Relationship between Insulin Sensitivity and Body Fat Composition, and Leptin Sensitivity in Non-Obese and Overweight/Obese Adults

Khin Mi Mi Lay[1], Sanda Kyaw[2] & Ohnmar[2]*

[1]Department of Medical Research
[2]University of Medicine (1), Yangon

Both insulin and leptin are major contributors for the body energy balance. Obesity is a state of energy imbalance and is also associated with changes in both insulin sensitivity and leptin sensitivity. The aim of this study was to find out the relationship between insulin sensitivity and body fat composition, and leptin sensitivity in non-obese and obese adults. A total of 86 adults participated: 42 non-obese and 44 over-weight/obese. Body fat (BF) percent was determined by skinfold method. Fasting plasma glucose was analyzed by glucose oxidase-phenol and 4 aminophenazone (GOD-PAP) method using spectro-photometer, fasting serum insulin and leptin concentrations by direct sandwich ELISA method and resting energy expenditure (REE) by indirect calorimetry. Leptin sensitivity index and insulin sensitivity were expressed as REE : Leptin ratio and homeostatic model assessment-insulin resistance (HOMA-IR), respectively. It was found that median value of HOMA-IR was significantly higher [2.93 *vs* 1.72, $p<0.01$] and leptin sensitivity was significantly lower [116.76 *vs* 265.66, $p<0.001$] in the overweight/obese adults than the non-obese adults, indicating that insulin sensitivity and leptin sensitivity were markedly reduced in overweight/obese adults in compare to non-obese adults. There was a moderate degree of positive relationship between HOMA-IR and BF only in the overweight/obese ($\rho=0.509$, $n=44$, $p<0.001$) and all adults ($\rho=0.39$, $n=86$, $p<0.001$). Similarly, a weak negative relationship between leptin sensitivity index and HOMA-IR was found in the overweight/obese ($\rho=-0.328$, $n=44$, $p<0.05$) and all adults ($\rho=-0.35$, $n=86$, $p<0.01$). It can be concluded that the insulin sensitivity was adiposity dependent, but, it did not depend on leptin sensitivity.

Keyword: Insulin sensitivity, Leptin sensitivity, Obese adults

INTRODUCTION

Obesity is a worldwide epidemic considered to be the fifth leading risk for global deaths. Obesity is caused by an energy imbalance between calories consumed and calories expended. The high-fat food consumption and the low physical activity result in excessive fat accumulation, which increases the risk of non-communicable diseases such as cardiovascular diseases, diabetes mellitus, musculoskeletal disorders and some cancers such as endometrium, breast, and colon.[1]

Many studies have focused on the inter-relationship of body fat composition, leptin and insulin sensitivity in humans. These studies have demonstrated that serum leptin is correlated with BMI and adiposity in non-obese middle age women and is not correlated with insulin sensitivity in both non-obese women and obese individuals.[2] In

*To whom correspondence should be addressed.
Tel: +95-9402741523
E-mail: khnlay883@gmail.com
DOI: https://doi.org/10.34299/mhsrj.00950

contrast to this finding, Zuo and coworkers found that serum leptin levels in insulin resistant subjects were almost double compared to those subjects who were not insulin resistant in both men and women, at the same level of adiposity. Thus, the reports on the interrelationship of body fat composition, leptin sensitivity and insulin sensitivity in humans are still needed.[3] Leptin, the satiety hormone, is a hormone produced by the fat cells in the body, which regulates the amount of fat stored in the body. The major function of leptin in human physiology is to signal inadequate energy stores or balance rather than an overabundance of fat.[4] In addition, leptin sufficiency acts to permit energy expenditure by systems throughout the body, as well as to modulate appetite.[5]

Although obese subjects have high level of leptin, their energy expenditure and appetite are not sufficiently regulated. Hajer and coworkers reported that there is a hypo-thalamic leptin resistance, which may be responsible for the persistent hunger and the difficulty to lose weight in obese subjects.[6] Hence, obesity promotes hyperleptinemia, which in turn promotes leptin resistance and further obesity.[7]

