

Full Length Research Paper

Serum ghrelin levels in Syrian obese patients with diabetes mellitus type II

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The serum ghrelin levels had been studied in three groups of subjects: First group was normal weight (11 men and 9 women), non obese, non diabetic, 38 ± 12 years old, with BMI mean about 23.8 kg/m^2 and did not take any drugs. The second group was overweight (18 men and 13 women), non diabetic, 40.6 ± 9.9 years old, with BMI mean about 36.34 kg/m^2 . This group was apparently healthy and did not take any oral hypoglycemic drug. The third group was overweight type II diabetic (19 men and 16 women), 40 ± 10.9 years old, with BMI mean about 36.65 kg/m^2 . Patients in this group were taking at least one of the oral hypoglycemic drugs. In this regard, this study illustrated that ghrelin blood levels in type II overweight diabetics ($9.5 \pm 2.5 \text{ fmol/ml}$) were less than non diabetic overweight subjects ($11.3 \pm 2.8 \text{ fmol/ml}$), while normal weight subjects had the lowest levels of ghrelin ($16.8 \pm 4.6 \text{ fmol/ml}$). By studying the correlation between serum ghrelin levels and BMI, and the correlation between serum ghrelin levels and insulin, a statistically significant reverse relation between them was found, within each group. In conclusion, ghrelin concentrations were affected by obesity and diabetes mellitus type II.

Key words: Ghrelin, insulin, type II diabetes, obese, body mass index (BMI).

INTRODUCTION

Ghrelin's receptor was discovered in 1997 (McKee et al., 1997) while ghrelin itself was discovered in 2001 by the technique of the orphan receptor (Kojima et al., 2001). This hormone was purified from the stomach throughout four steps of chromatography: gel filtration, two ion-exchange HPLC steps, and a final reverse-phase HPLC (RP-HPLC) procedure (Miura et al., 2009; Kojima et al., 1999). Ghrelin consists of 28-amino acid peptide, in which the serine-3 (Ser3) is n-octanoylated, and this modification is essential for ghrelin's activity (Matsumoto, 2001; Kojima and Kangawa, 2005).

The discovery of ghrelin indicated that the release of GH from the pituitary is not regulated by hypothalamic growth hormone releasing hormone (GHRH) only, but also by ghrelin (Osterstock et al., 2010) which is secreted

from the stomach. On the other hand, appetite is stimulated by ghrelin by acting on the hypothalamic arcuate nucleus indicating that it transmits the hunger signal to the central nervous system from the periphery (Cowley et al., 2003). Furthermore, an intravenous injection of ghrelin has decreased the arterial pressure (Lin et al., 2004). Subsequently, ghrelin has been shown to stimulate insulin secretion in some studies (Adeghate, 2002), while inhibiting insulin release in others (Reimer et al., 2003). As a result of the detection of ghrelin receptor's mRNA in liver and kidney tissues by RT-PCR method, ghrelin may be involved in the regulation of gluconeogenesis *in vivo* (Uribe et al., 2008). Steady-state ghrelin levels correlate negatively with measures of adiposity, and they are low in overweight individuals (Tschöp, 2001). This observation is consistent with a compensatory rather than causal role for ghrelin in common obesity. However, the only known overweight condition associated with high ghrelin levels is the Prader-Willi

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Table 1. The titrated biochemical parameters in the study groups.

Column 1	Obese diabetic	Obese non-diabetic	Control	Unit
Ghrelin	9.5 ± 2.5	11.3 ± 2.8	16.8 ± 4.6	fmol/L
Insulin	7.4 ± 3.91	10.6 ± 6.4	6.4 ± 2.5	µu/ml
Glucose	262.4 ± 61.4	94.29 ± 9.9	89.68 ± 8.5	mg/dl
Cholesterol	225.12 ± 48.6	193.8 ± 40.1	165.68 ± 22	mg/dl
LDL	117.51 ± 33.2	107.94 ± 30.6	94.82 ± 21.6	mg/dl
HDL	43.95 ± 10.6	44.58 ± 12.2	42.23 ± 6.1	mg/dl
TG	266.25 ± 105.5	180 ± 81	157.6 ± 38	mg/dl

syndrome, the most common form of human syndromic obesity (Butler and Bittel, 2007). Finally, this study aims to define the correlation between ghrelin and other parameters which are contributed with insulin resistance, and the possibility of a negative correlation of ghrelin and obesity.

