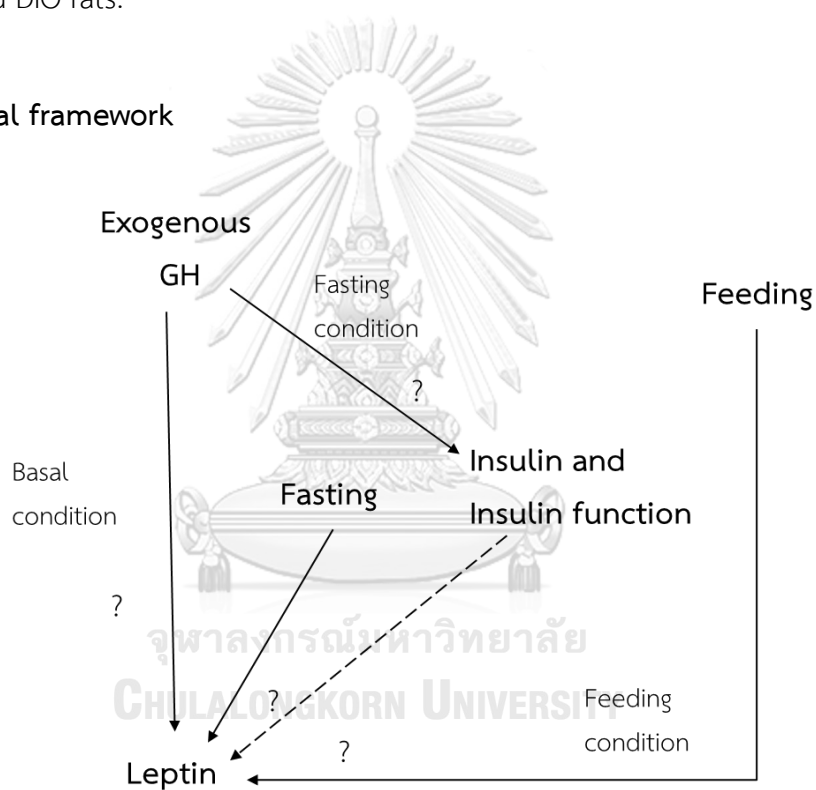
1. When would GH alter basal plasma leptin in control and DIO rats?
2. Can short-term GH administration affect basal, meal-induced and fasting plasma leptin in control, DR and DIO rats?
3. Can short-term GH administration affect insulin and insulin sensitivity in control, DR and DIO rats?
4. Can short-term GH administration influence body adiposity in control, DR and DIO rats?

The hypotheses of this study were:

1. The 5-day GH administration could decrease basal plasma leptin in DIO rats.
The higher body fat mass may be in part involved in this response.
2. The short-term GH administration could alter meal-induced plasma leptin in DIO rats.

3. The short-term GH administration increases fasting plasma leptin in control and DR rats.
4. The short-term GH administration increases plasma insulin and insulin resistance in control and DR rats.
5. The short-term GH administration does not alter body adiposity in control, DR and DIO rats.

Conceptual framework



CHAPTER II

LITERATURE REVIEW

A. Update of obesity problem and the initiation factors

Nowadays, obesity is rapidly increasing in worldwide population. It is considered as the major health problems and linked to other life-threatening diseases, including cardiovascular disease, hypertension, type II diabetes, liver diseases with cholesterol gallstones and sleep apnea (Grundy and Barnett, 1990; Poirier et al., 2006). Classification of obese individual is defined by BMI, which is calculated from the ratio of body weight (kg) and height². As World Health Organization (WHO) criteria, the BMI of normal subjects is between 20.0-25.0 kg/m², while the BMI of overweight subjects is defined as in between 25.1-29.9 kg/m² and for obese subjects is more than 30.0 kg/m². Obesity is associated with hypertriglyceridemia (Grundy et al., 1979) and hypercholesterolemia which derived from elevated hepatic lipoprotein production, especially very low density lipoprotein (VLDL) (Kesaniemi and Grundy, 1983; Egusa et al., 1985; Kesaniemi et al., 1985). Additionally, peripheral insulin resistance (IR) is the major consequence of obesity. This issue may cause by the high level of free fatty acids (FFA) either in diet composition or excess body adiposity (Felber and Vannotti, 1964; Ruderman et al., 1969; Boden, 1998). In this regard, hyperinsulinemia in obese individuals has down-regulate insulin receptor in peripheral tissue, resulting in worsening IR (Bar et al., 1979).

The leading causes of obesity have been studied for long time. It should be noted that sedentary lifestyle and imbalanced diets are contributed to obesity (Jebb and Moore, 1999; Hu, 2003; Manson et al., 2004). The less physical activity in

children and adult persons is related to increasing the prevalence of obesity (Manson et al., 2004). Additionally, imbalanced diets such as cafeteria diet, hypercaloric diet and soft drink are globally increasing distribution with the lower price than that of organic food or healthy drink (Friel et al., 2007). These issues affect health problem; thus, it is necessary to study the pathophysiology of obesity and prevent obesity epidemic.

B. Animal model of obesity research

In order to study the pathophysiology of obesity, many researchers have focused on the investigation of mechanisms in obese animal models, which were induced by many factors. One of the ways to study these issues is developing animal models, such as genetic abnormalities or hypothalamic lesions in rodents (Bray and York, 1971; Sclafani and Springer, 1976). Genetic obesity was characterized mainly as autosomal dominant or recessive-inherited obesity. The dominant-inherited obesity was defined as yellow obese mice, which encoded by the agouti locus (Bray and York, 1971). Moreover, the recessive type has been more studied for investigating the pathogenesis and abnormalities of obesity than that of yellow obese rodents. For instance, obese (*ob/ob*) mouse (Westman, 1968; Chlouverakis et al., 1970; Czech et al., 1977), adipose (*ad/ad*) mouse (Falconer and Isaacson, 1959), fatty (*fa/fa*) rat (Zucker and Zucker, 1961; Zucker and Zucker, 1963; Zucker, 1972) and diabetes (*db/db*) mouse (Coleman and Hummel, 1967; Coleman and Hummel, 1975; Coleman, 1978) were used as animal models. Inbred obesity was also recognized as genetic obesity, such as New Zealand obese (NZO) mouse (Crofford and Davis, 1965; Herberg et al., 1970) and Japanese KK mouse (Nakamura and Yamada, 1963; Nakamura and Yamada, 1967). The rodents with genetic obesity

had developed obesity progressively since the age of 4-5 weeks. For the case of obesity due to hypothalamic lesions, the rodents develop obesity, which related to increasing food consumption and decreasing physical activity (Hetherington and Ranson, 1942; Brooks et al., 1946), feeding with high palatable diet is related to the enhanced intake when compared with normal chow diet (Hetherington and Ranson, 1942). Moreover, brain lesions, including hypothalamic area, were appeared after injection of monosodium glutamate (MSG) to newborn rodents, because MSG can destroy the neurons, leading to degenerative lesion (Olney, 1969). On the contrary with the previous cause of hypothalamic lesions, MSG-treated rodents didn't show the greater amount of food intake than that of control rodents (Olney, 1969). Other chemical agents that can destroy the hypothalamus, including gold thioglucose (GTG) (Brecher and Waxler, 1949; Mayer, 1953; Perry and Liebelt, 1961) and bipiperidyl mustard (Rutman et al., 1966); which injected to adult rodents, resulting in obesity. However, GTG and bipiperidyl mustard destroyed ventromedial nucleus of hypothalamus, which refers to satiety center. Therefore, those rodents had increased food consumption.

On one hand, the rapid increase of obesity in modern society is related to environmental factors, which disturb the body homeostasis. For instance, hypercaloric diets and physical inactivity are the major causes of obesity. Additionally, laboratory rodents are proper models for research, which can mimic the causes of human obesity. The rodents are used as gold standard for investigating dietary-induced obesity (Woods et al., 2003; Reuter, 2007). There are many factors which influences the stage of fattening period, such as age and sex. First, laboratory rodents get obese when they get old despite feeding with standard

chow (Kennedy, 1957). Also, when feeding with palatable hypercaloric diet to adult rodents leads to increase more adiposity than that of younger rats (Tschop and Heiman, 2001). In case of sex-dependent manners, male rats still gain weight throughout lifetime, whereas female rats tend to show the stable weight in adulthood (Stewart, 1916). The final body weight after feeding with hypercaloric diet in male rats is greater than that of female rats (Schemmel et al., 1969). Despite of this character, both male and female rats can be induced obesity by feeding with hypercaloric diet (Woods et al., 2003). Moreover, the effect of different strains of rats were studied and the result revealed that Osborne-Mendel and Wistar-Lewis rats gained 50% in body weight, while Sprague-Dawley rats gained around 25%. Likewise, body adiposity was greater (2-3 times) in these rats when compared with control rats (Schemmel et al., 1970).

Dietary macronutrient composition also affects the intake in the period for the development of obesity. It should be noted that diet with high fat has an influence on satiety effect, resulting in less satiety and more caloric intake compared with other macronutrients (Warwick and Weingarten, 1995; Blundell and Macdiarmid, 1997; Covasa and Ritter, 2000). This issue could be explained in term of high fat diet has lesser volume than that of chow. In this regard, the degree of stomach distension is also lesser than chow (French and Robinson, 2003), involving the decreased fatty acid oxidation lead to stimulate intake (Kahler et al., 1999; Scharrer, 1999). Additionally, carbohydrate also leads to gain body weight and get obese, even though it has sweet taste or not (Sclafani and Xenakis, 1984; Sclafani, 1987). Liquid diets with sweet taste can induce higher caloric intake and obesity (Ramirez, 1987), because these moisture-containing diets can decrease osmotic

pressure in gut, leading to promote food intake (Ramirez et al., 1989). Currently, soft drinks have contributed to developing obesity in childhood and adulthood, because of the low satiety effect of these beverages (Malik et al., 2006). Moreover, the compensation of energy intake by the subsequent meal after consumption of soft drinks could be less effective than that of solid food (Mattes, 1996).

C. Leptin synthesis and adipose tissue

Leptin, a 16 kDa peptide hormone, is mainly synthesized by adipose tissue (Ahima and Flier, 2000; Russell et al., 2001), although it is found in gastric mucosa, placenta, muscle, brain in less portion (Masuzaki et al., 1997; Bado et al., 1998; Esler et al., 1998; Wang et al., 1998; Li et al., 1999). It is encoded by the *obese (ob)* gene. The *ob* gene expression shows the highest level in visceral (gonadal, perirenal) fat and the lower in subcutaneous (interscapular, inguinal) fat in rodents (Masuzaki et al., 1995a; Trayhurn et al., 1995b). However, the higher level of the *ob* gene is found in subcutaneous fat in humans (Masuzaki et al., 1995b; Hube et al., 1996; Montague et al., 1997). The *ob* gene expression and circulating leptin are related to body adiposity in both humans and rodents. On the one hand, *ob/ob* mice are obese with the abnormally high *ob* gene but do not have circulating leptin, the *db/db* mice and *fa/fa* rats show high *ob* gene and circulating leptin but they have dysfunctional leptin receptors (Masuzaki et al., 1995a; Hardie et al., 1996). The leptin receptors are located in the hypothalamus as the primary site of actions (Mercer et al., 1996; Friedman and Halaas, 1998; Rayner and Trayhurn, 2001). Leptin decreases food intake by decreasing neuropeptide Y (NPY) and agouti-related protein (AGRP) (Schwartz et al., 1996; Wang et al., 1997) together with

increasing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (Schwartz et al., 1997). The product of POMC genes, such as α -melanocyte stimulating hormone (α -MSH), and CART have anorexic effect (Kristensen et al., 1998). Moreover, the leptin receptors were located in peripheral tissue, such as pancreatic β -cells, heart, lung, lymph node, spleen and kidney (Fei et al., 1997; Lollmann et al., 1997; Kieffer and Habener, 2000). This information revealed that leptin has many roles in the body regulation and homeostasis.

Leptin synthesis is regulated by many factors, including fat cell size, energy status, nutritional and hormonal effects. The size of adipocyte has an influence on quantitation of leptin synthesis, as a bigger adipocyte contains greater leptin contents than a smaller one (Hamilton et al., 1995; Lonngvist et al., 1997; Guo et al., 2004; Skurk et al., 2007). The higher leptin biosynthesis in adipose tissue of obese individuals is related to higher leptin contents and fat cell size (Lee et al., 2007; Lee and Fried, 2009). The leptin protein in adipocytes are decreased by 18 h fasting condition and it can be rapidly restored after refeeding without any change in body fat mass (Becker et al., 1995; Saladin et al., 1995; Attoub et al., 1999). Energy deficit in fasting stage also markedly decreases the leptin gene expression which could be reversibly after refeeding (Becker et al., 1995; MacDougald et al., 1995; Saladin et al., 1995). Fasting is related to stimulate the sympathetic activity of adipose tissue and release fatty acids as the energy for other tissues (Migliorini et al., 1997). The decreased leptin gene expression could be demonstrated by treatment with β -adrenergic receptor agonists (Moinat et al., 1995; Trayhurn et al., 1995a). Moreover, sex has influences on leptin regulation. Leptin is decreased by testosterone and increased by estrogens, humans show higher leptin in females;

whereas, rodents show higher leptin in males at the similar percentage of body fat (Shimizu et al., 1997; Wabitsch et al., 1997; Landt et al., 1998). The hormonal effects on leptin synthesis have been studied, especially insulin and glucocorticoid. Insulin increased leptin synthesis and secretion from adipose tissue (Barr et al., 1997) and β -adrenergic receptor agonists could be inhibited insulin-induced leptin secretion from adipocytes (Cammisotto and Bukowiecki, 2002). Furthermore, insulin, dexamethasone (synthetic glucocorticoid) or insulin and dexamethasone increased leptin gene expression in adipose tissue culture (Russell et al., 1998); however, some studies reported that insulin could not affect leptin mRNA but increased leptin secretion and revealed insulin-regulated leptin in posttranscriptional mechanism (Bradley and Cheatham, 1999; Roh et al., 2003; Lee et al., 2007). Dexamethasone increased both leptin mRNA and leptin secretion. The results revealed dexamethasone regulated leptin via transcriptional mechanism (Lee et al., 2007). In the case of incubation with insulin and dexamethasone, leptin content was increased higher than basal condition (Russell et al., 2001). In addition, tumor necrosis factor α (TNF- α) has influenced on increasing leptin secretion from 3T3-L1 adipocytes and increasing leptin mRNA together with circulating leptin in mice (Kirchgessner et al., 1997). Another study showed that TNF- α could be increased leptin mRNA in the adipocyte culture with dexamethasone (Trujillo et al., 2006). This information revealed the contribution from local glucocorticoid and inflammatory cytokines in the obese state play the roles in regulating leptin production and secretion.

D. The roles of leptin in physiology

Physiological roles of leptin are depended on pharmacokinetic of plasma leptin (Hill et al., 1998), consisting of 1) binding with the soluble receptors as a binding protein in circulation to increase its half-life (Lee et al., 1996; Sinha et al., 1996); and 2) leptin storage in tissue which has leptin binding sites (Hill et al., 1998). Circulating leptin in lean subjects was significantly greater in bound form; including both leptin and its binding protein, called soluble receptor than those of obese subjects (Sinha et al., 1996). Leptin soluble receptors regulate the free form of leptin and also act as the buffer of leptin bioactivity for prolonging its half-life (Chan et al., 2002). Bound form of plasma leptin had longer half-life about 20 times than that of another one, free form of plasma leptin (Hill et al., 1998). Obese individuals had greater amount of free form of leptin, which was the bioactive form of leptin, and this form had an effect on the regulation of food intake and energy balance (Sinha et al., 1996). Because of higher plasma leptin, the binding sites of its soluble receptor were completely saturated. Increased free form of leptin could produce leptin resistance state in obese subjects (Houseknecht et al., 1996).

