Role of Ghrelin, Leptin and Insulin Resistance in Development of Metabolic Syndrome in Obese Patients

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Abstract

Objective: Obesity and its complications including metabolic syndrome (MetS) have been increased in children and adolescents recently. Leptin is known to play an important role in the pathogenesis of obesity. The objective of this study was to evaluate the relationship between Leptin, Ghrelin and Insulin resistance in the development of metabolic syndrome in obese persons.

Methods: this study was carried out on fifty obese persons. All patients have BMI ≥ 30 Kg/m². Twenty of them have metabolic syndrome. Body Mass Index (BMI), Waist Circumference (WC), and blood pressure were measured. Fasting Plasma Glucose (FBG), two hours Post Prandial Blood Glucose (PPBG), Glycated hemoglobin A1c (HbA1c), triglyceride (TG), Total Cholesterol (TC), high and low density lipoprotein cholesterol (HDL-C and LDL-C), serum Leptin, Ghrelin and Insulin were done. HOMA-IR and HOMA- β were calculated.

Results: SBP, DBP, FBS, PPBG, HbA1c, HOMA-IR and HOMA-β were significantly increased in obese and MetS groups compared to control group. There was a significant increase in insulin and Leptin, serum TG, TC and LDL-C with a significant decrease in HDL cholesterol and Ghrelin in obese and MetS groups. A significant negative correlation between plasma Ghrelin and BMI, WC, SBP, DBP, and FBS, PPBG, serum TG, TC, LDL-C, HbA1c, and HOMA-β was observed while leptin showed a significant positive correlation with them and a negative correlation with HOMA-IR. Plasma Ghrelin was positively correlated with HDL-C while Leptin was positively correlated with it in obese and MetS groups. There was a significant negative correlation between plasma Ghrelin, Insulin and leptin with significant positive correlation between plasma insulin and leptin in obese and MS groups.

Conclusion: There are hormonal changes associated with clusters of metabolic abnormalities and elevation of blood pressure that may have a role in the development of MetS in obese persons and are major CHD risk factors. Insulin has stimulatory trophic effect on leptin secretion, but the effect of leptin on insulin is controversial as leptin may modulate insulin action and participates in the development of insulin resistance. As regard Ghrelin secretion, there are many suppressive factors: Insulin, Leptin and glucose. Our study produces a preliminary result, thus further studies with large number of patients are required.

Keywords: Ghrelin; Leptin; Insulin resistance; Obesity and metabolic syndrome

Introduction

Obesity is generally recognized as an increasingly important cause of childhood and adolescent morbidity worldwide and is a contributor to chronic diseases such as type 2 diabetes mellitus (T2DM) and Coronary Heart Disease (CHD) [1]. Obesity has central role in Metabolic Syndrome (MetS), MetS has appeared with increasing frequency in children and adolescents, driven by the growing pediatric obesity epidemic [1,2]. Behavioral factors such as poor dietary habits, a sedentary lifestyle, and a social environment which encourages unhealthy behaviors are closely correlated with the prevalence of obesity and MetS in adolescents [3]. MetS is characterized by a clustering of metabolic abnormalities which leads to increased cardiovascular disease and all-causes mortality. The five generally accepted features of metabolic syndrome are obesity, insulin resistance, dyslipidemia [including increased triglycerides and decreased HDL], impaired glucose tolerance, and hypertension [4]. Recent studies suggest that MetS may be associated with subsequent risk of T2DM and CHD in young population, particularly in the overweight or obese. Considering recent global rise of childhood obesity prevalence, the overall prevalence of the metabolic syndrome in children and adolescents seems to be higher than what is estimated from previous studies [5].

Leptin is a peptide produced by differentiated adipocytes. It is thought to be a key hormone in the regulation of body fat stores. This peptide controls energy metabolism at the level of hypothalamus by supporting the food intake and stimulating energy expenditure [6]. The relationship between serum Leptin and body mass index has been well established [7]. Leptin is also proposed to be associated with insulin resistance and diabetes in human [8]. However, potential relationship between metabolic syndrome and leptin has not been sufficiently addressed in previous studies, particularly in children [9]. Ghrelin is a novel 28-amino acid residue peptide hormone predominantly produced by the stomach. Substantially lower amounts were detected in bowel, pancreas, kidneys, the immune system, placenta, testes, pituitary, lung, and hypothalamus [10]. Ghrelin displays strong Growth Hormone (GH)-releasing activity, which is mediated by the activation of the so-called GH secretagogue (GHS) receptor type 1a (GHS-R 1a) [11]. At the hypothalamic level, Ghrelin acts via mediation of GHRH-secreting neurons as indicated by pretreatment with GHRH antagonists, reduces their stimulatory effect on GH secretion [12]. An increased release of GHRH in portal blood of the pituitary after