MATERIALS AND METHODS

Subjects

We investigated 86 subjects attending the out-patient clinic of Al-Assad University Hospital and Diabetes Center, Damascus, Syrian Arab Republic, from February 2009 to April 2009. We divided the subjects into 3 groups according to their BMI and fasting blood glucose levels:

1. Obese diabetic group (n = 35: [19 women and 16 men], age: 40.6 ± 9.9 years, BMI: 36.65 ± 5.46 kg/m²) taking at least one of oral anti-diabetic drugs, and they were diagnosed with diabetes by an experienced endocrinologist for more than 6 months, with fasting blood glucose >110 mg/dl.
2. Obese non-diabetic group (n = 31: [18 women and 13 men], age: 40 ± 10.9 years, BMI: 36.34 ± 4.2 kg/m²); healthy subjects with no symptoms of any disease, and they were not taking any medications, with fasting blood glucose <110 mg/dl.
3. Control group (n = 20: [9 women and 11 men], age: 38 ± 12 years, BMI: 23.8 ± 3.5 kg/m²) with fasting blood glucose <110mg/dl.

Subsequently, patients with cardiovascular disease (CVD), nephritic syndrome and BMI of <30 kg/m² were excluded. Besides, patients taking insulin along with oral anti-diabetic drugs were also excluded.

In this regard, informed consents were obtained from Al-Assad University Hospital Administration, Diabetes Center Administration, and all subjects were given a full explanation of the study.

Samples and biochemical analysis

Overnight fasting samples were collected at 8:00 to 10:00 am on Vacutainer® dry tubes, and directly were centrifuged (4000 rpm) for 5 min, then serum was allocated into four eppendorf tubes (300 µl in every tube); one of them contained 150 µl of 1N hydrochloric acid. All samples were stored at -80°C for less than 4 months.

First, ghrelin concentrations were measured using Linco's Human ghrelin (total) ELISA kit (RIA Kit LINCO Research, St. Charles, Missouri, United States). The assay sensitivity was 30 pg/ml (0.1 fmol/L) when using 20 µl of the sample. The specificity is

very high in the presence of other like components in the sample matrix, and the assay procedures were performed according to the supplier's instructions.

Secondly, insulin concentrations were determined by radioimmunoassay using Linco's Ultra Sensitive Human Insulin Radioimmunoassay (RIA Kit LINCO Research, St. Charles, Missouri, United States). The assay sensitivity was 0.2 µU/ml when using 100 µl of the sample.

Additionally, analysis of glucose, cholesterol, high and low density lipoproteins, and triglyceride were performed on the 911 Hitachi auto analyzer (Hoffmann-La Roche-BM, Germany), using glucose oxidase method for glucose, glycerol 3-phosphate PAP GPO/PAP method for triglyceride, cholesterol oxidase method for total cholesterol, and direct method for high-density lipoprotein HDL, while low density lipoprotein (LDL) was calculated using Friedewald et al.'s (1972) method.

Statistical analysis

SPSS V 10 program (Statistical Package for Social Sciences Version 10) for windows was used for the statistical evaluation of the results. Results were expressed as mean ± SD. Correlations between variables were based on Pearson's correlation coefficient using regression modeling. T student test was performed to determine differences between variables. A p-value of <0.05 was considered as statistically significant.

RESULTS

First, all of the following biochemical parameters: ghrelin, insulin, glucose, TC (total cholesterol), LDL-C (low density lipoprotein-cholesterol), HDL-C (high density lipoprotein-cholesterol), and TG (triglyceride) were titrated in the study groups (Table 1).