The primary roles of leptin are inhibition of food intake and stimulation of energy expenditure. Leptin regulates the food intake by acting as satiety factor via its receptors in hypothalamus (Mercer et al., 1996; Friedman and Halaas, 1998; Cammisotto and Bukowiecki, 2002). After a meal, plasma leptin could not alter rapidly. However, it could play a role in the long-term regulation of intake rather than the short-term regulation (Friedman and Halaas, 1998). However, leptin that produced by gastric mucosa has been involved in the short-term regulation of intake (Friedman and Halaas, 1998). Acute administration of exogenous leptin

reduces food intake and body weight, yet it increases energy expenditure of normal subjects (Barr et al., 1997; Szkudelski et al., 2005; Myers et al., 2010). Moreover, leptin decreases body fat mass by the stimulation of apoptotic process in adipocytes (Qian et al., 1998). Leptin plays a role in the absorption of sugar in small intestine, which inhibits active transport (sodium glucose cotransporter-1; SGLT1) but not modifies passive absorption of sugar (Lostao et al., 1998). Leptin appears to regulate lipid metabolism by increasing the lipid oxidation via stimulating the gene expression of lipid oxidation enzymes (Shimabukuro et al., 1997). Besides the lipid oxidation, leptin decreases triglyceride formation in pancreas, skeletal muscle and liver as well (Shimabukuro et al., 1997). In addition, leptin plays a role in the regulation of immunity, including T-cell proliferation and cytokines production from helper T-cells. The results reveal that the body immune function was suppressed during starvation and it could reverse when exogenous leptin was injected (Lord et al., 1998). Leptin also regulates the onset of puberty and acts as the signal which links between adiposity and reproduction. Leptin treatment in female mice leads to accelerating of the reproductive system maturation and the onset of estrous cycle (Ahima et al., 1997; Chehab et al., 1997).

Furthermore, as aforementioned in the previous part; leptin could inhibit basal and glucose-stimulated insulin secretion via leptin receptors which located at pancreatic β -cells, the latter effect was demonstrated as dose-dependent of leptin treatment (Emilsson et al., 1997). Thus, the theory of adipoinsular axis has been proposed (Kieffer and Habener, 2000). Adipoinsular axis is the feedback loop between adipose tissue and pancreas (Kieffer et al., 1996; Kieffer and Habener, 2000). In *ob/ob* or *db/db* mice, which are leptin-deficient or resistance,

respectively, have hyperinsulinemia and obesity with insulin resistance (Genuth, 1969; Genuth et al., 1971; Coleman and Hummel, 1974; Dubuc, 1976; Chen and Romsos, 1995; Chen et al., 1997). Due to the defect of leptin system, these mice have hyperinsulinemia because leptin plays a role in blunted insulin secretion in normal subjects. Acutely lower plasma insulin level after exogenous leptin injection for 10 minutes in *ob/ob* mice was demonstrated (Kulkarni et al., 1997) and was not related with plasma glucose. By contrast, for long-term leptin treatment, these results revealed that leptin could normalize plasma glucose concentration in hyperglycemic rodents (Halaas et al., 1995; Pelleymounter et al., 1995; Weigle et al., 1995; Levin et al., 1996; Muzzin, 1996; Schwartz et al., 1996; Harris et al., 1998). These effects were possibly because of the increased insulin sensitivity.

E. Regulation of plasma leptin levels

According to secrete leptin by adipocytes, plasma leptin is in proportion to the adipose tissue mass (Considine et al., 1996; Considine and Caro, 1997). Plasma leptin has circadian variation with low level at 0730 h and begins to reach the peak at 0130 h in adult rats fed *ad libitum* (Mastronardi et al., 2008). Circulating level has been maintained at the basal value during the light phase and begins to increase after the dark onset, then reach the peak after the first meal of dark phase (Bodosi et al., 2004). Diurnal variation of plasma leptin was related to feeding time, when the diet was provided only at the light phase; the rhythm of plasma leptin also shifted and the nighttime peak of plasma leptin was abolished. Normal feeding increased plasma leptin from basal level, this issue was occurred after the

nighttime feeding for 4-5 h (Bodosi et al., 2004). This result was similar to that of study in humans, which showed the shifted diurnal rhythm of plasma leptin according to meal (Schoeller et al., 1997). These data revealed that energy status plays a role in the regulation of circulating leptin levels. The energy deficit in fasting stage, which related to activating sympathetic activity (Trayhurn et al., 1998; Fried et al., 2000), leads to decrease plasma leptin despite a less decrease in body fat mass (Trayhurn et al., 1999). After refeeding, plasma leptin has restored to baseline level (Weigle et al., 1997). Moreover, the size of adipocyte affects plasma leptin because the larger adipocyte can secrete leptin rapidly with the bigger pulse than that of the smaller adipocyte (Licinio et al., 1997). Thus, both fed stage and the size of fat cells have influenced on plasma leptin. Feeding and fasting models define the roles of nutrient composition and insulin on plasma leptin levels (Coleman and Herrmann, 1999; Bodosi et al., 2004). Overfeeding has led to stimulating plasma leptin greater than those of normal feeding (Kolaczynski et al., 1996; Wang et al., 2001). There is an evidence that plasma leptin is regulated by macronutrient composition in diet including fasting condition. Its level is higher after intake the high-carbohydrate diet, especially high-sugar diet, than those of high-starch or high-fat diet (Dirlewanger et al., 2000; Raben and Astrup, 2000). The high-carbohydrate, low-fat meals lead to increase glucose and insulin greater than the high-fat, low-carbohydrate meal and also increase greater plasma leptin in humans (Havel et al., 1999); therefore, the eating plasma leptin was according to the level of glucose and insulin after feeding (Walker et al., 2002). Glucose has been shown to rapidly regulate leptin mRNA (Mizuno et al., 1996) and increase leptin secretion from cultured rat adipocytes (Levy and Stevens, 2001; Cammisotto

et al., 2005). The *in vivo* effect of glucose infusion during fasting could maintain normoglycemia and prevent fasting effect on plasma leptin (Boden et al., 1996).

Additionally, plasma leptin was influenced by plasma insulin. Many studies have focused on the effect of plasma insulin on plasma leptin in various conditions. Fasting stage decreases both plasma leptin and insulin; however, overfeeding and obese stage increases both hormones (Cammisotto and Bukowiecki, 2002). There are controversial issues about the effect of insulin on plasma leptin. Some studies found that insulin increases plasma leptin (Kolaczynski et al., 1996; Malmstrom et al., 1996; Saad et al., 1998), while other studies reported that insulin does not acutely modify leptin gene expression (Vidal et al., 1996) and plasma leptin (Dagogo-Jack et al., 1996; Clapham et al., 1997). The information related with insulin and leptin has been defined. A previous study showed the increased plasma leptin after consuming a high-carbohydrate diet for 3-5 h (Coppack et al., 1998) and suggested that insulin acts as metabolic signal which probably mediates the leptin production associated with caloric intake (Saad et al., 1998). Euglycemic-hyperinsulinemic clamp study showed the increased plasma leptin after 4 h in rats. Moreover, prolonged insulin treatment led to increase plasma leptin in days 5, 6 and 7 compared with pre-treatment day; whereas food intake was decreased (Koopmans et al., 1998). These results suggested that circulating leptin was related to long-term feeding regulation. However, in the subjects with insulin resistance, the effect of insulin on plasma leptin is blunted. There are many variations in plasma leptin levels in the subjects with similar amount of fat mass, the results reveal that the insulin resistant subjects have lower plasma leptin (Maffei et al., 1995; Saad et al., 1998). As similar to that observed in insulin resistant rodents,

Zucker rats, insulin could not increase basal plasma leptin level. On the contrary insulin could be increase plasma leptin in the lean littermates which has lower basal plasma leptin (Pagano et al., 1997). The experimental-induced insulin resistant stage, hyperinsulinemic-hypoglycemic clamps, was studied and reported that plasma leptin could not response to the effect of insulin (Wellhoener et al., 2000; Fruehwald-Schultes et al., 2002). The results revealed that glucose metabolism could modulate plasma leptin. Increased glucose utilization in adipose tissue led to stimulate plasma leptin according to the effect of insulin (Mueller et al., 1998).

Apart from the effect of insulin on plasma leptin, glucocorticoid is another hormone that affected on plasma leptin. Plasma leptin was correlated with cortisol levels over 24 h and low-dose dexamethasone treatment in humans led to increasing plasma leptin, despite body mass index (BMI) was not changed (Elimam et al., 1998). Dexamethasone treatment increased plasma insulin and decreased insulin sensitivity. Thus, the short-term dexamethasone treatment-induced increase in plasma leptin was independent of insulin sensitivity and the pattern of plasma leptin response was not altered by hyperinsulinemia (Larsson and Ahren, 1996; Kolaczynski et al., 1997). Exogenous glucocorticoid could increase plasma leptin in the stage of ample energy which the effect was blunted in fasting stage (LaferrÈre et al., 1998; Elimam and Marcus, 2002). These results suggested that insulin and glucose may be necessary for glucocorticoid effect on plasma leptin. However, the effect of glucocorticoid is to increase circulating glucose in both feeding and fasting stage. Thus, the results revealed that glucose was unlikely mediated plasma leptin after glucocorticoid administration because glucocorticoid

decreased glucose utilization by fat cells (Larsson and Ahren, 1996; Laferrère et al., 1998). Increased plasma leptin after glucocorticoid treatment was likely to derive from the combination effects of glucocorticoid and insulin. The *in vitro* studies showed the greater leptin secretion from human fat cells that treated with both glucocorticoid and insulin than those fat cells treated only with glucocorticoid or insulin (Wabitsch et al., 1996; Russell et al., 1998).

Plasma leptin is influenced by sympathetic nervous system as well. Fasting stage or cold exposure can induce sympathetic activity especially in adipose tissue which can mobilize fatty acids in order to provide energy source and maintain body temperature (Garofalo et al., 1996; Migliorini et al., 1997). Sympathetic nervous system affected plasma leptin under fasting not fed conditions. Treatment with β -antagonists has led to attenuate the effect of fasting on plasma leptin, but this effect was not found under fed condition (Trayhurn et al., 1998; Rayner and Trayhurn, 2001). Isoproterenol, β -adrenergic receptor agonist, could decrease plasma leptin. The effect was greater in dose-dependent manner over 120 mins of infusion (Donahoo et al., 1997). Treatment with β -3 adrenergic receptor agonist in lean mice has led to decrease plasma leptin and leptin gene expression (Trayhurn et al., 1996). On the contrary the responses to β -3 adrenergic receptor agonist, cold exposure and fasting were impaired in *ob/ob* mice and *fa/fa* rats because they had lower β -3 adrenergic receptors in white adipose tissue and blunted the adenylyl cyclase activity (Muzzin et al., 1991; Collins et al., 1994; Bégin-Heick, 1995; Hardie et al., 1996). Exercise-induced sympathetic activity has led to secrete catecholamines (Ji et al., 1986). Acute exercise could reduce leptin gene expression in retroperitoneal fat, which was independent of body adiposity and

size of adipocytes, but acutely exercise could not alter plasma leptin despite the higher level of corticosterone in exercise rats than those of control rats (Bramlett et al., 1999). However, training exercise for long-term decreased leptin gene expression and plasma leptin according to the decreased body adiposity (Zachwieja et al., 1997). In case of treatment with β -3 adrenergic receptor antagonist together with acute exercise, the results revealed that leptin gene expression was reduced and β -3 adrenergic receptor antagonist blunted the effect of exercise on leptin gene expression. On the one hand, plasma leptin was higher in acute exercise rats than those of control rats, which was independent of the effect of β -3 adrenergic receptor antagonist (Bramlett et al., 1999). Other studies reported that prolonged exercise with more than 60 mins resulting in decreased plasma leptin (Landt et al., 1997) or no change (Racette et al., 1997), the different results depended on the various conditions and intensity of exercise training. Additionally, decreased activity of sympathetic nervous system was demonstrated in obese individuals (Peterson et al., 1988; Tataranni et al., 2012) in spite of higher plasma leptin; thus, this issue should develop leptin resistance state.

F. Leptin in obesity

Circulating leptin shows a marked correlation with relative percentage of body fat mass, this level is also correlated with BMI in humans (Maffei et al., 1995; Caro et al., 1996; Considine et al., 1996; Ma et al., 1996; McGregor et al., 1996). The increased leptin production, secretion and its gene expression are reported in obese subjects (Hamilton et al., 1995; Lonqvist et al., 1995; Turban et al., 2002; O'Doherty and Nguyen, 2004). However, obesity is linked with leptin resistance in

regulation of food intake and energy balance (Sinha and Caro, 1998). The previous study showed that DIO mice with HF diet presented a lot of body fat mass and increased circulating leptin, despite unaltered body weight and food intake. This issue was reflected leptin resistance state due to the attenuation of leptin signaling pathway (Frederich et al., 1995). The defects of leptin signaling that linked with fat storage and energy metabolism were occurred in rodents after feeding with HF diet just a week (El-Haschimi et al., 2000; Munzberg et al., 2004). Leptin resistance state was induced when rodents get hypercaloric intake and get more adiposity; firstly, they could also respond to leptin injection peripherally. After that, they could not respond to peripheral leptin, but they could respond to central leptin injection. Finally, continuous feeding rodents with HF diet for long-term had led to the leptin resistance state both central and peripheral ways (El-Haschimi et al., 2000). The central leptin resistance probably occurred by the saturation of the transport system of leptin into the cerebrospinal fluid (CSF) because there was a rate-limiting step of leptin transport to brain and CSF, and hyperleptinemia in obese subjects led to down-regulate of leptin transporter as well (Banks et al., 1996; Caro et al., 1996; Schwartz et al., 1996), thus the capability of the leptin uptake in the brain of obese individuals is lower than that from the lean individuals. For the peripheral leptin resistance, leptin receptors were found in various tissues, including liver, pancreas, muscle and fat tissue (Tartaglia et al., 1995; Lee et al., 1996; Houseknecht et al., 1998), which has been desensitization (Martin et al., 2000). However, it should be noted that obese rodents can reverse into leptin sensitivity state after switching the HF diet with chow diet (Enriori et al., 2007).

The leptin signaling has started after its molecule binds to leptin receptor long isoform (LepRb) and activates the janus-kinase 2 (JAK2) then activates signal-transducer-and-activator-of-transcription 5 (STAT5), which leads to regulating other gene transcription (Banks et al., 2000; Munzberg et al., 2005). Leptin also activates suppressor-of-cytokine-signaling 3 (SOCS3) and phosphotyrosine phosphatase-1B (PTP1B) for feedback regulation (Bjorbaek et al., 2001). For leptin resistance state, it has been reported that the gene expression of LepRb and protein expression in hypothalamus were downregulated (Martin et al., 2000). This cellular signaling through LepRb is blunted due to the higher of SOCS3 or PTP1B or both (Howard et al., 2004; Howard and Flier, 2006; White et al., 2009). Other ways regulated leptin resistance is the chronic inflammation in obese subjects. The consequence of this issue is endoplasmic reticulum (ER) stress in hypothalamus which lead to insulin resistance state and inhibit cellular signaling of leptin (Ozcan et al., 2004; Zhang et al., 2008; Ozcan et al., 2009). After treatment with chemical agents which relieved ER stress in hypothalamus, the leptin sensitivity was enhanced (Hosoi et al., 2008; Ozcan et al., 2009). The causes that linked ER stress and leptin resistance in obese subjects were the excess level of FFA in hypothalamus, especially palmitoyl-CoA, which led to ER stress and induced apoptosis of the neurons in hypothalamus (Benoit et al., 2009; Posey et al., 2009; Choi et al., 2010; Mayer and Belsham, 2010). The effect of ER stress could lead to blunt the function of melanocortin system through reduction of the receptor of α -MSH, MC4R (Cragle and Baldini, 2014). Because the ER has an important function for processing the protein, thus the effect of leptin on the stimulation of α -MSH release is also blunted (Bjørnbæk, 2009; Diano et al., 2011; Gamber et al., 2012). The ER stress can induce SOCS3 and PTP1B

expression in the neurons of hypothalamus (Cakir et al., 2013), resulting in reduction of leptin signaling pathway, and becoming leptin resistance stage.

