

Effect of Ramadan Fasting on Circulating Leptin and Adiponectin Levels and
Metabolic Profiles in Healthy Men Thai Muslim Population

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ผลของการถือศีลอดต่อระดับฮอร์โมนเลปติน อะดิโปเนกตินและระบบเมตาบอลิซึมในชายไทยมุสลิม
ที่มีสุขภาพดี



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การถือศีลอดเป็นพิธีทางศาสนาอิสลามที่มีขึ้นในเดือนรอมฎอน เดือน 9 ตามปฏิทินทางจันทรคติ มุสลิมที่มีสุขภาพดีทุกคนต้องงดเว้นจากการรับประทานอาหาร ดื่มน้ำหรือเครื่องดื่มต่างๆ งดสูบบุหรี่และงดกิจกรรมทางเพศในช่วงเวลาระหว่างพระอาทิตย์ขึ้นจนถึงพระอาทิตย์ตกดินของทุกวันในเดือนรอมฎอน การศึกษาวิจัยเกี่ยวกับการถือศีลอดซึ่งเป็นการอดอาหารในช่วงเวลาสั้นมีมากกว่า 5 ทศวรรษแล้ว ผลการศึกษาเกี่ยวกับผลของการถือศีลอดต่อสุขภาพและโรคบางชนิดรายงานผลแตกต่างกัน การศึกษานี้จึงมีวัตถุประสงค์เพื่อประเมินผลของการถือศีลอดในช่วงรอมฎอนที่มีต่อระดับของฮอร์โมนเลปตินและอะดิโปเนกตินในเลือดและภาวะเมตาบอลิซึมในผู้ชายมุสลิมที่ถือศีลอดเป็นเวลา 29 วัน ผู้ชายมุสลิมที่มีสุขภาพดี อายุระหว่าง 19-31 ปี จำนวน 27 คนเข้าร่วมการศึกษาในครั้งนี้ เพื่อเปรียบเทียบผลของการถือศีลอดต่อฮอร์โมนเลปตินและอะดิโปเนกติน น้ำตาลก่อนอาหาร อินซูลินไขมันในเลือด องค์ประกอบของร่างกาย การกินอาหารและการออกกำลังกายโดยเปรียบเทียบก่อนและหลังการถือศีลอด ผลการศึกษาพบว่าการถือศีลอดไม่มีผลต่อการเปลี่ยนแปลงของระดับฮอร์โมนเลปตินและอะดิโปเนกติน ระดับคอเลสเตอรอลและเอชดีแอลคอเลสเตอรอลลดลงอย่างมีนัยสำคัญทางสถิติ ระดับน้ำตาลในเลือด อินซูลิน แอลดีแอลคอเลสเตอรอล ไตรกลีเซอไรด์ และ HOMA-IR ไม่มีการเปลี่ยนแปลง ในขณะที่น้ำหนักตัว ดัชนีมวลกาย มวลไขมัน มวลกายไร้ไขมัน มวลกล้ามเนื้อ คาร์บอนไฮเดรตและไขมันที่อวัยวะได้รับต่อวันลดลงอย่างมีนัยสำคัญทางสถิติในขณะที่การออกกำลังกายไม่เปลี่ยนแปลง สรุปได้ว่าถึงแม้ว่าจะมีการเปลี่ยนแปลงของระบบเมตาบอลิซึมและการองค์ประกอบร่างกายที่ดีขึ้น การถือศีลอดไม่มีผลทำให้ระดับเลปตินและอะดิโปเนกตินเปลี่ยนแปลงอย่างไรก็ตามควรมีการศึกษาต่อไปเพื่อยืนยันและอธิบายความสำคัญทางคลินิกของการถือศีลอดในประชากรที่มีสุขภาพดี

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GUNTARI PRASETYA: Effect of Ramadan Fasting on Circulating Leptin and Adiponectin Levels and Metabolic Profiles in Healthy Men Thai Muslim Population. ADVISOR: ASST. PROF. SUWIMOL SAPWAROBOL, DrPH, RD, 106 pp.

Ramadan fasting, the 9th month of Islamic lunar calendar is one of the religious worship among Muslims. All healthy adult Muslims must refrain from eating, drinking, smoking, and sexual relationship from dawn to dusk. Ramadan fasting, a unique model of intermittent fasting has been investigated since the last 5 decades and the heterogeneous findings regarding to the impact of this Islamic fasting in health and certain diseases gain more investigations. Aim of this study is to determine the effect of Ramadan fasting on circulating leptin and adiponectin levels and metabolic profiles in healthy men. One month (29 days) of Ramadan fasting in a year of 2015 was conducted (before and after three weeks of Ramadan fasting). Twenty seven healthy men (19-31 years) were eligible and completed study. The outcomes measured in comparison at before and after three weeks of Ramadan fasting were including biochemical parameters (circulating serum leptin and adiponectin and metabolic profiles: fasting plasma glucose, insulin, and lipid profile); anthropometry, body composition, dietary intake and physical activity. Ramadan fasting did not pose significant changes on circulating leptin and adiponectin levels. Serum total cholesterol and high-density lipoprotein cholesterol (HDL-c) were decreased significantly, while fasting plasma glucose, serum insulin, low-density lipoprotein cholesterol (LDL-c), triglycerides, and homeostasis model assessment of insulin resistance (HOMA-IR) index were remained unchanged. A significant reduction in body weight, BMI, fat mass, fat free mass, muscle mass, and waist and hip circumferences were noted after three weeks of Ramadan fasting. Energy, carbohydrate, and fat intake were decreased significantly, whereas physical activity was remained unchanged. In conclusion, despite of modulation in metabolic profiles and improvement in body composition, Ramadan fasting did not significantly alter circulating leptin and adiponectin levels. Further extensive studies is needed to validate and determine the clinically effect of Ramadan fasting in healthy men.

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CONTENTS

	Page
THAI ABSTRACT	iv
ENGLISH ABSTRACT	v
ACKNOWLEDGEMENTS	vi
CONTENTS	vii
LIST OF TABLE	x
LIST OF FIGURE.....	xi
LIST OF APPENDIX.....	xii
CHAPTER 1 INTRODUCTION	1
1.1 Rationales	1
1.2 Objectives	8
1.3 Research Question	9
1.4 Hypothesis	9
CHAPTER 2 LITERATURE REVIEW.....	10
2.1 Glucose homeostasis.....	10
2.2 Intermittent Fasting (IF).....	12
2.3 Ramadan Fasting	15
2.3.1 Characteristic of Ramadan fasting	15
2.3.2 Impact of Ramadan fasting on metabolic and health	15
2.3.3 Effect of Ramadan fasting on body weight and body composition in healthy individuals	17
2.3.4 Effect of Ramadan fasting on fasting blood glucose and lipid profile in healthy individuals	18
2.4 Leptin.....	19

	Page
2.4.1 Biology of leptin.....	19
2.4.2 Factors that involved in the regulation of leptin.....	20
2.4.3 Role of leptin in regulation of food intake and energy homeostasis.....	23
2.5 Adiponectin	25
2.5.1 Biology and regulation of adiponectin	25
2.5.2 Circadian pattern of adiponectin and leptin in healthy men.....	27
2.5.3 Relationship between leptin and adiponectin on AMP-activated protein kinase.....	29
2.6 Ramadan fast and studies on circulating leptin and adiponectin	32
CHAPTER 3 METHODOLOGY	36
3.1 Study Design.....	36
3.2 Population and Study Participants	36
3.2.1 Target population	36
3.2.2 Study participants.....	36
3.3 Methods	38
3.4 Outcome measurements.....	39
3.4.1 Biochemical parameters.....	39
3.4.2 Anthropometry indices and body composition parameters	41
3.4.3 Energy and macronutrient intake.....	42
3.4.4 Daily physical activity.....	42
3.5 Statistical Analysis.....	43
CHAPTER 4 RESULTS	44
4.1 Baseline characteristics of study participants.....	44

	Page
4.2 Circulating serum leptin and adiponectin.....	46
4.3 Metabolic profiles	47
4.4 Anthropometry and body composition.....	48
4.5 Energy and macronutrient intake.....	50
4.6 Daily physical activity	51
CHAPTER 5 DISCUSSION.....	52
CHAPTER 6 CONCLUSION	68
CHAPTER 7 LIMITATION AND FUTURE STUDIES	69
7.1 Limitation of study	69
7.2 Future studies.....	69
7.3 Application.....	69
REFERENCES	70
VITA.....	106

LIST OF TABLE

	Page
Table 1 Key metabolic event during fed and fasted state.....	10
Table 2 Effect of Ramadan fasting on metabolism and different organs in healthy individuals.....	16
Table 3 Factors regulating circulating leptin levels.....	22
Table 4 Studies on Ramadan fast and serum leptin and adiponectin concentrations in healthy volunteers.....	33
Table 5 Method for analysis metabolic profiles parameters	41
Table 6 Baseline characteristics of study participants.....	45
Table 7 Circulating serum leptin and adiponectin levels before and after three weeks of Ramadan fasting.....	46
Table 8 Metabolic profiles before and after three weeks of Ramadan fasting.....	47
Table 9 Anthropometric and body composition parameters before and after three weeks of Ramadan fasting.....	49
Table 10 Energy and macronutrient intake before and after three weeks of Ramadan fasting	50
Table 11 Daily physical activity before and after three weeks of Ramadan fasting.....	51
Table 12 Comparison of present study with other previous studies of the effect of Ramadan fasting (RF) on circulating leptin and adiponectin.....	56

LIST OF FIGURE

	Page
Figure 1 Pivotal roles of the nervous and endocrine systems as mediators of adaptive responses of major organ systems to intermittent fasting	14
Figure 2 Effects of leptin in states of energy excess and energy deficiency.....	20
Figure 3 Effect of leptin in glucose and lipid metabolism.....	21
Figure 4 Role of leptin in energy homeostasis-satiety-related stimuli.....	24
Figure 5 Diurnal variations of serum adiponectin, leptin, sOB-R, and cortisol.....	28
Figure 6 Role of AMP-activated protein kinase in whole-body energy homeostasis ...	30
Figure 7 Adiponectin & leptin levels under fasting (left) and refeeding (right).....	31
Figure 8 Conceptual framework: Effect of Ramadan fasting on circulating leptin and adiponectin levels and metabolic profiles in healthy men Thai Muslim population.....	35
Figure 9 Study design and outcome measurements	39
Figure 10 Flowchart the selection of study participants.....	44

LIST OF APPENDIX

	Page
APPENDIX 1 ETHICAL APPROVAL.....	89
APPENDIX 2 HUMAN LEPTIN ELISA ASSAY KIT AND PROCEDURE.....	94
APPENDIX 3 HUMAN ADIPONECTIN ELISA ASSAY KIT AND PROCEDURE.....	95
APPENDIX 4 BODY COMPOSITION MEASUREMENT.....	96
APPENDIX 5 DIETARY AND PHYSICAL ACTIVITY RECORD.....	97



CHAPTER 1

INTRODUCTION

1.1 Rationales

One of the Islamic rules for Muslims is fasting during Ramadan.¹ Ramadan, the 9th month of the Islamic lunar calendar, is a unique model of long-term intermittent fasting and known to be the religious duty of all healthy adult Muslims. A whole month of intermittent fasting includes avoidance of drinking liquids, eating foods, smoking, and sexual relations, from dawn to dusk is practicing every year. The duration of fasting varies from 11 to 18 h/day for 29 or 30 days per month.²

Ramadan is associated with physiological, behavioral changes including body weight and body mass index; circadian pattern of sleep, energy expenditure, and body temperature; food intake; and physical activity/performances.³⁻⁹ Food and fluid intake become nocturnal during Ramadan and the common practice is to eat one large meal after sunset and one lighter meal before dawn.¹⁰ During this period, glucose homeostasis, a condition by which serum glucose levels within normal limits is maintained by meals taken before dawn and by liver glycogen stores.

Ramadan fasting may affect not only on physiological standpoint but also metabolic alteration influenced by genetic and environmental factors including nutritional habit, the length of fasting day, and timing of the rest–activity cycle and meals. Because Ramadan fasting affects a huge population, numerous studies were performed in the last two decades to show the effect of Ramadan fasting on various parameters in healthy subjects such as changes in metabolic profile and weight; blood lipid levels; positive effect of Ramadan fasting on proinflammatory cytokines, coagulation markers, cardiovascular disease risk factors (homocysteine, C-reactive protein, and HDL risk factor), B vitamins (folate and vitamin B12); blood pressure and hematological parameters; body composition; nutritional intake; and some indices of

insulin resistance and components of the metabolic syndrome in healthy male adult.^{3,11-19}

Many studies have assessed the effects of Ramadan fast on different anthropometric and metabolic parameters, but have noted conflicting results, particularly regarding lipid profiles. Several studies have found an increase in high-density lipoprotein-cholesterol (HDL-c) levels and a decrease or no change in low-density lipoprotein-cholesterol (LDL-c) levels.^{16,17,20-22}

Two available systematic review and meta-analysis have been carried out to determine the effect of Ramadan fasting on body weight, body composition, and metabolic profile parameters in healthy subjects. First, pooled meta-analyzed data showed that fasting during Ramadan resulted in significant weight loss (- 1.24 kg; 95% confidence interval (CI) - 1.60, - 0.88 kg) and these result showed a substantial heterogeneity ($I^2 = 79.1\%$). However, weight reduction at the end of Ramadan was followed by a significant increase in weight after Ramadan (0.72 kg, 95% CI 0.32, 1.13 kg; $P < 0.001$) which also had substantial heterogeneity ($I^2 = 81.7\%$).²³

When findings were meta-analyzed separately for men and women, weight loss at the end of Ramadan was significant in both genders (- 1.5 kg for men and - 0.92 kg for women) in which during post-Ramadan weeks, men gained 1.02 kg in weight (95% CI = 0.42, 1.63 kg; $P < 0.001$), whereas weight in women remained unchanged.²³ Second, another meta-analysis found a similar finding, weight loss during Ramadan was observed in men (standardize weighted mean difference (SMD) = - 0.24, 95% CI = - 0.36, - 0.12, $P = 0.001$) and the overall pooled SMD for body weight was - 0.17, 95% CI = - 0.26, - 0.07, $P = 0.001$) and there was no heterogeneity across studies for weight loss ($I^2 = 0\%$, $P = 0.82$).²⁴

Moreover, not only in healthy subjects, effect of Ramadan fasting have been studied in unhealthy subjects with certain disease. First study, in stable cardiac

patients (n = 56) of those, 80.4% and 19.6% were male and female, respectively. Most of patients (67.9%) were aged >50 years. All patients fasted completely during Ramadan. Follow-up study for 3 months was taken in cardiology outpatient with clinical and electrocardiography examinations were measured in three times: before, during, and after Ramadan. No report in both cardiac and non-cardiac morbidity/mortality during Ramadan. Significant reduction in serum HDL-c was observed during Ramadan compared to before Ramadan ($P = 0.012$), whereas LDL-c was increased significantly compared to before Ramadan ($P = 0.022$). Total cholesterol (TC), triglycerides (TG), serum leptin, and high-sensitivity C-reactive protein (hs-CRP) were not significantly change.²⁵

Second study in nineteen patients (14 female, 5 male), aged 37.1 ± 12.5 years were encouraged for lifestyle changes during Ramadan fasting and data was collected 1 week before and in the fourth week of Ramadan. Neither complications nor symptoms of hypoglycemia were observed during Ramadan. The results show that daily energy consumption remained similar, although the fat intake increased. Body weight (103.9 ± 29.8 vs. 102.1 ± 29.0 kg, $P = 0.001$) and waist circumference (123 ± 14 vs. 119 ± 17 cm, $P = 0.001$) decreased significantly. Ramadan fasting has exert a small significant increases in fasting plasma glucose (6.3 ± 1.7 vs. 6.8 ± 2.0 mmol/L, $P = 0.024$) and hemoglobin-A_{1c} (6.3 ± 0.9 vs. $6.5 \pm 0.9\%$, $P = 0.003$) after 4 weeks. However, the others parameters such as fasting plasma insulin, lipid profiles, and HOMA-IR remained unchanged.²⁶

Third, study in two groups: non-diabetic subjects (ND) and diabetic patients (D) group (n = 40 each; age 55 ± 5 years) determined the metabolic effect of Ramadan fasting and its influence on oxidative stress in diabetic patients within 11 week period (1 week before, 4 weeks during, and 6 weeks post-Ramadan). During Ramadan, insignificant reduction (5.8%) on fasting plasma glucose was observed in

the ND group, while reduced significantly (23.0%) in the D group. Significant lower levels of serum triglycerides (TG) and malondialdehyde (MDA) were found in both ND and D group (TG: 22.8 and 22.1%; MDA: 54.3 and 46.6%). HDL-c (6.7%) was found to be elevated significant in ND group, while raised insignificantly (2.2%) in D group. Blood glutathione (GSH) was increased significantly by 52.6 and 59.4% in the ND and D groups, respectively. At 6 weeks post-Ramadan fasting, the GSH levels were remained higher than pre-Ramadan fasting values by 26.3 and 31.3% in the ND and D groups, respectively.²⁷

Fourth, study in hypertensive (HT) and control (C) group (n = 40 each; age 55 ± 5 years), the arterial pulse pressure (PP), lipid profile, and oxidative stress parameters were assessed during Ramadan compared to the pre-Ramadan values. Ramadan fasting elicited an improvement in PP and lipid profile in HT group. A decrease in PP was observed significantly in HT during Ramadan, compared to the pre-Ramadan values, whereas in C group, PP was decreased insignificantly. Insignificant lower levels of TC were observed in both groups during Ramadan.

The others two lipid parameters, TG and MDA were decreased significantly by: TG: 24.5% and 22.8%; MDA: 45.6% and 54.3%; and Ramadan fasting also ameliorates oxidative stress as evidenced by GSH elevated by 56.8% and 52.6% in the HT and C groups, respectively. HDL-c increased significantly by 33.3% and insignificantly by 6.7% in the HT and C groups, respectively; whereas LDL-c decreased significantly by 17.7% and insignificantly by 4.0% in the HT and C groups, respectively. At 6 weeks post-Ramadan fasting, MDA remained significantly lower than the pre-Ramadan fasting values by 24.3% and 25.7%, and GSH higher by 30.2% and 26.3% in the HT and C groups, respectively, while PP and TC returned to pre-Ramadan fasting values in both groups. HDL-c was significantly higher by 20.3% and LDL-c lower by 12.0% than the fasting levels in the HT patients.²⁸

In general, metabolic alterations during Ramadan fasting has been marked with the glycogenolysis of the liver and some degree of gluconeogenesis in longer fasting day, since only 1,200 calories are stored as carbohydrate in the liver, providing the basal requirement for glucose for only 5–6 h. In normal adults, a slight decrease in serum glucose of between 3.3 and 3.9 mmol/L (60–70 mg/dL) occurs within a few hours after fasting. These changes are a result of a fall in insulin and rises in glucagon and sympathetic activity.²⁹ Fasting serum glucose may decrease slightly in the first few days of Ramadan fasting, normalizing by the 20th day and showing a slight rise by the 29th day.³⁰

Despite of insulin and glucagon as fuels regulator hormones, there are adipocytes-derived hormones or called as adipokines known to be particularly of importance in maintenance of energy homeostasis: leptin and adiponectin. Leptin is a small peptide (16kDa) that indicates common structural and functional properties, belonging to the interleukin-6 (IL-6) family of cytokines.³¹⁻³³ The Ob gene expressed by adipocytes encoded it. It is anorexigenic peptide, increases energy expenditure, and is primarily cleared from plasma by the kidney through glomerular filtration followed by proteolytic degradation in the renal tubules. The rate of leptin secretion and its plasma concentrations are correlated with total fat mass.³⁴⁻³⁶ Therefore, this hormone circulates as an internal signal indicating the size of body fat stores.