Differences among groups according to the biochemical parameters

It was clear that the levels of fasting serum ghrelin were high in control group (16.8 ± 4.6 fmol/ml) compared to obese non-diabetic group (11.3 ± 2.8 fmol/ml) (P = 0.018), and to those in obese diabetic group (9.5 ± 2.5 fmol/ml) (P = 0.010). Consequently, these concentrations were higher in obese non-diabetic group than obese diabetic group (P = 0.031). These differences were found to be statistically significant (Figure 1).

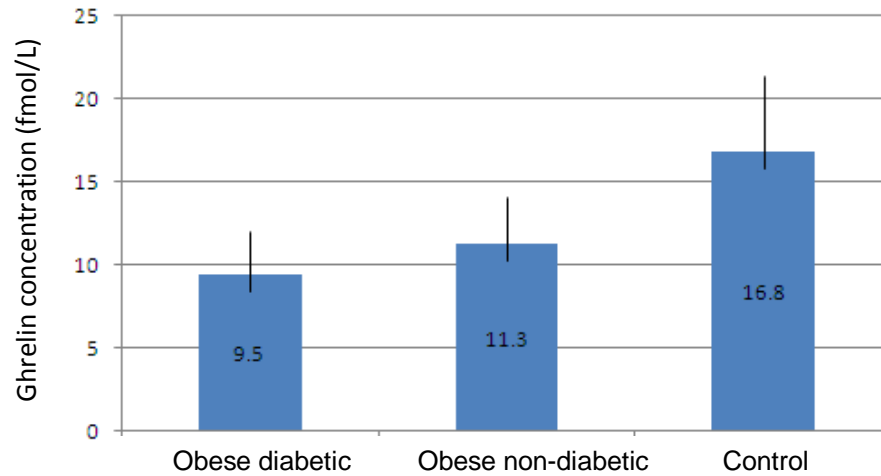


Figure 1. Ghrelin concentrations in the study groups.

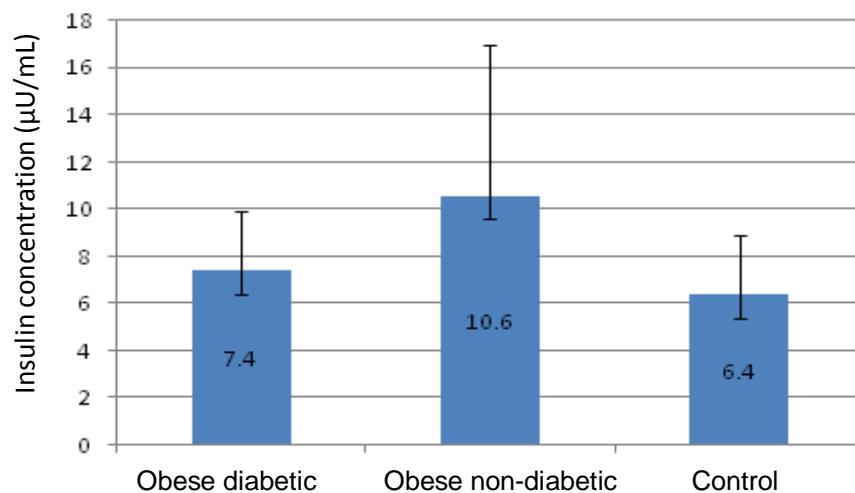


Figure 2. Insulin concentrations in the study groups.

Next, insulin levels in obese non-diabetics (10.6 ± 6.4 µU/ml) were significantly higher than those in obese diabetics (7.4 ± 3.9 µU/ml) ($p = 0.019$), and also in control group (6.4 ± 2.5 µU/ml) ($p = 0.033$). Additionally, a significant difference between obese diabetics and control group was detected ($p = 0.010$) (Figure 2).

With regard to the other biochemical parameters: serum glucose, cholesterol, LDL-C and triglyceride in obese diabetics were significantly higher than in obese non-diabetics ($p < 0.00001$, $p = 0.046$, $P = 0.040$, $P = 0.040$). There were also a significant differences between obese diabetics and control group ($p < 0.00001$, $p = 0.031$, $P = 0.039$, $p = 0.021$). In contrast, we found no significant differences in HDL-c values among the three groups.