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Ghrelin administration has also been shown in animals [13]. Ghrelin enhances appetite and increases food intake by increasing agouti-related protein (AGRP) and neuropeptide Y (NPY) expression in the hypothalamus. Also, it reduces fat utilization and causes adiposity in rodents. The release of Ghrelin may stimulate the release of orexigenic peptides and neurotransmitters, thus representing a novel regulatory circuit controlling energy homeostasis and neurotransmitters release [14]. Ghrelin is able to modify glucose and insulin metabolism, blood adipogenesis and inflammatory processes in experimental conditions [15].

The present study aims to evaluate such a relationship between plasma leptin, Ghrelin and insulin resistance with the development of metabolic syndrome in obese patients.

Patients and Methods

The present study was carried out on fifty obese persons (obesity was defined according to WHO criteria 2010 as BMI greater than or equal to 30 kg/m²) with matched age range 24-45 years 20 of them has metabolic syndrome selected from Outpatient Clinics of Internal Medicine Department, Tanta University Hospital. The metabolic syndrome was diagnosed according to WHO criteria. Fifteen apparently healthy volunteers’ hospital staff with average body weight (7 female, 8 male), with matched age range 34-52 years, and were enrolled in the study as a control. Persons with a waist circumference equal to or above 90 percentile of their age and height were invited to attend and participate in the study. All persons had similar lifestyle with no significant physical training program before beginning the study.

Exclusion criteria

- Persons with a known history of primary hyperlipidemia, diabetes or secondary obesity.
- Patients receiving medication that could affect the metabolic profile (e.g., beta blocker, steroids and diuretics).
- Menopausal women.

After their consent, all subjects were subjected to:

- Full history taking with particular emphasis on age, family history, history of any systemic diseases e.g. diabetes, hypertension, dyslipidemia or history of any associated diseases and any drug intake.
- Thorough clinical examination with special stress on:
  - Systolic and diastolic blood pressure (SBP & DBP).
  - Body weight was measured to the nearest 0.5 Kilograms (kg) with subjects barefooted and wearing light indoor clothes. Body height was recorded to the nearest 0.5 centimeter (cm).
  - BMI was calculated as the ratio of body weight to body height squared expressed as Kg/m².
  - Waist circumference (WC) was measured at the distal third of the line from the xyphoid process to the umbilicus.

Participants were classified to overweight or obese using national sex and age specific BMI cut-offs recommended by the International Obesity Task Force (IOTF) [9].

Blood sampling and analysis

After overnight fasting, 7 ml blood was collected from a vein in the antecubital fossa between 8 and 9 AM after overnight fasting in clean tubes. 1.8 ml were collected in EDTA tube and preserved at refrigerator after gentle mix for assay of Hb A1c. The other 5.2 ml were collected in plain tube, incubated for 20 minutes, then centrifuged at 3000 rpm for 10 minutes. Serum was separated in another clean tube and stored at -20 till the assay time. Fasting and two hours post prandial blood glucose was measured according to glucose-oxidase method [16]. Glycated hemoglobin A1c was measured using a column chromatography method by commercial kit provided from Biosystem Company. After final elution the result was determined photometrically using Biosystem photometer (Reference range 3.1-6.4%). Serum triglycerides (TG), Total Cholesterol (TC) and high density lipoprotein (HDL cholesterol).were measured photo metrically using commercial kit provided from Spinreact, and LDL was calculated by the equation (LDL=cholesterol - triglycerides/5 –HDL).

Insulin level: was estimated according to Angel (1988) [17] using a commercially available ELISA kit which was modified for use in microtiter plates. The adapted assay, is based on the binding of porcine anti-guinea pig insulin antibodies to microtiter plates and uses insulin-peroxidase conjugate as displacer.

