

Anthropometric Measurements and Appetite Related Hormones in Obesity Patients

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Abstract: Obesity and hyperlipidemia are two of the most highly distributed disorders that commonly associated with a cluster of progressive pathogenesis of many public health problems. This nutritional diseases increase the morbidity and the mortality of other diseases as cardiovascular diseases, atherosclerosis, hypertension, type2 diabetes, osteoarthritis and certain types of cancer. Thus the goal of the present study was to investigate the anthropometric measurements and biochemical parameters in case of obese patients before and after dietary treatments as well as the obesity related hormones in obese and in comparison with their corresponding healthy subjects. Serum leptin concentration increased significantly in obese subjects associated with a significant decrease in serum ghrelin concentration in obese group only. After dietary treatment serum leptin concentration decreased significantly in obese subjects while serum ghrelin increased significantly when compared with their values before treatments. There was a significant increase in serum glucose, insulin and insulin resistant (IR) value in obese subjects. While after dietetic treatment for obesity, there was a significant decrease in serum glucose, insulin and IR associated with a significant increase in serum adiponectin levels. In conclusion, the main features of obesity are leptin resistance and insulin resistance. Other hormonal influences are ghrelin and adiponectin which could be pathogenic factors for obesity. Hyperghrelinemia lead to hyperphagia and morbid obesity. While hypoadiponectinemia correlated to insulin resistance and diabetes type 2. So reducing body weight and controlling of hyperlipidemia enhancing insulin and leptin sensitivity through increase in adiponectin secretion, which has known as anti-diabetic, anti-inflammatory and anti-atherogenic hormone. [Nature and Science 2010;8(7):12-19]. (ISSN: 1545-0740).

Keywords: Obesity; hyperlipidemia; anti-diabetic; anti-inflammatory; anti-atherogenic; hormone

1. Introduction

Obesity is the increased accumulation of fat cells in the body in the form of adipose tissues. It is associated with many diseases, particularly cardiovascular diseases, type 2 diabetes, breathing difficulties during sleep, certain types of cancer, and osteoarthritis. Obesity is most commonly caused by a combination of excessive dietary calories, lack of physical activity, and genetic susceptibility, though a limited number of cases are due solely to genetics, medical reasons, or psychiatric illness. Overeating either due to hormonal imbalance or to bad food behavior and some others diet problems, increase body weight and deposition of fat in the body, when the increment of fat exceed the normal weight by 20% lead to the production of obesity *Haslam and James, (2005) & Yu et al., (2006)*.

Adipocytes take up and store free fatty acids as neutral fat. Body fat is located in the subcutaneous adipose tissue, and in visceral adipose tissues such as peritoneal adipose tissues. There are two types of adipose tissues in the body the white and brown adipose tissues. The white adipose tissue has been considered to stored fat and it is reported to have a secreting function for various chemical factors as leptin hormone, adiponectin hormone and tumor necrosis factor TNF- α , these chemical factors are called adipocytokines. Brown adipocytes; possess

mechanisms that regulate metabolic pathways that are under the control of the sympathetic nervous system or endocrine system. *Kawada et al., (2001), Ahima, (2006) & James, (2008)*.

Leptin hormone is produced by white adipose tissue and it is the product of obese (ob) gene. When there is a lot of adipose tissue, production of leptin increase to activate the satiety centers in the hypothalamus and reduce food intake. Conversely, when the reserves of adipose tissue decreases due to limited availability of food, leptin levels decrease and appetite increases *Martin et al., (2008)*. So leptin is important as a regulator for appetite, whole body energy balance and body composition. So leptin administration has been shown to reduce fat mass, food intake hyperglycemia and hyperinsulinemia. *Martin et al., (2008) & Paz-Filho et al., (2009)*.