Correlation between ghrelin and other parameters

The negative correlation between body mass index and ghrelin values were statistically significant within obese diabetic and obese non-diabetic groups ($r = -0.7$; $r = -0.546$, [$p < 0.05$]) (Figures 3 and 4). In contrast, there were no correlations between ghrelin concentrations and the other assayed values including: glucose, total cholesterol, LDL, HDL and TG.

In control group, a reverse correlation was found to be statistically significant between ghrelin concentrations and the concentrations of each of the following: LDL($r = -0.735$), TG ($r = -0.607$), glucose ($r = -0.630$) (Figures 8, 9 and 10). In contrast, a positive correlation between ghrelin and HDL concentrations was statistically

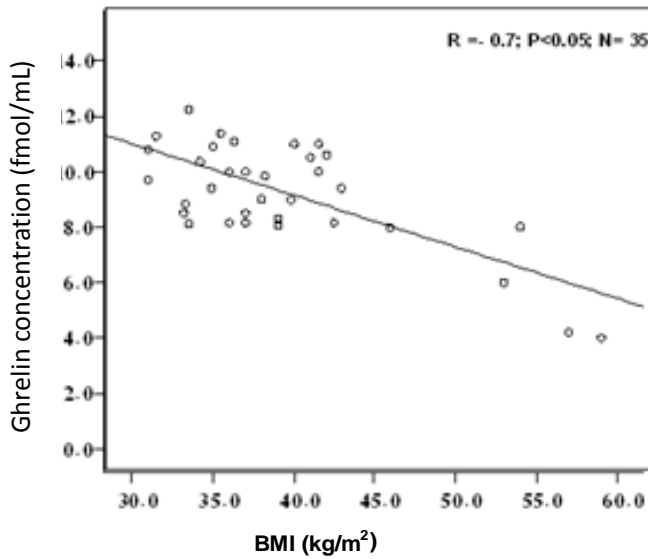


Figure 3. Correlation of ghrelin concentration and body mass index among diabetic obese subjects.

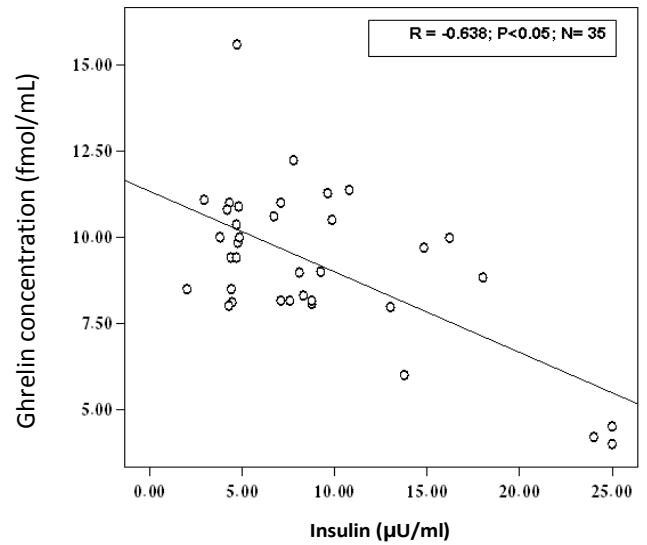


Figure 5. Correlation of ghrelin concentration and insulin concentration among diabetic obese subjects.