Leptin receptor is expressed in brain regions (hypothalamus, cerebral cortex, cerebellum, choroid plexus), lung, kidney, liver, skeletal muscle, adipose tissue, pancreas, and adrenal medulla.³⁷⁻⁴¹ Obese individuals have higher leptin levels compare to lean individuals. Overfeeding increase circulating leptin levels, while fasting decrease it, and insulin is a potent stimulator of leptin synthesis and secretion.⁴²⁻⁴⁴ In other words, leptin synthesis and secretion is dependent on insulin-mediated glucose metabolism so that increasing glucose availability and metabolism

lead to increases leptin levels. Despite of insulin, plasma leptin concentrations can be acutely modulated by several other hormones such as catecholamines, glucocorticoids, thyroid hormone, growth hormone, and gonadal steroid.⁴⁵

Adiponectin is a large (30 kDa) protein produced by adipocytes, related to the complement 1q family and contains a carboxy-terminal globular domain and an amino-terminal collagenous domain and also share extensive sequence homology with collagen VIII and X. This adipokine circulates in three isoforms: a trimer, of low-molecular weight (LMW), a hexamer (trimer-dimer) of medium molecular weight (MMW), and a multimer high molecular weight (HMW) isoform.⁴⁶ Circulating adiponectin levels are resulting from a complex, highly regulated secretory pathway in adipocytes.⁴⁷ Glucocorticoids are known may suppress human adiponectin mRNA expression and secretion.⁴⁸ In plasma, adiponectin has minor fluctuation (~20%) from the 24-hour mean, levels declining during the night and reaching a nadir values in the early morning.⁴⁹ It is evident that diurnal variation in circulating adiponectin is less than that of observed from leptin.⁵⁰

Adiponectin circulates at high concentrations in human serum between 0.5 and 30 μ g/mL, which is ~0.01% of total plasma proteins.⁵¹ Females have higher concentrations of adiponectin compared to males, this is appears to be due to a selective increase in the HMW oligomer of the hormones. Central adiponectin/leptin signaling may represent the physiological pathway to regulate food intake. Leptin may act to inhibit food intake, while adiponectin in another way increase food intake. Circulating serum adiponectin levels are inversely related to serum leptin levels during fasting and refeeding condition.⁵²

Evidence-based research or literature findings to date are still in infancy to shows the establish effect of Ramadan fasting towards circulating levels of leptin and adiponectin in healthy men. Some studies in Islamic countries have shown that

Ramadan fasting contributed to the circadian rhythm (24-h mean concentrations) of certain endocrine and neuroendocrine hormones including cortisol, melatonin, insulin, leptin, adiponectin, and ghrelin with emphasize on either meal or sleep pattern as the synchronizer, in which reduced frequency of meal and delayed the onset of sleep were observed during Ramadan.⁵³⁻⁵⁷ One of Ramadan circadian study in healthy individuals showed that Ramadan fasting modified the circadian profile of several biological variables including gastric pH, plasma gastrin, insulin, glucose in 10th day, and calcium on the 24th day. Post-Ramadan (one month after), insulin circadian profile were remained altered, and the others biological variables, except plasma insulin had a change in their 24-h mean concentrations during Ramadan.⁵⁷

In regards with adiponectin level, report that no significant change of adiponectin levels existed after Ramadan fasting in trained young men.⁵⁸ Contrary, one study in twenty three healthy volunteers (18 males, 5 females), aged 18-42 years found that Ramadan resulted a reduction in mean morning serum adiponectin levels (11.62 ± 0.8 vs. 8.80 ± 0.57 $\mu\text{mol/mL}$, $P = 0.001$) followed by significant elevation in mean morning serum leptin levels (7.01 ± 2.27 vs. 16.36 ± 4.35 ng/mL , $P = 0.001$). While, evening levels of both serum adiponectin (8.61 ± 0.67 vs. 9.28 ± 0.78 $\mu\text{mol/mL}$, $P = 0.405$) and leptin levels (9.11 ± 2.66 vs. 8.03 ± 2.85 ng/mL , $P = 0.451$) were not significantly change from pre-Ramadan values. However, during Ramadan, in comparison between morning and evening levels of serum leptin showed statistically significant decreased (16.36 ± 4.35 vs. 8.03 ± 2.85 ng/mL , $P = 0.001$).⁵⁵ Two of Ramadan circadian study found no change in 24-h mean leptin concentrations, with either significant shift of 5 h 30 min in peak of serum leptin or nocturnal reduction of plasma leptin at 22:00 h were observed in each study, respectively.^{54,56}

Despite of levels in circulating leptin and adiponectin, the ratio of leptin to adiponectin (L/A) is also important as markers to detect metabolic syndrome (MetS)

and insulin resistance.^{59,60} Study in general Japanese population (678 Japanese subjects: 208 men and 470 women; mean age $58 \pm$ (SD) 14.4 years; mean body mass index: $23.6 \pm 3.3 \text{ kg/m}^2$) reported that L/A was significantly higher in subjects with MetS than that of without MetS, regardless of gender, and the cut-off point of the L/A to detect MetS was 0.59 (sensitivity: 0.72, specificity: 0.70) in men and 1.04 (sensitivity: 0.72, specificity: 0.69) in women.⁵⁹ It is reported that L/A has a better correlation to insulin resistance than the level of leptin or adiponectin alone which can serve as an excellent indicator of obesity and could be useful marker for the progression of arterial sclerosis because the levels of the 2 hormones changes in the opposite direction depending on the amount of visceral fat.^{61,62} However, regarding to the studies in Ramadan fasting, yet have no study evaluate the ratio of leptin to adiponectin during Ramadan fasting.

Systematic review and meta-analyses have been reported to conclude the effect of Ramadan fasting on body weight and some markers in glucose and lipid metabolism.^{23,24} However, studies on leptin and adiponectin in Ramadan fasting are still growing in more evidence and inconclusive yet. According to the rationale aforementioned, more studies are required to provide more evidence related to the effect of Ramadan fasting on circulating leptin and adiponectin levels in healthy individuals as the endocrine hormones which responsible for changes in food intake and energy homeostasis regulation. In order to see in what extent Ramadan fasting may effect on circulating leptin and adiponectin, it is essential to evaluate metabolic profiles, anthropometry, body composition, and energy intake in the study.

1.2 Objectives

The study aims to determine the effect of Ramadan fasting on circulating serum leptin and adiponectin levels and metabolic profiles in healthy men Thai Muslim. Metabolic profiles biomarkers including fasting plasma glucose, serum insulin,

serum lipid profile, and homeostasis model assessment of insulin resistance (HOMA-IR). Specifically, the objectives are:

1. To determine the effect of Ramadan fasting on circulating serum leptin and adiponectin levels in healthy men
2. To analyze the effect of Ramadan fasting on metabolic profile including fasting plasma glucose, serum insulin, serum lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides), and HOMA-IR in healthy men
3. To analyze the effect of Ramadan fasting on anthropometry indices, body composition, and energy intake in healthy men

1.3 Research Question

1. Does Ramadan fasting pose a significant effect on circulating serum leptin and adiponectin levels in healthy men
2. Does Ramadan fasting pose a significant effect on metabolic profiles as measured by fasting plasma glucose, serum insulin, serum lipid profile, and HOMA-IR in healthy men
3. Does Ramadan fasting pose a significant effect on anthropometry indices, body composition, and energy intake in healthy men

1.4 Hypothesis

The hypothesis from this study is Ramadan fasting elicit some improvement in body composition and modulate metabolic profiles by altering circulating serum leptin and adiponectin levels as an adaptive response to the natural intermittent fasting in healthy men.

CHAPTER 2
LITERATURE REVIEW

2.1 Glucose homeostasis

One of physiological conditions which may alter some metabolic disturbances is fasting state. Body has the ability to regulate the biochemical responses during starved-fed cycle, begins after an evening meal and during overnight fast. Glucose homeostasis, a constant blood glucose level is the major target within this period. Importantly, there are some key metabolic events occur during both fed and fasted state as mention in Table 1.

Table 1 Key metabolic event during fed and fasted state

Fed state	Fasted state
Primary importance of control glycemia	Maintain blood glucose level
Replenish liver glycogen	Increasing hepatic glucose output
Generate reducing equivalents for ATP production via electron transport chain	Use more muscle amino acids for glucose production
Convert carbohydrate to fatty acids and transfer for storage	Mobilize fatty acids from storage
Clear dietary lipids into various organs	Increase ketone bodies provision during prolong fasting
Deaminate amino acids and produce urea	

Adapted from⁶³

There are three stages in nightly-starved fed cycle, the post-absorptive state after meal, early fasting state during the night, and the re-fed state after breakfast.⁶⁴

The well-fed or post-absorptive state

This state occurs acutely after evening meal ingestion. Digestion and absorption of foods is process in gastrointestinal tract and nutrient such glucose and amino acids transported from intestine to the blood. Beta-cell pancreas releases insulin as response of rising postprandial blood glucose levels. Multiple insulin signaling pathways are responsible for the certain conditions including fed state, fuels storage, and protein synthesis. Glycogen synthesis is triggered by insulin via insulin-mediated protein kinase cascade in both muscle and liver, while suppressed gluconeogenesis in the liver. Glycolysis also stimulated by insulin which promotes fatty acid synthesis in the liver.⁶⁴

The early fasting state

In several hours after meal, lower blood glucose levels signals a decrease level of insulin secretion. On the other hand, glucagon plays a major role to mobilize glycogen stores. Glycogen breakdown and inhibition of glycogen synthesis are mediated by cyclic adenosine-monophosphate (cAMP) cascade leading to the phosphorylation and activation of phosphorylase and the inhibition of glycogen synthase. A reliance on fatty acids as fuel in both muscle and liver is pre-dominantly occurred in response to lower blood glucose levels. In order to maintain the constant rate of blood glucose level (kept at or above 80 mg/dl), three major factors are occurred during this state: (1) Glycogen mobilization and the release of glucose by the liver, (2) the release of fatty acids by adipose tissue, and (3) the shift in the fuel used from glucose to fatty acids by muscle and the liver.⁶⁴

The re-fed state

The re-fed state is occurred as follow by breakfast, play a role to generate a newly synthesized glucose to replenish the liver glycogen stores. As the blood-glucose levels continue to rise, the liver completes the replenishment of its glycogen stores and begins to process the remaining excess glucose for fatty acid synthesis.⁶⁴

2.2 Intermittent Fasting (IF)

Intermittent fasting (IF) is individuals fasting strategy within certain periods of time.⁶⁵ In humans, IF in the form of alternate day fasting and Ramadan fasting are beneficial in the improvement of some parameters in anthropometric, lipid profile; and reduction in inflammatory markers.^{16-18,66} Aksungar and coworkers¹⁶ demonstrated the effect of Ramadan fasting as a nature form of IF in 24 healthy volunteers (12 males, 12 females; aged 21-35 years) on serum lipid markers, coagulation status, and plasma homocysteine levels. Ramadan fasting resulted in elevated serum high-density lipoprotein-cholesterol (HDL-c) levels significantly in both male (49 ± 15.25 vs. 56 ± 16.31 mg/dL, $P < 0.001$) and female (57.4 ± 13.63 vs. 66.5 ± 11.79 mg/dL, $P < 0.001$), and this elevation was persisted to 20 days after Ramadan ($P < 0.05$) without any significant changes on the average of body weight. Whilst, total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-c) levels were not significantly change. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer levels were in the physiologic limits, significantly low at the end of Ramadan in both male (159.3 ± 21.6 vs. 89.2 ± 27.7 ng/mL, $P < 0.001$) and female (167.3 ± 35.2 vs. 73.5 ± 26.7 ng/mL, $P < 0.001$). Plasma homocysteine levels, still in reference range were low during Ramadan in both males and females ($P < 0.05$).¹⁶

Moreover, another study by the same author reported some positive effect of Ramadan fasting on inflammatory status and cardiovascular risk factors including lipid markers, interleukin-6 (IL-6), C-reactive protein (CRP), and homocysteine levels in comparison between 40 healthy normal-weight of fasting group (20 males, aged 23-39 years and 20 females, aged 20-38 years; body mass index (BMI) < 25 kg/m²); and 28 healthy volunteers of non-fasting group (14 males, aged 22-40 years and 14 females, aged 20-36 years; BMI < 25 kg/m²). No significant changes were observed in

serum TC, TG, LDL-c in the fasting group during Ramadan. While, HDL-c was significantly higher in females during Ramadan (48.51 ± 11.68 vs. 56.46 ± 8.07 mg/dL, $P < 0.01$) compared to the before Ramadan levels, and remained significantly higher than non-fasting group (45.29 ± 23.71 vs. 56.46 ± 8.07 mg/dL, $P < 0.05$). Since, the levels of HDL-c were increased in both genders, although not significant in males, HDL-c risk factor (TC/HDL ratio) was decreased significantly during and after Ramadan in both genders in the fasting group. Compared to the pre-Ramadan values, levels of IL-6 ($P < 0.001$), CRP ($P < 0.001$), and homocysteine ($P < 0.01$) were significantly low during Ramadan in the fasting subjects of both genders.¹⁷

In metabolic and neuroendocrine perspective, intermittent fasting provides a complex system with majors organs in which energy metabolism, food intake and neuroendocrine are being connected along brain-peripheral functions. Herein Figure 1 is illustrated the brain communication with all peripheral organs related with energy metabolism, and metabolic and endocrine systems in condition of intermittent fasting (IF).

The impact of intermittent fasting (IF) is associated with the modification in brain neurochemistry and neuronal network which are involved in brain function and peripheral metabolism. The adaptive response to IF is connected with four brain regions: the hippocampus (cognitive processing), striatum (control of body movements), hypothalamus (Hyp, control of food intake and body temperature) and brainstem (control of cardiovascular and digestive systems).

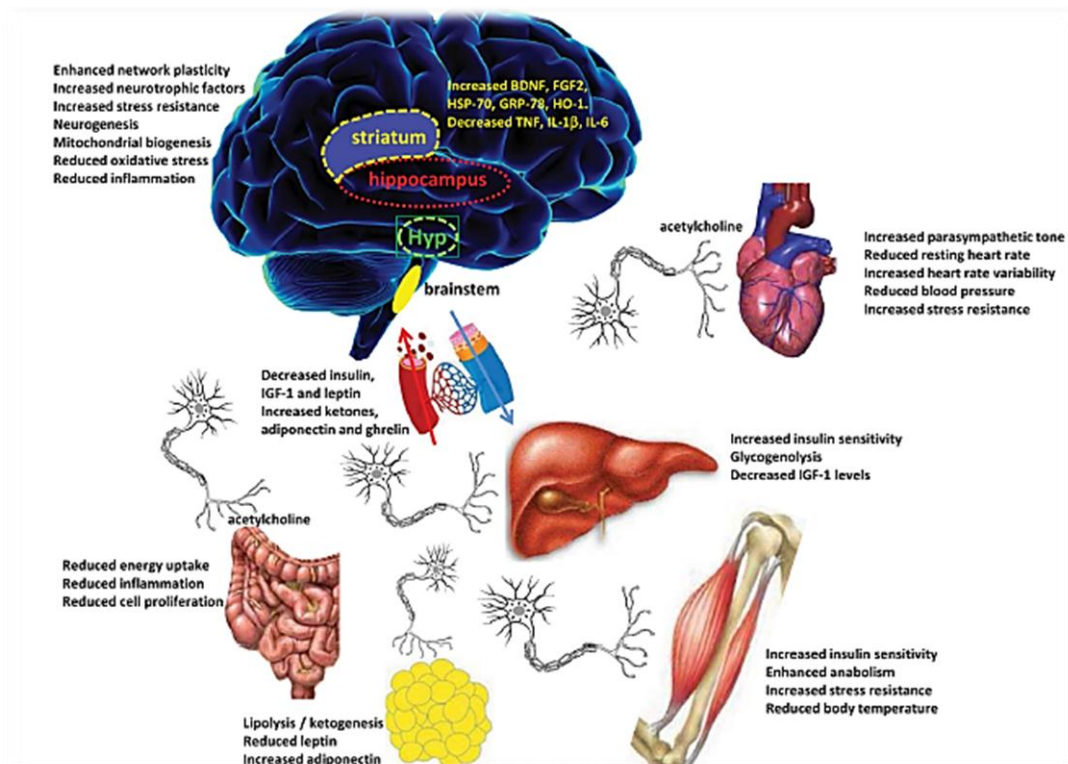


Figure 1 Pivotal roles of the nervous and endocrine systems as mediators of adaptive responses of major organ systems to intermittent fasting

Adapted from⁶⁷

Fasting stimulates parasympathetic activity (mediated by the neurotransmitter acetylcholine) in the autonomic neurons that innervate the gut, heart and arteries, resulting in improved gut motility and reduced heart rate and blood pressure. Fasting affected to promote glycogen depletion in the liver and production of ketone bodies leading to reduced body fat through lipolysis. In the peripheral organ muscle and liver, insulin sensitivity is enhanced following IF and decreases production of insulin-like growth factor-1 (IGF-1). Moreover, the levels of oxidative stress and inflammation markers are also reduced throughout the body and brain. Leptin and adiponectin as two important adipocytes-derived hormones are also affected by IF in which the values being decreased and increased during or after IF, respectively.⁶⁷

2.3 Ramadan Fasting

2.3.1 Characteristic of Ramadan fasting

Ramadan is the holiest month in the Islamic lunar calendar and Muslims fast during this month. All healthy adult Muslim must refrain from eating, drinking, smoking and sexual relations from sunrise to sunset during the month of Ramadan. Ramadan fasting is associated with the reduction in meal frequency (meals were taken exclusively at nighttime as pre-dawn and evening meal) and sleep deprivation. Study by Aksungar and coworkers reported the sleep pattern of the fasting group compared to the non-fasting group was delayed by approximately 2-3 h on average,¹⁷ and another one study showed delayed sleep phase syndrome as evidenced by marginally significant delay of bedtime (large effect size) and significant delay of wake-up time.⁶

2.3.2 Impact of Ramadan fasting on metabolic and health

The physiological and metabolic changes in Ramadan are not well known yet. Study in males (n = 55, age 34.1 (SD. 8.9) years) with metabolic syndrome (according to waist circumference ≥ 90 cm ; blood pressure $\geq 130/85$ mmHg; fasting glucose ≥ 1100 mg/L or 6.1 mmol/L; triglycerides ≥ 1500 mg/L or 1.70 mmol/L; and high-density lipoprotein-cholesterol, HDL-c < 400 mg/L or 1.04 mmol/L) showed that total energy intake was decreased by 234.6 (SD 88.2) kJ/d in the fasting period ($P = 0.005$) followed by significant decrease in body weight (80.69 ± 12.27 kg to 78.73 ± 12.05 kg, $P < 0.001$) and waist circumference (94.81 ± 7.8 cm to 91.98 ± 7.70 cm, $P < 0.001$) of both 2.4% reduction were observed.¹⁴

Insulin resistance index indicated by HOMA-IR was not declined significantly by Ramadan fasting ($P = 0.412$). Fasting plasma glucose had decreased significantly after Ramadan fasting, whereas did not significantly altered fasting plasma insulin ($P =$

0.77). Moreover, correlation has been found between waist circumference and HOMA-IR, with decreasing waist circumference, HOMA-IR levels decreased linearly (HOMA-IR: $0.15 \times \text{waist} - 11.7$; $P = 0.007$).¹⁴

The other beneficial result was the increased of HDL-c significantly after Ramadan fasting (428.7 ± 54.5 mg/L to 462.4 ± 55.0 mg/L, $P = 0.004$) and decreased rate of systolic (115.00 ± 13.57 mmHg to 108.93 ± 11.57 mmHg, $P < 0.001$) and diastolic blood pressure (76.66 ± 7.97 mmHg to 73.78 ± 6.46 mmHg, $P < 0.001$).¹⁴ Indeed, Ramadan fasting have several effects on metabolism and different organs in healthy individuals as shown in Table 2.²

Table 2 Effect of Ramadan fasting on metabolism and different organs in healthy individuals

Metabolism/organ	Effects
Carbohydrate	Glycogenolysis in the liver, some degree of gluconeogenesis in longer fasting days
Lipids	Variable, depending on the quality and quantity of diet and weight change
Body weight	Variable, mostly decreased or unchanged
Liver	Slight increase in indirect bilirubin in the first half of Ramadan fasting
Kidney	Small, insignificant changes in serum urea, creatinine and uric acid
Hematological profile	Small decrease in both iron and total iron binding capacity
Neurophysiatrics	Changes in chronotype and sleep patterns; increase in the prevalence of headaches; decrease in parasulcide
Endocrine glands	Slight changes in protein binding of T4 and T3 and in serum calcium concentration Small reversible shifts in cortisol, testosterone and prolactin secretions
GI tract, heart, lungs and eyes	None

Adapted from²

2.3.3 Effect of Ramadan fasting on body weight and body composition in healthy individuals

The available studies regarding effect of Ramadan fasting on body mass have been reported since last 50 years back and those remains inconclusive. The latest two of systematic review and meta-analysis found that Ramadan fasting resulted in a greater weight loss in men by $- 1.51$ kg (95% CI $- 2.04, - 0.98$) and $- 0.24$ kg (95% CI $- 0.36, - 0.12, P = 0.001$), respectively.^{23,24} Indeed, some studies noted decreases in body weight and body mass index (BMI) during the Ramadan fast,^{3,11-13,18} while others noted no significant differences in these same variables when assessed prior to, during, and/or following the Ramadan fast.^{16,17,68} The cohort study reported, body weight (men: 68.7 ± 12.1 kg to 67.5 ± 10.8 kg, $P = 0.001$; women: 55.7 ± 5.8 kg to 54.6 ± 5.9 kg, $P = 0.002$) were decreased significantly during Ramadan fasting in both genders in 81 healthy volunteers (male/female ratio of 41:39).³

Recently, two studies have been investigated the effect of Ramadan fasting on body composition parameters in healthy individuals.^{12,13} One study by Fahrial Syam et al¹² in 43 healthy subjects (84% female, aged 34.19 ± 11.25 years), found that body weight ($- 0.874 \pm 0.859$ kg, $P < 0.001$), BMI ($- 0.360 \pm 0.371$ kg/m², $P < 0.001$), body fat ($- 0.484 \pm 0.597$ kg, $P < 0.001$), body water ($- 0.293 \pm 0.46$ kg, $P = 0.001$) had decreased significantly by 28th day of Ramadan fasting and returned to the same values as on the first day, while calorie intake did not change significantly (12.94 ± 760.608 kcal, $P = 0.082$).