Serum ghrelin level: was measured by a commercially available ELISA kit (The DSL- 10-33700) Human Ghrelin ELISA Kit; Diagnostic System laboratories, Webster, Texas). This assay is an enzymatically amplified “one-step” sandwich-type immunoassay. In the assay, standards, controls and unknown plasma samples are incubated with anti-Ghrelin antibody in microtitration wells which have been coated with another anti-Ghrelin monoclonal antibody. After incubation and washing, the wells are incubated with the substrate tetramethylbenzidine (TMB), an acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured is directly proportional to the concentration of total Ghrelin present. A set of total ghrelin standards is used to plot a standard curve of absorbance versus total Ghrelin concentration from which the total Ghrelin concentrations in the samples were calculated [18].

Serum leptin level: was measured by a commercially available ELISA kit (The DSL- 10-23100 Human Leptin ELISA Kit; Diagnostic System laboratories, Webster, Texas). This assay is a direct Sandwich ELISA based, sequentially, on capture of human leptin molecules from samples to the wells of a microtiter plate coated by pre-titered amount of polyclonal rabbit anti-human leptin antibodies, and wash away of unbound materials from samples, then, binding of a biotinylated monoclonal antibody to the captured human leptin, and conjugation of alkaline phosphatase to biotinylated antibodies, after that, wash away of free antibody-enzyme conjugates. Finally, quantification of immobilized antibody-enzyme conjugates by monitoring alkaline phosphatase activities in the presence of the substrate p-nitrophenyl phosphate. The enzyme activity is measured spectrophotometrically by the increased absorbency at 405 nm due to production of the yellow colored product p-nitrophenol. Since the increase in absorbency is directly proportional to the amount of captured human leptin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human leptin [19].

Homeostasis model assessment: HOMA is an arithmetic way of deriving indices of pancreatic endocrine function HOMA- β and HOMA-IR from fasting plasma samples [20]. This model assumes that plasma glucose and insulin in the fasting state is controlled by a feedback loop between the pancreas, liver, and insulin-sensitive and insulin-insensitive peripheral tissues. HOMA correlates well with and
P values ˂ 0.05 were considered significant.

HOMA-IR and HOMA-β are derived using the formulae:

HOMA-IR = [glucose (mg/dL) × insulin (mU/l)] / 405
HOMA-β = [360 × insulin (mU/l)] / [glucose (mg/dL) – 63]

IR is insulin resistance and β is the β-cell function. Glucose and insulin are both during fasting. In an "ideal" reference population of young, healthy subjects HOMA-β and HOMA-IR are 100% and 1 (arbitrary units), respectively.

Statistical Analysis

Values were expressed as mean ± SD. The statistical analysis of the results was carried out according to the conventional standard statistical procedures using computed statistical analysis by SPSS, version 20.0 for Microsoft Windows XP. All variables were tested for normality of distribution, Fisher's exact test; independent samples t-test (t value) and ANOVA was applied to detect the difference between two arithmetic distributions, respectively.

Results

Table 1 showed basic clinical data of the studied groups, patients with metabolic syndrome were significantly more obese than others. There is no significant difference between studied groups as regard age and gender. WC, BMI, SBP and DBP were significantly increased in obese and metabolic syndrome group.

Table 5 showed a significant negative correlation between plasma Ghrelin and BMI (r=-0.7506), WC (r=-0.5616), SBP (r=-0.5464) and DBP (r=-0.6050) while Ghrelin is significantly decreased in obese and MetS groups compared to control group (p<0.0001) with significant difference between obese and MetS groups. Table 6 shows laboratory data of the studied groups, FBS, PPBG and HbA1c were significantly increased in obese and MetS groups compared to control group with a significant difference between obese and MetS groups (p<0.0001). HOMA-IR, was increased in obese and MetS groups with a significant difference only between control and MetS groups (p=0.0073). HOMA-β was significantly increased in obese and MetS groups compared to control group (p=0.002) with no significant difference between obese and MetS group. As regards lipid profile (Table 2), there was a significant increase of serum TG, TC and LDL cholesterol (p<0.0046, 0.0001 & 0.0001 respectively) in obese and MetS compared to control group with a significant difference between obese and MetS groups. As regards serum TC and TG. There was a significant decrease in the level of HDL cholesterol in obese and MetS groups (p<0.0001) compared to control group with no significant difference between obese and MetS group. Table 3 shows a significant increase of insulin and leptin (p<0.0001) while Ghrelin is significantly decreased in obese and MetS groups compared to control group (p<0.0001). Table 4 reveals a significant negative correlation between plasma Ghrelin and BMI (r=-0.7506), WC (r=-0.5616), SBP (r=-0.5464) and DBP (r=-0.6050) while leptin showed a significant positive correlation with them (r=0.5944, 0.5643, 0.3839 & 0.4250 respectively).