Ghrelin is the first circulating hunger hormone. Synthesis of ghrelin occurs predominantly in epithelial cells lining the fundus of the stomach, in the placenta, kidney, pituitary and hypothalamus *Munding et al., (2006). Mondal et al., (2005)* defined ghrelin, as the ligand for the growth hormone secretagogue receptor, potently stimulates secretion of growth hormone. Also ghrelin affects on energy metabolism and food intake, so it was identified as a prominent target for development of anti-obesity

treatments. Mondal et al., (2005), Mundinger et al., (2006) & Gueorguiev et al., (2009).

Insulin hormone is produced by β -cells in the pancreas. Its primary role is to regulate blood glucose levels. Insulin acts on liver to increase glucose uptake and formation of glucose-6-phosphate with activate glycogen synthesis. In adipose tissue, insulin stimulate glucose uptake and then glucose is converted to glycerol and combines with free fatty acids (FFAs) to form triacylglycerols. While in muscles, insulin stimulates uptake of glucose and amino acids to form glycogen and protein. Also decreases protein catabolism *Semenkovich, (2006)*.

Adiponectin is a new member of adipocytokines and it is produced solely in adipocytes then secreted into serum and in white adipose tissue. Adiponectin has anti-diabetic, anti-inflammatory and anti-atherogenic effects. It correlates negatively with percent body fat, waist/hip ratio and plasma insulin, and positively with insulin sensitivity. *Fu et al., (2005) & ADA, (2007)*.

The aim of the work is to find a relation between obesity related hormone and anthropometric measurements.

2. Material and Methods

This study was conducted with 30 male subjects, fifteen obese patients were selected from "Obesity Outpatient Clinic, while healthy normal subjects were random. All subjects were asked for the demographic, anthropometric, healthy and dietary information according to questionnaire form.

The selected subjects were as follow:-

1-Normal healthy control group: contain fifteen healthy adult males in normal body weight with Body Mass Index (BMI) ($22.01 \pm 0.32 \text{ kg/m}^2$) which was calculated from the equation according to Raatz et al., (2005). $\text{BMI} = \text{body weight in (kg)} / \text{body height in meter}^2$.

2- Obese group: contain fifteen obese adult males with BMI ($36.75 \pm 0.94 \text{ kg/m}^2$) at zero time of experiment.

Methods

Anthropometric measurements:- are the most commonly used methods for diagnosis of obesity. Body weight was measured to nearest 0.1kg and body height to nearest 0.5cm with a digital scale with a standimeter (scaletronix, Holtain Ltd, Crymmych, Pembs, UK). Subjects were shoeless during measuring. Body mass index (BMI) was calculated from the equation according to Raatz et al., (2005) as described before. Body fat mass (BFM) was calculated from the sum of 4 skinfold measurements, triceps, biceps, subscapular and suprailiac, as described by Durnin and Womersley, (1974). By

using skinfold dialmax caliper (Bel-Art Products Pequannock, USA)

Serum measurements:

Determination of glucose concentration:

Serum level of glucose was determined by enzymatic colorimetric method according to Teuscher and Richterich, (1971).

Determination of insulin concentration:-

Serum insulin level was determined by Radioimmuno-assay method according to Deberg et al., (1998) by using Sorin kit. Cat. No. P2796

Determination of insulin resistance (IR):-

Insulin resistance was estimated by using HOMA (homeostasis model assessment) Garces et al., (2005) from the following equation:

$$\text{IR} = \frac{\text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mmol/l)}}{22.5}$$

Determination of adiponectin concentration:-

Serum adiponectin level was determined by Enzyme immunoassay method according to Yamauchi et al., (2003) by using SPI-BIO Human adiponectin enzyme immunoassay kit.

Determination of leptin concentration:-Serum

leptin level was determined by Radioimmunoassay method according to Ahren, (1997) by using Linco's Human Leptin-RIA-Sensitive product

Determination of ghrelin concentration:

Serum ghrelin level was determined by Enzyme immuno-assay method according to Grassi and Pradelles, (1991) by using SPI-BIO Human ghrelin enzyme immuno-assay kit.