Another one study was a prospective study in comparison between age and sex,¹³ Subjects were grouped to age and sex: ≤ 35 years ($n = 83$, males: 31) and 36-70 years ($n = 158$, males: 127). Reduction in body weight and BMI were found in almost all subjects, and a greater reduction found in males ≤ 35 years [$- 2.2\%$, (standard

error mean, SE 2.2%), $P < 0.001$]. Waist and hip circumferences reduced in most subjects, except females aged 36-70 years.

Body composition parameters including fat mass, fat free mass, and percentage of body fat were decreased significantly after Ramadan fasting. A decrease in fat mass found in most subjects by 2.3% to 4.3% from baseline, except in females aged 36-70 years who did not experience a significant change. Fat free mass was decreased significantly in all subjects ($P < 0.001$). Dietary intake was similar between before and during Ramadan, except for protein intake which had reduced in males ($P = 0.032$).¹³

In males, body weight were decreased significantly (% change: - 2.2% (SE 2.2), $P < 0.001$) followed by reduction in BMI (% change: - 2.1% (SE 1.8), $P < 0.001$), fat mass (% change: - 4.3% (SE 4.7), $P = 0.001$), fat free mass (% change: - 2.1% (SE 1.8), $P < 0.001$), % body fat (% change: - 2.5% (SE 3.2), $P < 0.029$), waist (% change: - 1.3% (SE 1.3), $P = 0.004$) and hip (% change: - 1.9% (SE 1.0), $P < 0.001$) circumferences.¹³ It was suggested that Ramadan fasting lead to a significant decline in carbohydrate oxidation and a significant increase in fat oxidation, as well as a decline in diurnal energy expenditure which affected by the absence of post-prandial thermogenesis during fasting.^{69,70}

2.3.4 Effect of Ramadan fasting on fasting blood glucose and lipid profile in healthy individuals

Effect of the Ramadan fasting on plasma glucose levels had intensively reviewed by many research groups. The latest meta-analysis from 16 studies concluded that fasting blood glucose was decreased significantly during Ramadan fasting both in healthy male and female [standardized weighted mean difference, SMD: -1.11 (95% CI = -1.72 to -0.49, $P = 0.001$); -1.09 (95% CI = -2.11 to -0.06, $P = 0.040$).²⁴

Studies on the effects of Ramadan fasting on blood lipids, lipoproteins and apolipoproteins are scarce, and have given variable results and remain incomplete. Decreased levels of total cholesterol reported by some investigators following a Ramadan fast.^{15,21,71-74} Several other studies have found an increase in HDL-c levels and a decline or no change in LDL-c levels.^{16,17,20-22,73,75,76} Indeed, some studies have noted an increase in HDL-c levels by as much as 20%²⁰ and 30%.²² The majority of studies have found no difference in triglyceride levels,^{17,22} while one study noted a decrease following a period of Ramadan fasting.¹⁹

A systematic review and meta-analysis reported a significant decrease in LDL-c in both sex (SMD = - 1.67, 95% CI = - 2.48, - 0.86, $P = 0.001$). In males, total cholesterol (SMD = - 0.44, 95% CI = - 0.77, - 0.11, $P = 0.009$) and LDL-c (SMD = - 2.22, 95% CI = - 3.47, - 0.96, $P = 0.001$) were decreased significantly, with a small decrease in triglycerides levels (SMD = - 0.35, 95% CI = - 0.67, - 0.02, $P = 0.040$). While, in female subgroup, total cholesterol (SMD = 0.05, 95% CI = - 0.51, - 0.50, $P = 0.870$) and triglycerides (SMD = - 0.03, 95% CI = - 0.31, - 0.36, $P = 0.880$) were remained unchanged, while HDL-c levels were increased significantly (SMD = 0.86, 95% CI = 0.11 to 1.61, $P = 0.03$).²⁴

2.4 Leptin

2.4.1 Biology of leptin

Leptin is a 16 kDa with 167-amino acid peptide that is mainly expressed in white adipose tissue (WAT) or so called as *obese gene*, but is also found in a variety of tissues including placenta, mammary gland, ovary, skeletal muscle, stomach, pituitary gland, and lymphoid tissue.⁷⁷ Circulating leptin levels signals the status of long-term energy store by its direct correlation with the amount of body fat. Changes in calorie intake lead to the changes in leptin levels, starvation and obese state may decrease and increase leptin levels, respectively.⁷⁸ To date, the primary functional

role of leptin is on energy homeostasis, glucose and lipid metabolism, immune function, and other systems.⁷⁹

2.4.2 Factors that involved in the regulation of leptin

During fasting, leptin levels fall rapidly both in adipose tissue and circulation (Figure 2). Consequently, food intake increases by the expression of orexigenic neuropeptides and decreases the expression of anorexigenic neuropeptides.⁷⁸ Reduction in circulating leptin levels is found among overweight men post-exercise and calorie restriction which showed that circulating leptin levels is associated with energy status.⁸⁰

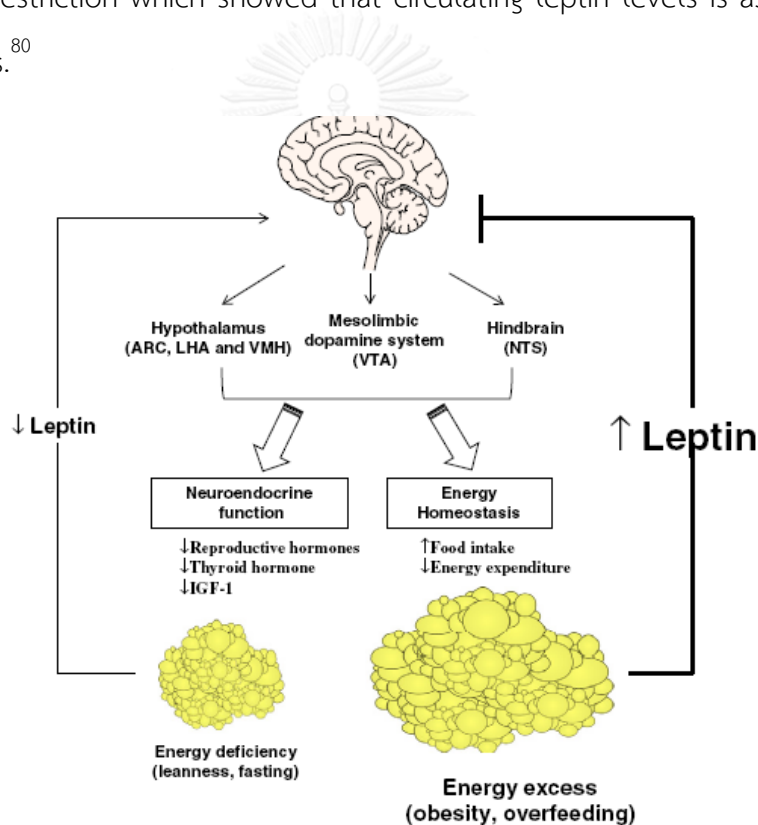


Figure 2 Effects of leptin in states of energy excess and energy deficiency

Adapted from⁷⁸

Contrary, excess amount of energy intake (e.g. obesity and overfeeding) may sense to increase circulating leptin levels; however, leptin resistance may altered the effects of leptin in the central nervous system.⁷⁸ In rodent models, leptin resistance has been attributed to multiple factors including: (a) an interference of leptin

signaling in hypothalamic and other CNS neurons, (b) a diminish process in leptin transport across blood brain barrier, (c) hypothalamic inflammation, (d) an endoplasmic reticulum stress, or (e) and autophagy.⁸¹⁻⁸³ Glucose homeostasis is affected by leptin in various pathways, including modulation of autonomic nervous systems (ANS), hepatic glucose production, muscle glucose uptake, and glucagon secretion from pancreatic α -cells (Figure 3). Leptin inhibits insulin secretion, and stimulates fatty acid oxidation. Leptin levels are stimulated by several factors such as insulin, glucocorticoids, and pro-inflammatory cytokines, and decreased by catecholamine.

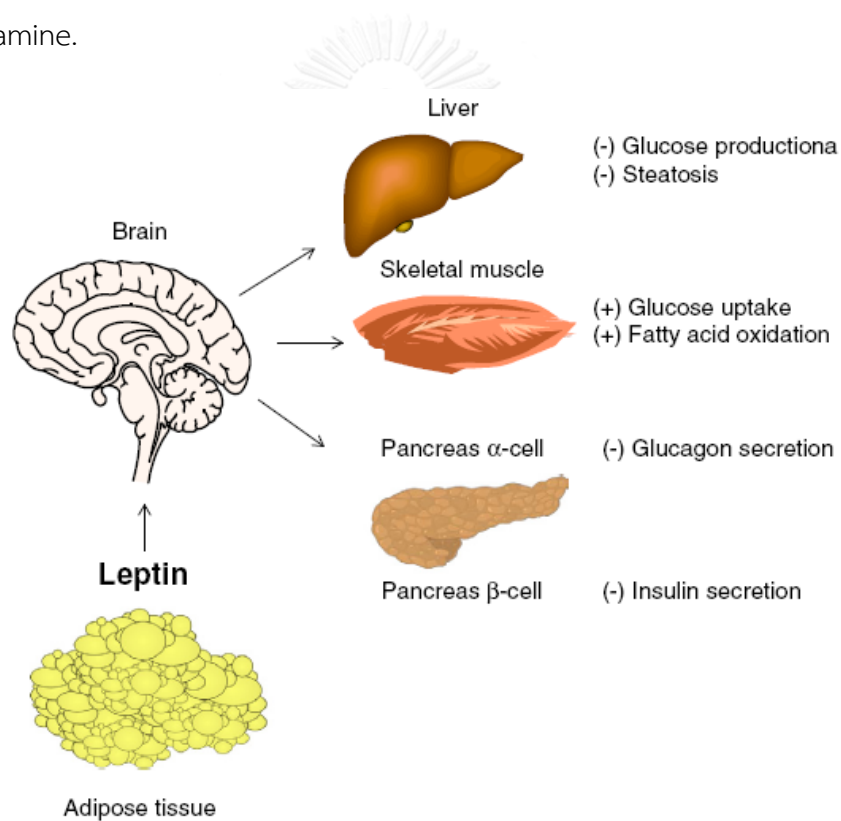


Figure 3 Effect of leptin in glucose and lipid metabolism

Adapted from⁷⁸

Lower circulating leptin levels during fasting induced some metabolic and hormonal responses in mice and humans,^{79,84} including hyperphagia, hypogonadotropic hypogonadism, and suppression of thyroid and growth hormone

(GH) levels, which are prevented by physiological doses of leptin.⁸⁵⁻⁸⁷ Table 3 show several factors contributed to the circulating leptin levels.

In humans, leptin is secreted in a pulsatile fashion and also displays a circadian rhythm in which nadir (lowest) level in midafternoon and zenith (highest) in the midnight. Between obese and lean subjects, leptin secretion showed similar pulsatile pattern, but has different pulse of amplitude.⁸⁸

Table 3 Factors regulating circulating leptin levels

Factors increasing leptin	Factors reducing leptin
Excess energy stored as fat (obesity)	Low energy states with decreased fat stores (leanness; lipoatrophy)
Overfeeding	Fasting
Glucose	Cold exposure, and adrenergic agonists
Insulin	Thyroid hormone
Estrogen	Testosterone
Pro-inflammatory cytokines (TNF- α , IL-6)	
Adapted from ^{78,80} ; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6	

Differences in circulating leptin levels through gender variation also have been identified, called as sexual dimorphism. Higher leptin level was found among women rather than men, it is likely partly attributable to the greater existence of subcutaneous adipose tissue distribution in women and even after the correction for the greater extent of adiposity in women. Moreover, the influencer such as hormone estrogen stimulated the synthesis of leptin, whereas testosterone inhibited leptin synthesis.⁸⁹⁻⁹¹

2.4.3 Role of leptin in regulation of food intake and energy homeostasis

Energy homeostasis is controlled by brain (central nervous system, CNS) mainly hypothalamic region through three physiological actions: 1) regulation of satiety, 2) regulation of energy balance (intake and expenditure), and 3) regulation of secretory hormone which involve in energy storage. There are two categories of signals to regulate energy homeostasis, (1) short-term signal (situational) which is related to portion and meal timing, controlled by some hormones/peptide in gastrointestinal tract and (2) long-term signal which comes from fat-derived hormone leptin (white adipose tissue, WAT) which regulates food intake, energy expenditure, and body adiposity.⁹²⁻⁹⁴

As a long-term signal in the regulation of food intake and energy homeostasis, both leptin and insulin plays critical role throughout the pathways link central nervous system (CNS) and peripheral tissues.⁹² In CNS, leptin interacts with a number of hypothalamic neuropeptides in response to feeding behavior and energy expenditure.^{93,95,96}

Hypothalamus acts as central in controlling energy metabolism with anabolic and catabolic signals. Anabolic signal related to nutrient intake and energy storage; while catabolic signal related to limitation of nutrient intake and energy expenditure. Hypothalamus is also regulator of energy balance. Stimulus from ventromedialis hypothalamic nucleus (VMH) as satiety center inhibit food intake. Lesions in VMH cause hyperphagia (overeating) and obesity. In contrast, lateral hypothalamic nucleus (LHA) as hunger center promote food intake and lesions in LHA may inhibit food intake.⁹⁴

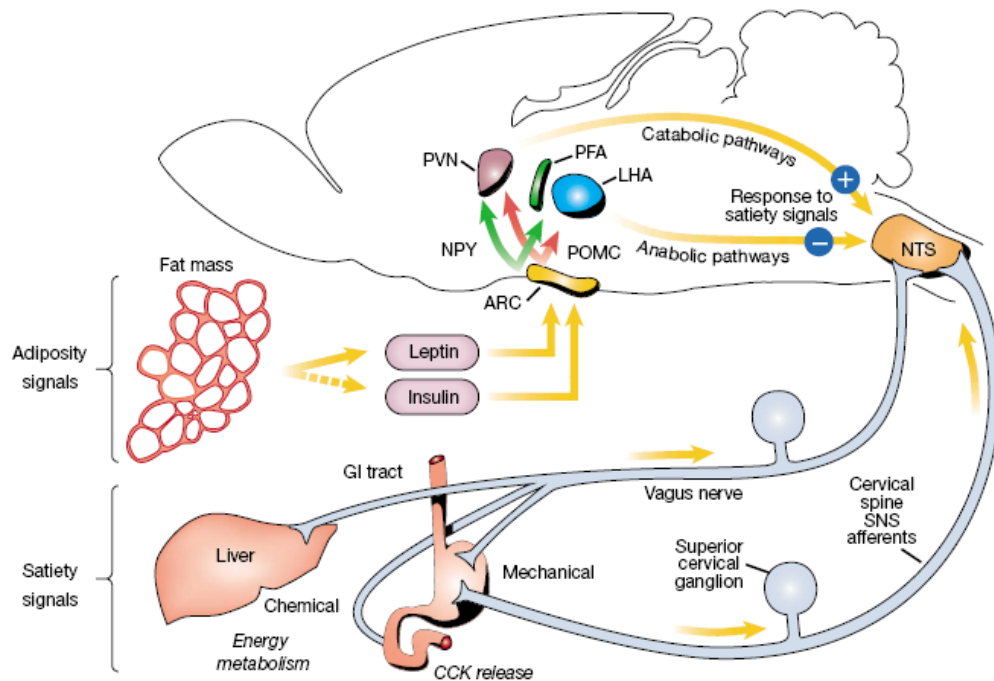


Figure 4 Role of leptin in energy homeostasis-satiety-related stimuli

Adapted from⁹⁶

Abbreviations: agouti-related protein (AGRP), arcuate nucleus (ARC), cocaine-and amphetamine-regulated transcript (CART), cholecystokinin (CCK), lateral hypothalamic area (LHA), nucleus of the solitary tract (NTS), neuropeptide-y (NPY), paraventricular nucleus (PVN), perifornical area (PFA), pro-opiomelanocortin (POMC).

Figure 4 illustrated the neuroanatomical model of pathways incorporated with adiposity signals leptin (secreted by adipocytes) and insulin (secreted by the endocrine pancreas in proportion to adiposity) to regulate meal size. Leptin and insulin are proposed to stimulate a catabolic pathway (POMC/CART neurons) and inhibit an anabolic pathway (NPY/AGRP neurons) that originates in ARC.⁹⁶ Evidence showed that leptin potentiates the effect of CCK to activate NTS neurons demonstrates clearly that signals involved in energy homeostasis modulate the response of NTS neurons to input related to satiety.⁹⁷

It is now well established that the adipocyte hormone leptin serves as a signal that provides information about the size of energy reserves to systems

regulating feeding, substrate utilization and energy balance through adipocytes-negative feedback mechanism. Proposed criteria for a negative-feedback signal include: (1) that it circulates at levels proportionate to body fat content and enters the brain; (2) that it promotes weight loss by acting on neuronal systems implicated in energy homeostasis; and (3) that blockade of these neuronal actions increases food intake and body weight. Although many nutrients (e.g. free fatty acids and glucose), cytokines (e.g. interleukin-6, tumor necrosis factor- α) and hormones (e.g. glucocorticoids) fulfill some of these criteria, only leptin and insulin satisfy all of them.⁹⁶

The association of leptin and insulin with body fat contents is underlie towards differential mechanisms.⁹⁸ Weight gain affected to reduction in insulin sensitivity may explain how insulin, but not leptin, varies according to body fat stores.⁹⁹ Naturally, an increase in body weight may in turn follow by an increase in insulin secretion both the basal state and in response to meals in order to compensate for insulin resistance if normal glucose homeostasis is to be maintained.^{100,101} While, mechanisms involved in leptin secretion are quite different. The rate of insulin-stimulated glucose utilization in adipocytes is a key factor linking leptin secretion to body fat mass.¹⁰² Glucose flux through the hexosamine pathways seems to be involved.¹⁰³ Acute changes of energy balance markedly affect adipocyte glucose metabolism, leptin secretion can become transiently dissociated from levels of total body fat.

2.5 Adiponectin

2.5.1 Biology and regulation of adiponectin

Adiponectin, a 30-kDa protein comprising 274 amino acid humans, is one of the most abundant adipokines encoded by the AdipoQ gene and adiponectin is involved in modulation of both glucose and fatty acid metabolism. Adipocytes are

the most frequent source of production for these active polypeptides.^{104,105} In the blood, adiponectin is one of the most concentrated hormones (between 1.9 and 17 mg/mL), thus accounting for 0.01% of total plasma proteins.¹⁰⁶ AdipoQ or adiponectin shares significant homology of subunits of complement factor C1q and adiponectin has a specific conformational structure consisting of four domains; an N-terminal signal sequence, a specific variable region, a collagen-like domain and a carboxy-terminal globular domain.¹⁰⁷

Adiponectin secretory is existed as full length (fAd) form, which can be cleaved into smaller globular (gAd) form-mediated the elastase from the activation of monocytes and/or neutrophils.^{108,109} Three different multimers can be assembly by the monomers of fAd, low-molecular weight (LMW) monomers or trimers; middle-molecular weight monomers or hexamers; and the high-molecular weight oligomeric species; which comprises 12-18 monomers in human circulation.^{110,111} Each form represented its own biological action through its binding site with specific receptors: two G-protein-independent, seven-transmembrane-spanning receptors called AdipoR1 and AdipoR2, and T-chaderin as a potential receptor for HMW adiponectin.^{112,113}

In serum, adiponectin found as two forms, a lower molecular weight trimer-dimer and high molecular weight complex. Higher affinity for AdipoR2 is shown in fAd, while gAd has higher affinity for AdipoR1, thus these different target tissues induce different signaling pathways.¹¹⁴⁻¹¹⁷ Both gAd and fAd in skeletal muscle activate AMP kinase (AMPK), thereby stimulating phosphorylation of acetyl-coenzymeA-carboxylase (ACC), fatty acid oxidation, and glucose uptake. In the liver, full length adiponectin is the only one type of adiponectin which stimulates AMPK-mediated reduction in molecules involved in gluconeogenesis, ACC phosphorylation, and fatty acid oxidation. The other biological action of adiponectin is the peroxisome

proliferator-activated receptor α (PPAR α) action which has a role to decrease triglyceride content in liver and skeletal muscle.^{116,118,119}

Adiponectin has a long half-life in serum being 2.5-6 h,^{110,120} and in plasma, 24-hour mean concentrations of adiponectin are fairly stable, only minor fluctuation was seen (~20%) along the day, display diurnal variation with a nocturnal decline and maximum levels in the late morning.⁴⁹ Regulation of adiponectin is particularly might take place at the transcriptional, the translational, and at the post-translational level, which involved in protein modification, secretion and oligomerization. Its degradation and excretion might be an important regulatory site for circulating adiponectin levels.