Table 5: Correlation between Ghrelin and leptin with lipid profile obese and metabolic syndrome group.

Table 6: Correlation between Ghrelin and leptin with lipid profile obese and metabolic syndrome group.
correlation between plasma Ghrelin and serum TG \((r=0.5291)\), cholesterol \((r=0.4768)\) and LDL cholesterol \((r=0.6695)\) with a positive correlation with HDL cholesterol \((r=0.6135)\). Leptin showed significant positive correlation with serum TG \((r=0.3609)\), TC \((r=0.3170)\) and LDL cholesterol \((r=0.5005)\) with significant negative correlation with HDL cholesterol \((r=-0.4346)\) in obese and MetS groups. Table 7 showed a significant negative correlation between plasma Ghrelin and insulin and leptin \((-0.6452\) and \(-0.7253\) respectively) with a significant positive correlation between plasma insulin and leptin \((r=0.5174)\) in obese and MS groups.

### Discussion

Obesity is generally recognized as an increasingly important cause of morbidity worldwide and contribute to chronic diseases such as T2DM and CHD [1]. MetS a clustering of obesity, impaired glucose metabolism, hypertension, and dyslipidemia, has been shown to be predictive for the development of diabetes and CHD in this young population, particularly in the overweight or obese [5]. In the present study, there were significant increases in BMI, WC, SLPB, DBP, FBS, PP BS and HbA1c in obese and MetS groups compared to control group. This in agreement with Yoon et al. [22], who found that MetS was positively associated with body weight, waist circumference, blood pressure and blood glucose levels.

HOMA-IR, is increased in obese and MetS groups with a significant difference only between control and MetS groups \((p=0.0073)\). HOMA-β is significantly increased in obese and MetS groups compared to control group \((p=0.002)\) with no significant difference between obese and MetS group. Garg et al. [23] found that subjects with MetS had more insulin resistance (HOMA-IR) and less insulin secretion (HOMA-β) than healthy controls. Yoon et al. [22] who found that MetS was positively associated with the HOMA-IR. As regards lipid profile, there was a significant increase in serum triglyceride, cholesterol and LDL cholesterol in obese and MS groups compared to control group with a significant difference between obese and MS groups with respect to serum cholesterol and triglyceride. There was a significant decrease in HDL cholesterol in obese and MetS groups \((p<0.0001)\) compared to control group with no significant difference between obese and MetS group. This in agreement with Yoon et al. [22] who found that MetS was positively associated with TC, TG and negatively associated with the HDL-cholesterol level. The degree of correlation between the MetS score and TG level was highest, followed by that with HDL cholesterol.

Our study revealed significant increase in serum Insulin and Leptin hormones concentrations in obese and MetS groups compared to control group. More evidence emerges that insulin can regulate Leptin expression. This is most evident from studies on isolated adipocytes, which showed that in vitro insulin clearly stimulates the mRNA expression and secretion of Leptin in cultured rat and human adipocytes. Another possibility, glucose metabolism seems to be an important determinant of Leptin expression and secretion. In human experiments, hyperinsulinaemia induced by clamp techniques leads to a rise in leptin concentrations [24]. One report on a patient with insulinoma reported markedly elevated leptin levels during chronic high insulinemia, which both dropped after surgical removal [25]. High leptin concentrations may in part be influenced by hyperinsulinaemia or impaired insulin sensitivity. This in agree with our results where HOMA-IR, was significantly decreased while, HOMA-β was non-significantly increased in obese and MetS groups compared to control group. Leptin showed significant positive correlation with HOMA-β and significant negative correlation with HOMA-IR.