Leptin resistance is a hallmark of obesity, but its etiology is unknown, and its clinical measurement is elusive. Lee and coworkers used leptin to BMI ratio as an index of leptin resistance. This index measured leptin level while controlling for the contribution of BMI.[8] However, Lustig and coworkers suggested that the REE to leptin ratio may provide a surrogate index of leptin sensitivity. A decrease in REE : Leptin ratio indicates leptin resistance. In fact, this concept of the REE to leptin ratio currently does not have external validation. Since leptin increases energy expenditure, the REE to leptin ratio is a more appropriate index of leptin sensitivity and thus, also of leptin resistance. However, no other clinical measures of leptin sensitivity have yet been advanced.[9] Leptin acts as an insulin sensitizer when leptin levels are at low and normal levels whereas it may contribute to insulin resistance when leptin is chronically elevated.[10] In addition, Boden and coworkers also reported that hyperinsulinemia causes hyperleptinemia and both lead to insulin resistance and leptin resistance.[11] Although insulin resistance and leptin resistance increase in parallel with the rise in adiposity, they differ in the timing of their development and their relationship to white adipose tissue mass.[11, 12] Thus, the relationship between body fat composition, insulin resistance and leptin resistance is still need to be clarified. Thus, this study aimed to find out the relationship between insulin sensitivity and body fat composition, and leptin sensitivity in non-obese and obese adults.

MATERIALS AND METHODS

A cross-sectional analytical study was done at the Physiology Research Division, Department of Medical Research (DMR) and Postgraduate Research Laboratory, Department of Physiology, University of Medicine (1), Yangon. Detailed procedure was explained and written informed consent was obtained. A total of 86 adults (13 male and 73 female), with age between 20-55 years, were voluntarily participated. Among them, 44 (6 male and 38 female) were overweight/obese (BMI $\geq$25 kg/m^2) and 42 (7 male and 35 female) were non-obese (BMI 18.5 and 25 kg/m^2). The adults with known history of chronic disease, acute illness and concurrent treatment, fasting blood glucose level >126 mg/dl (>7 mmol/l), pregnant women and menopause were excluded.

Body fat (BF) percent was determined by skinfold method. Fasting serum insulin level was determined by direct sandwich ELISA method and fasting plasma glucose level was determined by GOD-PAP method. Insulin sensitivity was expressed as homeo-static model assessment-insulin resistance (HOMA-IR). Fasting serum leptin level was determined by direct sandwich ELISA method and resting energy expenditure (REE) was measured by indirect calorimetry (Vista-MX-REE, Vacumed, USA). Leptin

sensitivity index was expressed as REE: Leptin ratio.

Procedure of the study

At the first enlistment at the Physiology Research Division, Department of Medical Research, the aim and detail procedures of the study were explained clearly to the participants and a written informed consent was obtained from all subjects before the study. Forty-two non-obese and forty-four overweight/obese adult subjects were selected according to inclusion and exclusion criteria.

Before experiment, detailed history taking, thorough physical examination, anthropometric measurements such as height, weight, waist circumference, triceps skinfold thickness (TSF) and biceps skinfold thickness (BSF thickness) were measured. BMI was calculated as kg/m^2 and body fat composition was calculated using the following equation.[13]

Body fat (%)=17.308 x Log (TSF+BSF) +1.120x(BMI)+6.137x(Sex)-27.149, Sex=1 for men, Sex=2 for women, Normal range: Adult men=15-20%; adult women=25-30%

They were instructed to come back to the laboratory after taking 10-hour overnight fasting from 10:00 p.m to 8:00 a.m.

At the morning of the experiment day (between 8:00 am and 9:00 am), before taking breakfast, REE was measured after resting for 30 minutes in lying position. Then, a venous blood sample (7 ml) was taken from the antecubital vein under aseptic condition with a sterile disposable syringe. Two millilitres of blood was kept into a test tube containing sodium fluoride anti-coagulant for determination of fasting plasma glucose and 5 ml of venous blood sample were collected in a test tube with no anticoagulant for determination of fasting serum insulin and fasting serum leptin levels. After collection, the blood samples were centrifuged at 3000 rpm for 15 minutes. The serum was stored in two separate tubes: one for serum leptin and another for serum insulin. The fasting plasma glucose was determined within 2 hours from blood collection. The serum samples for determination of fasting serum leptin and fasting serum insulin were stored at -20°C in the laboratory and they were determined within five months of sample collection. After all samples were collected, serum leptin and serum insulin were analyzed by using ELISA. Then, insulin resistance index and leptin sensitivity index were calculated.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 16 was used to analyse the data. Results were expressed as the mean±standard deviation (SD). For skewed results (leptin, leptin sensitivity index, insulin and HOMA-IR), median and inter-quartile range (IQR) was used. Then, the skewed data of the non-obese and obese adults were compared by using Mann-Whitney U test. Independent samples "t" test was used to determine the difference in values between the study groups for other variable with normal distribution. Spearman's coefficient of correlation was used to assess the relationship between two skewed parameters and between skewed and normally distributed parameters.