G. Growth hormone in obesity

Obesity is related to decreased GH level, both the endogenous pulsatile GH secretion and the stimulated secretion which responses to potential stimuli, such as insulin, arginine, galanin, and GH releasing hormones (GHRH) (Copinschi et al., 1967; Williams et al., 1984; Kopelman et al., 1985; Finkelstein et al., 1986; Kopelman and Noonan, 1986; Pertzalan et al., 1986; Loche et al., 1987; Kelijman and Frohman, 1988; Loche et al., 1990). The negative relationship between GH concentration and body adiposity has been reported in many studies (Veldhuis et al., 1991; Hartman et al., 1993; Rasmussen et al., 1995; Vahl et al., 1997; Coutant et al., 1998; Scacchi et al., 1999). The defects of GH in obese stage also resulted in both ways; decreased GH secretion and increased GH clearance (Veldhuis et al., 1991; Veldhuis et al., 1995). By comparing the character of GH secretion, the obese subjects showed the decreased GH half-life, the frequency of GH secretory bursts, and the rate of GH production (lower than that of normal subjects approximately 4-fold) (Veldhuis et al., 1991). These have been suggested as a result of the lower spontaneous secretion of GHRH and the higher somatostatin secretion (Davis, 1988). There was the higher GH binding protein (GHBP) in obese people when compared with normal subjects (Hochberg et al., 1992). However, circulating GHBP was lowered and returned to the normal level after caloric restriction (Rasmussen et al., 1996). The GH secretion also returned to normal level after lowering caloric intake with reduction of BMI (Argente et al., 1997). Additionally, the axis of GH-

insulin-like growth factor 1 (IGF-1) is affected in obese stage, for instance, it has an evidence that increased free IGF-1 in obese subjects can inhibit the secretion of GH as the negative feedback pathway (Scacchi et al., 1999). However, the concentration of plasma IGF-1 in obese subjects shows the conflicting trends, some studies reported the normal level of plasma IGF-1 (Frystyk et al., 1995; Postel-Vinay et al., 1995; Nam et al., 1997), higher level (Van Vliet et al., 1986; Loche et al., 1987; Hochberg et al., 1992), or lower level of IGF-1 (Minuto et al., 1988; Skaggs and Crist, 1991; Argente et al., 1997) than that from the healthy lean subjects. However, obese individuals usually increase the free form of IGF-1 in blood circulation (Frystyk et al., 1995; Argente et al., 1997; Nam et al., 1997) which can be derived from the decreased IGF-1 binding protein (IGFBP), especially IGFBP-1 and 2 (Frystyk et al., 1995; Argente et al., 1997; Nam et al., 1997). The role of IGFBP-1 has an effect as inhibitor of IGF-1 (Cox et al., 1994; Rajkumar et al., 1995).

Other mechanisms could mediate the secretion of GH, including nutritional status and insulin. Overfeeding in lean control individuals led to decrease GH secretion which occurred in several days (Cornford et al., 2011). On the contrary, short-term caloric restriction in obese subjects led to increasing GH secretion (Kasavubu et al., 2002). Further, hyperinsulinemia in obese subjects blunted the GH release, whereas IGF-1 was maintained because insulin could enhance IGF-1 production from liver, but IGFBP-1 was decreased (Argente et al., 1997; Nam et al., 1997). These issues could explain by inhibition of GH, GHRH receptor, GH secretagogues (GHS) receptor gene expression in pituitary by dose-dependent insulin administration. The higher insulin level, which presented in DIO rodents, decreased GH and GHRH receptor gene expression approximately 60% compared

with concurrent control rodents. For the gene expression of GHS receptor, it was maximally decreased 50% from the normal level in control animals. It should be noted that the pituitary also responds to the effect of insulin-inhibited GH secretion despite insulin resistance in peripheral organs (Luque and Kineman, 2006). The previous reports revealed that GH gene expression and GH secretion from the cell culture of pituitary was suppressed by insulin (Melmed et al., 1985; Yamashita and Melmed, 1986b; Yamashita and Melmed, 1986a). There was markedly inversed relationship between plasma insulin and GH (Cornford et al., 2011). Additionally, the FFA level also plays as the potential factor on GH secretion. Many studies reported that FFA could inhibit GH release from pituitary (Imaki et al., 1986; Casanueva et al., 1987; Kennedy et al., 1994; Perez et al., 1997; Luque et al., 2006). The GH secretion was suppressed in both basal and GHRH-stimulated pathways. This issue was probably in part mediated by the effect of somatostatin to inhibit GH secretion (Imaki et al., 1986; Casanueva et al., 1987). The elevated FFA level after fasting leads to decrease GH secretion in rats because of the increased somatostatin level (Mlekusch et al., 1975; Tannenbaum et al., 1978). However, fasting could raise GH secretion in human despite the increased FFA level. This result also points out the species-specific to the fasting response. In order that the secretion of GH has related with the other hormonal effect, for instance, hyperleptinemia in obese subjects, the previous study concluded that plasma leptin was not influenced GH secretion in human subjects (Ozata et al., 2003). Nevertheless, the decreased GH secretion in obese subjects was affected due to the various potential factors as mentioned before.

H. Effects of growth hormone on leptin levels

The primary roles of GH on body composition is the reduction of body fat mass. The linkage between GH and body adiposity has been demonstrated; GH-deficient (GHD) patients had higher body adiposity than that of healthy lean subjects (Binnerts et al., 1992; Amato et al., 1993; Rosen et al., 1993; Snel et al., 1995; Fisker et al., 1997), and exogenous GH was given to those patients for improvement of body composition. When GH reduces the body fat mass, it also affects the leptin level because this leptin level is proportional to the body adiposity as well. This issue brings many researchers in the field of obesity to investigate the effect of GH on the leptin production and secretion from adipose tissue (Florkowski et al., 1996; Fisker et al., 1997; Fain and Bahouth, 2000). However, there is a controversial result of GH effect on leptin levels. Some studies reported that GH has effect on circulating leptin level in GHD patients, as aforementioned, GH decreased plasma leptin because it decreased body adiposity (Florkowski et al., 1996; Fisker et al., 1997; Janssen et al., 1997; Nystrom et al., 1997; Rauch et al., 1998). Moreover, there was the study which demonstrated the effect of GH treatment could decrease plasma leptin before the alteration of body composition in GHD subjects (Elimam et al., 1999). On the contrary, treatment with GH increased plasma leptin in GHD patients with elevated plasma IGF-1 and insulin (Bianda et al., 1997), yet plasma leptin was not associated with plasma IGF-1 or insulin (Gill et al., 1999). Additionally, in healthy subjects, acute GH treatment increased plasma leptin within 24 h, then decreased it after the GH bolus for 72 h (Lissett et al., 2001). In other species, GH-treated goats have increased circulating leptin which occurred after 4 days of GH injection (Thammacharoen et al., 2014). For the study

in rodents, it has an evidence that GH may alter plasma leptin in short-term period because short-term GH treatment resulted in anorexia in rats which was the similar ways of leptin action (Malmlof et al., 2002; Malmlof and Johansen, 2003; Malmlof et al., 2011); however, it has been not concluded in the effect of GH on plasma leptin. The anorexia effect after GH treatment may in part mediated by the increased plasma leptin.

When focusing on the mechanisms in which GH regulated leptin production and secretion, many researchers also reported the effect of GH on leptin by using rodents' adipose tissue. It has been demonstrated that GH increased leptin production, by stimulation of leptin mRNA expression, and increased leptin release in part which mediated by dexamethasone or insulin-stimulated leptin release (Hardie et al., 1996; Fain and Bahouth, 2000; Houseknecht et al., 2000; Lee et al., 2001). Interestingly, GH could increase leptin release in which the lipolysis effect did not occur. For instance, GH increased leptin release in the condition that presented insulin and triiodothyronine (T3) (Fain and Bahouth, 2000). From this point, the effect of GH on leptin levels may occur indirectly via modifying other hormonal effects.

I. Effects of growth hormone on plasma insulin and insulin sensitivity

Normally, GH has the significant effect on plasma insulin due to the proliferation of β -cell mass of pancreatic islet (Liu et al., 2004; Kushner, 2006). A previous study reported that the deficiency of GH receptor could lead to diminishing of β -cell mass, also insulin secretion (Liu et al., 2004). The mechanism in which GH has potential role on β -cell mass due to cellular pathway via STAT

(Nielsen et al., 2001). The signal from STAT, especially STAT5, has antiapoptotic function (Socolovsky et al., 1999), also maintain cells and protect from inflammatory pathway (Jensen et al., 2005). Additionally, STAT5 has influenced on cyclin D2 gene expression and protein level which regulate cell cycle, resulting in proliferation of β -cell mass (Friedrichsen et al., 2003). However, obese state has related to insulin resistance which the body response could compensate by elevated insulin secretion. There is an evidence of GH effect on β -cell mass regulation in obese subjects (Wu et al., 2011). It has been noted that GH enhances β -cell proliferation in which respond to glucose-stimulated insulin secretion (GSIS) in hypercaloric-diet feeding rodents (Wu et al., 2011).

The effect of GH on insulin sensitivity has been reported in various pathways. Firstly, GH blunted the disposal of glucose in skeletal muscle (Nørrelund et al., 2003). Therefore, the higher insulin level is required for maintain blood glucose which leads to insulin resistance. Moreover, GH increases circulating FFA because of its lipolytic effect (Jorgensen et al., 1993). Elevated FFA is correlated with muscle lipid accumulation and insulin resistance (Krssak et al., 1999; Perseghin et al., 1999; Boden et al., 2001). The previous study suggested that the high level of circulating FFA plays a role in insulin resistance in muscle (Randle et al., 1963). The mechanism could explain by the substrate competition between glucose and FFA, being an energy source of muscle and adipose tissue. Excess circulating FFA could impair glucose metabolism, including glucose phosphorylation and pyruvate oxidation (Randle et al., 1963). It should be noted that GH effect leads to increase the level of circulating FFA, resulting in the reduction of insulin clearance by liver, also reduction of glucose uptake by muscle (Nam and Marcus, 2000).

Decreased insulin sensitivity after the state of GH excess could be defined by downregulation of insulin-signaling pathway, phosphatidylinositol (PI 3)-kinase (Barbour et al., 2004; Barbour et al., 2005; del Rincon et al., 2007). This cellular pathway plays an important role in the insulin effect to stimulate glucose uptake and inhibit lipolysis in adipocytes (Okada et al., 1994). Furthermore, the structure of PI 3-kinase has 2 subunits, p85 regulatory and p110 catalytic subunits, which combine as dimer. The p85 regulatory subunit controls PI 3-kinase pathway and acts negatively on insulin effect (del Rincon et al., 2007). For instance, rodents with p85 α knockout increased insulin sensitivity (Terauchi et al., 1999; Mauvais-Jarvis et al., 2002) and overexpression of p85 subunit reduced glucose uptake via PI 3-kinase (Ueki et al., 2000). The role of GH which regulates p85 α subunit was reported. The effect of GH could upregulate p85 α gene expression in rodents' adipose tissue and muscle (Barbour et al., 2004; Barbour et al., 2005; del Rincon et al., 2007). Therefore, GH decreases insulin signaling pathway, resulting in blunting of insulin-induced insulin receptor substrate 1 (IRS-1) which is related to decreasing PI 3-kinase pathway (Barbour et al., 2004; Barbour et al., 2005). Furthermore, GH could impair the insulin receptor phosphorylation, also decrease the activity of tyrosine kinase (Smith et al., 1997). On the one hand, it should be noted that some evidence reported GH effect could stimulate tyrosine phosphorylation of IRS-1 in liver and adipose tissue which was not similar as insulin effect because there were phosphorylated at different sites, also not fully activated of PI 3-kinase pathway; therefore, GH treatment could alter cellular signaling (Yamauchi et al., 1998; Takano et al., 2001).

CHAPTER III

MATERIALS AND METHODS

All experimental methods were performed at Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

1. Approvals:

All experimental procedures conducted on animals were approved by Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Science, Chulalongkorn University (protocol #1531026).

2. Animals and animals procedures:

The adult male Wistar rats (n = 28 and 33, for study part 1 and 2, respectively), aged 12 weeks, were purchased from National Laboratory Animal Center (NLAC), Mahidol University. All rats were adapted to the laboratory environment in temperature ($22 \pm 1^\circ\text{C}$) and humidity (55-60%) controlled room with a 12-hour light and dark period (light on 0600 – 1800 h, light off 1800 - 0600 h) at least 1 week prior to the start of the experiment. The rats were individually housed in conventional hanging cage with stainless steel wire-mesh floors (33 x 18 x 20 cm). At this period, the rats were fed with chow (STD diet, CP mice feed 082, Perfect Companion Group Ltd., Samutprakarn, Thailand) and water *ad libitum*.

After the adaptation period, the rats were randomly divided into 2 groups, control and hypercaloric-diet (HC) feeding rats. Both chow and HC were prepared freshly in our laboratory. Daily food intake (23 h FI, ± 0.1 g corrected for spillage) was measured throughout the whole period of experiment. The rats were fed for 6 weeks, also known as fattening period, only HF diet were given during the first 2 weeks, then HF and HFHC diet were given for the last 4 weeks. Then, intraperitoneal glucose tolerance test (IPGTT) were performed in control and DIO rats. This procedure was shown in figure 1.



Figure 1 The HC feeding rats were fed with HC diet for 6 weeks, only HF diet was fed during the first 2 weeks, then HF and HFHC were fed during the last 4 weeks. After 6-week period, IPGTT was performed.

3. Diet preparation:

Pellets of STD diet were cracked into 2-3 pieces/pellet and divided into 2 parts, first part was used for feeding the control rats and another was prepared for high-fat (HF) diet. The HF diet was prepared by mixing the cracked pellet with melting lard (27 g lard/ 73 g STD diet). For HC-feeding rats, there were two types of diet, HF and high-fat and high-carbohydrate (HFHC) diet. For the HFHC diets, they were composed of the sugar-based diet; cookie (KCG Corporation Ltd., Bangkok,

Thailand), and the starch-based diet; cornmeal snack (Pepsi-Cola (Thai) Trading Co., Ltd., Bangkok, Thailand). The nutrient composition of all diets was shown in table 1.

Table 1 Diet composition in this study

Diet	STD chow (CP mice feed 082)	Hypercaloric diet		
		HFD	Cookie	Cornmeal snack
• Protein (%)	24	17.52	4	6
• Carbohydrate (%)	42	30.66	60	62.5
• Fat (%)	4.5	30.3	32	31.25
• Energy (kcal/g)	3.04	4.65	5.6	5.625
• Energy from fat (%)	13	60	50	50

To achieve the objectives of this study, the current work was divided into 2 parts as followed:

Study part 1: The effects of short-term GH administration on basal plasma leptin levels in control and DIO rats

1. Experimental procedures:

When the rats were reaching the aged of 14 weeks, they were divided into 2 groups, control group (n = 14) and the HC group (n = 14). Control rats were fed with cracked STD diet and the HC rats were fed with HF and HFHC diets for 6 weeks as aforementioned. Daily FI was measured by weighing the remaining diet in the food cup and collecting the diet that left on the floor cage before the next

feeding (23 h FI), after that the preweighed-diet was given. Both groups of rats received water *ad libitum*.