3. Results

Table (1) Anthropometric measurements in human subjects showed a significant increase in body weight, BMI and BFM in obese subjects compared with healthy control subjects and after dietary treatment their values decreased significantly by 16.39%, 25.44% and 16.70% respectively compared with corresponding values before treatments.

Table (2) showed the concentration of serum glucose, insulin, insulin resistance (IR) and adiponectin in human subjects. Concerning to the concentration of fasting serum glucose and of insulin, it recorded $82.47 \pm 0.61 \text{ mg/dl}$ & $10.09 \pm 0.24 \mu\text{IU/ml}$ respectively in normal healthy subjects. There was a significant increase in serum glucose by 15.19%, insulin by 79.68% and IR by 108.96% in obese subjects compared with healthy

control subjects associated with a significant decrease in serum adiponectin levels by 39.38% in the same manner. While after dietetic treatment for obesity there was a significant decrease in serum glucose, insulin and IR associated with a significant increase in serum adiponectin levels in comparison with the corresponding values before treatments. Consuming the diet regimen for reducing weight in association with reduced blood glucose to no significant difference with the control, while insulin level decreased significantly ($P < 0.01$) compared to its normal level, the percentages of decrement were 11.08% and 24.98% for glucose and insulin respectively of treated obese subjects in comparison with their corresponding values of obese before treatment.

Table (3): The results leptin and ghrelin in human subjects showed that serum leptin concentration increased significantly in obese and

subjects by 202.75% and 181.79% respectively, associated with a significant decrease in serum ghrelin concentration in obese group only by 42.52% in comparison with the corresponding values of healthy control subjects. The normal ratio of the two-serum appetite hormones leptin: ghrelin was about 1:12 in healthy subjects. Their mean values were recorded 7.25 ± 0.19 ng/ml and 87.79 ± 10.27 pg/ml, respectively. After dietary treatment serum leptin concentration decreased significantly while serum ghrelin increased significantly in obese subjects when compared with their values before treatments. When obese subjects received the dietetic treatment of obesity. The value of leptin decreased significantly (28.75%), while ghrelin level significantly increased (39.38%) in comparison with the values before treatment (15.64 ± 0.41 ng/ml), (70.01 ± 16.12 pg/ml) respectively.

Table (1): Anthropometric measurements; body weight (kg) body mass index BMI (kg/m^2) and body fat mass BFM (%) of obese male subjects before and after treatments compared with healthy male subjects.

Groups Parameters	Healthy group	Obese group		Obese group
		before	Before	
Body Weight (Kg)	81.80 \pm 2.45 ^b	101.67 \pm 1.92 ^a	85.00 \pm 1.78 ^b	13.56
BMI (kg/m^2)	22.01 \pm 0.32 ^c	36.75 \pm 0.94 ^a	27.40 \pm 0.63 ^b	4.78
BFM (%)	19.36 \pm 0.30 ^c	29.82 \pm 0.40 ^a	24.84 \pm 1.62 ^b	4.09

Values expressed as mean \pm S.E. n=15

There was no significant difference between means have the same superscript in the same row at $P < 0.01$

Table (2): Concentrations of serum glucose (mg/dl), insulin ($\mu\text{Iu}/\text{ml}$), insulin resistance (IR) and adiponectin ($\mu\text{g}/\text{ml}$) of obese male subjects before and after treatments compared with healthy male subjects.