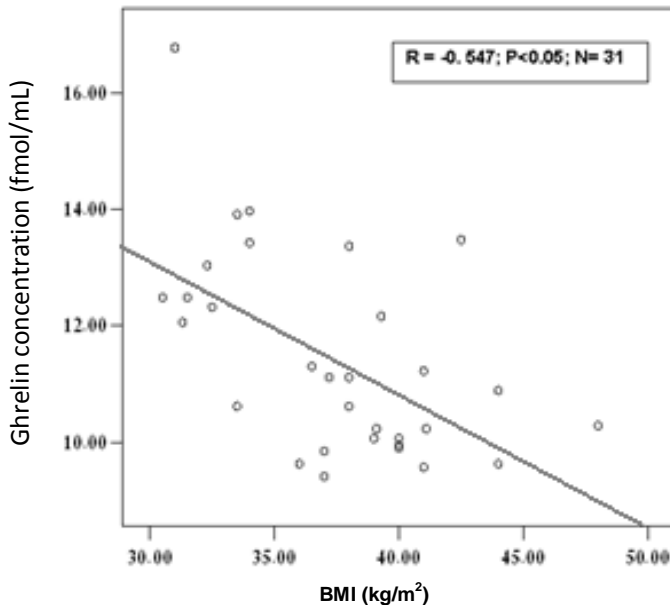


Figure 4. Correlation of ghrelin concentration and body mass index among non diabetic obese subjects.

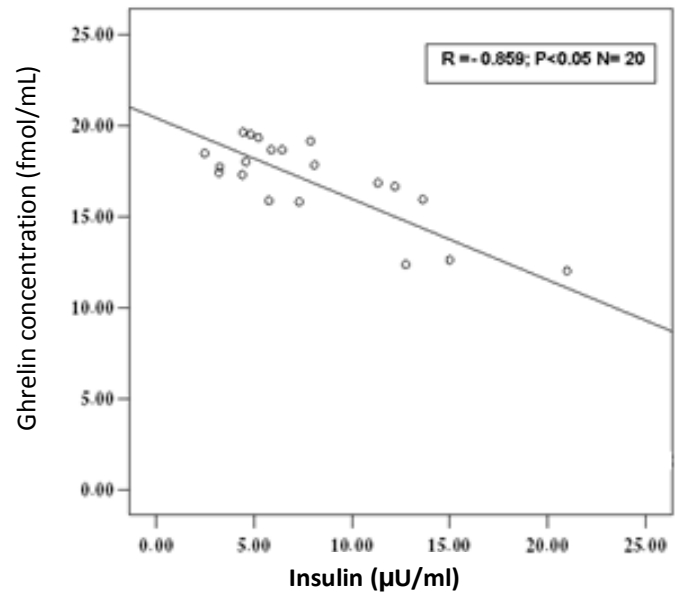


Figure 6. Correlation of ghrelin concentration and insulin concentration among non diabetic obese subjects.

significant ($r = +0.576$) (Figure 11).

We also found a statistically significant negative correlation between ghrelin values and insulin in the three groups ($r = -0.638$; $r = -0.568$; $r = -0.859$ [$p < 0.05$]) (Figures 5, 6 and 7).

However, there were no correlations between the

concentrations of TC (total cholesterol) and BMI, and ghrelin's concentrations in the studied groups.

DISCUSSION

First, our data suggest that diabetes and/or obesity might

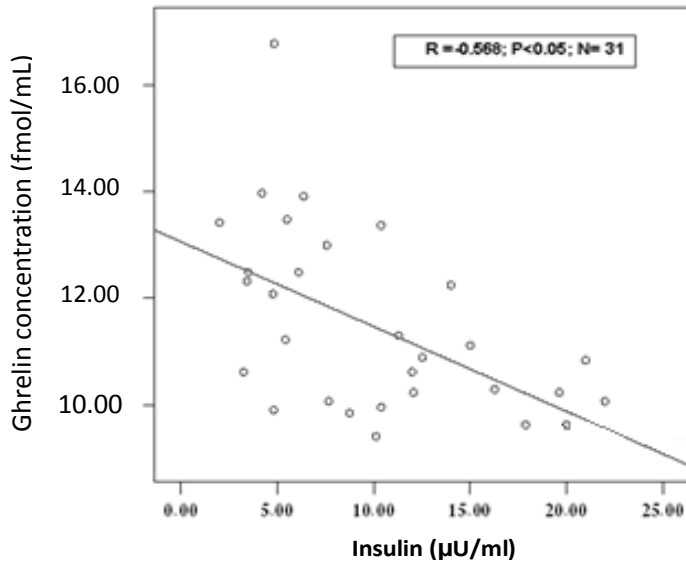


Figure 7. Correlation of ghrelin concentration and insulin concentration among control subjects.