After the fattening period, HC rats were selected as the diet-induced obesity (DIO) rats, separated from the diet-resistant (DR) rats. The characteristic of DR rats was that the body weight (BW) gain and final BW were similar to control rats, despite receiving HC diet (Levin and Keesey, 1998). The DR rats were excluded from this experiment. The IPGTT was then performed in control and DIO rats (n = 9, each) for measuring the insulin sensitivity. For IPGTT, the rats were fasted for 16 h overnight and blood glucose was measured. Then, the rats was injected with glucose solution (50% glucose solution, A.N.B. Laboratories Co., Ltd., Bangkok, Thailand) intraperitoneally (2 g/kg BW) (Beguinet and Nigro, 2012). Blood glucose was measured at 15, 30, 60, 90 and 120 minutes following glucose injection using glucose meter (Accu-check Performa, Roche diagnostic (Thailand)). The blood was collected using tail-clipping method. Calculation of the area under the glucose curve (AUC) was performed for determination of the glucose tolerance and the insulin sensitivity (Figure 2).

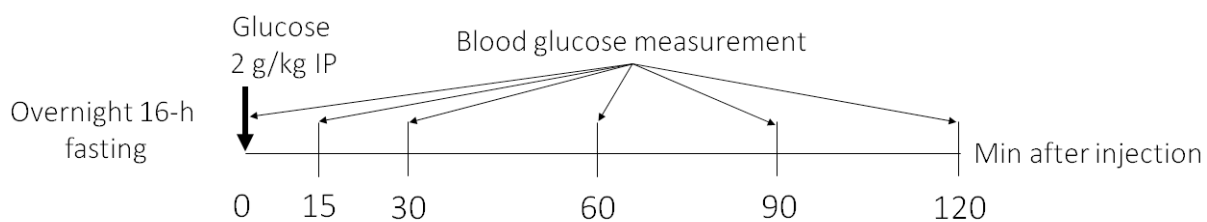


Figure 2 Experimental procedure of IPGTT. First, the rats were fasted for 16 h overnight and blood was collected for glucose measurement. Then, glucose solution (2g/kg) was injected intraperitoneally and blood glucose was measured at 15, 30, 60, 90 and 120 minutes following glucose injection.

Both control and DIO rats were injected with GH (GenHeal, Shanghai United Cell Biotechnology Co., Ltd., Shanghai, China) at a dose of 1 mg/kg, twice daily (0800 h and 1600 h) for 5 days. Daily FI was measured during GH administration. Blood samples (0.3 ml) were collected from the ventral tail artery at 0730 h before GH (day 1), during GH (day 3 and 5) and after GH (day 7) administration for measurement of plasma leptin and IGF-1 (Figure 3). After day 7, the rats were injected with the anesthetic drug (pentobarbital 50 mg/kg IP, Ceva sante animale, Libourne, France) then perfused transcardially with 0.1 M Phosphate buffer saline (PBS, pH 7.4). Body fat, including subcutaneous (interscapular and inguinal fat pads) and intra-abdominal fat (mesenteric, epididymal, perirenal and retroperitoneal fat pads), and liver were dissected and weighed.

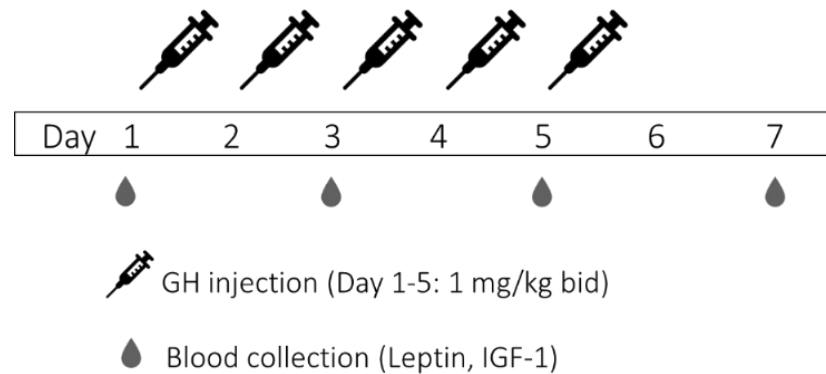


Figure 3 Experimental procedure, GH was injected to control and DIO rats (n = 9, each) from day 1-5. Blood was collected for measuring plasma leptin and IGF-1 at day 1 (before GH injection), day 3, 5 (during GH injection) and day 7 (after stopping GH injection).

2. Analytical procedures for measurements of blood samples

Blood sample preparation

Blood sample was collected in the microtube containing EDTA. The plasma was separated by centrifugation at $3,000 \times g$ for 15 minutes at 4°C and transferred into 3 separate tubes and stored at -20°C for measurement of leptin and IGF-1 using commercial enzyme-linked immunosorbent assay (ELISA) kit (Leptin; EZRL-83K, Merck Millipore, MA, USA and IGF-1; MG100, Quantikine ELISA, R&D Systems, MN, USA).

ELISA method

Plasma leptin and plasma IGF-1 levels were measured by using sandwich ELISA technique. A 96-well flat bottom plate was coated with primary capture antibody (Ab), which binds the antigen (Ag) epitope specifically for rat leptin or IGF-1, respectively. For leptin ELISA kit, assay buffer

(0.05 M Phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.08% sodium azide, 0.5% Triton X-100 and 1% bovine serum albumin (BSA); 40 μ l) and matrix solution (0.08% sodium azide; 10 μ l) were orderly added, followed by plasma samples (10 μ l) and anti-rodent leptin serum (50 μ l). The microplate was incubated for 2 h at room temperature and washed with wash buffer (50 mM Tris buffered saline containing Tween-20). Next, the biotinylated anti-mouse leptin antibody (100 μ l) was added and incubated for 1 h at room temperature. Horseradish peroxidase (HRP)-conjugated streptavidin was added to the wells and incubated for 30 minutes at room temperature. Then, the substrate of this enzyme, tetramethylbenzidine (TMB; 100 μ l) was added and protected from light. After that, stop solution (0.3 M hydrochloric acid (HCl)) was added as the final step. Quantification of the leptin concentration by reading the absorbance at 450 nm and 590 nm was performed by using microplate reader within 5 minutes. For IGF-1 ELISA kit, plasma samples (10 μ l) were diluted with calibrator diluent RD5-38 (buffer BSA solution with preservative) at 1:1000. Then, calibrator diluent RD5-38 (50 μ l) and the diluted sample solution (50 μ l) were added to the wells. The microplate was incubated for 2 h at room temperature and washed with wash buffer. After that, HRP-conjugated mouse/rat IGF-1 Ab (100 μ l) was added and further incubated for 2 h at room temperature. Color reagent A (stabilized hydrogen

peroxide) and color reagent B (TMB) were mixed together and protected from light, then added (100 μl) to each well and further incubated for 30 minutes. Stop solution (0.5 M HCl; 100 μl) was added as the final step. Quantification of the IGF-1 concentration by reading the absorbance at 450 nm and 570 nm was performed by using microplate reader within 30 minutes.

3. Data analysis:

Statistical analysis

The data were presented as mean \pm standard error of mean (SEM). Statistical analysis was performed using GraphPad Prism v.7.00 (GraphPad Software, California, USA). The data of blood glucose from IPGTT at each time point after glucose injection and AUC were compared between groups using unpaired T-test. The amount of FI and EI from control and DIO rats were compared between before and during GH injection using two-way ANOVA, with the main effect of groups and GH treatment, followed by Bonferroni post hoc analysis. Plasma leptin and IGF-1 levels between control and DIO rats were compared before GH (day 1) injection using unpaired T-test. The leptin and IGF-1 levels throughout experiment period within group were compared using one-way repeated measured analysis of variance (ANOVA) and followed by Bonferroni post hoc analysis. A probability value of <0.05 was considered as a significance.

Study part 2: The effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels and insulin function in control, DR and DIO rats

1. Experimental procedures:

All experimental procedures were similar to part 1. Control rats (n = 12) were fed with cracked STD diet and HC rats (n = 21) were fed with HC diet, including HF and HFHC diets, for 6 weeks (Figure 1). Daily FI was measured by weighing the remaining diet in the food cup and collecting the diet that left on the floor cage before the next feeding (23 h FI), after that the preweighed-diet was given. All rats received water *ad libitum*.

After feeding for 6 weeks, HC rats were selected as DIO rats, which were separated from DR rats as similar to those of part 1. In this experiment, DR rats were used to study the short-term GH effect on plasma leptin. Then, IPGTT was performed in control (n = 12), DIO (n = 12) and DR (n = 9) rats for measuring the insulin sensitivity at 1 week before GH administration. The steps of IPGTT was shown in Figure 2. Calculation of AUC for determination of the glucose tolerance and identification of insulin sensitivity.

Control, DR and DIO rats were divided into 2 subgroups, the first subgroup was injected with 0.9% normal saline solution (NSS) and another was injected with GH (GenHeal, Shanghai United Cell Biotechnology Co., Ltd., Shanghai, China) at a dose of 1 mg/kg, twice daily (0800 h and 1600 h) for 3 days. The duration was selected based on results from study part 1, which basal plasma leptin was altered. Blood

samples (0.3 ml) were collected from ventral tail artery for measurement of plasma leptin levels in 3 different conditions. Firstly, blood sample was collected at 0730 h of day 2 during GH administration (at 24 hours after the first GH administration), basal plasma leptin. Next, all rats were fasted for 2 hours before the beginning of dark phase (1800 h), after that they were fed for 2 hours. Blood sample was collected before (pre-meal, 1800 h) and after feeding for 2 hours (post-meal, 2000 h), meal-induced plasma leptin levels (Leibowitz et al., 2006). Lastly, the rats were fasted overnight (16-hour fasting period) then blood sample was collected at the mid-light phase of day 3, fasting condition (Figure 4). The blood sample was collected and measured for fasting plasma insulin and non-esterified fatty acids (NEFA). Then all rats received anesthetic drug (pentobarbital 50 mg/kg IP, Ceva sante animale, Libourne, France) and perfused transcardially with 0.1 M Phosphate buffer saline (PBS, pH 7.4). Body fat, including subcutaneous (interscapular and inguinal fat pads) and intra-abdominal fat (mesenteric, epididymal, perirenal and retroperitoneal fat pads), and liver were dissected and weighed.

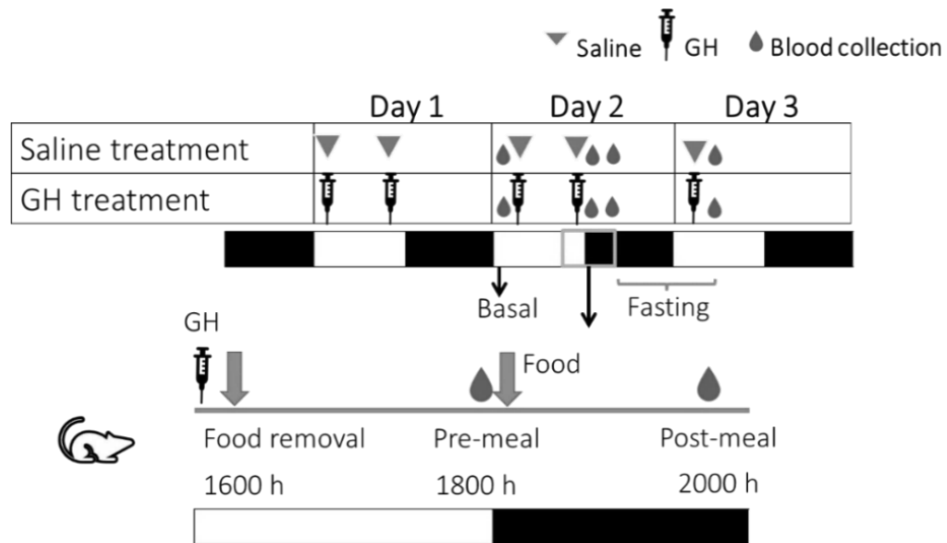


Figure 4 Experimental procedure, either GH or saline was injected to control, DR and DIO rats for 3 days. Blood was collected for measuring plasma hormones, glucose and NEFA:

1. Basal plasma leptin was measured at day 2 (24 h after the first GH injection).
2. Meal-induced plasma leptin was measured at pre- and post-meal (after feeding for 2 h) at 32 and 34 h after the first GH injection, respectively.
3. Fasting plasma leptin, insulin, glucose and NEFA were measured after fasting for 16 h at mid-light phase of day 3.

2. Analytical procedures for measurements of blood samples

Blood sample preparation

Blood sample was prepared using the similar method as study part 1. For measurement of leptin and insulin, commercial ELISA kits, leptin (EZRL-83K, Merck Millipore, MA, USA) and insulin (EZRMI-13K, Merck Millipore, MA, USA) were performed as in study part 1. For NEFA measurement, plasma samples were analyzed by colorimetric assay kit (ab65341, Abcam, Cambridge, UK).

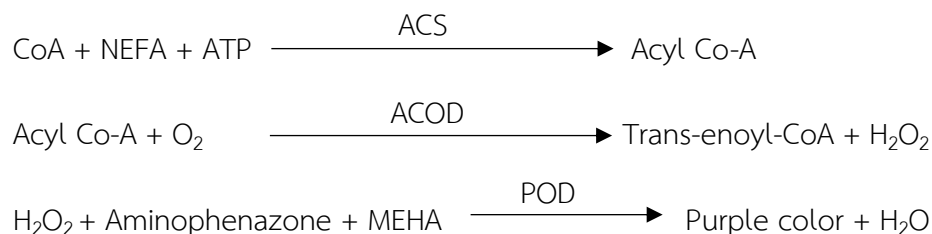
ELISA method

Plasma leptin levels were measured by using sandwich ELISA technique, which was similar to study part 1. For plasma insulin measurement, the 96-well flat bottom plate was coated with anti-rat insulin Ab. Firstly, assay buffer (0.05 M Phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.08% sodium azide, and 1% BSA; 10 μ l) and matrix solution (charcoal stripped pooled mouse serum; 10 μ l) were orderly added. Then, plasma samples (10 μ l) and biotinylated anti-insulin Ab (80 μ l) were added. The microplate was incubated for 2 h at room temperature and washed with wash buffer. Next, HRP-conjugated streptavidin was added and incubated for 30 minutes at room temperature. Then, substrate (TMB; 100 μ l) was added and protected from light. Lastly, stop solution (0.3 M HCl) was added. Quantification of the hormonal concentration by using microplate reader was performed by reading the absorbance at 450 nm and 590 nm within 5 minutes.

The measurement of NEFA

Plasma NEFA was measured using colorimetric assays. Firstly, coenzyme A (CoA) is acylation by NEFA with acyl-CoA synthetase (ACS) in the presence of ATP to form acyl-CoA. Then, acyl-CoA was oxidized by the enzyme acyl-CoA oxidase (ACOD) to form hydrogen peroxide in the presence of peroxidase (POD). Oxidative condensation of 3-methyl-N-ethyl-N-(β -hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form the product in purple color which will be

measured by colorimetric assay at 570 nm (as below). Colorimetric assays for plasma NEFA could detect the C-8 and longer fatty acids.



The procedures of the assay were consisted of several steps. Firstly, plasma sample (50 μl) was added to each well. The reagent of ACS was added and incubated at 37°C for 30 minutes, followed by reaction mix (assay buffer, fatty acid probe, enzyme mix and enhancer; 50 μl). The microplate was further incubated at 37°C for 30 minutes and protected from light. Finally, the concentration was measured by using a microplate reader with the absorbance at 570 nm.