The up-regulation of adiponectin level by caloric restriction (CR) has been highlighted as a key underlying mechanism for CR-induced improvement of metabolism. In human, approximately 10 to 20% weight reduction by CR in obese subjects significantly increased adiponectin gene expression in WAT and circulating adiponectin levels.^{51,121-123} In addition, anorexic patients who had very low calorie intake exhibited 30 % increase in circulating adiponectin levels.¹²⁴

A mouse study examined the roles of adiponectin in the hypothalamus and found that adiponectin is involved in the regulation of energy homeostasis by regulating food intake and energy expenditure. Energy intake plays a key role in adiponectin expression, serum and cerebrospinal fluid levels of adiponectin, hence increased the levels during fasting and reduced post-refeeding.¹²⁵

2.5.2 Circadian pattern of adiponectin and leptin in healthy men

There are several terms used to identify how certain hormones in particular of endocrine system may express various functional properties throughout 24-h of body clock, called as circadian rhythm or less than that as ultradian manner. A

variety of endocrine and neuroendocrine hormones exhibit these dynamic patterns such as adiponectin, leptin, soluble leptin receptor, and cortisol.

Adipokine, adipocytes-secreted protein known having capacity to regulate insulin sensitivity.⁹³ Available evidence reported that adiponectin an abundant serum adipokine plays an important role in insulin sensitivity.^{126,127} In humans, obesity and diabetes may decrease levels of serum adiponectin,^{51,128} and low levels correlate with the decrease of insulin sensitivity.^{129,130} Another adipokine, leptin has an important role in the regulation of energy homeostasis.¹³¹ The biological activity of leptin is modulated by its main binding protein in the serum, known as the soluble cleaved extracellular part of the leptin receptor (sOB-R).¹³² Serum leptin levels showed ultradian and circadian rhythmicity throughout the body clock,^{50,88,133} whereas differ from leptin, sOB-R shows a diurnal variation.¹³⁴

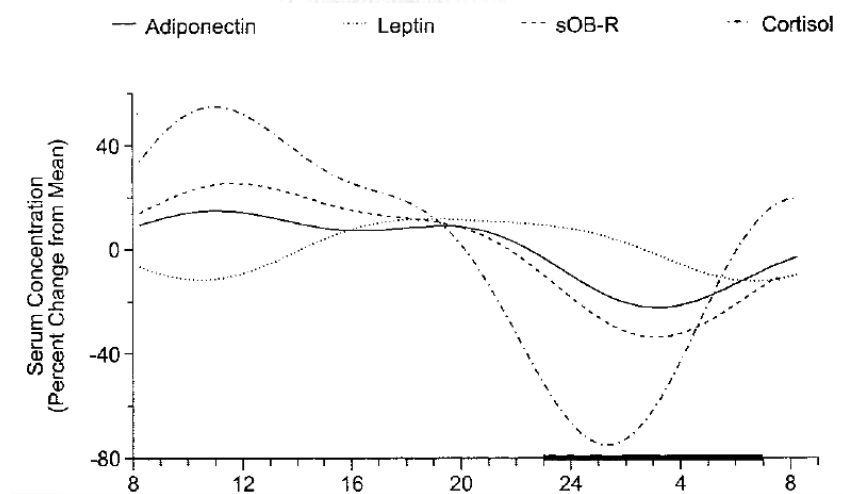


Figure 5 Diurnal variations of serum adiponectin, leptin, sOB-R, and cortisol

Adapted from⁴⁹

Based on findings by Gavrilu et al⁴⁹ as shown in Figure 5 serum adiponectin has a clear diurnal variation characterized by a nocturnal decline begins in the late evening and continuing throughout the night to reach a nadir in the early morning. Adiponectin levels were higher during the day, with a peak in the late morning.

Adiponectin levels decreasing slightly during the early afternoon and then plateauing until late evening. In contrast with adiponectin, leptin is progressively increased during the daytime, reaching peak levels in the evening or early night, declining during sleeping hours to a nadir in early morning. Differ from leptin phase, soluble-leptin receptor (sOB-R) profiles showed a striking similarity and tracked closely with adiponectin over 24 h, reaching their minimum and maximum levels at about the same time.

2.5.3 Relationship between leptin and adiponectin on AMP-activated protein kinase

AMP-activated protein kinase (AMPK) plays a crucial role in the control of energy balance, as it activates the cell processes that promote energy production (i.e. fatty acid oxidation and glucose uptake) while inhibits those that take energy (i.e. lipogenesis, protein synthesis and gluconeogenesis).^{135,136} AMPK is a major element in the integration of inter-tissue signaling events (Figure 6).

Leptin and adiponectin (or Acrp30) are the most abundant adipokines. Both of these adipokines modify glucose and lipid metabolism, insulin sensitivity, food intake, inflammatory processes and cardiovascular function. Leptin secretion and its plasma levels are proportional to the extent of whole-body adipose tissue and mean fat cell size. While plasma adiponectin concentrations are inversely related to adiposity. The metabolic and insulin-sensitizing effects of leptin and adiponectin can be at least partially explained by their direct activation of AMPK. Their actions converge on the final control of AMPK activity in skeletal muscle, liver, and adipose tissue, leading to increased fatty acid oxidation and the prevention of triacylglycerol accumulation and lipotoxicity in these tissues.^{117,137}

In addition to the regulation of insulin sensitivity, insulin has been mediated the action of leptin in the alteration of glucose homeostasis. Insulin gene expression and glucose-stimulated insulin secretion are inhibited by leptin and these showed

the adaptive actions of glucose levels to body fat stores.^{138,139} In turn, both leptin synthesis and secretion are stimulated by insulin, thus resembling an adipose-islet axis.¹⁴⁰

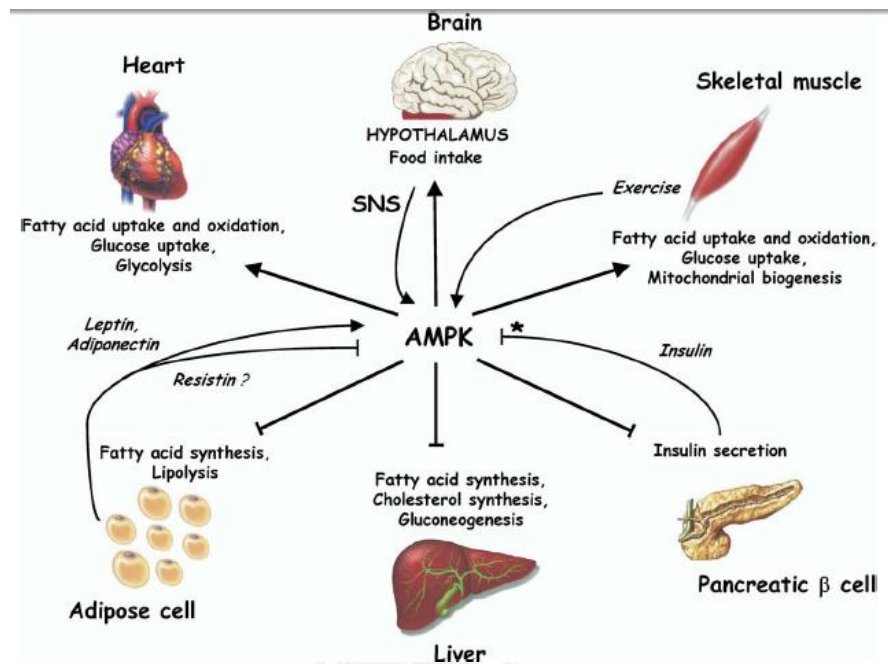


Figure 6 Role of AMP-activated protein kinase in whole-body energy homeostasis

Adapted from¹³⁵

Indeed, adiponectin was primarily investigated as insulin-sensitizer by suppressing gluconeogenesis and increasing fatty acid oxidation, thus reducing triglyceride accumulation in the liver.^{116,141-143} Hepatic insulin sensitivity in humans is associated with circulating adiponectin concentrations,¹⁴⁴⁻¹⁴⁶ the amount of adiponectin in plasma is negatively correlates with endogenous glucose production in healthy,¹⁰⁶ severely obese,^{107,147} and type 2 diabetic individuals.^{51,144,146}

Study by Høeg et al¹⁴⁸ reported that serum adiponectin was significantly associated with leg glucose uptake in healthy, young, lean men, but the association was absent in women. Additionally, serum adiponectin was significantly associated with AMP-activated protein kinase (AMPK) phosphorylation in skeletal muscles of men but not in women. These findings suggested that adiponectin play a modulating

role of human skeletal muscle insulin sensitivity via activation of AMPK. Others¹¹⁶ have described that adiponectin decreased insulin resistance by decreasing muscular lipid content in obese mice.

Adiponectin and leptin may trigger AMP-activated protein kinase both in peripheral and central route in the control of whole-body energy homeostasis. In peripheral site, these two adipokine may propose AMPK stimulates fatty acid oxidation and prevention of triacylglycerol accumulation in the liver, skeletal muscle and adipose tissue as described above.

In central line, they may act on hypothalamus as response of either fasting or refeeding state to control energy balance. Figure 7 below illustrated the hypothetical model based on in vivo studies in mice showed adiponectin and leptin levels in serum and cerebrospinal fluid, and AdipoR1 and AdipoR2 expression in the arcuate nucleus hypothalamus (ARH).⁵²

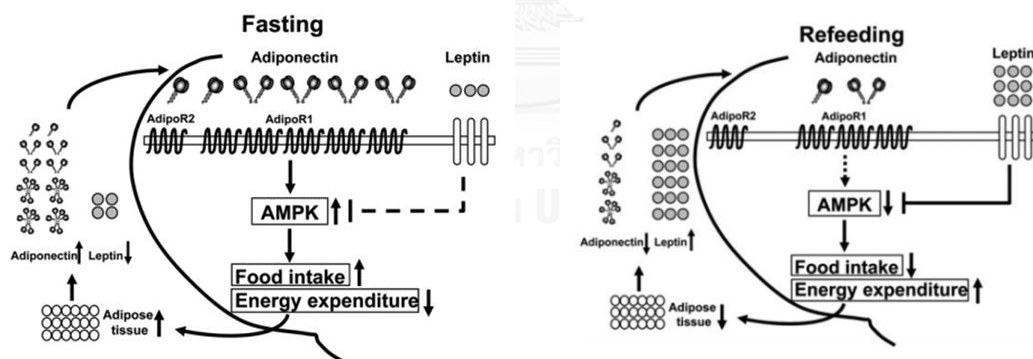


Figure 7 Adiponectin & leptin levels under fasting (left) and refeeding (right)

Adapted from⁵²

Under fasting condition, serum and CSF adiponectin levels and AdipoR1 expression in the ARH increase. As consequences, hypothalamic AMPK is activated, which stimulates food intake and suppresses energy expenditure, hence, promoting fat storage. After refeeding, on the other hand, serum and CSF adiponectin levels

and AdipoR1 expression in the ARH decrease; thereafter decreases hypothalamic AMPK activity, resulting in reduced food intake and increased energy expenditure.⁵²

On the other hand, leptin suppresses hypothalamic AMPK activity and food intake, as opposed to the action of adiponectin. Thus, central adiponectin/leptin signals may represent the physiological pathway by which hypothalamic AMPK activity and food intake are stimulated under fasting conditions and suppressed by refeeding. The fundamental roles of leptin and adiponectin seem to be to preserve an adequate fat reserve: leptin acts as a satiety signal, and adiponectin acts as a starvation signal.⁵²

2.6 Ramadan fast and studies on circulating leptin and adiponectin

Evidence on circulating leptin and adiponectin and Ramadan fasting have been studied little and questions remain. Some studies have investigated circulating plasma or serum leptin and adiponectin in relation with weight loss, metabolic alteration, as well as its role in the regulation of energy homeostasis during Ramadan fasting. Table 4 summarized four available studies on Ramadan fast and serum leptin and adiponectin in healthy subjects.

One study evaluated the health impact of fasting in Saudi Arabia during Ramadan with emphasize on disturbed in circadian rhythm, metabolic and sleep pattern among twenty three healthy volunteers (18 male, 5 female, mean age \pm SEM: 23.2 ± 1.2 years). Fasting serum glucose levels was kept within normal range, with a significant increase in the morning. Mean morning serum concentration of leptin was significantly higher than pre-Ramadan values (7.01 ± 2.77 ng/mL to 16.36 ± 4.35 ng/mL, $P = 0.001$). Contrary, mean morning serum of adiponectin was significantly lower (11.62 ± 0.80 μ mol/mL to 8.80 ± 0.57 μ mol/mL, $P = 0.001$),⁵⁶ and it was similar with another one study by Gnanou et al¹⁴⁹ reported decrease of fasting plasma adiponectin at 8 h after morning meals during Ramadan fasting.

Serum leptin has the circadian rhythm which has been demonstrated in normal subjects, with peak averages between 22:00 and 03:00 hours.^{133,150} Morning leptin levels were relatively high; however, a dramatic reduction in plasma leptin was noted after breakfast, and the lowest level was between 08:00 and 17:00.¹³³ Moreover, plasma leptin profiles were higher in obese than in lean subjects and higher in women than in men.¹⁵¹

Table 4 Studies on Ramadan fast and serum leptin and adiponectin concentrations in healthy volunteers

Studies	(n;sex;age)	Conclusion
Bogdan, Bouchareb, Touitou ⁵⁴	10 male mean age \pm SD (34 \pm 3.7 years)	No significant change have found in amplitude or 24-hour mean serum leptin concentrations; but significant shift of 5 h 30 min in peak and trough serum leptin concentrations were found on the 23 rd day of Ramadan
Alzogaibi et al ⁵⁵	8 male mean age \pm SEM (26.6 \pm 4.9 y)	a significant reduction in plasma leptin levels at 22:00; the nocturnal reduction in plasma leptin levels during fasting may be the result of the changes in meal times during fasting
Ajabnoor et al ⁵⁶	(18 male, 5 female) mean age \pm SEM (23.16 \pm 1.2 y)	Mean morning concentration of serum leptin was significantly higher than pre-Ramadan values (p = 0.001); while adiponectin was significantly lower (p<0.001)
Gnanou et al ¹⁴⁹	(20 male) (19-23 y)	Significant decrease of fasting plasma adiponectin (- 45.6%), glucose (- 12.3%), and insulin (- 52.8%) levels.

Bogdan, Bouchareb, and Touitou⁵⁴ reported the circadian pattern of serum leptin concentrations in ten healthy male volunteers before and on the twenty-third day of Ramadan daytime fasting. Although no changes in the 24 h mean concentrations of serum leptin during Ramadan daytime fasting, a significant shift

(approximately 5 h delay) in the rhythm was observed. Even though a meal was given at 19.00 hours in both situations, it has to be noted that the concentrations of serum leptin at this time point were significantly lower on the Ramadan day than the control day. Another study supported the finding of decrease serum leptin levels during Ramadan in 8 healthy male. From the circadian rhythm study, there was a significant reduction in plasma leptin levels at 22:00 (194.2 ± 177.2 pg/mL vs. 132.6 ± 130.4 pg/mL, $P < 0.05$).⁵⁵

From the available studies, acute sleep deprivation did not alter plasma leptin, but day/night reversal such as time zone shift caused a 12 ± 2 h shift ($P < 0.01$) in the timing of zenith and nadir. When meals were shifted 6.5 h without changing the light or sleep cycles, the plasma leptin rhythm was shifted by 5-7 h ($P < 0.01$).¹⁵² It is important to note that levels of leptin may vary in its physiologic variations,⁴⁹ (a) leptin levels vary exponentially, not linearly with fat mass; its circulating levels are higher between midnight and early morning, (b) diurnal rhythm of circulating leptin levels may be modified by meal-timing; decreases by sleep deprivation, and increased by emotional stress, (c) leptin levels is decreases after short-term fasting (24-72 h), even when changes in fat mass are not observed.

Leptin plays a crucial role in the adaptive response to starvation. A significantly greater proportion of total leptin circulate as bound leptin in lean subjects may correspondent to its greater response of resting energy expenditure rather than body fat mass. On the other hand, free leptin is a major fraction of circulating leptin in obese subjects and related to the changes in body fat mass.¹⁵³

Conceptual Framework

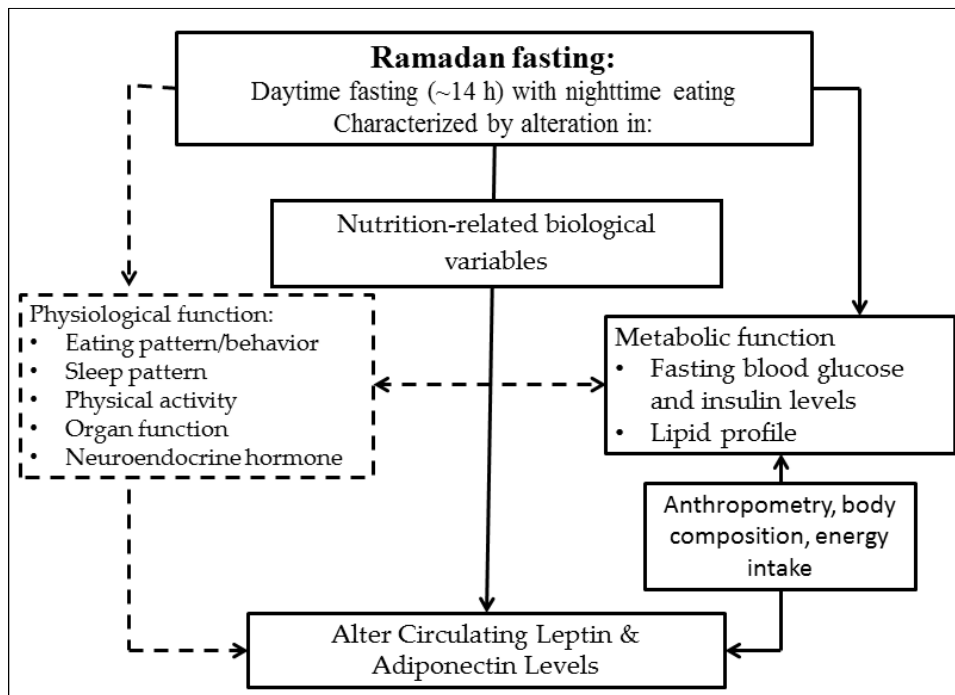
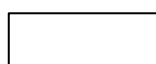


Figure 8 Conceptual framework: Effect of Ramadan fasting on circulating leptin and adiponectin levels and metabolic profiles in healthy men Thai Muslim population

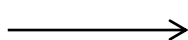
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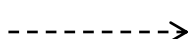
collecting variables



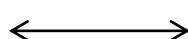
non-collecting variables



one direction of relationship between variables



one direction of relationship between variables (unmeasured)



two direction of relationship between variables



two direction of relationship (unmeasured)

CHAPTER 3

METHODOLOGY

3.1 Study Design

The study was conducted in 29 days of period of Ramadan fasting in year 2015. This observational study has two time point before (beginning day of Ramadan) and after three weeks of Ramadan fasting. Study was carried out in Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand.

3.2 Population and Study Participants

3.2.1 Target population

Target population in this study was healthy adult Muslim men in Bangkok, Thailand who fast during the month of Ramadan in year 2015. In accordance with Islamic rule, in Ramadan period, fasting is an obligatory practice for all adult Muslims except for pregnancy, lactation or menstruation in women, or any illness in both men and women.

3.2.2 Study participants

Participant was selected by purposive sampling in community-based Muslims population in Central Bangkok area, Thailand. All participants in this study were fasted (refrain from eating and drinking from dawn to dusk), free-living, and had been advised to maintain their habitual activity. The inclusion and exclusion of study participants are as follows:

Inclusion criteria:

- Aged 19-40 years
- Muslim men fasted completely in the month of Ramadan

- No known of any metabolic-related diseases

Exclusion criteria:

- Chronic disease: metabolic disease, cardiovascular disease, liver or kidney disease
- Intake of any medication or dietary supplement two weeks before the study
- Smoking and alcohol drinking

Determination of number of study participants (sample size)

$$n = \frac{(t_{n-1,\alpha/2} + t_{n-1,\beta})^2}{d^2}$$

$$n = \frac{(1.96 + 0.84)^2}{\left(\frac{1.5\sqrt{24}}{6.6}\right)^2} = 12.25 = 12$$

Where,

- n = Minimum sample size
- α = The Type I error probability for a two sided test. This is the probability that we will falsely reject the null hypothesis. In this study $\alpha = 5\%$ or $t_{n-1,\alpha/2} = 1.96$
- β = Probability of correctly rejecting the null hypothesis of equal population means given n pairs of subjects, a Type I error probability α and a true difference in population means of δ . In this study $\beta = 80\%$ or $t_{n-1,\beta} = 0.84$
- d = $\left((\delta \div \sigma) \times \sqrt{\frac{n}{2}}\right)$, where δ is difference means, σ is standard deviation, and n is sample size of reference study
- δ = An expected difference between before and after fasting means (body weight in men: - 1.5 kg from pooled studies by Sadeghirad et al.²³ study used $n = 24.3 \pm 13.4$ sample size)
- σ = The within group standard deviation (SD = 6.6 kg from Sadeghirad et al.²³ study)

The minimum sample size for this study is 12 participants. Numbers of participants were calculated based on an expected difference mean of body weight found in pooled studies by Sadeghirad et al.²³ Because of adjusting for 20% dropping out rate by using formula $n_1 = n / (1-d)$; with $n=12$ and $d=0.2$, the minimal total number should be 15 participants.