Ethical consideration

Ethical approval was taken from Ethical Review Committee, University of Medicine (1), Yangon.

RESULTS

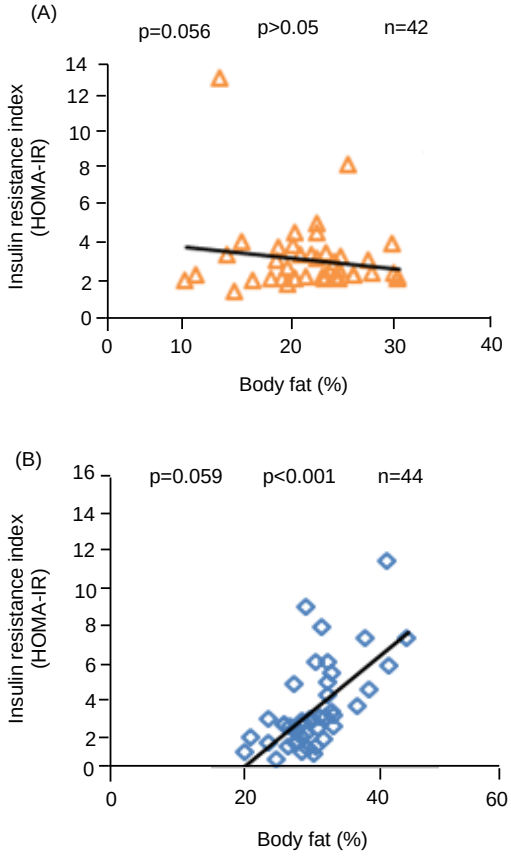
In the present study, a total of 86 adults were recruited. Among the 42 non-obese adults, 7(16.67%) were males and 35(83.33%) were females, and in the 44 overweight/obese adults, 6(13.64%) were males and 38(86.36%) were females. The mean ages of the non-obese and overweight/obese adults were 33.9±7.89 years and 37.98±8.32 years, respectively. The mean values (±SD) and median (interquartile range) of body fat percent, fasting plasma glucose, fasting serum insulin, HOMA-IR, REE, fasting serum leptin and REE : Leptin of the non-obese and overweight/obese adults are summarized in Table 1.

Table 1. Metabolic parameters in non-obese and overweight/obese adults

	Non-obese (n=42)	Overweight/ obese (n=44)	p value
Body fat (%)[a]	18.3 ±10.11	28.02 ±10.01***	<0.001
Fasting plasma glucose (mmol/l)[a]	5.06 ±3.59	5.48 ±4.16*	<0.05
Fasting serum insulin (µIU/ml)[b]	8.22 (6.21-13.63)	12.45 (7.39-18.65)*	<0.05
HOMA-IR[b]	1.72 (1.35-2.65)	2.93 (1.77-4.15)**	<0.01
REE (kcal/day)[a]	2047.24 ±522.38	2786.93 ±842.25***	<0.001
Fasting serum leptin (ng/ml)[b]	8.23 (4.47-11.6)	22.71 (14.53-4.32)***	<0.001
REE: Leptin[b]	265.66 (166.3-89.71)	116.76 (77.28-02.94)***	<0.001

[a]Independent sample "t" test and expressed as mean±SD. [b]Mann-Whitney U test and expressed as median (interquartile range), HOMA-IR[b]=Homeostatic model assessment insulin resistance, REE=Resting energy expenditure