3. Data analysis:

Calculation

The effect of short-term GH administration on meal-induced plasma leptin was represented by the normalized plasma leptin of post-meal to pre-meal levels (%).

The surrogate indexes of insulin resistance were calculated as follows:

The homeostasis model of insulin resistance (HOMA-IR) was calculated from the following equation (Cacho et al., 2008):

$$\text{HOMA-IR} = \frac{\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mg/dL)}}{2,430}$$

This HOMA-IR indicates the insulin resistance to maintain glucose homeostasis. The greater values represent the worsen insulin sensitivity.

Index of adipose tissue insulin resistance (Adipo-IR) was calculated from the following equation:

$$\text{Adipo-IR} = \text{fasting NEFA (mmol/l)} \times \text{fasting insulin } (\mu\text{U/ml})$$

This equation was derived from the linear relationship between increased fasting plasma insulin and decreased fasting plasma NEFA in normal human beings (Groop et al., 1989). Normally, insulin was able to restrict the NEFA which released from adipose tissue. Higher plasma insulin and NEFA in the same time showed the markedly defect of insulin function (Gastaldelli et al., 2009; Lomonaco et al., 2012). This Adipo-IR indicates the insulin resistance to inhibit adipose tissue lipolysis. The greater values represent the worsen insulin sensitivity at adipose tissue.

Statistical analysis

The data were presented as mean \pm SEM. Statistical analysis were performed using GraphPad Prism v.7.00 (GraphPad Software, California, USA). The analysis of basal plasma leptin, pre-meal plasma leptin and the energy intake for 2 h were done using two-way ANOVA, with the main effect of groups

and GH treatment. Fasting plasma leptin was compared to basal plasma leptin within group using paired T-test. Additionally, the analysis of blood glucose from IPGTT between groups at each time point was done using one-way ANOVA. Plasma insulin, glucose and NEFA were analyzed by two-way ANOVA, with the main effect of groups and GH treatment. The significant main effects were followed using a Bonferroni post-test. A probability value of <0.05 was considered as a significance.



CHAPTER IV

RESULTS

Study part 1: The effects of short-term GH administration on plasma leptin levels in control and DIO rats

A. Characterization of DIO and DR rats

Following 6-week fattening period, there were two groups of rats i.e. DIO and DR. The characters of DIO rats were composed of higher BW, BW gain and body fat mass when compared with control rats; while DR rats had similar BW compared with control rats and lower BW gain than that of DIO rats. In this study, there were 9 rats considered as DIO (64%) and 5 rats considered as DR (36%) as shown in figure 5. The DR rats were excluded. The final BW and BW gain of DIO rats were markedly higher than those of control rats (Table 2; $t_{16} = 3.22$ and 7.08 ; $P < 0.01$ and $P < 0.001$, respectively). The results from IPGTT showed the non-significant difference of blood glucose at fasting state (time 0; $t_{16} = 1.95$, $P > 0.05$), while higher blood glucose after glucose injection in DIO rats was occurred when compared with control rats (Figure 6; $t_{16} = 3.46, 2.74, 3.37, 2.71, 2.48$ for 15, 30, 60, 90, 120 minutes after glucose injection, respectively; $P < 0.05$ for 30, 90, 120 minutes and $P < 0.01$ for 15, 60 minutes). Additionally, DIO rats showed higher AUC than that of control rats (Table 2; $t_{16} = 3.34$; $P < 0.01$).

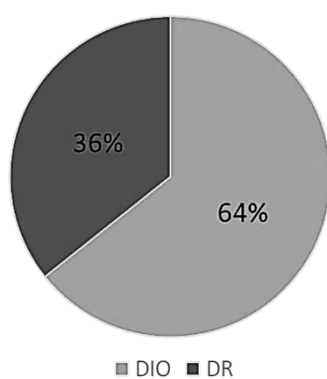


Figure 5 Percentage of DIO (n = 9/ 14, 64%) and DR (n = 5/ 14, 36%) rats from study part 1.

Table 2 The characteristic of control and DIO rats.

	Control (n = 9)	DIO (n = 9)
Initial BW (g)	423.52 ± 11.68	434.70 ± 11.39
Final BW (g)	500.82 ± 15.79	575.94 ± 17.14**
BW gain (g)	77.30 ± 5.58	141.24 ± 7.10***
BW gain/ day (g/day)	1.93 ± 0.14	3.53 ± 0.18***
Body fat mass (%)	10.24 ± 0.80	17.20 ± 0.60***
AUC-IPGTT	17503 ± 1574	27296 ± 2472**

** $P < 0.01$, *** $P < 0.001$ when compared to control using unpaired t-test

AUC-IPGTT: area under the curve from intraperitoneal glucose tolerance test

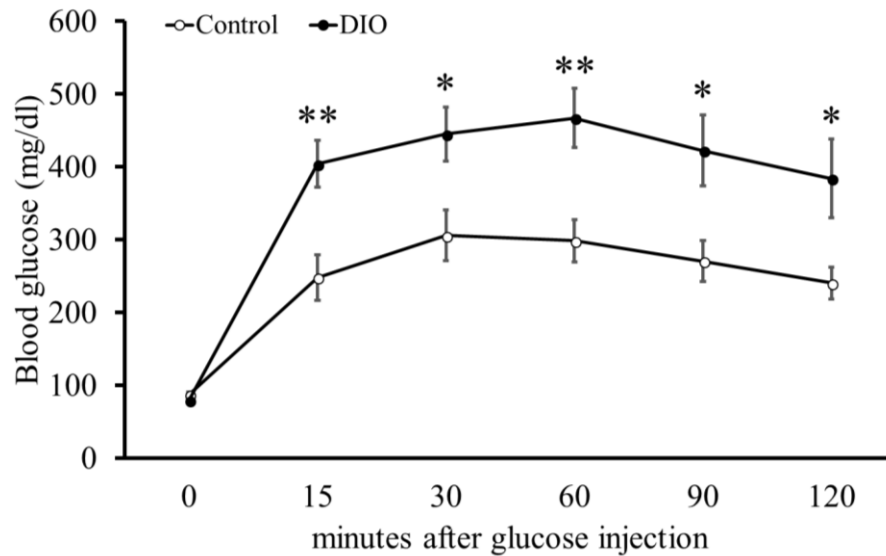


Figure 6 Glucose curve from IPGTT. From 15 minutes to 120 minutes after glucose injection, blood glucose from DIO rats was significantly higher than that of control rats. * $P < 0.05$, ** $P < 0.01$ when compared to control using unpaired *t*-test.

B. Effects of short-term GH administration on plasma leptin in control and DIO rats

Both control and DIO rats showed the positive correlation of plasma leptin levels and body adiposity (Figure 7; $r = 0.93$, $P < 0.001$). Additionally, DIO rats had higher plasma leptin than that of control rats before GH treatment (Figure 8; $t_{16} = 7.19$; $P < 0.001$). During the period of GH treatment, plasma leptin levels were decreased in DIO rats (Figure 8; $F_{3,24} = 5.79$; $P < 0.01$), which rebounded after stopping GH administration. The significant effect was occurred at day 3 and day 5 after GH administration (Figure 8; $t_{24} = 2.66$ and 3.38 ; $P < 0.05$, respectively). On the contrary, plasma leptin levels of control rats did not alter throughout the period of GH injection (Figure 8; $F_{3,24} = 2.50$; $P > 0.05$).

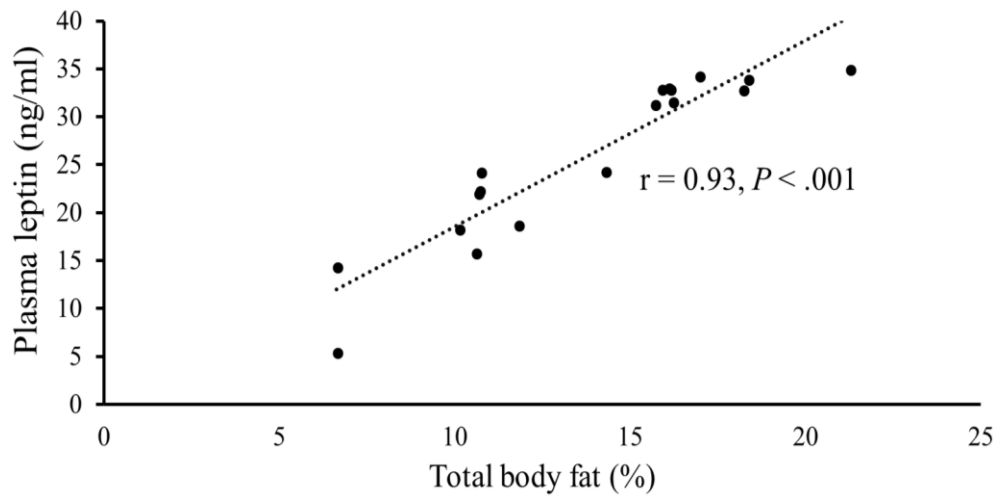


Figure 7 The correlation between plasma leptin and relative body fat mass (%) in control and DIO rats.

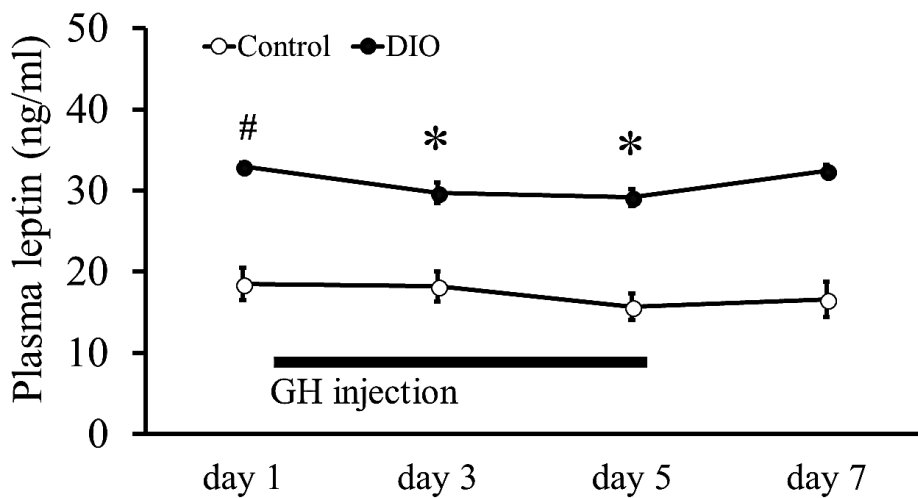
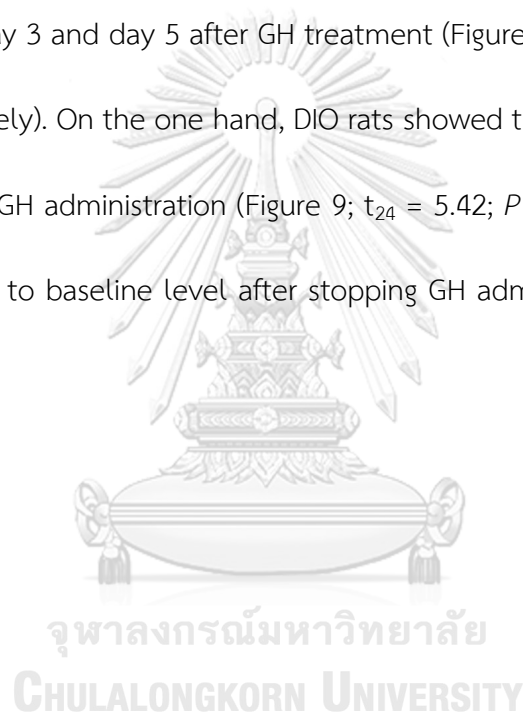


Figure 8 Basal plasma leptin in control and DIO rats before GH treatment (day1), during GH treatment (day 3 and 5) and after stopping GH (day 7). Short-term GH treatment decreased basal plasma leptin significantly at day 3 and day 5 in DIO rats. The black bar indicates the duration of GH injections. * $P < 0.05$ when compared to day 1 within group using one-way repeated measured ANOVA followed by Bonferroni post-hoc. # $P < 0.01$ when compared to control at day 1 using unpaired t-test.

C. Effects of short-term GH administration on plasma IGF-1 in control and DIO rats

Circulating IGF-1 at baseline level, before GH injection, was not significantly different between control and DIO rats (Figure 9; $t_{16} = 2.10$; $P > 0.05$). Plasma IGF-1 in control and DIO rats were increased (Figure 9; $F_{3,24} = 24.05$ and 11.93 ; $P < 0.05$) after short-term GH administration. In control rats, increased plasma IGF-1 was detected at day 3 and day 5 after GH treatment (Figure 9; $t_{24} = 4.15$ and 2.78 ; $P < 0.05$, respectively). On the one hand, DIO rats showed the increased plasma leptin at day 5 after GH administration (Figure 9; $t_{24} = 5.42$; $P < 0.05$). Moreover, plasma IGF-1 returned to baseline level after stopping GH administration in both control and DIO rats.



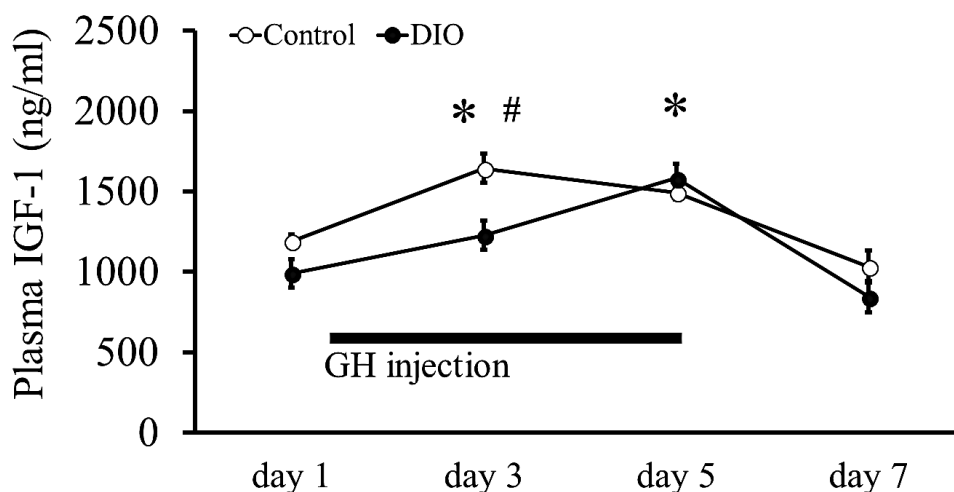


Figure 9 Plasma IGF-1 was increased after GH treatment in control and DIO rats. In DIO rats, plasma IGF-1 was increased significantly at day 5. However, plasma IGF-1 was increased at day 3 and 5 in control rats. On day 7, plasma IGF-1 from both groups was decreased to the pre-treatment level of day 1. The black bar indicates the duration of GH injections. * $P < 0.05$ when compared to day 1 within group using one-way repeated measured ANOVA followed by Bonferroni post-hoc. # $P < 0.05$ when compared to control at day 3 using unpaired t-test.

D. Effects of short-term GH administration on food intake and energy intake in control and DIO rats

The effect of short-term GH treatment on decreased FI was reported (Figure 10; $F_{1,32} = 27.51$; $P < 0.001$). The significant effect was found in control (Figure 10; $t_{32} = 4.50$; $P < 0.001$) and DIO rats (Figure 10; $t_{32} = 2.92$; $P < 0.05$). It was noted that control rats showed higher FI than that of DIO rats (Figure 10; $F_{1,32} = 4.50$; $P < 0.05$) before GH treatment. Further, energy intake (EI) was also decreased after short-term GH administration (Figure 11; $F_{1,32} = 25.13$; $P < 0.001$). The significant effect was found in control (Figure 11; $t_{32} = 3.46$; $P < 0.01$) and DIO rats (Figure 11; $t_{32} =$

3.63; $P < 0.01$). There was markedly significant higher EI in DIO rats than that of control rats (Figure 11; $F_{1,32} = 108.40$; $P < 0.001$) before and after GH treatment.