The study was approved by the office of Ethics Review Committee for Research Involving Human Research Subjects, Human Science Group, Chulalongkorn University (COA no. 136/2558, *see Appendix 1*). Every participant signed the informed consent before being involved in this study.

3.3 Methods

Baseline characteristics of participant were including age, ethnicity, education, occupation, and body mass index (BMI). All participants were underwent two time research visit, before and after three weeks of Ramadan fasting (Figure 9) in order to collect the data as follows:

1. Biochemical parameters including fasting serum leptin and adiponectin levels; the ratio of leptin to adiponectin (L/A); and metabolic profiles including fasting plasma glucose, serum insulin, and serum lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides)
2. Anthropometry indices and body composition including weight (kg), body mass index or BMI (kg/m^2), waist and hip circumferences (cm), fat mass (kg), fat (%), fat free mass or FFM (kg), fat free mass (%), muscle mass (kg), muscle mass (%), total body water or TBW (kg), TBW (%), basal metabolic rate (kcal), extracellular water or ECW (kg), intracellular water or ICW (kg), and ratio ECW to TBW (%)
3. Energy and macronutrient intake before and after three weeks of Ramadan (3 days/week of dietary).

4. Daily physical activity before and after three weeks of Ramadan (3 days/week of daily physical activity record).

Figure 9 below represented the study design and outcome measurements.

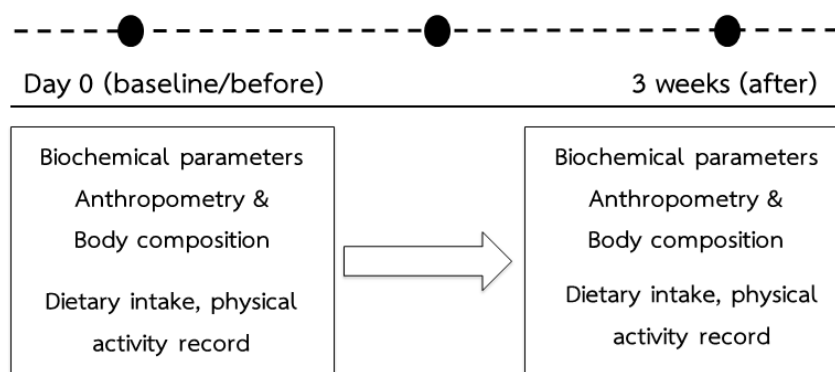


Figure 9 Study design and outcome measurements

3.4 Outcome measurements

3.4.1 Biochemical parameters

Fasting venous blood samples were collected after 9-10 hours of daytime fasting (at 1-2 P.M.) before and after three weeks of Ramadan fasting. Blood samples were collected at the Clinical Laboratory in Faculty of Allied Health Sciences, Chulalongkorn University for analyzing metabolic profiles parameters. Samples of collected serum for determination of leptin and adiponectin were kept at -80°C until analysis.

Fasting serum leptin assay

Serum leptin levels was measured by the enzyme-linked immunoassays (ELISA) method using a commercially human leptin ELISA kit (Peprotech, Inc., Rocky Hill, NJ, USA; Cat. no; 900-K90), which uses a polyclonal antibody against recombinant human leptin, raised in rabbits.

Principle of the assay is based on sandwich ELISA (see **Appendix 2**). Leptin coated on a 96-well plate. Standards and samples are pipetted into the wells and leptin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated antihuman Leptin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, ABTS liquid substrate solution is added to the wells and color develops in proportion to the amount of leptin bound, reflecting from green color, and the intensity of the color is measured at 405 nm with wavelength set at 650 nm.

Fasting serum adiponectin assay

Serum adiponectin was measured by the ELISA method using a commercially human adiponectin ELISA kit (Enzo Life Sciences, Inc., Farmingdale, NY, USA; Cat. no; ALX-850-377). This assay employs a quantitative sandwich enzyme immunoassay technique that measures adiponectin in less than 3 hours (see **Appendix 3**).

A polyclonal anti-human adiponectin antibody has been pre-coated onto a 96-well microplate with removable strips. Adiponectin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for adiponectin, which is recognized by a horseradish-peroxidase conjugate (HRP). All unbound material is then washed away and a peroxidase enzyme substrate solution (TMB) is added. The color development is stopped and the intensity of the color (in yellow) is measured. The absorbance is proportional to the concentrations of adiponectin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Metabolic profiles

Metabolic profiles parameters including fasting plasma glucose, serum insulin, lipid profiles (total cholesterol, triglycerides, HDL-c, and LDL-c levels) were analyzed as shown in Table 5. Additionally, homeostasis model assessment of insulin resistance (HOMA-IR as fasting serum insulin ($\mu\text{IU/mL}$) \times fasting plasma glucose (mmol/L)/22.5) was calculated as indicator to determine insulin resistance.¹⁵⁴

Table 5 Method for analysis metabolic profiles parameters

Test	Laboratory	Method	Analyzer
Plasma Glucose	AHS - CU	Enzymatic	Vitalab Flexor XL ®
Serum total cholesterol	AHS - CU	Enzymatic colorimetric	Vitalab Flexor XL ®
Serum triglycerides	AHS - CU	Enzymatic colorimetric	Vitalab Flexor XL ®
Serum HDL-c	AHS - CU	Enzymatic clearance assay	Vitalab Flexor XL ®
Serum LDL-c	AHS - CU	Estimated by the equation*	
Serum Insulin	PCT**	Chemiluminescence Immunoassay (CLIA)	-

Note: Fasting plasma glucose, serum total cholesterol, triglycerides, HDL-c, LDL-c in mg/dL; Serum Insulin ($\mu\text{IU/mL}$). Abbreviations: HDL-c, high-density lipoprotein cholesterol, LDL-c, low-density lipoprotein cholesterol, AHS – CU, Allied Health Sciences – Chulalongkorn University. * LDL-c: $\text{TC} - \text{HDL-c} - (\text{TG}/5)$.¹⁵⁵ **PCT Laboratory Service, Co.,Ltd. (Bangplad, Bangkok).

3.4.2 Anthropometry indices and body composition parameters

Anthropometry indices (body weight, BMI) and body composition parameters were identified and measured by Bioelectrical Impedance Analysis (MC-980 MA), see **Appendix 4**. Waist and hip circumferences were measured by using a standard rounded tape at a point right above the iliac crest on the mid-axillary line at minimal

respiration (for waist) and in a horizontal plane at the level of the maximal extension of the buttocks (for hip) to the nearest 1.0 cm.

3.4.3 Energy and macronutrient intake

Dietary intake and type of physical activity were recorded in diary handbook (as study-provided) by each participant for 3 days/week (2 week-day and 1 week-end) at before and during three weeks period of Ramadan fasting (see **Appendix 5**). Dietary record provides information of meal-timing, food menu, foodstuff/beverages composition and its portion size in the household unit. The data of foodstuff/beverages in household unit were translated into the amount of food (in gram or mL of food and beverages intake, respectively). Daily energy intake was calculated in kcal (calorie), while carbohydrate, fat, and protein intake were calculated both in mass value (gram) and percentage of calorie contribution (% of calorie) by using food composition database in INMUCAL-Nutrients Program from Institute of Nutrition, Mahidol University. ¹⁵⁶⁻¹⁵⁸

3.4.4 Daily physical activity

Qualitative evaluation on daily physical activity (type of activity with or without exercise) was carried out before and after three weeks of Ramadan fasting in 3 days/week of consecutive records (2 week-day and 1 week-end). The classification of daily physical activity is according to the information has been recorded by each study participants, including: (1) 'no exercise' defined as daily routine activity such as work or study without any exercise; (2) 'exercise (< 1 h)' defined as daily routine activity with exercise holds for less than 1 h; and (3) 'exercise (1-2 h)' defined as daily routine activity with exercise holds for 1-2 h.

3.5 Statistical Analysis

Data were expressed as frequency or percentage for descriptive (categorical data); mean \pm standard error mean (SEM), mean percentage change (% change) \pm SEM for the continuous-normally distributed data; and median (range) for continuous-non normally distributed data. Paired student t-test, two related sample (Wilcoxon) test were employed for comparison of normally distributed and non-normally distributed data, respectively.

Differences were considered as statistically significant for two-tailed of probability value or *P* less than 0.05. Pearson-correlation coefficient was used to determine the correlation between significant values. All analyses were performed in Statistical Package for Social Sciences (SPSS) software version 16.0.

CHAPTER 4

RESULTS

4.1 Baseline characteristics of study participants

Twenty nine volunteers were eligible and participated in the baseline time. During study period, two of study participants were excluded due to incomplete study and another one taken supplement for weight reduction. At last, 27 of study participants were completed the study (Figure 10). None of study participants (n = 27) were smoking, alcohol drinking, use any medication and/or dietary supplement.

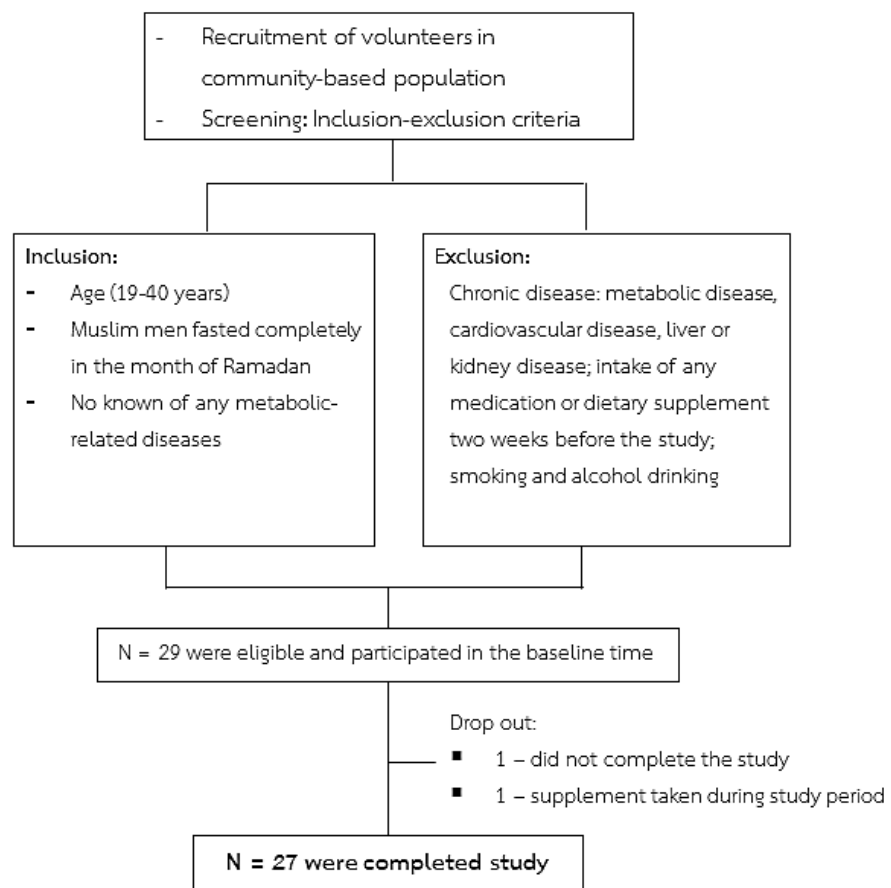


Figure 10 Flowchart the selection of study participants

Study participants were within the age range of 19-31 years with the mean of age was 24.3 ± 0.7 years. Most of them (n=22, 81.5%) were Thai origin, and some others (n=5, 18.5%) were Indonesian who lived in Bangkok Province, Thailand. The others baseline characteristics including education, occupation, and body mass index were mentioned in Table 6.

Table 6 Baseline characteristics of study participants

	Values (n = 27)
Age, years: mean \pm SEM (range)	24.3 \pm 0.7 (19-31)
Ethnicity (n, %)	Thai (22, 81.5%) Indonesian (5, 18.5%)
Education (n, %)	University (27, 100%)
Occupation (n, %)	Students (18, 66.7%) Employee (6, 22.2%) Government officer (2, 7.4%) Other (1, 3.7%)
BMI, kg/m ² : mean \pm SEM (range)	22.3 \pm 0.6 (16.2 – 29.2)

Note: *BMI, body mass index based on Asian criteria.¹⁵⁹

All participants were Ramadan observance, fasted completely during the daytime for approximately 14 h each day in the month of Ramadan. Observance is allowed to have their meals during nighttime, before dawn and shortly after sunset, and have been advised to maintain habitual activity as before Ramadan fasting, and record it together with dietary intake into the handbook as provided.

4.2 Circulating serum leptin and adiponectin

Table 7 shows circulating serum leptin and adiponectin levels did not differ significantly between before and after three weeks of Ramadan fasting with median (range) of serum leptin were 1.35 (0.46-1.94) ng/mL vs. 1.13 (0.46-2.09) ng/mL, whereas serum adiponectin were stable on the levels of 23.93 (16.31-37.13) $\mu\text{g/mL}$ vs. 23.84 (14.66-34.79) $\mu\text{g/mL}$, hence the ratio of leptin to adiponectin ($n = 15$) were remained unchanged after three weeks of Ramadan fasting (Before: median 0.05 (0.02 – 0.10) vs. after: median 0.04 (0.02 – 0.14), $P = 0.968$). In addition, according to participant's body mass index (BMI), $n = 16$ for analyzing serum leptin were having BMI of $22.73 \pm 0.79 \text{ kg/m}^2$ at before and $22.52 \pm 0.79 \text{ kg/m}^2$ after three weeks of Ramadan fasting ($P = 0.078$); whereas $n = 24$ for analyzing serum adiponectin were having BMI of $22.19 \pm 0.72 \text{ kg/m}^2$ at before and $21.85 \pm 0.73 \text{ kg/m}^2$ after three weeks of Ramadan fasting (% change: -1.56 ± 0.39 , $P = 0.0001$). However, BMI in participants for analyzing both serum leptin and adiponectin levels ($n = 15$) were not significantly different ($22.89 \pm 0.83 \text{ kg/m}^2$ vs. $22.66 \pm 0.84 \text{ kg/m}^2$, $P = 0.070$).

Table 7 Circulating serum leptin and adiponectin levels before and after three weeks of Ramadan fasting

Parameters	Before	After	<i>P</i> value
Leptin (ng/mL)	1.35 (0.46 - 1.94)	1.13 (0.46 – 2.09)	0.196
Adiponectin ($\mu\text{g/mL}$)	23.93 (16.31 – 37.13)	23.84 (14.66 – 34.79)	0.587
Ratio of leptin to adiponectin	0.05 (0.02 – 0.10)	0.04 (0.02 – 0.14)	0.968

Notes: Data are expressed as **median (range)**. $P < 0.05$ considered as statistically significant. Serum leptin ($n = 16$), adiponectin ($n = 24$), and ratio of leptin to adiponectin ($n = 15$).

4.3 Metabolic profiles

Table 8 shows the results of metabolic profiles values before and after three weeks of Ramadan fasting. A constant rate of fasting plasma glucose has been observed, kept at above 80 mg/dL that is 87.92 ± 1.28 mg/dL versus 88.55 ± 1.23 mg/dL at before and after three weeks of Ramadan fasting, respectively. Total cholesterol (204.4 ± 7.07 mg/dL to 193.70 ± 5.90 mg/dL, % change: -4.46 ± 1.74 , $P = 0.012$) and HDL-c (52.52 ± 2.29 mg/dL to 46.74 ± 2.16 mg/dL, % change: -10.89 ± 1.41 , $P = 0.0001$) were decreased significantly, and changes in levels of total cholesterol were positively correlated with changes in HDL-c ($r = 0.556$, $P = 0.003$).

Table 8 Metabolic profiles before and after three weeks of Ramadan fasting

Parameters	Before	After	% changes from baseline	P value
Plasma glucose (mg/dL)	87.92 ± 1.28	88.55 ± 1.23	1.15 ± 1.81	0.689
Serum insulin (μ U/mL)	5.37 ± 0.57	4.68 ± 0.58	-5.59 ± 10.05	0.154
Total cholesterol (mg/dL)	204.40 ± 7.07	193.70 ± 5.90	-4.46 ± 1.74	0.012
HDL-c (mg/dL)	52.52 ± 2.29	46.74 ± 2.16	-10.89 ± 1.41	0.0001
LDL-c (mg/dL)	133.77 ± 5.44	131.49 ± 4.79	-0.32 ± 2.56	0.553
Triglycerides (mg/dL)	90.59 ± 12.01	77.37 ± 9.65	-10.79 ± 5.98	0.058
HOMA-IR (unit)	1.19 ± 0.14	1.04 ± 0.14	-2.45 ± 11.86	0.218

Notes: Data are expressed as **mean \pm SEM**. $P < 0.05$ considered as statistically significant. Fasting plasma glucose, total cholesterol, HDL-c, LDL-c, and triglycerides (n = 27); fasting serum insulin and HOMA-IR (n = 22). Abbreviations: HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance.

Whereas, serum insulin levels (5.37 ± 0.57 $\mu\text{IU/mL}$ to 4.68 ± 0.58 $\mu\text{IU/mL}$, $P = 0.154$), LDL-c (133.77 ± 5.44 mg/dL to 131.49 ± 4.79 mg/dL, $P = 0.553$) and triglycerides levels (90.59 ± 12.01 mg/dL to 77.37 ± 9.65 mg/dL, $P = 0.058$) were not different significantly from baseline values.

4.4 Anthropometry and body composition

Details of anthropometric and body composition parameters before and after three weeks of Ramadan fasting have shown in Table 9. A significant decreased on body weight was observed after Ramadan fasting (65.33 ± 2.14 kg to 64.23 ± 2.13 kg, % change: -1.71 ± 0.38 , $P = 0.0001$). The initial mean of BMI was 22.30 ± 0.66 kg/m^2 and decreased to the value of 21.93 ± 0.65 kg/m^2 with % change from baseline was -1.66 ± 0.38 . A reduction in body weight is likely due to a significant decreased on body fat mass (11.72 ± 1.12 kg to 11.27 ± 1.05 kg, % change: -3.84 ± 1.84 , $P = 0.03$), fat free mass or FFM (53.60 ± 1.19 kg to 52.96 ± 1.20 kg, % change: -1.21 ± 0.36 , $P = 0.004$), and muscle mass (50.82 ± 1.13 kg to 50.21 ± 1.14 kg, % change: -1.21 ± 0.36 , $P = 0.004$) after three weeks of Ramadan fasting.

A decrease in body weight was positively correlated with decrease in fat mass ($r = 0.602$, $P = 0.001$), FFM ($r = 0.619$, $P = 0.001$), and muscle mass ($r = 0.621$, $P = 0.001$). Ramadan fasting did not pose any significant effect on body fat percentage and total body water. While, the others anthropometric parameters including waist (81.82 ± 1.49 cm to 78.82 ± 1.53 cm, % change: -3.63 ± 0.79 , $P = 0.0001$) and hip (96.83 ± 1.44 cm to 94.20 ± 1.52 cm, % change: -2.69 ± 0.70 , $P = 0.001$) circumferences were decreased significantly without any significant changes on waist-to-hip ratio.

Basal metabolic rate were decreased significantly (1533.74 ± 35.82 kcal to 1513.85 ± 35.84 kcal, % change: -1.21 ± 0.36 , $P = 0.002$) and it was positively correlated with a decrease in body weight ($r = 0.673$, $P = 0.0001$) and BMI ($r = 0.641$,

$P = 0.0001$), respectively. Eventually, a little loss of extracellular water volume has been observed (% change: -1.01 ± 0.37 , $P = 0.017$) and it was positively correlated with a decrease in body weight ($r = 0.609$, $P = 0.001$) and BMI ($r = 0.576$, $P = 0.002$), respectively.