Relationship between body fat composition and insulin sensitivity



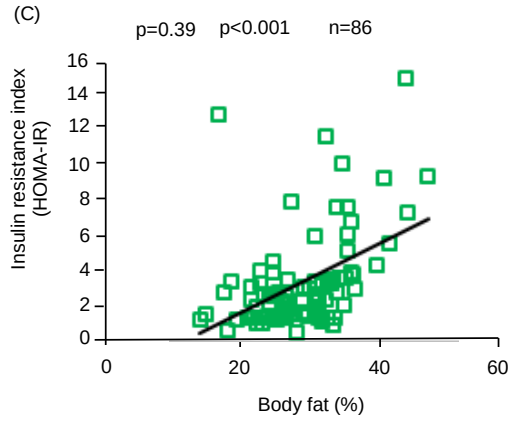
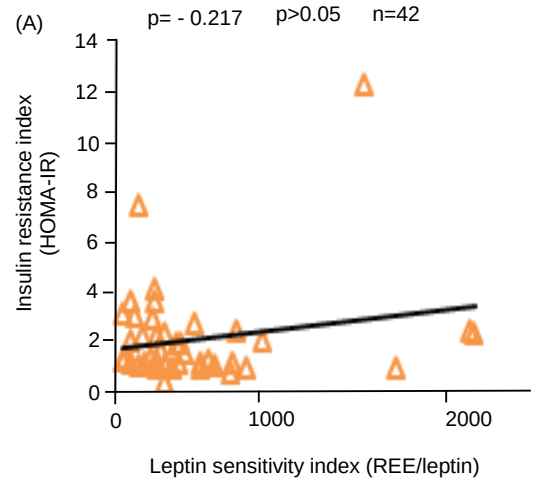


Fig. 1. Relationship between body fat composition and insulin resistance index of non-obese (A), overweight/obese (B) and all (C) adults

There was no correlation (Spearman's coefficient of correlation) between body fat (%) and insulin resistance index (HOMA-IR) in non-obese adults (ρ=0.06, n=42, p>0.05). But, moderate degree of positive correlation between body fat (%) and insulin resistance index (HOMA-IR) was found in overweight/obese (ρ=0.51, n=44, p<0.001) and all adults (ρ=0.39, n=86, p<0.001) (Figure 1).

Relationship between leptin sensitivity and insulin sensitivity

There was no significant correlation (Spearman's coefficient of correlation) between leptin sensitivity index (REE/Leptin) and insulin resistance index (HOMA-IR) (ρ=-0.22, n=42, p>0.05) in the non-obese



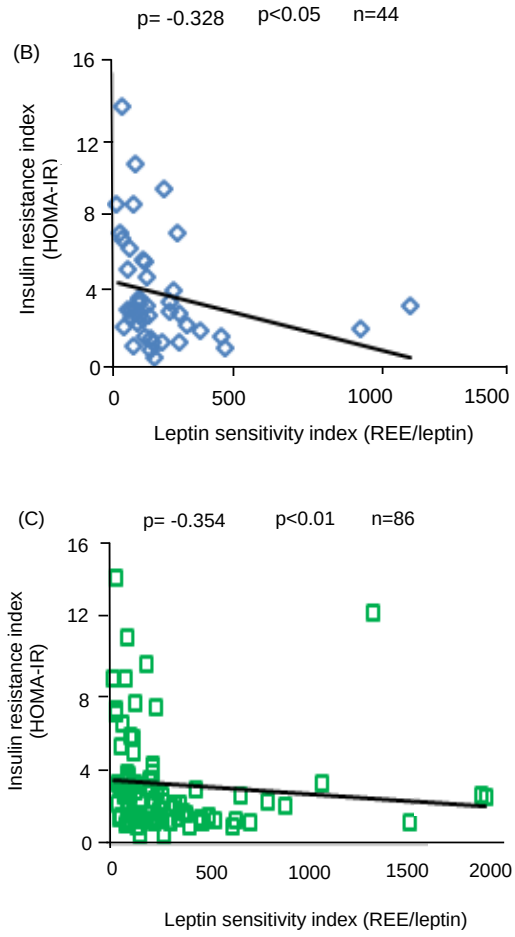


Fig. 2. Relationship between leptin sensitivity and insulin resistance index in the non-obese (A), overweight/obese (B) and (C) all adults

adults. However, weak negative correlations were found between leptin sensitivity index and insulin resistance index (HOMA-IR) of the overweight/obese adults (ρ=-0.33, n=44, $p<0.05$) as well as of all adults (ρ=-0.35, n=86, $p<0.01$) (Figure 2).