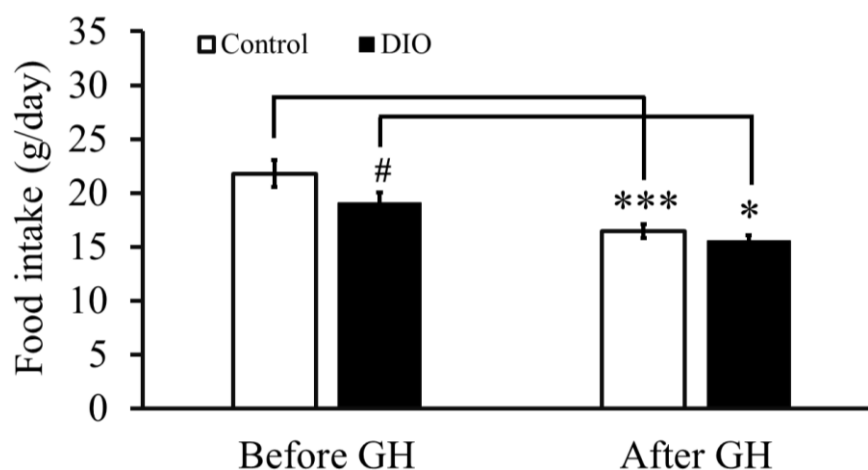


Figure 10 Short-term GH treatment decreased FI in both control and DIO rats. ** $P < 0.01$, *** $P < 0.001$ when compared to before GH treatment within group using two-way ANOVA followed by Bonferroni post-hoc. # $P < 0.05$ when compared to control at the same time point using two-way ANOVA followed by Bonferroni post-hoc.

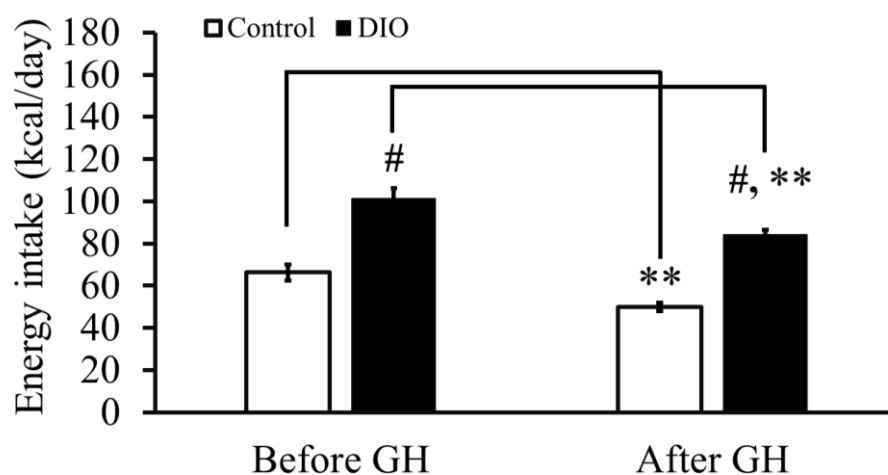


Figure 11 Short-term GH treatment decreased EI in both control and DIO rats. Additionally, EI was significantly lower in control rats than that of DIO rats. ** $P < 0.01$ when compared to before GH treatment within group using two-way ANOVA followed by Bonferroni post-hoc. # $P < 0.001$ when compared to control at the same time point using two-way ANOVA followed by Bonferroni post-hoc.

E. Body adiposity and liver of GH-treated control and DIO rats

Adipose tissue and liver of all rats were weighed and collected. The results revealed that DIO rats had higher total and relative weight of adipose tissue than that of control rats (Table 3; $t_{16} = 5.31$ and 6.97 ; $P < 0.001$, respectively). Likewise, DIO rats had higher wet weight and relative weight of liver than that of control rats (Table 3; $t_{16} = 3.99$ and 3.19 ; $P < 0.01$, respectively). The gross appearance of adipose tissue and liver in control and DIO rats was shown in figure 12.

Table 3 Body adiposity and liver weight of control and DIO rats.

	Control (n = 9)	DIO (n = 9)
Total body fat (g)	53.39 ± 5.90	102.07 ± 7.00***
Relative fat mass (%)	10.24 ± 0.80	17.20 ± 0.60***
Liver weight (g)	13.34 ± 0.33	17.83 ± 1.08**
Relative liver weight (%)	2.62 ± 0.06	3.02 ± 0.11**

** $P < 0.01$, *** $P < 0.001$ when compared to control using unpaired t-test.

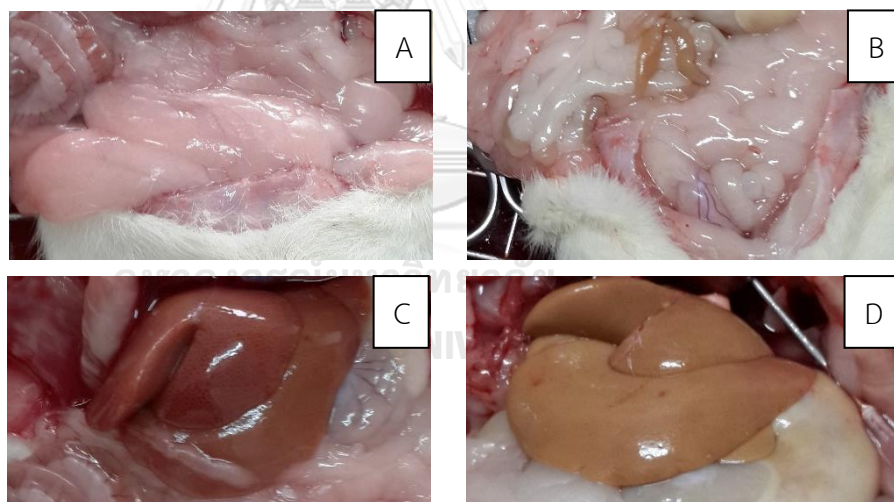


Figure 12 Gross appearance of adipose tissue and liver in control (A, C) and DIO (B, D) rats. Higher amount of fat mass and enlarged liver with pale color was found in DIO rats.

Study part 2: The effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels and insulin function in control, DR and DIO rats

A. Characterization of DIO and DR rats

Similar to the previous study, there were 12 DIO and 9 DR rats (57% and 43%, respectively; figure 13). The final BW and BW gain of DIO rats were significantly higher than those of DR and control rats (Table 4; $F_{2,30} = 38.26$; $t_{30} = 6.90$ and 7.96 respectively; $P < 0.001$). Additionally, the results from IPGTT revealed the markedly difference of blood glucose at 60 and 90 minutes after glucose injection (Figure 14; $F_{2,30} = 4.83$ and 3.33 respectively; $P < 0.05$), and DIO rats had higher blood glucose concentration than that of control rats (Figure 14; $t_{30} = 2.74$ and 2.54 at 60 and 90 minutes after glucose injection, respectively; $P < 0.05$). The AUC of DIO rats tended to be greater than control rats but it was not reach to significant difference (Table 4; $F_{2,30} = 3.30$; $t_{30} = 2.18$; $P = 0.05$). Furthermore, the result from IPGTT in DR rats revealed that were not significantly difference of blood glucose concentration and AUC when compared with control rats.

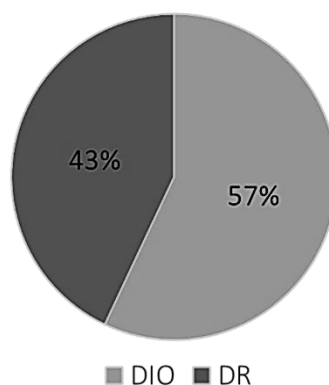


Figure 13 Percentage of DIO (n = 12/ 21, 57%) and DR rats (n = 9/ 21, 43%) from study part 2.

Table 4 The characteristic of control, DR and DIO rats.

	Control (n = 12)	DR (n = 9)	DIO (n = 12)
Initial BW (g)	441.02 ± 2.93		435.70 ± 5.29
Final BW (g)	503.68 ± 3.41 ^b	508.27 ± 7.73 ^b	574.52 ± 7.98 ^a
BW gain (g)	62.67 ± 1.93 ^c	88.08 ± 4.68 ^b	127.19 ± 5.80 ^a
BW gain/ day (g/day)	1.57 ± 0.05 ^c	2.20 ± 0.12 ^b	3.18 ± 0.14 ^a
Body fat mass (%)	11.25 ± 0.30 ^c	12.92 ± 0.41 ^b	14.97 ± 0.31 ^a
AUC-IPGTT	24667 ± 1303	24304 ± 1198	28411 ± 1242

The different letters (a, b, c) represent the significant difference within a row, $P < 0.05$, using one-way ANOVA followed by Bonferroni post-hoc.

AUC-IPGTT: area under the curve from intraperitoneal glucose tolerance test

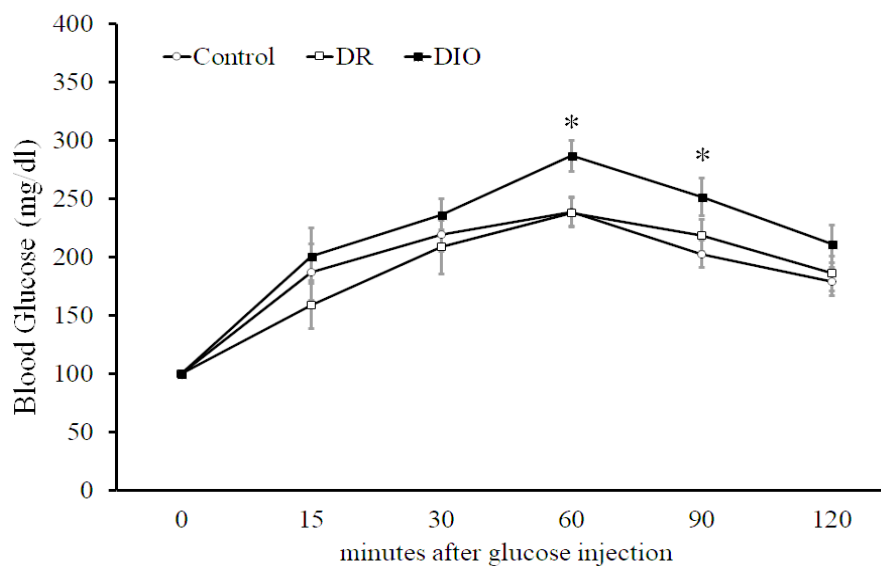


Figure 14 Glucose curve from IPGTT. Blood glucose from DIO rats was significantly higher than that of control rats at 60 and 90 minutes after glucose injection. * $P < 0.05$ when compared to control rats at the same time point using one-way ANOVA followed by Bonferroni post-hoc.

B. Effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels in control, DR and DIO rats

Plasma leptin at basal condition was measured at 24 h after the first GH administration. Additionally, plasma leptin at 32 h (pre-meal) was considered as basal condition, because the levels of plasma leptin in light period were still in the range of basal plasma leptin (Ahren et al., 2000; Bodosi et al., 2004). For basal condition, two main effects, different time points and GH treatment, were considered in control, DR and DIO rats. In GH-treated control rats, plasma leptin at 24 h and 32 h after the first injection was not significantly different (Table 5; $F_{1,10} = 0.45$; $P > 0.05$). Likewise, there was no significant effect of GH treatment (Table 5;

$F_{1,10} = 0.03$; $P > 0.05$). In DR rats, there was also no effect of time points and GH treatment on basal plasma leptin (Table 5; $F_{1,7} = 0.86$ and 0.76 , respectively; $P > 0.05$). However, DIO rats showed the significant effects of time points and GH treatment (Table 5; $F_{1,10} = 40.84$ and 7.36 , respectively; $P < 0.05$). The effect of time points was found in saline and GH-treated DIO rats (Table 5; $t_{10} = 5.77$ and 3.27 , respectively; $P < 0.05$). The effect of GH was pronounced at 32 h after the first GH injection (Table 5; $t_{20} = 3.20$; $P < 0.05$). Furthermore, the normalized basal plasma leptin to body fat mass was decreased by GH treatment only in DIO rats (Figure 15; $F_{1,27} = 7.74$; $t_{27} = 3.43$; $P < 0.05$) but not in control and DR rats (Figure 15; $t_{27} = 0.53$ and 0.98 , respectively; $P > 0.05$).

For the meal-induced condition, two main effects, meal and GH treatment, were considered in control, DR and DIO rats. Only DIO rats showed the significant effect of meal on plasma leptin (Figure 16; $F_{1,10} = 20.10$; $P < 0.05$). However, the effect of GH on meal-induced plasma leptin was analyzed by the normalized plasma leptin from post- and pre-meal. The result revealed that GH did not affect meal-induced plasma leptin in all rats (Figure 17; $F_{1,27} = 0.01$; $P > 0.05$). Moreover, the 2-h energy intake of all rats was analyzed, DIO rats had higher EI than that of control and DR rats (Figure 18; $F_{2,27} = 3.66$; $P < 0.05$) and GH did not affect the 2-h EI in all groups (Figure 18; $F_{1,27} = 0.26$; $P > 0.05$).

Table 5 The effect of short-term GH treatment on basal plasma leptin at 24 h and 32 h after GH injection in control (n = 6, each), DR (n = 4 for saline and n = 5 for GH) and DIO rats (n = 6, each).

Time after the first injection	Basal plasma leptin (ng/ml)		
	Control	DR	DIO
Saline			
● 24 h	16.98 ± 0.7	18.93 ± 2.7	21.16 ± 1.0
● 32 h	17.94 ± 1.2	18.47 ± 1.9	29.20 ± 1.8*
GH			
● 24 h	16.89 ± 0.8	15.74 ± 0.9	17.00 ± 1.8
● 32 h	17.44 ± 2.5	18.38 ± 0.8	21.55 ± 2.0* [#]

* $P < 0.05$ when compared between different time points within group using two-way ANOVA followed by Bonferroni post-hoc.

$P < 0.05$ when compared to saline treatment at the same time within group using two-way ANOVA followed by Bonferroni post-hoc.

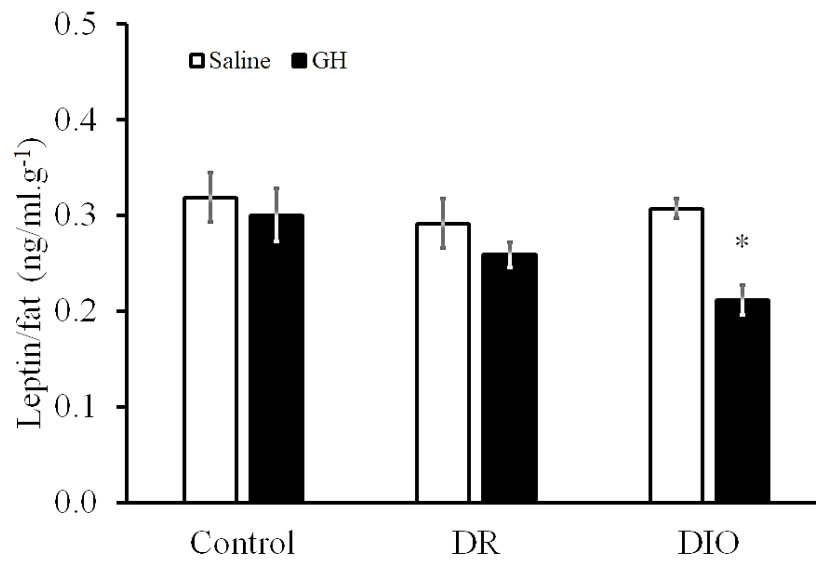


Figure 15 The effect of short-term GH treatment on plasma leptin per fat mass. There was the significant effect of GH on leptin per fat mass in DIO rats. * $P < 0.05$ when compared to saline treatment using two-way ANOVA followed by Bonferroni post-hoc.