Table 9 Anthropometric and body composition parameters before and after three weeks of Ramadan fasting

Parameters	Before	After	% change from baseline	<i>P</i> value
Weight (kg)	65.33 \pm 2.14	64.23 \pm 2.13	- 1.71 \pm 0.38	0.0001
BMI (kg/m ²)	22.30 \pm 0.66	21.93 \pm 0.65	- 1.66 \pm 0.38	0.0001
WC (cm)	81.82 \pm 1.49	78.82 \pm 1.53	- 3.63 \pm 0.79	0.0001
HC (cm)	96.83 \pm 1.44	94.20 \pm 1.52	- 2.69 \pm 0.70	0.001
WHR	0.85 \pm 0.01	0.84 \pm 0.01	- 0.91 \pm 0.77	0.223
Fat mass (kg)	11.72 \pm 1.12	11.27 \pm 1.09	- 3.84 \pm 1.84	0.03
Fat (%)	17.11 \pm 1.16	16.69 \pm 1.15	- 2.28 \pm 1.66	0.14
Fat free mass (kg)	53.60 \pm 1.19	52.96 \pm 1.20	- 1.21 \pm 0.36	0.004
Fat free mass (%)	82.89 \pm 1.16	83.31 \pm 1.15	0.53 \pm 0.33	0.131
Muscle mass (kg)	50.82 \pm 1.13	50.21 \pm 1.14	- 1.21 \pm 0.36	0.004
Muscle mass (%)	78.57 \pm 1.10	78.98 \pm 1.09	0.52 \pm 0.33	0.127
TBW (kg)	36.05 \pm 0.86	35.73 \pm 0.85	- 0.84 \pm 0.67	0.242
TBW (%)	55.83 \pm 1.07	56.28 \pm 1.06	0.91 \pm 0.68	0.234
BMR (kcal)	1533.74 \pm 35.82	1513.85 \pm 35.84	- 1.21 \pm 0.36	0.002
ECW (kg)	13.90 \pm 0.28	13.76 \pm 0.28	- 1.01 \pm 0.37	0.017
ICW (kg)	22.15 \pm 0.60	21.97 \pm 0.59	- 0.71 \pm 0.88	0.415
Ratio ECW to TBW (%)	39.05 \pm 0.41	38.59 \pm 0.29	- 0.99 \pm 0.88	0.262

Notes: Data are expressed as **mean \pm SEM**. $P < 0.05$ considered as statistically significant. Abbreviations: BMI, body mass index; WC, waist circumference; WHR, waist-to-hip ratio; TBW, total body water; BMR, basal metabolic rate; ECW, extracellular water; ICW, intracellular water.

4.5 Energy and macronutrient intake

Dietary records were obtained from participants for 3 days/week (2 weekday and 1 weekend) before and after three weeks of Ramadan fasting. Energy and macronutrient intake data were presented in energy intake (calorie per day); carbohydrate, fat, and protein intake (gram per day); and % of daily calorie contributed from carbohydrate, fat, and protein. Daily energy intake (1538.73 ± 63.13 kcal to 1292.12 ± 44.92 kcal, % change: -12.68 ± 4.51 , $P = 0.003$), carbohydrate intake (203.29 ± 9.71 g to 173.26 ± 9.27 g, % change: -11.15 ± 5.10 , $P = 0.018$), and fat intake (50.56 ± 3.20 g to 41.53 ± 2.37 g, % change: -9.24 ± 8.51 , $P = 0.028$) were decreased significantly after three weeks of Ramadan fasting; whereas daily protein intake and % of daily calorie contributed from carbohydrate, fat, and protein were not different significantly (Table 10).

Table 10 Energy and macronutrient intake before and after three weeks of Ramadan fasting

	Before	After	% change from baseline	<i>P</i> value
Energy (kcal)	1538.73 ± 63.13	1292.12 ± 44.92	-12.68 ± 4.51	0.003
Carbohydrate (g)	203.29 ± 9.71	173.26 ± 9.27	-11.15 ± 5.10	0.018
Carbohydrate (%)	52.94 ± 1.45	53.53 ± 1.74	2.97 ± 4.05	0.794
Fat (g)	50.56 ± 3.20	41.53 ± 2.37	-9.24 ± 8.51	0.028
Fat (%)	29.30 ± 1.10	28.41 ± 1.11	2.45 ± 6.05	0.752
Protein (g)	67.93 ± 3.81	57.64 ± 2.97	-2.04 ± 14.03	0.064
Protein (%)	17.82 ± 0.92	18.06 ± 0.79	8.20 ± 8.58	0.833

Notes: Data are expressed as **mean \pm SEM**. $P < 0.05$ considered as statistically significant.

4.6 Daily physical activity

Daily physical activity (type of activity with or without exercise) was recorded by each of study participants during study period and compiled as 3 days/week record (2 week-day and 1 week-end) at before and after three weeks of Ramadan. Three classifications were used to classify type of daily physical activity: (1) no exercise defined as daily routine activity such as work or study without any exercise; (2) exercise < 1-h defined as daily routine activity with exercise holds for less than 1 h; and (3) exercise 1-2 h defined as daily routine activity with exercise holds for 1-2 h. Type of exercise varies such as stretching and gym training (sit-up, push-up, skipping, weight training, and cardio exercise) sports performances (bike, swim, run, football, badminton, tennis).

Most of participants (n=18, 66.7%) have no exercise before and during Ramadan fasting (Table 11). A few number of participants holds exercise < 1-h (n = 3, 11.1% versus n = 5, 18.5%); and exercise 1-2 h (n = 6, 22.2% versus n = 4, 14.8%). Additionally, in the nighttime, Ramadan observance had practiced a routine praying called as 'Taraweeh' in the mosque together with others Muslims. However, we have no determination in this factor with physical activity levels.

Table 11 Daily physical activity before and after three weeks of Ramadan fasting

Distribution of daily physical activity n (%)			
	No exercise	Exercise (< 1 h)	Exercise (1-2 h)
Before	18 (66.7)	3 (11.1)	6 (22.2)
After	18 (66.7)	5 (18.5)	4 (14.8)

CHAPTER 5

DISCUSSION

This observational study has been carried out in men since any possible absence from fasting (non-fasting state during menstrual period) in women may induce more heterogeneity on the number of fasting days and body adaptation. On the other hand, sex differential may be act as confounders on study outcomes such as adiponectin and leptin hormones because both sex and age provide differences of nutrient oxidation and changes in diurnal energy expenditure during the fasting period. Variation in ages, sex, life style (smoking habit), dietary and physical activity factors have been identified in this study. As the results, we are able to discuss in depth of any factors associated with Ramadan fasting in healthy men.

Three weeks of daytime fasting in the month of Ramadan did not pose significant changes on circulating serum leptin and adiponectin levels; serum total cholesterol and high-density lipoprotein cholesterol (HDL-c) were decreased significantly; while have no remarkable changes in fasting plasma glucose, serum insulin, low-density lipoprotein cholesterol (LDL-c), triglycerides, and homeostasis model assessment of insulin resistance (HOMA-IR) index. Weight reduction ($- 1.7 \pm 0.38\%$) were noted accompanied by reduction in fat mass ($- 3.84 \pm 1.84\%$), fat free mass ($- 1.21 \pm 0.36\%$), and muscle mass ($- 1.21 \pm 0.36\%$).

Reduced energy intake, carbohydrate, and fat intake were also noted following three weeks of Ramadan fasting. There are several plausible factors and proposed mechanisms explaining the occurrence of the available results from this study which is discussed within three parts (1) effect of Ramadan fasting on circulating leptin and adiponectin in healthy men; (2) effect of Ramadan fasting on

metabolic profiles in healthy men; (3) effect of Ramadan fasting on anthropometry indices, body composition, and energy intake in healthy men.

1. Effect of Ramadan fasting on circulating leptin and adiponectin in healthy men

Serum leptin and adiponectin on third week of Ramadan fasting did not differ significantly with baseline fasting (earlier days of Ramadan). In comparison with previous available studies, we notice discrepancies among the results due to different in study population and protocol design as summarized in Table 12.

Former studies by Bogdan et al⁵⁴ and Alzoghbaibi et al,⁵⁵ measured serum and plasma leptin in 24-h span of mean concentrations by the cosinor rhythmicity analysis at before and during Ramadan (either 2nd or 3rd week of Ramadan). No changes in 24-h mean concentrations of serum/plasma leptin with shifts in 5 h 30 minute of nocturnal peak of serum leptin were observed in Bogdan et al,⁵⁴ while another one study showed a reduction of plasma leptin at 22:00 h.⁵⁵ Bogdan et al⁵⁴ have identified the primary possible confounding factors to the changes on circadian rhythm of leptin including meal-timing and composition, so that all meals were quantitatively and qualitatively standardized by a nutritionist and were eaten at fixed hours both before and during Ramadan. Moreover, Alzhoghaibi et al,⁵⁵ have identified sleep-wake cycles, sleep duration and quality, physical activity, light exposure, and energy expenditure in sleep disorder center (SDC) as others potential confounding factors. From this study, sleep pattern were not different when it measured at SDC, while at home (by a wrist actigraphy) it showed different in mean bedtime (baseline: 00:36 vs. Ramadan: 02:42, $P = 0.004$) and mean wake-up (baseline: 05:30 vs. Ramadan: 08:48, $P = 0.034$), respectively; whereas energy expenditure did not differ from pre-Ramadan values.

Latter study by Ajabnoor et al⁵⁶ reported serum leptin and adiponectin levels measured at morning and evening time at before and 2 week into Ramadan fasting which is differ from the former studies on its protocol, in this study morning serum leptin levels were higher compared to the value at before Ramadan (16.36 ± 4.35 ng/mL vs. 7.01 ± 2.27 ng/mL, $P = 0.001$), while morning serum adiponectin levels were lower during Ramadan (11.62 ± 0.80 μ mol/mL vs. 8.80 ± 0.57 μ mol/mL, $P = 0.001$). The changes of leptin and adiponectin could be attributed to the sleeping/feeding pattern-associated with Ramadan which has effect on diurnal variation of these hormones. Another plausible explanation for the outcome of the study by Ajabnoor et al,⁵⁶ was in the morning time during Ramadan, leptin may function in response to the last meal (pre-dawn meal or called as 'Sahur') typically increased its circulating levels during 4-7 h after meal to suppress food intake. While before Ramadan (non-fasting day), nadir or low levels were noted in the morning after overnight fast which has been previously studied by Gavrilu et al.⁴⁹ On the other hand, adiponectin has inverse diurnal variation with those of leptin in healthy men. Additionally, we noticed that in study by Ajabnoor et al,⁵⁶ there was variation in gender (18 male and 5 female) which may effect to the available results.

In regards with the findings from this present study, serum leptin levels showed unremarkable changes compared to baseline fasting day of Ramadan. According to the literature, mid-afternoon circulating levels of leptin reach its nadir values, and in our study time intervals from the last meal was 9-10 h, therefore we were able to prevent the bias from acute effect of meal towards circulating leptin levels. It is important to note that single measurement of circulating leptin levels has sensitivity to the variation of time when the blood has been withdrawal,¹⁶⁰ hence, either acute or chronic effect of the state between feeding and fasting may influence circulating leptin levels.

Overall, in our knowledge, our study has some strength points in order to prevent the possible confounding/bias from baseline characteristics of study participants, including study participants all of were men; baseline BMI range were shorter than previous study; and blood withdrawal between before (earlier days of Ramadan) and after three weeks of Ramadan fasting were taken at the same time and same environmental condition of study participants, in which study participants fasted in daytime hours, had last meal as pre-dawn meal, and had activity in the morning time either study for students or working for employees, then blood withdrawal taken in the afternoon (1 PM \pm 1 h).

Additionally, we aim to see effect of Ramadan fasting in free-living individuals, in the assumption of usual activity is maintained and participants were slept at similar night hours with baseline time, so that others factors might be attributable to the establish effect, such as intra-individual variability in response to meal-time and portion size changes eaten during Ramadan; energy expenditure, and variation of the initial leptin concentrations due to body mass index variation (lean men have less circulating leptin compared to obese; and circulating leptin were associated with resting energy expenditure in lean men, while in obese more reflected to the amount of fat mass). Although, mean body mass index (BMI) in our study was at the rate for normal-weight, we could not discard these possibilities of variation in BMI to the available results of circulating serum leptin and adiponectin levels.

To date, very few studies have estimated circulating adiponectin levels during Ramadan fasting and yet the available studies have shown conflicting results of either no change or decrease on its levels with or without changes in body mass or body fat during Ramadan.^{56,149} As aforementioned, from Ajabnoor et al,⁵⁶ morning serum adiponectin was lower in second week of Ramadan fasting and it was correspondent with higher homeostasis model assessment of insulin resistance index

(HOMA-IR) in morning and evening both at before and during Ramadan fasting, respectively.^{56,161} We notice that these probably happened due to wide variation in baseline BMI (18.6 – 41.1 kg/m²) in that study may confounds the outcomes.

Table 12 Comparison of present study with other previous studies of the effect of Ramadan fasting (RF) on circulating leptin and adiponectin

Parameters	Present study	Ajabnoor et al ⁵⁶	Alzhoghaibi et al ⁵⁵	Bogdan et al ⁵⁴	Gnanou et al ¹⁴⁹
Time	Days 2-3 of RF (Before-baseline fasting) After 3-wk of RF	Before RF 2-wk into RF	(1) Baseline (2) Baseline fasting (3) 2-wk of RF	1-wk before RF 23 rd day of RF	1-wk before RF 4-wk of RF
Outcome	Serum leptin, adiponectin	Serum leptin, adiponectin	Plasma leptin	Serum leptin	Plasma adiponectin
Type of test	Single	(AM, PM)	24-h span	24-h span	Single
Subjects					
Healthy	Yes	Yes	Yes	Yes	Yes
Male	27	18	8	10	20
Female	-	5	-	-	-
Age (y)	19 – 31	18 – 42	20 - 35	32 - 40	19 – 23
BMI (kg/m ²)	22.3 ± SEM (0.6) (16.2 – 29.2)	24.6 ± SEM (1.1)* (18.6 – 41.1)	23.7 ± SEM (3.5)	-	22.2 ± SD (2.2)
Weight changes	Yes (- 1.7%)	No	No	-	Yes (- 2.4%)
Leptin changes	No change	AM: Increased	Nocturnal reduction	5 h 30 min shifts in peak	-
Adiponectin changes	No change	AM: Decreased	-	-	Decreased

Note: *reported in earlier study by Bahijri et al.¹⁶¹

Another one study by Gnanou et al¹⁴⁹ measured plasma adiponectin levels at the before (1 week prior to Ramadan) and 4th week of Ramadan, as a result, mean plasma adiponectin levels were decreased (- 45.6%) followed by decreased levels of fasting plasma glucose (- 12.3%) and insulin levels (- 52.8%) in 20 healthy young male (19 – 23 years). Study participants from this previous study were healthy Malaysian students who lived in the same dormitory with mean BMI of 22.2 ± SD (2.2). In addition, body weight (- 2.4%), BMI (- 4.5%), and mid-upper arm circumferences (- 3.2%) were also noted to be decreased during fourth week of Ramadan, compared to the pre-Ramadan values.

Indeed, Ramadan fasting has been reviewed by many research groups, in particular of its impact on circadian context.⁵ Circadian, is a word from Latin root 'circa and 'diem' means 'about a day.' A modification in circadian rhythms of some hormone secretion has been found in Ramadan circadian study, for example delayed in the timing of both morning rise of cortisol and nighttime melatonin peak.⁵³ Rest-activity cycle or light/dark cycle and meals play a part in the synchronization of individuals to the 24-h day which may contribute to the alteration in certain biological variables. Adipocyte-derived hormone leptin also has circadian rhythm and oscillatory pattern. It is demonstrated that the nocturnal increase of leptin secretion is entrained by mealtime, probably as a result of the cumulative hyperinsulinemia that occurs over the entire day.^{50,152}

Circulating leptin and adiponectin levels have found no viable change following three weeks of Ramadan fasting. Although not significant, most participants has experienced decreases levels of circulating leptin in third week of Ramadan, compared to the earlier days of Ramadan. It is important to discuss the biological function of leptin under fasting condition. From literature point of view, the biological effect of leptin on lipolysis and fatty acid oxidation is known to be

independently of food intake and/or weight loss. A rapid fall of circulating leptin found during fasting obviously did not affect its action on leptin-mediated lipolysis and fatty acid oxidation in peripheral tissues. Leptin binds to adipocytes to selectively counteract insulin-stimulated lipogenesis and activate lipolysis and lipid utilization in WAT,¹⁶² especially in its visceral compartment. Another explanation is due to role of catecholamine and growth hormones facilitate lipolysis and lipid utilization to systemic signals of energy deficit.^{163,164} Moreover, changes in hormone sensitivities and responses are greater to more rapid rather than to gradual or prolonged reductions in energy availability. Declines in leptin concentration are greater during faster weight loss over a two-day food restriction¹⁶⁵ than to a slower but cumulatively larger energy deficit extended over a 4-¹⁶⁶ or 7-d period.¹⁶⁷

Alteration/changes in nutrient availability are sensed and targeted for multiple biological pathways in multiple tissues. Feeding state also induces adipocytes-mediated secretion of counter-regulatory hormone, leptin to suppress feeding, increase metabolic rate, and limit weight gain. Contrary, under fasting condition, circulating leptin levels is rapidly decreased by reducing energy intake, greater than would be expected for smaller reductions of body adiposity. On the other hand, the entrainment in meal-timing or composition in the rate of decrease energy intake may alter circulating leptin levels.^{78,152} Acute effect of eating on leptin concentrations has been demonstrated, although early studies shown no acute effect, later studies demonstrated that meals and insulin acutely affect leptin concentrations.¹⁶⁸ Indeed, Leptin has dual regulation, in regular eating cycles or during period of weight maintenance, when energy balance is pronounced, leptin reflects the proportion of adipose tissue or total body fat mass,^{169,170} show an exponential relationship. However, in such condition of negative or positive energy balance, the changes of leptin concentrations function as sensor of energy imbalance.

In the experimental short-term fasting show that within 24 h of fasting, leptin concentrations decrease to ~30% of initial basal values and increases leptin ~50% of initial basal values by massive overfeeding over a 12-h.¹⁵⁰ However, Ramadan fasting is differ from that of experimental fasting. In Ramadan, short-term fasting (~14-h) is separated by two-meals before dawn and another one shortly after sunset. Fasters were free-living and another factor despite of eating habit is probably cues on physiological and metabolic condition. In lean individuals, the majority of leptin circulates in the bound form, whereas in obese, circulating leptin is present in the free form.¹⁵³ During short-term fasting, a rapid decrease of free-leptin is greater found in lean individuals than those in obese. In food deprivation or relatively small energy store, leptin circulating predominantly in the bound form and therefore may limits its availability to hypothalamic leptin receptors to induce the inhibitory effect of leptin to food intake and/or energy metabolism.¹⁵³ In addition, under fasting conditions, fatty acids may increase glucagon secretion indirectly by limiting somatostatin's inhibitory effect on islet α -cell exocytosis, and fasting-induced decreases in leptin secretion may act to relieve leptin's inhibitory effect on glucagon secretion. Thus, these conditions provide a link between adipocyte and islet in order to maintain glucose homeostasis.¹⁷¹

In terms of circulating adiponectin levels, insignificant decrease levels of circulating leptin is followed by unchanged circulating adiponectin levels following three weeks of Ramadan fasting. It may assume that compared to leptin, adiponectin level is under a constant rate reflecting a stable of energy reservoir in particular of the size of adipose tissue. According to the literature revealed that circulating adiponectin has no great fluctuations meaning that the release of adiponectin into circulation is regulated by long-term metabolic changes, and its circulating diurnal variation is much less than that of observed in leptin.¹⁷² Changes in blood levels of

metabolic hormones usually occur within a few minute to hours following a meal. However, circulating adiponectin levels appear not to acutely respond to meal. Study has demonstrated, when subjects fed 3 undefined meals over 22 h, there was no significant fluctuation in circulating adiponectin levels.^{51,173} Another study showed that postprandial plasma adiponectin levels were unchanged over 180 min in lean subject after breakfast.¹⁷⁴ Consistent with these studies, glucose infusion of 200 mg/m²/min for 48 h in normal-weight, insulin-sensitive humans affected to increase plasma glucose, insulin, and leptin concentrations, but did not change circulating adiponectin concentrations.¹⁷⁵

The available results showed that circulating adiponectin levels were remained unchanged. Small reduction in body weight is insufficient to induce elevation on adiponectin in the circulation. Another reason is an intra-individual variability that may cause variation on its circulating levels, hence, either decrease or increase of circulating adiponectin levels during short-term fasting did not reflect to its role as insulin-sensitizers in lean individuals, but more as a hormone related to the regulation of energy homeostasis.