DISCUSSION

In the present study, all metabolic parameters in Table 1 were significantly higher in the overweight/obese adults than the non-obese adults. According to the previous studies, serum leptin level in healthy non-obese subjects was 9.6±7.2 ng/ml,[14] 7.4± 0.16 ng/ml[15] and that of the obese adult women was 26.78±21.06 ng/ml.[16] Although there was no definite reference range for non-obese and obese adults, it was found that the mean (±SD) serum leptin levels (9.2±8.87 ng/ml in non-obese and 29.17 ±25.04 ng/ml in overweight/obese) were comparable with those of the non-obese and overweight/obese subjects of other studies.

In the present study, the relationship between body fat percent and insulin resistance index (HOMA-IR) was analyzed and moderate positive correlations were found in the overweight/obese (ρ=0.51, $p<0.001$, n=44) and all (ρ=0.39, $p<0.001$, n=86) adults. But, there was no relationship in the non-obese (ρ=0.06, $p>0.05$, n=42) adults. It seems that there is no relationship within normal adiposity, but insulin resistance increases with an increase in body fat percent beyond normal adiposity limit.

Ruige, *et al*. reported that the location of adipose tissue influenced the development of insulin resistance, with abdominal deposits being strongly associated with insulin resistance than peripheral (gluteal or sub-cutaneous) fat deposits.[17] In the present study, the relationship between HOMA-IR and central obesity (waist circumference, WC) was also analyzed and found that a poor positive relationship between WC and HOMA-IR in all adults (ρ=0.33, $p<0.001$, n=86) but no significant relationship was found after dividing into the non-obese (ρ=0.2, $p>0.05$, n=42) and overweight/obese (ρ=0.25, $p>0.05$, n=44) group. The observed relationship among HOMA-IR and central adiposity and peripheral fat deposits (body fat percent) in the present study also pointed out that not only central adiposity, but also body fat percent should be regarded as an alternative indicator of insulin resistance especially in obese subjects.

Regarding the relationship between insulin sensitivity and leptin sensitivity in the non-obese and overweight/obese adults, a significant but weak negative correlation between leptin sensitivity and HOMA-IR of the adults was seen in all adults (ρ=-0.35, $p<0.01$, n=86) in the present study. When analysis was done after dividing into two groups, a weak negative correlation between

leptin sensitivity and HOMA-IR was also found in the overweight/obese adults (ρ=0.33, $p<0.05$, n=44) and no significant relationship was found in the non-obese adults (ρ=-0.22, $p>0.05$, n=42). These findings indicated that the association between leptin sensitivity and HOMA-IR was also dependent on the adiposity level.

Al-Sultan and Al-Elq (2006) found a significant positive correlation between leptin and HOMA-IR (r=0.344, p=0.001, n=89) in non-diabetic healthy subjects with normal BMI between 20 and 25 kg/m^2 (n=43) and obese subjects with BMI >30 kg/m^2 (n=46).[18] Their findings were consistent with the present findings, however, Myat Mon Khine (2013) found a positive correlation between serum leptin and HOMA-IR in non-obese women (r=0.52, $p<0.001$, n= 38).[19]

The present finding indicated that increased serum leptin level in overweight/obese adults was associated with the reduction of leptin sensitivity. Cohen and colleagues suggested that secretion of leptin by enlarged store of adipose tissue might cause insulin resistance because of insulin-antagonizing effect of leptin. Thus, in leptin resistance state, the insulin-antagonizing effect of leptin was decreased, resulting in hyperinsulinemia and insulin resistance in obese adults.[20] These findings support the evidence that insulin resistance and leptin resistance coexist in the majority of obese subjects.

Conclusion

In the present study, leptin sensitivity and insulin sensitivity were markedly reduced in overweight/obese adults in compared to non-obese adults. A moderate degree of positive correlation was found between body fat and HOMA-IR only in the overweight/obese adults but not in non-obese adults. However, a negative relationship between leptin sensitivity index and HOMA-IR found in obese adults was weak. It can be concluded that the insulin sensitivity was adiposity dependent, but, it did not depend on leptin sensitivity in all adults.