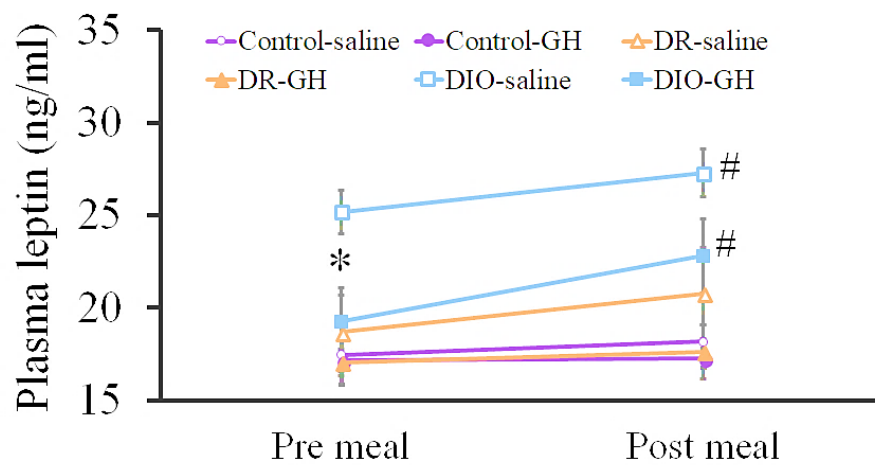


Figure 16 Meal-induced plasma leptin in control, DR and DIO rats. There was the significantly effect of meal on plasma leptin only in DIO rats. * $P < 0.05$ when compared to saline treatment at the same time using two-way ANOVA followed by Bonferroni post-hoc. # $P < 0.05$ when compared to plasma leptin at pre meal using two-way ANOVA followed by Bonferroni post-hoc.

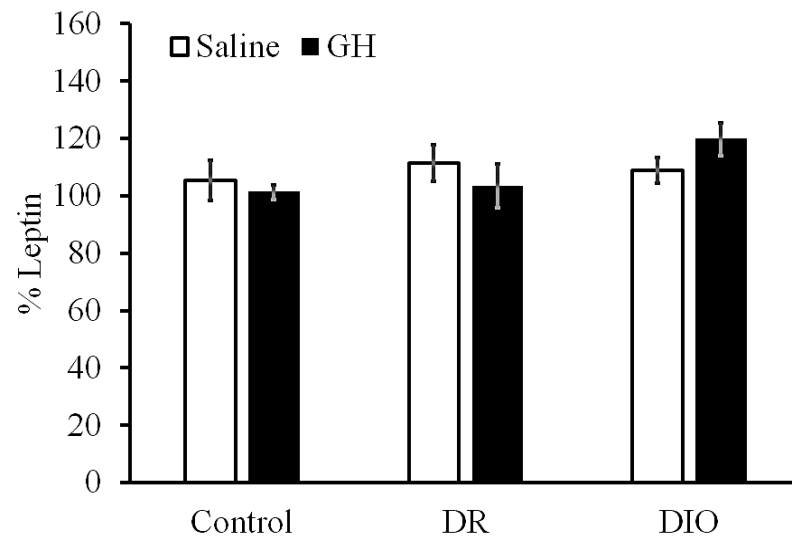


Figure 17 The effect of short-term GH treatment on meal-induced plasma leptin in control, DR and DIO rats was shown as the normalized leptin of post- and pre-meal (%). There was no effect of GH on meal-induced plasma leptin in all rats.

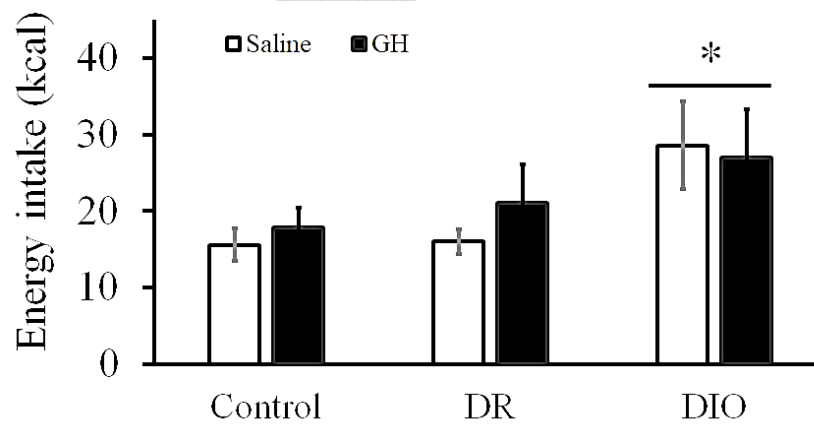


Figure 18 The energy intake from meal-induced plasma leptin study revealed that DIO rats had higher energy intake over both control and DR rats. * $P < 0.05$ when compared between groups using two-way ANOVA followed by Bonferroni post-hoc.

For the 16 h fasting condition, fasting plasma leptin was significantly lowered than that of basal condition in saline-treated control (Table 6; $t_5 = 7.76$, $P < 0.001$) and DR rats (Table 6; $t_3 = 10.86$, $P < 0.01$), except in DIO rats (Table 6; $t_5 = 0.78$, $P > 0.05$). Moreover, GH blunted the fasting effect of plasma leptin in control and DR rats, thus fasting plasma leptin was not different from basal plasma leptin (Table 6; $t_5 = 1.51$ and $t_4 = 1.94$ for control and DR rats, respectively; $P > 0.05$). For GH-treated DIO rats, there was not different between basal and fasting plasma leptin (Table 6; $t_5 = 0.15$, $P > 0.05$).

Table 6 The effect of short-term GH treatment on fasting plasma leptin in control ($n = 6$, each), DR ($n = 4$ for saline and $n = 5$ for GH) and DIO rats ($n = 6$, each).

Treatment	Plasma leptin (ng/ml)		
	Control	DR	DIO
Saline			
• Basal	16.98 ± 0.7	18.93 ± 2.7	21.16 ± 1.0
• Fasting	11.91 ± 0.9***	12.82 ± 2.8**	19.59 ± 2.1
GH			
• Basal	16.89 ± 0.8	15.74 ± 0.9	17.00 ± 1.8
• Fasting	15.29 ± 1.5	17.62 ± 1.0	17.15 ± 1.6

** $P < 0.01$, *** $P < 0.001$ when compared to basal condition within group using paired t-test.

C. Effects of short-term GH administration on insulin function in control, DR and DIO rats

a. Effects of short-term GH administration on plasma insulin, glucose and NEFA

There was significantly different of short-term GH effect on fasting plasma insulin in all rats (Table 7; $F_{1,27} = 12.92$; $p < 0.05$). However, there was no significantly different of GH effect on fasting plasma glucose and NEFA (Table 7; $F_{1,27} = 0.04$ and 2.29 , respectively; $P > 0.05$) in all rats.

b. Effects of short-term GH administration on HOMA-IR and Adipo-IR

The GH-treated group had higher calculated HOMA-IR than that of saline-treated group (Figure 19; $F_{1,27} = 12.59$; $p < 0.05$), especially in DR rats (Figure 19; $t_{27} = 2.76$; $P < 0.05$), but not in control (Figure 19; $t_{27} = 2.46$, $P > 0.05$) and DIO rats (Figure 19; $t_{27} = 0.83$; $P > 0.05$). The calculated Adipo-IR from saline-treated group was also significantly different when compared with GH-treated group (Figure 20; $F_{1,27} = 9.04$; $P < 0.05$). This GH effect was pronounced in DR rats (Figure 20; $t_{27} = 2.91$; $P < 0.05$), but not in control (Figure 20; $t_{27} = 1.16$; $P > 0.05$) and DIO rats (Figure 20; $t_{27} = 0.96$; $P > 0.05$).

D. Effects of short-term GH administration on body adiposity and liver weight in control, DR and DIO rats

The short-term GH administration had no effect on body adiposity of all rats (Table 8; $F_{1,27} = 0.85$; $P > 0.05$), while DIO rats had the higher fat mass than that of DR and control rats (Table 8; $F_{2,27} = 33.17$; $P < 0.05$). Likewise, GH had no effect

on liver weight (Table 8; $F_{1,27} = 3.02$; $P > 0.05$), while DIO rats had higher weight and relative weight of liver than that of DR and control rats (Table 8; $F_{2,27} = 18.21$ and 5.81, respectively; $P < 0.05$).

Table 7 The effect of short-term GH treatment on fasting plasma insulin, glucose and NEFA in control (n = 6, each), DR (n = 4 for saline and n = 5 for GH) and DIO rats (n = 6, each).

	Control	DR	DIO
Insulin (ng/mL)			
● Saline	2.96 ± 0.5	2.39 ± 0.7	5.12 ± 0.8
● GH	6.09 ± 1.0 [†]	6.26 ± 1.4 [†]	6.92 ± 1.2 [†]
Glucose (mg/dL)			
● Saline	131.50 ± 6.8	126.00 ± 6.0	131.50 ± 4.0
● GH	135.20 ± 3.0	126.80 ± 12.8	123.30 ± 7.7
NEFA (mmol/L)			
● Saline	0.55 ± 0.13	0.29 ± 0.06	0.60 ± 0.14
● GH	0.45 ± 0.10	0.81 ± 0.12	0.67 ± 0.17

[†] $P < 0.05$ significant main effect for comparison of GH and saline treatment using two-way ANOVA followed by Bonferroni post-hoc.

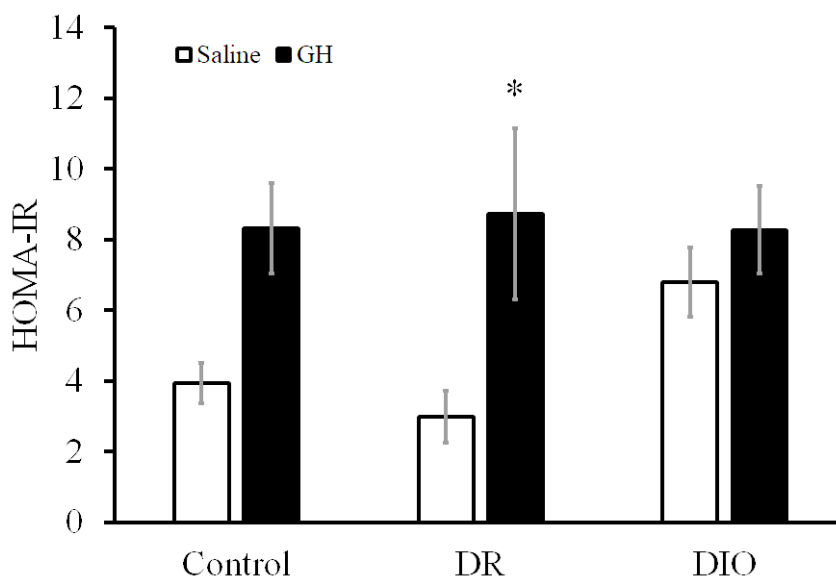


Figure 19 The effect of short-term GH treatment on HOMA-IR. It was noted that GH effect induced insulin resistance in DR rats. * $P < 0.05$ when compared to saline treatment using two-way ANOVA followed by Bonferroni post-hoc.

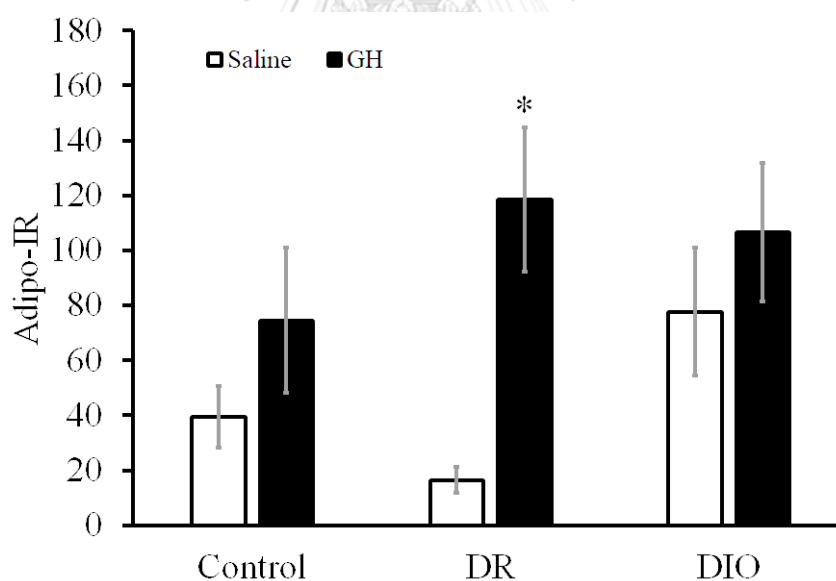


Figure 20 The effect of short-term GH treatment on Adipo-IR. The result revealed that the effect of GH can induce insulin resistance in DR rats. * $P < 0.05$ when compared to saline treatment using two-way ANOVA followed by Bonferroni post-hoc.

Table 8 The effect of short-term GH treatment on body adiposity and liver weight of control (n = 6, each), DR (n = 4 for saline and n = 5 for GH) and DIO rats (n = 6, each).

	Control	DR	DIO
Total body fat (g)			
● Saline	55.63 ± 2.53 ^c	64.28 ± 5.52 ^b	82.07 ± 3.45 ^a
● GH	57.58 ± 2.35	66.24 ± 2.83	90.47 ± 3.39
Relative body fat (%)			
● Saline	11.25 ± 0.46 ^c	12.73 ± 0.78 ^b	14.59 ± 0.37 ^a
● GH	11.24 ± 0.42	13.07 ± 0.48	15.36 ± 0.47
Liver weight (g)			
● Saline	12.32 ± 0.28 ^b	12.90 ± 0.73 ^b	14.83 ± 0.67 ^a
● GH	12.32 ± 0.24	14.14 ± 0.68	15.87 ± 0.51
Relative liver weight (%)			
● Saline	2.49 ± 0.05 ^c	2.56 ± 0.08 ^b	2.64 ± 0.08 ^a
● GH	2.40 ± 0.03	2.79 ± 0.14	2.69 ± 0.07

The different letters (a, b, c) represent the significant main effect for comparison between groups ($P < 0.05$) using two-way ANOVA followed by Bonferroni post-hoc.

CHAPTER V

DISCUSSION

Study part 1: The effects of short-term GH administration on plasma leptin levels in control and DIO rats

Obesity is metabolic syndrome that markedly identified by increasing of body fat mass. Feeding the rats with HC diets, both HF and HFHC diets, for 6 weeks could induce obesity. However, outbred laboratory rats displayed 2 characters after HC feeding, first was DIO rats and another was DR rats. DIO rats had higher BW gain, final BW and body fat mass than that of control rats. By contrast, DR rats had the similar final BW as control rats and the lower BW gain and body fat mass than that of DIO rats.