At a glance, in molecular perspective, there is a proposed mechanism in action to identify the regulation in the energy homeostasis during fasting. Evidence-based studies become more increasingly clear to determine the important role of nutrients (nutrient sensing) in particular of macronutrients, carbohydrates, protein, and lipids towards regulation of energy metabolism.¹⁷⁶ The available results may imply that the regulation in energy homeostasis during the intermittent fasting is pronounced by activating fatty acid oxidation rather than carbohydrate oxidation to provide fuels in liver and skeletal muscle, and Ramadan is known to be one of unique natural model to study the intermittent fasting.⁶⁵

Ramadan fasting (~14 hours of fasting time) is categorized as stage 2 from the five metabolic stages between the post-absorptive state and near-steady state of prolonged starvation.^{177,178} In this stage, the origins of blood glucose are glycogen and some degree of hepatic gluconeogenesis. Fatty adipose tissue lipolysis is prominent under fasting condition, and both fasting and leptin independently increases adipose tissue lipolysis.¹⁷¹ Aside from adipocytes, in the liver, leptin also prevents lipogenesis while activating β -oxidation of fatty acids. Leptin receptors are abundantly expressed in the liver and their expression is increased in response to leptin administration and short-term fasting.¹⁷⁹ The release of intracellular fatty acids by stimulation of lipolysis potently inhibits stimulated leptin secretion. It is suggested that endogenous free fatty acids in a condition of stress such as physical activity and less food availability inhibits leptin secretion.⁴⁴

2. Effect of Ramadan fasting on metabolic profiles in healthy men

A meta-analysis reported that in men, Ramadan fasting resulted in weight loss, together with a substantial reduction in total cholesterol, LDL-cholesterol, and a small decrease in triglycerides levels.²⁴ Findings from this study showed three weeks of Ramadan fasting induced reduction in total cholesterol levels and HDL-c. Fasting plasma glucose, serum insulin, LDL-c, and triglycerides levels, and homeostasis model assessment of insulin resistance (HOMA-IR) index were not decreased significantly. Decrease of total cholesterol was likely due to decrease in energy intake and dietary fat intake, and reduction in HDL-cholesterol may be reflected by some causal-factors such as lower physical activity level, lower in quality of dietary fat intake, and stress factor. In terms of dietary intake, a decrease level of the total energy intake and type of dietary fat (less polyunsaturated fatty acid or PUFA) may contribute to the lower HDL-c levels. However, we did not evaluate fatty acid composition and profiles in this study. From our study, energy and macronutrient

intake (carbohydrate and fat), except protein were decreased after three weeks of Ramadan fasting.

Previous study by Faris et al reported nutrient intake profile during Ramadan compared to the pre-Ramadan values. Energy and macronutrient intake (total protein, total carbohydrate), PUFA, fatty acids, trans-fat, cholesterol, α -carotene, vitamin C, and lycopene did not differ significantly.¹⁸ While, water intake and folate ($P < 0.001$); total fat, saturated, and monounsaturated fats ($P < 0.01$); and β -carotene intake ($P < 0.05$) were decreased significantly during Ramadan.¹⁸ However, this previous study evaluated the effect of Ramadan fasting in both male and female ($n = 50$, 21 male, 29 female), so that it may affected to the overall results in nutrient intake and blood lipid profile due to the interruption of fasting during the menstrual period (5 ± 2 days). While, Ziaee et al,³ indicated Ramadan fasting led to a decrease in fasting plasma glucose, body weight, and HDL-c. In contrast with the available results, a meta-analysis found that in relation to macronutrient composition, meals are often composed of more fat and less carbohydrate during Ramadan compared with the rest of the years.^{21,74}

Another plausible explanation is metabolic flexibility, a concept in which homeostatic adaptation is pronounced under fasting state. Over the last 5 decades, it was reported that in healthy lean volunteers, skeletal muscle chiefly relies on fat oxidation after just an overnight fast.¹⁸⁰ During fasting, a high rate extraction of plasma free fatty acid by skeletal muscle occurred approximately for 40%.¹⁸¹ Indeed, metabolic flexibility is not a new concept in normal physiological but recently has gain more attention in investigations due to its link with the etiology, prevention, and treatment of insulin resistance in obesity and type 2 diabetes.^{182,183}

In case of physical activity or exercise factors, even so during Ramadan, each participant tended to maintain their daily physical activity as experienced, we could

not clearly defined their levels of physical activity during Ramadan. It may be lower than usual day for such an active exercise. Thus, may reasonable to induce a reduction in HDL-cholesterol levels. In contrast with this findings, previous study reported a beneficial effect of physical activity during Ramadan fasting, being moderately active during Ramadan helped to maintain or even gain some fitness adaptations, and that fasted individuals who are active seemed to cope better with Ramadan fasting as have been shown in two studies by Trabelsi et al.¹⁸⁴ These studies reported that, a group of physically active men who performed their fasting regimen lowered their body mass and body fat percentage and increased their HDL-c to a greater extent compared to the one who were active but did not fast.

3. Effect of Ramadan fasting on anthropometry indices, body composition, and energy intake in healthy men

Weight loss of $-1.71 \pm 0.38\%$ were noted after three weeks of daytime fasting in the month of Ramadan. This modest reduction in body weight was accompanied by decreased on body composition parameters in particular of fat mass, fat free mass, and muscle mass. Weight loss was positively correlated with a reduction in fat mass (kg) followed by fat free mass (kg) and small change in muscle mass (kg). A decrease amount of fat free mass and muscle mass is possibly caused by an increased rate of protein breakdown i.e. muscle protein in relation to the protein synthesis in the post-absorptive state (8-16 hours after meal) leading to the loss of lean tissue.¹⁸⁵ This present study was similar with findings from previous studies by Faris et al,¹⁸ Fahrial Syam et al,¹² and Nourouzy et al,¹³ that fasting in Ramadan resulted in decreased on body weight, BMI, body fat mass, fat free mass, waist and hip circumferences in healthy individuals and these were consistent with the latest available systematic review and meta-analysis on body weight in healthy men.

Faris et al,¹⁸ demonstrated the effect of Ramadan fasting on anthropometric indices (body weight, body mass index or BMI, waist and hip circumferences, body fat percentage) in 50 healthy volunteers (21 men aged 18-49 years; 29 women aged 18-51 years; mean age, 32.7 ± 9.5 years). The outcomes were compared within three time point: 7 days before Ramadan, after 21 days of Ramadan, and 1 month after the end of Ramadan fasting month with the range of fasting hours was about 14 to 15 h a day. A significant reduction in body weight (71.82 ± 13.41 kg to 70.58 ± 13.2 kg, $P < 0.001$), BMI (26.30 ± 5.01 kg/m² to 25.85 ± 4.91 kg/m², $P < 0.001$), and % body fat ($24.12 \pm 12.60\%$ to $20.38 \pm 11.32\%$, $P < 0.001$) were noted during Ramadan, compared to the pre-Ramadan values.

Similar findings also reported by Fahrial Syam et al¹² in 43 healthy subjects (84% female, aged 34.19 ± 11.25 years). During Ramadan (28th day), weight loss (-0.874 ± 0.859 kg, $P < 0.001$) was followed by decreased in BMI (-0.360 ± 0.371 kg/m², $P < 0.001$), body fat (-0.484 ± 0.597 kg, $P < 0.001$), body water (-0.293 ± 0.46 kg, $P = 0.001$), without any significant changes on calorie intake (12.94 ± 760.608 kcal, $P = 0.082$). However, this previous study was conducted in majority of female subjects. While, in Norouzy et al,¹³ in comparison across age and sex, weight loss was found greater in male than female and especially in the age range ≤ 35 years. In males, body weight were decreased significantly (% change: -2.2% (SE 2.2), $P < 0.001$) followed by reduction in BMI (% change: -2.1% (SE 1.8), $P < 0.001$), fat mass (% change: -4.3% (SE 4.7), $P = 0.001$), fat free mass (% change: -2.1% (SE 1.8), $P < 0.001$), % body fat (% change: -2.5% (SE 3.2), $P < 0.029$), waist (% change: -1.3% (SE 1.3), $P = 0.004$) and hip (% change: -1.9% (SE 1.0), $P < 0.001$) circumferences.¹³

A systematic-review and meta-analysis of 35 observational studies in Ramadan found a mean reduction in body weight of -1.24 kg; 95% CI $-1.60, -0.88$ kg. A greater reduction is found among men rather than women. Across differences in

ethnicity, proportions in dietary macronutrient intake were not clearly associated with weight loss during Ramadan and the duration of fasting was not associated with changes in body mass.²³ Various possible factors have been reported to contribute the discrepancies among the results; the length and the temperature of fasting days,^{186,187} meal-timing and frequency,¹⁸⁶ eating behaviors and the quality and quantity of food intake,^{74,188} fluid intakes,^{189,190} sleeping patterns and duration,⁵ physical activity,^{188,191} and age.⁷⁴ Meta-regression model have investigated the effects of baseline BMI, age, fasting time, and energy intake on changes in body weight; however, none of these variables had a considerable effect on the weight reduction during and after Ramadan.²³

Moreover, the possible impact of Ramadan fasting has been reviewed by Leiper, Molla and Molla¹⁸⁹ revealed that fluid restriction during Ramadan fasting induced a little loss of body tissue due to a decrease in glycogen-bound water stores, a moderate hypohydration state, and extracellular water contraction secondary to the lower sodium intake. It is possible that the weight loss, in part, could be attributed to reduction in abdominal fat since waist and hip circumferences were also decreased. Study by Shariatpanahi et al¹⁴ and Saleh et al¹⁹² reported a similar reduction in waist circumference during Ramadan fasting among subjects with metabolic syndrome and healthy volunteers, respectively. Reduction in waist circumference usually correlates with improvement in insulin sensitivity,¹⁹³ which however, HOMA-IR index were not differ from the baseline values in this present study.

In this present study, with the assumption of maintained activity level throughout Ramadan, weight loss could be attributable to the better utilization of energy stores as evidenced by reducing fat mass, fat free mass, and muscle mass. In this context, glucose homeostasis is undoubtedly play a key role in body adaptive

response under less nutrient availability. It is necessary to preserve a constant rate of fasting blood glucose level to provide an adequate glucose for the brain and other vital tissues, such as red blood cell, peripheral nerves and renal medulla. According to the duration of fasting time (~14 h/day), the main plausible mechanism during this period is (1) glycogen mobilization and the release of glucose by the liver, (2) the release of fatty acids by adipose tissue (lipolysis), and (3) at the diminish rate, a fuel replacement used from glucose to fatty acids by muscle and liver.^{64,178} The large amount of glucose as the hydrolysis product of glucose 6-phosphate derived from glycogen is released from the liver into the circulation. The entry of glucose into muscle and adipose tissue decreases in response to a low insulin levels, while glucagon levels is actively secreted to encounter some pathways in response to lower blood glucose levels.⁶⁴

The absence of food and drink in the daytime is compensated by nighttime eating may be influence some physiological and behavioral changes. In terms of eating behavior, no doubt that food reward/perception may differ from non-Ramadan fasting day, hence may possible to provide different stimuli on hunger and appetite. Study on appetite (1998)¹⁹⁴ reported that during Ramadan fasting, rated hunger were increased substantially following the daily fast and in the earlier days it was higher among women and later, on average similar in both women and men. It is suggested that men tended to less exposed by eating-related cues (external cue control of hunger) during the daily fast. More importantly, a sudden inversion of usual diurnal cycle of food intake may stimulate further adjustments in eating behaviors.

From this present study, energy intake, carbohydrate, and fat intake were decreased after three weeks of Ramadan fasting by - 12.68%, -11.15%, and - 9.24%, respectively. In free-living condition, dietary intake was assessed by using 3 days

dietary record, in which each participant reported their daily meals/beverages intake into food diaries provided by researcher. According to the result of daily energy intake (before: 1538.73 ± 63.13 kcal vs. after: 1292.12 ± 44.92 kcal), we noticed possible minor underreporting on dietary intake record may be occurred and interfered the actual daily energy intake, especially in the baseline time. However, overall data showed that decrease of daily energy intake were consistent with decrease of daily carbohydrate and fat intake.

To date, heterogeneous findings were noted among the available studies aim to determine the effect of Ramadan fasting on energy and macronutrient intake. The latest systematic review and meta-analysis reported the number of studies with increased total daily energy intake was equal to the number of studies with decreased total daily energy intake during Ramadan. Overall, carbohydrate was the food group with the largest consumption (>55% of total energy intake) before, during, and after Ramadan. During Ramadan, in both West and Asian populations, reduction in daily energy intake (by 600 - 900kJ/d or 143.4 - 215.1 kcal) was found due to a decline in carbohydrate consumption.²³

CHAPTER 6

CONCLUSION

This present study showed that Ramadan fasting did not pose significant changes on circulating leptin and adiponectin levels. Serum total cholesterol, high-density lipoprotein cholesterol (HDL-c) were decreased significantly, while fasting plasma glucose, serum insulin, low-density lipoprotein cholesterol (LDL-c), triglycerides, and homeostasis model assessment of insulin resistance (HOMA-IR) index were remained unchanged. Reduction in total cholesterol and HDL-c, were thought to be affected by decrease of energy intake, lower in quality of fat intake, lower physical activity level, and possibility of stress factor. Weight reduction was noted significantly accompanied by decrease of body composition parameters (fat mass, fat free mass, and muscle mass). Energy and macronutrient intake were decreased, while physical activity were qualitatively evaluated to be similar with that of observed before Ramadan. In regards with the outcome of circulating serum leptin and adiponectin, several factors, such as meal time and composition, sleep-activity cycle, and intra-individual variability (variation in body mass index, energy expenditure, hunger and appetite) may possibly confounds the available results.

To date, studies on Ramadan fasting and circulating leptin and adiponectin are still infancy and questions remain. We notice the discrepancies among the available results and those related to the differences in study population, study design, duration of fasting time and Ramadan-associated life style in different country-origin study. However, in our knowledge, our study is one of the foremost studies to determine the effect of Ramadan fasting on circulating leptin and adiponectin together with metabolic profiles and body composition parameters in healthy men with free-living condition.

CHAPTER 7

LIMITATION AND FUTURE STUDIES

7.1 Limitation of study

This study has some limitations: (1) have no repeated measurement in post-Ramadan period; (2) have no evaluation in compliance for dietary intake and physical activity records; (3) have no quantitative evaluation on physical activity level. Several others physiological and behavioral factors-associated with Ramadan fasting were not identified in this study, such as sleep pattern, energy expenditure, hunger and appetite rates, and food consumption to reflect eating habits and quality of nutrient intake profile, all of these may possibly act as confounding factors to the outcomes.

7.2 Future studies

Further extensive study is needed to validate and determine the long-term and clinically significant effects on physiological and metabolic changes during Ramadan fasting in healthy men. Future studies are recommended to address or more emphasize on the aspects of: (1) time series in baseline non-fasting, baseline fasting, during, and post-Ramadan; (2) qualitative and quantitative evaluation in nutrient intake and daily physical activity before and during Ramadan.

7.3 Application

It is suggested that Ramadan fasting is a unique model to study the effect of intermittent fasting on physical and metabolic health, therefore its physiological and behavioral changes associated with Ramadan fasting i.e. eating habit and physical activity are need to be well-managed among Ramadan observances in order to preserve a wellness and better metabolic status during and after Ramadan period to be applied further as part of lifestyle modification.

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ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย

ชื่อโครงการวิจัยผลของการถือศีลอดต่อระดับฮอร์โมนเลปติน อะดิโปเนกตินและระบบเมตาบอลิซึมในชายไทยมุสลิมที่มีสุขภาพดี

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ขอเรียนเชิญท่านเข้าร่วมในการวิจัยก่อนที่ท่านจะตัดสินใจเข้าร่วมในการวิจัย มีความจำเป็นที่ท่านควรทำความเข้าใจว่างานวิจัยนี้ทำเพราะเหตุใด และเกี่ยวข้องกับอะไร กรุณาใช้เวลาในการอ่านข้อมูลต่อไปนี้อย่างละเอียดรอบคอบ และสอบถามข้อมูลเพิ่มเติมหรือข้อมูลที่ไม่ชัดเจนได้ตลอดเวลา

รายละเอียดโครงการ

เป็นที่ทราบกันดีว่า ในระหว่างการถือศีลอด มุสลิมจะงดอาหารและเครื่องดื่ม และการเสพกาม ตั้งแต่รุ่งอรุณ (พระอาทิตย์ขึ้น) จนกระทั่งถึงเวลาหลังตะวันตกดิน ในระหว่างนั้นมุสลิมจะกินอาหาร 2 มื้อหลัก คือ มื้อก่อนพระอาทิตย์ขึ้น และหลังพระอาทิตย์ตก ซึ่งการปฏิบัติตนในลักษณะนี้แตกต่างจากคำแนะนำทางโภชนาการที่แนะนำให้รับประทาน อาหารวันละ 3 มื้อหลัก รวมถึงการดื่มน้ำวันละ 6-8 แก้ว ผู้วิจัยจึงสนใจศึกษาการเปลี่ยนแปลงของระบบเมตาบอลิซึม การเผาผลาญสารอาหาร การสังเคราะห์และการหลั่งฮอร์โมนที่เกี่ยวข้องกับกระบวนการเมตาบอลิซึมที่อาจเปลี่ยนแปลงไปในขณะถือศีลอด

วัตถุประสงค์

การศึกษาในครั้งนี้จึงมุ่งศึกษาการเปลี่ยนแปลงของระบบเมตาบอลิซึมและระบบฮอร์โมนที่เกี่ยวข้องในระหว่างการถือศีลอดของมุสลิมในประเทศไทย

รายละเอียดการศึกษา

การศึกษานี้ทำในอาสาสมัครชาย จำนวน 40 คน ศึกษาการเปลี่ยนแปลงก่อนและหลังการถือศีลอด โดยแบ่งการศึกษา ออกเป็น 4 ช่วงดังนี้

ก่อนการถือศีลอด ทำการเจาะเลือดและวิเคราะห์องค์ประกอบร่างกาย ในวันแรก (วันที่ 0) ของการถือศีลอด
ระหว่างการถือศีลอด วิเคราะห์องค์ประกอบร่างกาย ในวันที่ 14 (วันที่ 14) ของการถือศีลอด
ภายหลังการถือศีลอด ทำการเจาะเลือดและวิเคราะห์องค์ประกอบร่างกาย ในวันสุดท้าย (วันที่ 30) ของการถือศีลอด

ภายหลังการถือศีลอด 4 สัปดาห์ ทำการเจาะเลือดและวิเคราะห์องค์ประกอบร่างกาย ภายหลังวันสุดท้ายของการถือศีลอด 4 สัปดาห์ (วันที่ 60)

ในทุกช่วงอาสาสมัครจะได้รับการวิเคราะห์ตัวชี้วัดการเปลี่ยนแปลงของระบบการเผาผลาญสารอาหารดังนี้

1. การวิเคราะห์องค์ประกอบร่างกาย โดยเครื่องวิเคราะห์องค์ประกอบร่างกาย วิเคราะห์พฤติกรรมกรกินอาหารและพลังงานที่ได้รับต่อวันโดยการใช้ ตารางการจดบันทึกซึ่งอาสาสมัครต้องจดบันทึกอาหารที่กิน สัปดาห์ละ 3 วัน
2. การวิเคราะห์ดัชนีชี้วัดระบบเมตาบอลิซึม ได้แก่ ไขมันในเลือด น้ำตาลในเลือด อินซูลิน ฮอร์โมน leptin และ adiponectin โดยการเจาะเลือด



แผนที่โครงการวิจัย..... 097-1/58
วันที่รับรอง..... - 6 ก.ค. 2558
วันหมดอายุ..... - 5 ก.ค. 2559

AF 04-07

ท่านสามารถเข้าร่วมโครงการได้ ก็ต่อเมื่อ

1. ท่านเป็นชายอายุระหว่าง 18 - 50 ปี
2. มีดัชนีมวลกายอยู่ในช่วงปกติ ($18.5 - 22.9 \text{ kg/m}^2$)
3. ไม่มีโรคประจำตัวไม่มีประวัติการใช้ยาที่มีผลต่อน้ำตาลและระดับคลอเลสเตอรอลในเลือดอย่างน้อย 3 เดือนก่อนการศึกษา
4. ไม่สูบบุหรี่และไม่ดื่มเครื่องดื่มแอลกอฮอล์
5. ไม่มีประวัติการเจ็บป่วยใดๆ ก่อนเข้าร่วมการวิจัยอย่างน้อย 3 เดือน
6. ได้รับอนุญาตให้ถือศีลอดตามหลักการของมุสลิม คือ บรรลุนิติภาวะตามศาสนบัญญัติ หรือที่เรียกว่า บรรลุศาสนภาวะ มีสติสัมปชัญญะครบบริบูรณ์ ไม่เมาหรือหมดสติไม่เจ็บป่วย