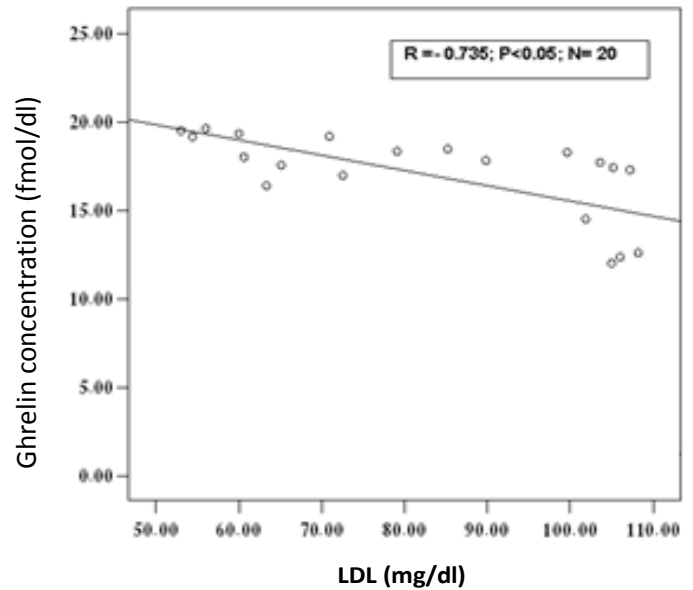


Figure 9. Correlation of ghrelin concentration and LDL concentration among control subjects.

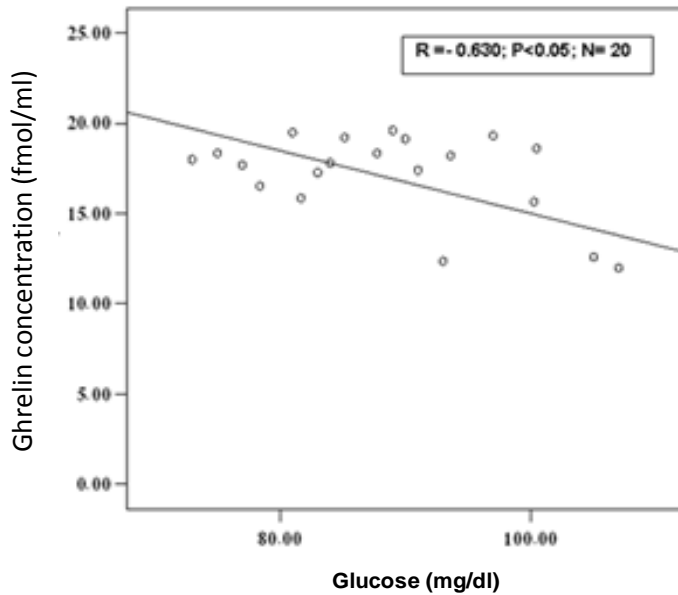


Figure 8. Correlation of ghrelin concentration and glucose concentration among control subjects.

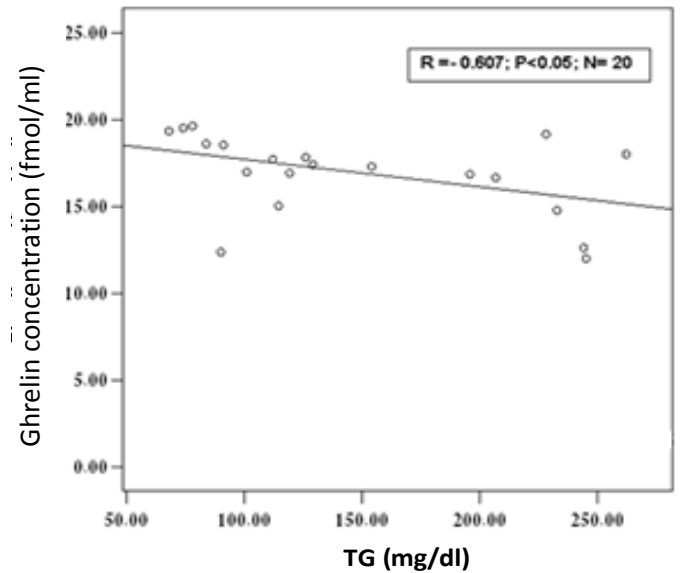


Figure 10. Correlation of ghrelin concentration and TG concentration among control subjects.