By using GH as exogenous hormonal stimulus, the current findings revealed the different ways controlling basal plasma leptin. Basal plasma leptin from DIO rats, but not from control rats, was decreased on day 3 after GH administration. Our results are consistent with the chronological study of acute GH treatment on basal plasma leptin in healthy humans (Lissett et al., 2001). Unfortunately, many experiments that have been done in rodents were considered as a long-term GH effect on plasma leptin or as cross-sectional information (Boni-Schnetzler et al., 1999; Malmlof and Johansen, 2003; Malmlof et al., 2011). The results suggested that GH had an influence on basal plasma leptin which depended in part on the different amount of adipose tissue. Circadian variation of plasma leptin has been well demonstrated in rodents. Plasma leptin is maintained at the basal level during the light phase and gradually increases

after the first meal of dark onset (Ahren et al., 2000; Bodosi et al., 2004). The single peak of plasma leptin in rodents at the dark phase could be strongly influenced by eating (Ahren et al., 2000; Bodosi et al., 2004). Both insulin and glucose are essential for these issues (Levy et al., 2000; Cammisotto and Bukowiecki, 2002; Walker et al., 2005). Although many experiments have been done to study nighttime plasma leptin, the mechanism controlling basal plasma leptin remains unclear.

The effect of short-term GH treatment increased plasma IGF-1 in both groups of rats. However, this result was different from the GH effect on basal plasma leptin in 2 ways. First, plasma IGF-1 responded to GH treatment was occurred in all rats, whereas basal plasma leptin significantly decreased only in DIO rats. Second, plasma IGF-1 was elevated in DIO rats later than decreased basal plasma leptin. Although it had been shown that IGF-1 rather than GH infusion decreases plasma leptin (Boni-Schnetzler et al., 1999), this information is different from our present experiment in many ways, and importantly, recombinant human IGF-1 infusion failed to suppress IGF-1, similar to other information (Isozaki et al., 1999). Therefore, it is unlikely that the short-term effect of GH treatment on basal plasma leptin was mediated by IGF-1 dependent pathway.

Short-term GH administration led to decrease FI and EI in control and DIO rats. This result is consistent with our previous finding in goats (Thammacharoen et al., 2014) and supports the previous studies in GH-treated obese rats (Malmlof et al., 2002; Malmlof and Johansen, 2003; Malmlof et al., 2011). Additionally, our study found the

similar trend of decreased FI after GH administration in control rats. The previous study reported that the reduced FI in GH-treated rats was derived from accelerated lipid oxidation rate (Malmlof et al., 2011). The GH effect was not only protein anabolic effect but also calorogenic and lipolytic effect (Moller and Jorgensen, 2009). Therefore, the decreased FI effect was occurred in subjects in which positive energy balance and low protein anabolic drive, as adulthood. By contrast, the increased FI effect was occurred in young-growing rats treated with GH because they have high protein anabolic drive (Roberts et al., 1995; Malmlof et al., 2011). It can be concluded that adult rats fed diet *ad libitum* in which excess ATP and low protein anabolic drive have decreased FI after GH administration. The results suggest that GH prefer calorogenic and lipolytic effect in adult rats. Additionally, the short-term effect of GH treatment on FI and energy intake is apparently due to the direct effect of GH rather than to the actions of GH-induced plasma leptin. For the characteristics of adipose tissue and liver weight, DIO rats had higher weight of fat mass and liver. It should be noted that fat accumulation was found in adipose tissue and liver, leading to nonalcoholic fatty liver disease in DIO rats (Carmiel-Haggai et al., 2005).

Study part 2: The effects of short-term GH administration on basal, meal-induced and fasting plasma leptin levels and insulin function in control, DR and DIO rats

Our results showed that short-term GH administration decreased basal plasma leptin only in DIO rats. However, the short-term GH treatment could not affect meal-induced plasma leptin in control, DR and DIO rats. Additionally, fasting plasma leptin

in control and DR rats was influenced by GH treatment. The results suggested that the effect of short-term GH treatment on plasma leptin in rats depended in part on body adiposity and energy status.

The present experiment aimed at investigating the effect of short-term GH treatment on plasma leptin in different conditions. For basal condition, we measured plasma leptin at the light-phase after 24 h of the first GH injection. The result revealed that GH had no effect on basal leptin. However, the level of pre-meal plasma leptin in GH-treated DIO rats was lower than that of saline-treated DIO rats. It should be noted that all rats were not allowed to access the food approximately for an hour before the onset of darkness during the maintenance period every day. Therefore, all rats were fasted only 2 h for pre-meal plasma leptin measurement. We consider that the level of pre-meal plasma leptin is still in the range of basal plasma leptin (Ahren et al., 2000; Bodosi et al., 2004). Thus, the effect of GH on pre-meal plasma leptin could represent the GH effect on basal plasma leptin in which demonstrated only in DIO rats. In this regard, body adiposity apparently affects basal plasma leptin. Leptin secretion and its content have been correlated with the size of adipocyte (Levy et al., 2000; Lee et al., 2007). However, the reason which short-term GH treatment influenced basal plasma leptin only in DIO rats still remains unknown.

We reported that short-term GH treatment had no effect on meal-induced plasma leptin by analysis of leptin difference. Meal-induced plasma leptin is one phenomenon that has been studied in rodents, which related to nighttime peak of

plasma leptin. It should be noted that the peak of plasma leptin was related to eating behavior (Ahren et al., 2000; Bodosi et al., 2004). If the diet were provided only in light period, the diurnal pattern of plasma leptin was switched according to eating behavior of rodents (Bodosi et al., 2004). The important factors that regulate meal-induced plasma leptin are insulin and glucose (Levy et al., 2000; Walker et al., 2005). This results further inform, but not prove, that short-term GH treatment had no effect on insulin-stimulated leptin secretion (or meal-induced plasma leptin) in DIO rats. Unfortunately, we could not address whether GH had no effect on meal-induced plasma leptin in control and DR rats, because we could not see meal-induced plasma leptin in control and DR rats with our conditions. The reasons might be due to the amount of energy intake during 2h in control and DR rats which lower than that of DIO rats (Leibowitz et al., 2006).

Next, we demonstrated the effect of GH on fasting leptin. The mechanisms which regulate fasting plasma leptin apparently involve with basal and meal-induced plasma leptin in 2 ways. First, fasting leptin secretion derived from the basal pool of leptin vesicle in adipocyte. Another is the decreased plasma leptin in fasting condition was occurred due to the absence of insulin-stimulated leptin secretion (Walker et al., 2005; Lee and Fried, 2009). Decreased plasma insulin with decreased glucose uptake and oxidation in adipose tissue were reported during fasting (Kasuga et al., 1977; Ahima et al., 1996). Moreover, lipolysis of adipose tissue provides FFA and blunts insulin-stimulated leptin secretion (Cammisotto et al., 2003). In fasting condition, the result

revealed that saline-treated control and DR rats had markedly decreased plasma leptin when compared with GH-treated control and DR rats. This information suggested that GH could attenuate fasting effect, which might be mediated by increased plasma insulin. For DIO rats, the fasting effect was blunted in saline-treated DIO rats. Therefore, plasma leptin levels in basal and fasting condition were not different. Short-term GH treatment did not affect plasma leptin as well. It should be noted that DIO rats had higher amount of adipose tissue, which might be influenced on fasting effect of plasma leptin. Moreover, short-term GH treatment increased plasma insulin, while fasting glucose and NEFA was not affected. Thus, higher level of plasma insulin was required to maintain plasma glucose and NEFA during fasting period. The results suggested that short-term GH treatment induced insulin resistance, which agreed with previous studies (Hettiarachchi et al., 1996; Thirone et al., 1997). When we calculated the parameters into HOMA-IR and Adipo-IR indexes, we found that GH induced insulin resistance by using these indexes in DR rats. Since HOMA-IR and Adipo-IR have focused on insulin resistance with different organs, liver and adipose tissue. Furthermore, short-term GH treatment did not alter body adiposity and liver weight in all rats. It should be noted that higher fat accumulation was found in adipose tissue and liver of DIO rats than that of control and DR rats.

In conclusion, the current study revealed the evidence of short-term GH administration on plasma leptin. First, GH treatment decreased basal plasma leptin in DIO rats. This response apparently depends on body fat mass. Next, GH treatment had

no effect on meal-induced plasma leptin (or insulin-stimulating leptin secretion) in DIO rats during the state of ample energy. Finally, GH could attenuate fasting effect on plasma leptin in DR and control rats, which had lower body adiposity than that of DIO rats.



CHAPTER VI

SUMMARY

A. Conclusions

This current study emphasized the role of short-term GH treatment on plasma leptin in 3 conditions from the animal models with different amount of body adiposity (figure 21). We concluded from our current work that short-term GH administration decreased basal plasma leptin only in DIO rats, which had more body fat mass. This finding apparently mediated by IGF-1 independent pathway, because plasma IGF-1 responded to GH treatment in control and DIO rats, whereas basal plasma leptin was altered only in DIO rats and the increased plasma IGF-1 in DIO rats was occurred later than that of decreased plasma leptin. The effect of GH treatment on decreased FI in both control and DIO rats was apparently due to the direct effect of GH rather than the indirect action of GH-induced plasma leptin because decreased FI might not occur while the levels of plasma leptin was lowered or unchanged in DIO and control rats, respectively. Moreover, short-term GH treatment had no effect on meal-induced plasma leptin (or insulin-stimulated leptin secretion) based on the normalized value of post- and pre-meal leptin in all rats. We propose from these results, but not prove, that short-term GH on basal plasma leptin may be independent of insulin-stimulated leptin secretion.

Additionally, in the stage of fasting, the mechanisms that regulate plasma leptin appears to link with basal and meal-induced plasma leptin, because fasting leptin had depended on leptin pool in adipocytes. However, short-term GH treatment increased plasma insulin, which could be explained to attenuate fasting effect on plasma leptin (dashed line that linked with insulin and leptin in figure

21). Hyperinsulinemia can stimulate leptin secretion from adipose tissue, resulting in increased circulating leptin, despite the insulin resistance state which was induced by GH administration. The short-term GH effect on fasting plasma leptin occurred in DR and control rats, which had lower body fat mass than that of DIO rats. Insulin sensitivity was represented by HOMA-IR and Adipo-IR, which derived apparently from plasma insulin levels.

In conclusion, short-term GH effect on plasma leptin was linked with body adiposity and energy status. In basal condition, GH affected plasma leptin in DIO rats, this result suggested that it could be mediated independently of insulin action. On the one hand, the attenuation of fasting effect on plasma leptin in control and DR rats appeared to mediate indirectly via GH-stimulated insulin secretion. Thus, the levels of basal and fasting plasma leptin were not different in GH-treated control and DR rats.

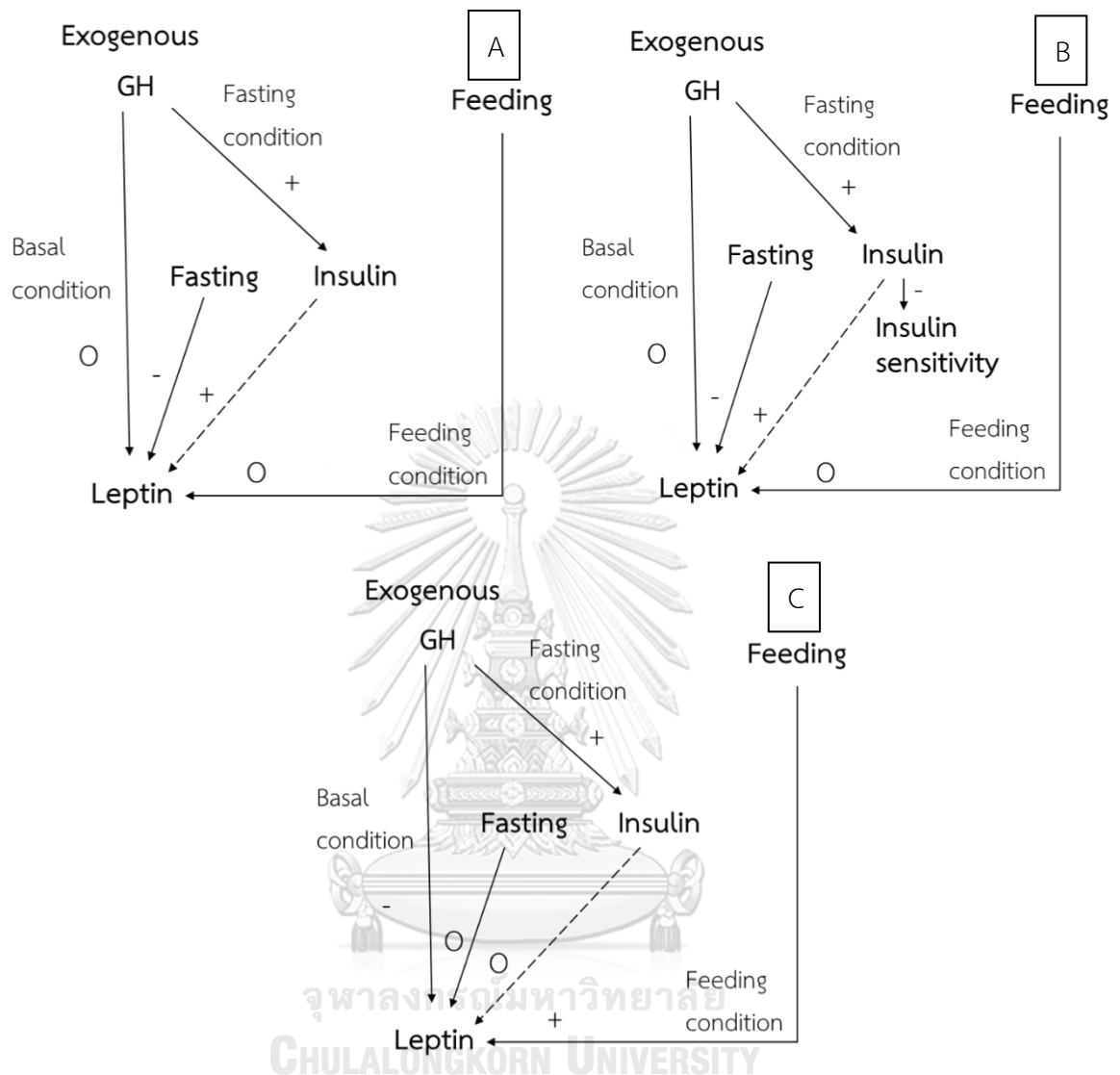


Figure 21 Diagrams represent the pathways, which short-term GH administration altered plasma leptin in basal, meal-induced and fasting condition in control (A), DR (B) and DIO rats (C) (+ : increase, - : decrease, O : unchanged, dashed line indicated that insulin could alter plasma leptin in fasting condition).

B. Implication

There is an evidence of short-term GH treatment on regulating of plasma leptin in which depends on body adiposity. In obese subjects, which has insulin

and leptin resistance, short-term GH treatment could be able to decrease plasma leptin. However, the decreased FI during short-term GH treatment is apparently due to the direct effect of GH. Next, the attenuation of fasting effect occurred in non-obese subjects, which might independent of induced-insulin resistance stage. This result suggests that the role of GH in regulation of fasting plasma leptin may mediate via insulin-stimulated leptin secretion. Overall, the current findings would contribute to the mechanisms of leptin secretion from adipose tissue and probably support the short-term GH treatment as an adjunctive therapy in obesity.

C. Further investigation

The current study demonstrated the evidence of short-term GH administration on plasma leptin in normal and obese subjects. Additionally, our study suggested that the GH effect on plasma leptin should be linked with insulin-independent pathway in basal condition, but not prove the direct way, so more experiment required to determine the pathway that regulate plasma leptin in this issue. Next, we demonstrated that GH could attenuate fasting effect on plasma leptin and it might be associated with insulin-dependent mechanism. Therefore, more study required to clarify this pathway. Finally, plasma leptin is secreted from adipose tissue, so its level may be related to leptin pool in adipocytes. Further studies also need to determine whether the short-term GH effect on plasma leptin may involve with adipose leptin pool.

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