Groups Parameters	Healthy group	Obese group		L.S.D 0.01
		Before	Before	
Glucose (mg/dl)	82.47 \pm 0.61 ^b	95.00 \pm 2.48 ^a	95.00 \pm 2.48 ^a	8.50
Insulin ($\mu\text{Iu}/\text{ml}$)	10.09 \pm 0.24 ^c	18.13 \pm 0.65 ^a	18.13 \pm 0.65 ^a	2.38
Insulin resistance (IR)	2.05 \pm 0.65 ^c	4.27 \pm 0.22 ^a	4.27 \pm 0.22 ^a	0.76
Adiponectin ($\mu\text{g}/\text{ml}$)	18.30 \pm 9.80 ^a	5.70 \pm 2.00 ^b	5.70 \pm 2.00 ^b	8.11

Values expressed as mean \pm S.E. n=15

There was no significant difference between means have the same superscript in the same row at $P < 0.01$

Table (3): Concentrations of serum appetite related hormones; leptin (ng/ml) and ghrelin (pg/ml) of obese male subjects before and after treatments compared with healthy male subjects.

Groups Parameters	Healthy group	Obese group		L.S.D 0.01
		Before	Before	
Leptin (ng/ml)	7.25 \pm 0.19 ^d	21.95 \pm 0.30 ^a	15.64 \pm 0.41 ^c	2.23
Ghrelin (pg/ml)	87.79 \pm 10.27 ^a	50.23 \pm 12.03 ^c	70.01 \pm 16.12 ^b	10.03

Values expressed as mean \pm S.E. n=15

There was no significant difference between means have the same superscript in the same row at $P < 0.01$

4. Discussion:

Body mass index (BMI) (kg/m^2) is useful anthropometric method and correlates closely with adiposity. Therefore, BMI increases in obese patient due to increase in adipose tissues mass. In addition, skin fold thickness as measured at different sites mainly in triceps, biceps, subscapular and superailiac, then Body fat mass (BFM) measured to give an account on the percentage of fat in body. These parameters are important for diagnosis of obesity. Reducing in body weight is positively correlates with (BMI) and (BFM).

Our results indicated that Body weight, BMI and BFM significantly increased in obese subjects compared with healthy control subjects and after dietary treatment their values decreased significantly compared with corresponding values before treatments. There was association between obesity with body mass index BMI. BMI and BFM were significantly higher in obese, while after weight reduction program there was a significant decrease in both BMI and BFM compared to normal healthy control. Hu et al., (2001), Mendez-Sanchez et al., (2002) & Mohn et al., (2004). Another study of Gonzalez et al., (2007) reported that the obese subjects had significantly higher ($P < 0.003$) weight, BMI and percentage of body fat than normal healthy control group.

After weight reduction regimen, there significant changes in body composition variables as body weight, and BMI in obese subjects when compared with normal healthy control subjects. In addition, plasma insulin reduced. Patalay et al., (2005) & Shih et al., (2006). The reduction of insulin level after weight reduction may be decrease inhibition of hormone-sensitive lipase within the adipocytes, this lead to increase release of fat from the adipocytes, and this associated with decrease in skinfold thickness, a measurement of body fat mass (BFM).

Concerning to the concentration of fasting serum glucose and of insulin. The result showed a significant increase in the glucose and insulin levels, the percentages of increment were being 15.19% & 79.68% for glucose and for insulin respectively, compared to the mean values of healthy control subjects. From the results of fasting serum glucose, insulin and insulin resistance (IR) in human, it is clear that, serum glucose level normally maintained within narrow limits by mainly release of insulin in case of normal body weight. High serum fasting glucose associated with the change of insulin levels and is a definite sign of IR, which associated with marked overweight and dietary impairment factors that increase serum lipids profile. Insulin resistance commonly observed in obesity and

this may be due to increased plasma free fatty acids. Insulin resistance cause lack in appropriate insulin signaling which develop abnormalities in glucose metabolism and decrease glucose uptake into cells (hyperglycemia). Hyperglycemia is sensed by pancreatic beta cells, which increase insulin secretion to compensate for elevated blood glucose level, this lead to hyperinsulinemia characteristic of IR in obesity. Weight reduction in obese subjects, reduced IR and increase insulin sensitivity, which enhance glucose uptake into cells and control hyperglycemia and hyperinsulinemia.