decrease the concentrations of ghrelin which could be related to the compensatory mechanism of obesity to control the overweight of the body, by the inhibition of appetite, while in diabetes this compensatory mechanism of the body tries to decrease the overdose of carbohydrates and fats to control the overload of these compounds in blood. This outcome assents many previous

studies (Tschöp et al., 2001; Ravussin et al., 2001; Jürimäe et al., 2009).

Second, the high concentrations of insulin at obese subjects reflect the compensatory action of body to decrease the insulin resistance of the lipid tissue. But these trials have failed at diabetics, because of the limited ability of β cells to produce the appropriate

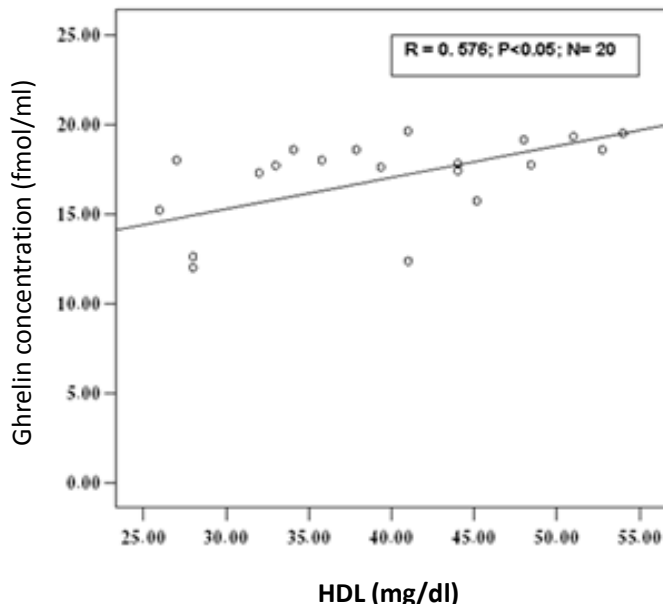


Figure 11. Correlation of ghrelin concentration and HDL concentration among control subjects.

amounts of insulin. As a result, high concentrations of insulin (compared to control subjects) did not give the desired response, so the concentrations of glucose subsequently increased.

Third, high concentrations of each of LDL, TC, and TG, at obese diabetic and obese non diabetic subjects, showed the effect of homeostasis model assessment of insulin resistance resulting in a metabolic syndrome, which became worse in the presence of diabetes. However, diabetes and obesity did not affect the concentrations of HDL.

Furthermore, ghrelin concentrations have correlated negatively to body mass index among diabetic obese and non diabetic obese subjects which emphasizes the controlling role of ghrelin on weight. This correlation was absent among control group according to the limited range of body mass index in this group.

On the other hand, among control group, ghrelin had a negative correlation with LDL and TG, while it had a positive one with HDL. These results stated the role of ghrelin on the metabolism of lipids and lipoproteins, and their distribution in the body. These correlations were absent among the two other groups, which might be related to the different factors that affect the metabolism rates of those parameters according to the high mass of lipid tissues of those subjects.

The most obvious correlation we found was between ghrelin and insulin as there was a negative correlation among the three study groups. These results might indicate the major association of ghrelin with insulin resistance, which disagrees with previous studies

(Adeghate and Ponery, 2002; Lee et al., 2002; Date et al., 2002) and agrees with others (Broglia et al., 2001; Reimer et al., 2003). The differences among the different studies may return to the genetic diversity of the studied groups.

In conclusion, overweight had a negative effect on ghrelin secretion, while ghrelin had a negative association with insulin levels.

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