In the present study, we observed a significant decrease in plasma Ghrelin concentration in obese and MetS groups compared to control group. The exact mechanism of decreasing Ghrelin secretion is not fully understood. There are many possibilities: the first one, hyperinsulinemia is an inhibitor of Ghrelin secretion [26]. The mechanism of inhibitory effect of insulin on Ghrelin secretion is unclear. The gastrointestinal tract can be regarded as an insulin-sensitive tissue; therefore, the insulin–mediated decrease in plasma Ghrelin could result from inhibition of Ghrelin release in the stomach cells [27]. The second possibility, there is substantial evidence that leptin can exert an inhibitory effect on gastric Ghrelin release [28]. Leptin has satiety–inducing effects which include suppression of gastric orexigenic signals and disruption of a potential feedback mechanism between body weight changes and plasma Ghrelin in lean adult rats [29]. This is in agreement with our results where there were increase in plasma insulin and leptin in obese and MetS groups. The third possibility, reduction of plasma Ghrelin level may be associated with higher BMI [30]. This is agreement with our results where Ghrelin was significantly lower in obese and MetS groups compared to control group with a negative correlation between Ghrelin and BMI, WC, SLPB and DBP. Some studies revealed that low Ghrelin was associated with the prevalence of hypertension [31] in some human studies, fasting plasma Ghrelin negatively correlated with BMI. The fourth possibility, suppression of plasma Ghrelin level may be due to hyperglycemia in T2DM patients [32]. This in accordance with our data where obese and MetS groups had higher blood glucose and Insulin levels suggesting that hyperinsulinenia associated with insulin resistance decreases serum levels of Ghrelin in obese patients. Also, there was a negative correlation between serum Ghrelin and FBS, PPBS, HbA1c and HOMA-β with positive correlation with HOMA-IR while there was a negative correlation between plasma Ghrelin and insulin level and leptin.

Leptin showed significant positive correlation with BMI, WC, SLPB and DBP in obese and MetS groups. In this agreement with Adil Omar et al. [33] who found that Leptin concentrations were high in both obese and diabetic obese group and showed a direct positive relation with BMI and waist circumference. Aanoo and Jie [34] found that, higher plasma leptin levels are associated with hypertension both among women as well as men in a representative sample of adults. Leptin showed significant positive correlation with FBS, PPBS, HbA1c, and HOMA-β with significant negative correlation with HOMA-IR. Maria et al. [35] stated that elevated serum leptin, particularly in obese individuals, should be taken as a warning sign of energy imbalance, poor diet, hyperinsulinemia, insulin resistance, or changes in other metabolic risk factors that are strongly associated with cardiovascular disease and T2DM. Leptin receptors are present on human hepatocytes, and Leptin was shown to modulate several insulin induced activities in these cells. Leptin antagonizes insulin signaling by decreasing insulin-induced tyrosine phosphorylation of Insulin Receptor Substrate (IRS), it increases phosphoenolpyruvate-carboxykinase and decreases gluconeogenesis expression leading to increased gluconeogenesis and decreased glycolysis [36]. The hepatic effects of high Leptin levels may thus contribute to hepatic insulin resistance. Some studies indicate that Leptin is able to modulate insulin action (on glucose uptake and/or lipid synthesis) in adipocytes and muscle cells, but other studies found no effect on peripheral glucose uptake [37]. Leptin may acts at different

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* Means significant.
intracellular levels, including transcription to membrane permeability to inhibit insulin synthesis as well as secretion. Functional Leptin receptors also present in insulin-secreting pancreatic β-cells [38]. The insulin-lowering effect of Leptin administration could thus be mediated through these receptors. Recently, a direct effect of Leptin on insulin gene transcription in pancreatic β-cells was shown, with a reduction of preproinsulin mRNA by 50% [39]. On the other hand, impaired insulin sensitivity may both induce increased insulin levels and lead to raised Leptin levels by down-regulation of the hypothalamic Leptin receptors or the subsequent satiety response to Leptin. Insulin resistance has been investigated as a potential CHD risk factor in the general population [40]. This in agreement of our results where a significant positive correlation between insulin and Leptin was found.

As regards lipid profile, there was a significant negative correlation between plasma Ghrelin and serum TG, TC and LDL cholesterol with a positive correlation with HDL cholesterol while Leptin showed a significant positive correlation with serum TG, TC and LDL cholesterol with significant negative correlation with HDL cholesterol. Hiroshi et al. [41] found that Leptin was positively correlated with SBP, DBP, FPG, TC, TG, LDL-C and as negatively correlated with HDL-C. Al-Hakeim and Ali [42] found that Ghrelin levels were negatively correlated with BMI.

In conclusion, there are hormonal changes associated with clusters of metabolic abnormalities and elevation of blood pressure that may have a role in the development of MetS in obese persons and are major CHD risk factors. Insulin has stimulatory trophic effect on leptin secretion, but the effect of leptin on insulin is controversial as leptin may modulate insulin action and participates in the development of insulin resistance. With regard to Ghrelin secretion, there are many suppressive factors: insulin, Leptin and glucose. Our study produces a preliminary result, thus further studies with large number of patients are required.

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