Moreover, from our results it is clear that, serum adiponectin level positively correlates with insulin sensitivity and negatively with insulin resistance. Since obesity is associated with hyperinsulinemia and insulin resistance adiponectin level decreases. Weight reduction as it modulates insulin resistance and increases sensitivity of insulin seems to enhance secretion of adiponectin from fat cells. Also adiponectin level decreased due to the presence of IR. After controlling by diet and medication insulin sensitivity increases and subsequently enhance adiponectin secretion. Increase plasma fatty acids as in obesity lead to insulin resistance. The mechanism of IR production explained as impairing the insulin-signaling pathway due to increasing accumulation of triacylglycerols in tissue. Moreover, through the metabolism of triacylglycerols intermediate metabolites formed such as diacylglycerols that is a potent activator of Protein Kinase-C (PKC-) and (NF-KB) pathways. PKC is an enzyme that phosphorylates serine residue in insulin receptor and/or (IRS-1), the phosphorylation of these molecules lead to its destruction and impairs insulin signaling pathway and result in IR. Boden, and, Laakso, (2005). The results of Kristina and Steven, (2006) confirmed that obesity was associated with elevated plasma free fatty acids (FFAs) that lead to insulin resistance. The mechanisms whereby fatty acid as induce insulin resistance mediated by translocation of the PKC- isoform from the cytosol to the membrane compartment resulting in impairment of insulin receptor substrate-associated phosphatidylinositol-3-kinase activity (IRS-PI3-K) which, is important in activation of insulin receptor and insulin signaling pathway. Lowering plasma fatty acid as level by controlling diet or regular exercise prevents activation of NF-KB pathway and had a benefit to increase insulin sensitivity and improving regulation of glucose level.

Our results indicated that serum adiponectin level was low in individuals with obesity compared with healthy control subjects, and this may be due to decreased adiponectin gene expression in these diseases. While weight reduction was associated with

increase in circulating adiponectin and may improve lipids profile in obese subjects. Schulze et al., (2005). Boden and Laakso, (2005) confirmed that the plasma adiponectin level negatively correlated with obesity, percentage of body fat and plasma insulin level. But, it positively correlated with insulin sensitivity and insulin-mediated glucose uptake. The hyperinsulinemia caused by obesity-induced insulin resistance, together with enhanced TNF- expression from adipose tissue may contribute to reduce adiponectin secretion. It has been suggested that visceral adipose tissue as in abdominal obesity may produce substances that decrease adiponectin mRNA. This reduction may be due to decreased expression of adiponectin receptor R1 in adipose tissue, which recorded to increase by weight reduction. Maria et al., (2006) & Lin et al., (2007). Fasting glucose, insulin and HOMA-IR significantly increased when compared with control group. The produced hyperglycemia was explained by impairment of glucose metabolism as measured by the two lipogenic genes (glycerol-3-phosphate dehydrogenase and fatty acids synthetase) since their level seemed to increase by high fat diet compared to control. Milagro et al., (2006), Gonzalez et al., (2007), Rector et al., (2007) & Shargorodsky et al., (2009). The hypothesis of hyperglycemia associated with obesity-induced diets, which can produce type 2 diabetes. This was explained as accumulation of adipose tissues in obesity increased the secretion of inflammatory cytokines from adipocytes as TNF- and IL-1, which responsible for decrease insulin sensitivity and affect β -cells function. In addition, hyperleptinemia present in obesity inhibited the auto defense of pancreatic cells against inflammatory cytokines, which subsequently produced IR. Sauter et al., (2008).

The results showed that serum leptin concentration increased significantly, associated with a significant decrease in serum ghrelin concentration, but after dietary treatment serum leptin concentration decreased significantly while serum ghrelin increased significantly in obese subjects when compared with their values before treatment.