Competing interest

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

We are particularly grateful to Associate Professors and lecturers form Department of Physiology (University of Medicine-1) and staffs from Department of Medical Research for their kind help. Our deepest thanks to all participants who gave consent willingly for the present study.

REFERENCES

1. World Health Organization. Obesity and Overweight fact sheet N°311. 2013 [Updated March 2013]**.** Available from: http://www. who.int/mediacentre/ factsheets/ fs311/en/ index. html
2. Clampham JC, Smith SA, Moore GB, Hughes MG, Azam H, Scott A & Jung RT. Plasma leptin concentrations and OB gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinemia in lean and obese human. *International Journal of Obesity* 1997; 21(3): 179-183.
3. Zuo H, Shi Z, Yuan B, Dai Y, Wu G & Hussain A. Association between serum leptin con-centrations and insulin resistance: A population-based study from China. *PLOS ONE* 2013; 8(1): e54615. Available from doi: 10.1371/ journal. pone.0054615. Epub 2013 Jan 22.
4. Rosenbaum M & Leibel RL. Role of leptin in energy homeostasis in humans. *Journal of Endocrinology* 2014; 223(1): T83-T96.
5. Münzberg H, Björnholm M, Bates SH & Myers MG. Leptin receptor action and mechanisms of leptin resistance. *Cellular and Molecular Life Sciences* 2005; 62(6): 642-652.
6. Hajer GR, van Haeften TW & Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *European Heart Journal* 2008; 29(24): 2959-2971.
7. Martin SS, Qasim A & Reilly MP. Leptin resistance: a possible interface of inflame-mation and metabolism in obesity-related cardiovascular disease. *Journal of the*

American College of Cardiology 2008; 52(15): 1201-1210.

8. Lee JH, Reed DR & Price RA. Leptin resistance is associated with extreme obesity and aggregates in families. *International Journal of Obesity* 2001; 25(10): 1471-1473.
9. Lustig RH, Sen S, Soberman JE & Velasquez-Mieyer PA. Obesity, leptin resistance and the effects of insulin reduction. *International Journal of Obesity* 2004; 28(10): 1344-1348.
10. Franks PW, Loos RF & Brage S. Physical activity energy expenditure may mediate the relationship between plasma leptin levels and worsening insulin resistance independently of adiposity. *Journal of Applied Physiology* 2007; 102(5): 1921-1926.
11. Boden G, Chen X, Kolaczynski JW & Polansky M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. *Journal of Clinical Investigation* 1997; 100(5): 1107-1113.
12. Ritchie IR, Gulli RA, Stefanyk LE, Harasim E, Chabowski A & Dyck DJ. Restoration of skeletal muscle leptin response does not precede the exercise-induced recovery of insulin-stimulated glucose uptake in high-fat-fed rats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 2011; 300(2): R492-R500.
13. Kwok T, Woo J & Lau E. Prediction of body fat by anthropometry in older Chinese people. *Obesity Research* 2001; 9(2): 97-101.
14. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK & Considine RV. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 1996; 348(9021): 159-161.
15. Gijo´n-Conde T, Graciani A, Guallar-Castilló P, Aguilera MT, Rodrı´guez-Artalejo F & Jose´ RB. Leptin reference values and cut-offs for identifying cardiometabolic abnormalities in the Spanish population. *Revista Española de Cardiología* 2015; 68(8): 672-679.
16. Sanda Kyaw. The influence of physical exercise, dieting and mental stress on body weight, serum leptin and cortisol levels in obese subjects. [PhD *thesis*]. University of Medicine - 1: Yangon; 2009.
17. Ruige JB, Dekker JM, Blum WF, Stehouwer CDA, Nijpels G, Mooy J, *et al*. Leptin and variables of body adiposity, energy balance, and insulin resistance in a population-based study. *Diabetes Care* 1999; 22(7): 1097-44.
18. Al-Sultan AL & Al-Elq AH. Leptin levels in normal weight and obese Saudi adults. *Journal of Family & Community Medicine* 2006; 13(3): 97-102.
19. Myat Mon Khaing. Plasma ghrelin, leptin and insulin level in obese and non-obese women. [PhD *thesis*]. University of Medicine - 2: Yangon; 2013.
20. Cohen B, Novick D & Rubinstein M. Modulation of insulin activities by leptin. *Science*1996; 274(5290): 1185-1188.