การศึกษาครั้งนี้จะรับเฉพาะ อาสาสมัครที่อ่านออก เขียนได้ และสามารถสื่อสารเป็นภาษาไทยได้เท่านั้น เพื่อประโยชน์ในการสื่อสารได้สะดวก ผู้วิจัยจะไม่รับอาสาสมัครที่เป็นกลุ่มผู้ด้อยโอกาส/เปราะบาง ผู้ป่วยโรคจิต ผู้ต้องขัง เด็กอายุต่ำกว่า 18 ปีสตรีมีครรภ์ ผู้สูงอายุที่มีความจำบกพร่อง ผู้ป่วยสมองเสื่อม คนพิการ ชนกลุ่มน้อยผู้ย้ายถิ่น/อพยพปัญหาอ่อน ทหารเกณฑ์ และผู้ป่วยอาการหนักไม่สามารถให้คำยินยอมด้วยตนเองได้

ข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ของอาสาสมัครที่อาจนำไปสู่การเปิดเผยตัวของท่าน จะได้รับการปกปิดและจะไม่เปิดเผยแก่สาธารณชน ในกรณีนี้ผลการวิจัยได้รับการตีพิมพ์ ชื่อและที่อยู่จะได้รับการปกปิดอยู่เสมอ โดยเฉพาะรหัสประจำโครงการวิจัย ข้อมูลของอาสาสมัครจะผ่านกระบวนการต่างๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์และการรายงานเพื่อวัตถุประสงค์ทางวิทยาศาสตร์ การใช้ข้อมูลทางการแพทย์ในอนาคตหรือการวิจัยทางการแพทย์เท่านั้น

เมื่อเสร็จสิ้นการวิจัยแล้วข้อมูลที่เกี่ยวข้องกับผู้มีส่วนร่วมในการวิจัยจะถูกทำลาย เลื่อนที่นำไปใช้แล้ว จะถูกทำลายทิ้งตามกระบวนการทางวิทยาศาสตร์ หากผู้รับผิดชอบโครงการวิจัยตรวจพบความผิดปกติของค่าใดใดในเลือดของอาสาสมัคร ผู้วิจัยจะแจ้งให้อาสาสมัครทราบในทันที รวมทั้งให้คำแนะนำในกรณีที่อาสาสมัครต้องได้รับการรักษาต่อไป

ประโยชน์ที่ท่านจะได้รับ

ผู้เข้าร่วมโครงการจะได้รับการตรวจเลือดทางห้องปฏิบัติการ ได้แก่ ระดับไตรกลีเซอไรด์ คลอเลสเตอรอล ไขมันเลว (LDL) และไขมันดี (HDL) ซึ่งเป็นปัจจัยหลักในการเกิดโรคไม่ติดต่อเรื้อรังต่างๆ โดยเฉพาะโรคหัวใจและหลอดเลือด ดังนั้นผู้เข้าร่วมโครงการสามารถนำผลที่ได้มาพบแพทย์เพื่อประเมินภาวะสุขภาพอันจะเป็นประโยชน์แก่ผู้เข้าร่วมโครงการอีกทางหนึ่ง อีกทั้งมีการตรวจวัดองค์ประกอบของร่างกายซึ่งจะทำให้อาสาสมัครทราบข้อมูลเกี่ยวกับองค์ประกอบของร่างกายตนเองด้วย หากตรวจพบความผิดปกติของค่าใดในเลือด ผู้วิจัยจะแจ้งให้ทราบในทันที และให้คำแนะนำเพื่อการรักษาต่อไป

ผลข้างเคียงที่อาจเกิดขึ้น

ในการศึกษาครั้งนี้ ผู้เจาะเลือดเป็นพยาบาลวิชาชีพที่มีความเชี่ยวชาญและประสบการณ์ในการเจาะเลือด อย่างไรก็ตาม หากเกิดผลข้างเคียงจากการเจาะเลือดอันได้แก่ อาการเจ็บ เลือดออก ช้ำจากการเจาะเลือด อาการบวมบริเวณที่เจาะเลือดหรือหน้ามืดจะดำเนินการส่งต่อแพทย์ในสถานพยาบาลใกล้เคียงเพื่อรับการรักษาต่อ โดยผู้วิจัยจะรับผิดชอบค่ารักษาพยาบาลที่เกิดขึ้น และหากอาสาสมัครมีอาการหรืออาการแสดงที่มีผลต่อร่างกาย จิตใจ สังคม เศรษฐกิจ ความเชื่อของผู้มีส่วนร่วมในการวิจัย จะสามารถถอนตัวหรือจะเข้าร่วมหรือถอนตัวจากการวิจัยได้ทุกขณะ โดยไม่ต้องให้เหตุผลและไม่สูญเสียประโยชน์ที่พึงได้รับ

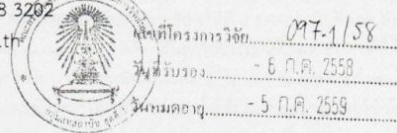
ศูนย์โครงการวิจัย..... 097-1/58
- 6 ก.ค. 2558
วันรับรอง.....
- 5 ก.ค. 2558
วันหมดอายุ.....

AF 04-07

ผู้วิจัยขอยืนยันว่าการเข้าร่วมเป็นกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัยเป็นโดยสมัครใจ และสามารถปฏิเสธที่จะเข้าร่วมหรือถอนตัวจากการวิจัยได้ทุกขณะ โดยไม่ต้องให้เหตุผลและไม่สูญเสียประโยชน์ที่พึงได้รับหากอาสาสมัครท่านใดมีข้อสงสัยให้สอบถามเพิ่มเติมได้โดยสามารถติดต่อผู้วิจัยได้ตลอดเวลา และหากผู้วิจัยมีข้อมูลเพิ่มเติมที่เป็นประโยชน์หรือโทษเกี่ยวกับการวิจัย ผู้วิจัยจะแจ้งให้ท่านทราบอย่างรวดเร็วเพื่อให้ท่านทบทวนว่ายังสมัครใจจะอยู่ในงานวิจัยต่อไปหรือไม่

ข้อมูลที่เกี่ยวข้องกับอาสาสมัครจะถูกเก็บเป็นความลับ การเสนอผลการวิจัยจะเสนอในภาพรวม จะไม่มีข้อมูลที่สามารถระบุถึงตัวอาสาสมัครได้การวิจัยในครั้งนี้ อาสาสมัครจะได้รับค่าพาหนะ รวมทั้งสิ้น 800 บาท หากท่านมีข้อสงสัยให้สอบถามเพิ่มเติมได้จากผู้วิจัยตลอดเวลา และหากผู้วิจัยมีข้อมูลเพิ่มเติมที่เป็นประโยชน์หรือโทษเกี่ยวกับการวิจัย ผู้วิจัยจะแจ้งให้ท่านทราบอย่างรวดเร็ว

หากท่านไม่ได้รับการปฏิบัติตามข้อมูลดังกล่าวสามารถร้องเรียนได้ที่
คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
254 อาคารจามจุรี 1 ชั้น 2 ห้อง 210-211 ถ.พญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพฯ 10330
โทรศัพท์/โทรสาร 02 218 3202
E-mail: eccu@chula.ac.th



หนังสือแสดงความยินยอมเข้าร่วมการวิจัย

ทำที่.....

วันที่.....เดือน.....พ.ศ.....

เลขที่ ประชากรตัวอย่างหรือผู้มีส่วนร่วมในการวิจัย.....

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย

ผลของการถือศีลอดต่อระดับฮอร์โมนเลปติน อะดิพอเนกตินและระบบเมตาบอลิซึมในชายไทยมุสลิมที่มีสุขภาพดี

ชื่อผู้วิจัย ผศ.ดร.สุวิมล ทรัพย์วโรบล ตำแหน่ง หัวหน้าภาควิชาโภชนาการและการกำหนดอาหาร

ภาควิชาโภชนาการและการกำหนดอาหาร คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

154 ซ. จุฬา 12 ถนนพระราม 1 แขวงวังใหม่ เขตปทุมวัน กรุงเทพฯ 10330

โทรศัพท์ (ที่ทำงาน) 02 218 1116 ต่อ 24 โทรศัพท์มือถือ 081 902 0953

E-mail : ssapwarobol@gmail.com

ข้าพเจ้า ได้รับทราบรายละเอียดเกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/อันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงผู้เข้าร่วมการวิจัย โดยตลอด และได้รับคำอธิบายจากผู้วิจัย จนเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้ ตามที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย โดยข้าพเจ้ายินยอมให้วิเคราะห์องค์ประกอบของร่างกาย ทั้งสิ้น 3 ครั้ง ได้แก่ ก่อนเริ่มถือศีลอด สัปดาห์ที่ 2 ของการถือศีลอด สัปดาห์ที่ 4 ของการถือศีลอด และ 4 สัปดาห์หลังการถือศีลอด และเจาะเลือดจำนวนทั้งสิ้น 3 ครั้ง ได้แก่ ก่อนเริ่มถือศีลอด สัปดาห์ที่ 4 ของการถือศีลอด และ 4 สัปดาห์หลังการถือศีลอด ในแต่ละครั้งข้าพเจ้าจะได้รับการเจาะเลือดครั้งละ 2 ซ่อนชา ณ ภาควิชาโภชนาการและการกำหนดอาหาร อาคารจุฬาพัฒน์ 3 คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย แต่ละครั้งใช้เวลาประมาณ ครึ่งชั่วโมงเมื่อเสร็จสิ้นการวิจัยแล้วข้อมูลที่เกี่ยวข้องกับผู้มีส่วนร่วมในการวิจัยจะถูกทำลาย เลือกที่นำไปใช้แล้ว จะถูกทำลายทั้งตามกระบวนการทางวิทยาศาสตร์ และข้าพเจ้าจะจดบันทึกอาหารที่กินในระหว่างอยู่ในการศึกษา สัปดาห์ละ 3 วัน (โดยเลือกวันที่สะดวกจากรวันจันทร์ถึงศุกร์ 2 วัน วันเสาร์ อาทิตย์ 1 วัน)

ข้าพเจ้ามีสิทธิถอนตัวออกจากการวิจัยเมื่อใดก็ได้ตามความประสงค์ โดยไม่ต้องแจ้งเหตุผล ซึ่งการถอนตัวออกจากการวิจัยนั้น จะไม่มีผลกระทบต่อการศึกษาหรือผลกระทบต่อข้าพเจ้าทั้งสิ้น

ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติต่อข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และข้อมูลใดๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะเก็บรักษาเป็นความลับ โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย 254 อาคารจามจรี 1 ชั้น 2 ห้อง 210-211 ถ.พญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพฯ 10330 โทรศัพท์/โทรสาร 02 218 3202 E-mail: eccu@chula.ac.th

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นสำคัญต่อหน้าพยาน ทั้งนี้ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงผู้เข้าร่วมการวิจัย และสำเนาหนังสือแสดงความยินยอมไว้แล้ว

ลงชื่อ.....
(.....)
ผู้วิจัยหลัก



ลงชื่อ.....
(.....)
ผู้มีส่วนร่วมในการวิจัย

เลขที่โครงการวิจัย..... 097-1/58..... พยาน

วันที่รับรอง..... - 6 ก.ค. 2558.....

วันหมดอายุ..... - 5 ก.ค. 2559.....

APPENDIX 2 HUMAN LEPTIN ELISA ASSAY KIT AND PROCEDURE

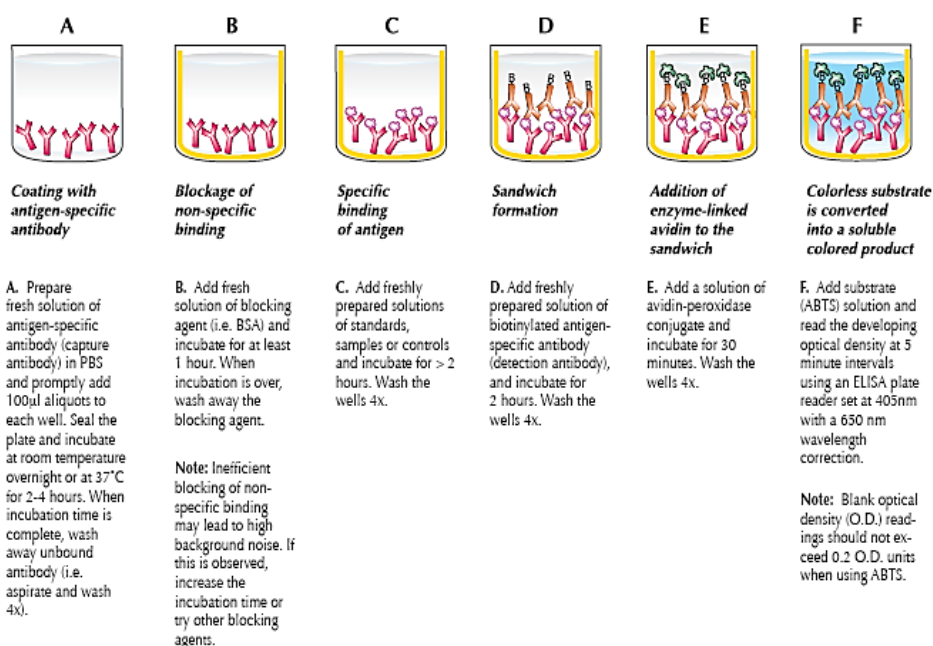
Human Leptin ELISA Development Kit & ELISA Buffer Kit

(PeproTech, Inc., Rocky Hill, NJ, USA; Cat. no; 900-K90 and Cat. no; 900-K00)

Kit components

Human Leptin ELISA Development Kit	ELISA Buffer Kit
Capture Antibody (Rabbit Anti-Human Leptin)	ELISA microplates-uncoated
Detection Antibody (Biotinylated Rabbit Anti-Human Leptin)	PBS Conc. (20x)
Human Leptin Standard (Recombinant Human Leptin)	Blocking Buffer (1x)
Avidin-HRP conjugate	Wash Buffer Conc. (20x)
	Diluent Buffer Conc. (20x)
	ABTS Liquid Substrate

Serum Leptin Assay Procedure: Sandwich ELISA



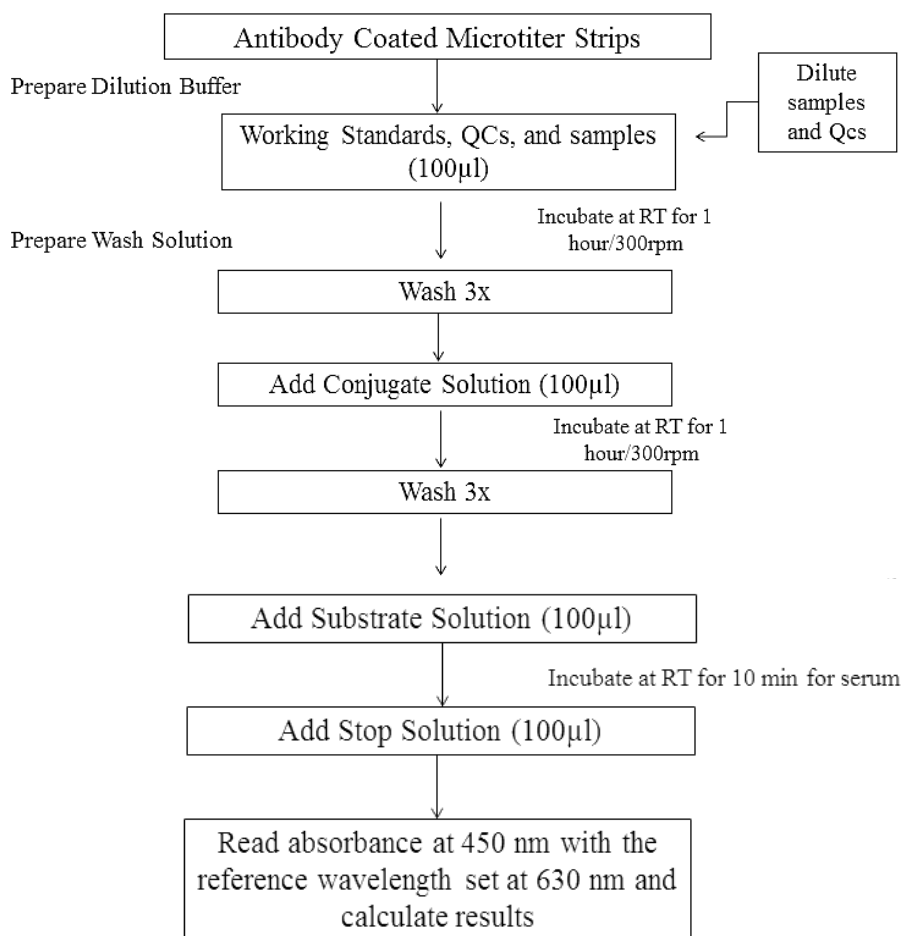
Source: Peprotech. Antibody Resources Guide. PeproTech Inc. Rocky Hill, New Jersey USA. 3rd Edition © 2012. www.peprotech.com

APPENDIX 3 HUMAN ADIPONECTIN ELISA ASSAY KIT AND PROCEDURE

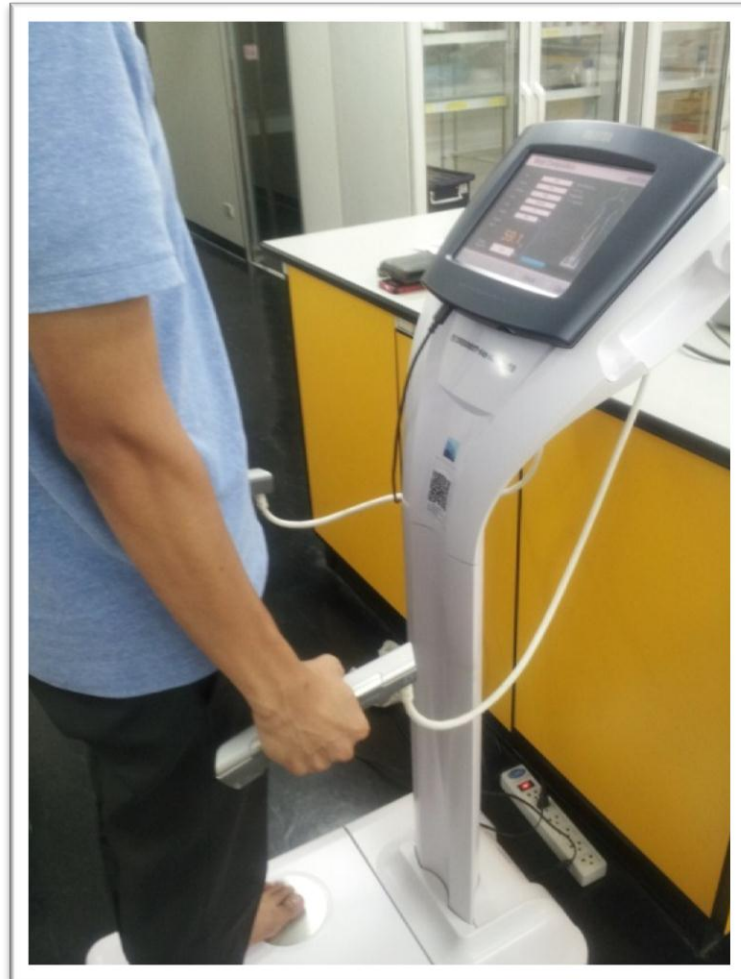
Adiponectin (human), ELISA kit (Farmingdale, NY, USA; Cat. no; ALX-850-377)

Kit components	Kit components
Antibody Coated Microtiter Strips	Dilution Buffer Conc. (10x)
Conjugate Solution	Wash Solution Conc. (10x)
Set of Standards	Substrate
Quality control HIGH	Stop Solution
Quality control LOW	

Assay procedure summary of human serum adiponectin assay



APPENDIX 4 BODY COMPOSITION MEASUREMENT
(BIA MODEL MC-980 MA)



APPENDIX 5 DIETARY AND PHYSICAL ACTIVITY RECORD

Page 1: Cover of handbook provided for study participants

**คู่มือผู้เข้าร่วมงานวิจัย:**

ผลของการถือศีลอดต่อระดับฮอร์โมนเลปติน อะดิโปเนกตินและ

ระบบเมตาบอลิซึมในชายไทยมุสลิมที่มีสุขภาพดี

จัดทำโดย:

สาขาโภชนาการและการกำหนดอาหาร คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ร่วมกับ:

มัสยิดขามี่อูล์ก้อยริยะห์

12 มิถุนายน 2558 — 10 กรกฎาคม 2558

VITA

Miss Guntari Prasetya was born on 7th January 1989 in Bogor, Indonesia. She graduated with Bachelor Degree of Science in Nutrition at Bogor Agricultural University in 2010. Then, she has worked as teaching assistant at Department of Community Nutrition, Faculty of Human Ecology, Bogor Agricultural University, Indonesia. In 2013, she pursued her Master of Science Degree in Food and Nutrition (major Medical Nutrition Therapy) at Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University, Thailand. After graduation, she will return to her hometown and planned to be a lecturer at Department of Community Nutrition, Faculty of Human Ecology, Bogor Agricultural University, Indonesia.