With respect to our findings of tested appetite related hormones in human subjects, it is clear that, serum leptin concentrations are positively correlates with BMI and BFM. Therefore, its level increases in obesity due to increase in body fat percentages. While by weight reduction, leptin level decreases due to reduce in BMI and BFM. Since hyperinsulinemia increase lipids deposition in adipose tissues which increase leptin secretion.

On the other hand, serum ghrelin concentration increases in case of negative energy balance. Since obese patients are usually in positive energy balance

thus ghrelin level decrease in obese patients. Another explanation is that leptin level increase in obesity and decrease ghrelin secretion from stomach, while by reducing weight, ghrelin level increase due to decrease in leptin level by weight reduction. Insulin resistance and hyperleptinemia closely correlated together, since IR with hyperinsulinemia increased lipids storage in adipose tissue with increased secretion of leptin from adipocytes. Chu et al., (2000) & Hintz et al., (2003).

Our results of serum leptin in association with the results of serum insulin and IR as shown in Table (2) go hand in hand with the results of Doehner et al., (2002) who reported that increase in plasma leptin level causes attenuation of insulin-induced activities in human hepatic cells and may leads to IR. Leptin down regulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), a key step of the insulin receptor-signaling cascade. Also leptin counteracted the other steps of this signaling cascade, like the association of insulin-receptor substrate-1 with the adapter molecule growth factor-receptor-bound protein 2. Leptin also inhibited glycogen synthesis in skeletal muscles. Furthermore, a functional leptin receptor was discovered in the pancreas, which exerts a direct inhibitory effect on insulin secretion. Obese subjects were almost hyperleptinemic, but the hypothalamus is unable to transduce this leptin signals to reduce body weight and termed leptin resistance. Leptin resistance also prevents exogenous leptin administration from promoting weight loss. Hewson et al., (2002).

Fasting plasma ghrelin concentration was significantly lower in obese subjects compared with healthy normal control subjects. Ghrelin negatively correlated with BMI, percent of body fat, fasting plasma insulin and leptin concentrations. Weight loss increased plasma ghrelin level and increased ghrelin secretion from the epithelial cell of stomach since ghrelin level positively correlated with negative energy balance. Once weight reduced through marked food restriction exogenous administration of leptin is able to increase energy expenditure and permit weight loss suggesting that some factors or processes associated with weight loss improve leptin sensitivity. Inui et al., (2004) & Straznicki et al., (2005).

The results obtained by Wang et al., (2005) support our data since they found hyperleptinemia in mice fed diet-induced obesity and indicated that this hyperleptinemia cannot deplete fat in adipocytes. The ability of adipocytes to undergo hypertrophy and hyperplasia despite hyperleptinemia implies that a powerful leptinergic blockage protects their vital fat-storing function from the antilipogenic action of leptin. This leptinergic blockage is essential for

obesity. Two likely mechanisms capable of blocking the paracrine action of increasing leptin level secreted by adipocytes, first, a large increase in expression of post receptor inhibitor of leptin, which appear in adipocytes by the sixth day of high fat feeding diet, at this point leptin level slightly elevated. Later, as the hyperleptinemia become more intense, the decline in leptin-b mRNA becomes substantial, and by the end of experiment leptin-b mRNA reaches undetectable levels. Thus, a combination of post receptor and receptor leptin blockage appears to minimize potential leptinergic interference with fat storage.

Leptin and adiponectin exert opposing effects on glucose metabolism, fat oxidation and insulin sensitivity. The ratio of leptin to adiponectin investigated as a potential atherogenic index; leptin to adiponectin ratio was 8-fold higher in obese subjects compared to non-obese and strongly correlated with other atherogenic metabolic markers such as BMI and skin-fold thickness. Koenig et al., (2007).

Another study of Huang et al., (2008) confirmed that obese subjects are almost hyperinsulinemic. Since insulin stimulates leptin production from adipocytes via an indirect mechanism dependent on lipogenesis, most reducing weight regimens (through caloric restriction) cause lipolysis and this lead to acute leptin suppression and increased leptin sensitivity.

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