

## The effect of metformin on ghrelin serum level in type 2 diabetes mellitus

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### ABSTRACT

**Objectives:** Ghrelin is an orexigenic peptide hormone. A great deal of evidence suggests that ghrelin is involved in the development of type 2 diabetes mellitus (DM). The aim of this study was to investigate the effect of metformin on ghrelin serum level in type 2 DM patients.

**Patients and methods:** This case control study was carried out in Al-Wafaa medical center for diabetic and endocrine disorder patients in Mosul from October 2011 to March 2012. In this study 50 type 2 diabetic patients and 20 control healthy subjects was participated. Those patients and subjects was divided into 4 groups. Blood samples had been collected from all subjects and patients and body mass index (BMI) had been calculated for each patient. Fasting blood sugar (FBS) level and ghrelin serum level were estimated for each patient.

**Results:** The study shows a non significant lower mean ghrelin serum level in control diabetic group in comparison to that of control healthy group. There is significant difference in ghrelin serum level between control diabetic patients group and diabetic patients' group using metformin 1000 mg daily at  $P < 0.05$ . Ghrelin serum level has a negative correlation with ages more than 30 years old and BMI in both healthy and diabetic individuals.

**Conclusion:** Ghrelin serum level in diabetic patients is tends to be lower when compared with healthy subjects. Metformin increase ghrelin serum level in diabetic patients and this increment was related to the dose and duration.

**Keywords:** Diabetes, Ghrelin, Metformin.

### الخلاصة:

**الخلفية:** الغريلين هو هرمون ببتيدي فاح للشهية. هناك قدر كبير من الأدلة تشير إلى أن هرمون الجريلين يشارك في تطوير مرض البول السكري من النمط الثاني. والهدف من هذه الدراسة هو دراسة تأثير الميتفورمين على مستوى هرمون الجريلين في المصل لدى مرضى السكري من النمط الثاني.

**المرضى وطرق العمل:** أجريت هذه الدراسة في مركز الوفاء الطبي لمرضى السكري وأمراض الغدد الصم في الموصل من تشرين الأول 2011 إلى آذار 2012. شارك في هذه الدراسة 55 مريض سكري من النمط الثاني و20 شخص سليم. وقد تم تقسيم هؤلاء المرضى والأشخاص إلى أربعة مجموعات. تم سحب عينات الدم من المرضى ثم تم حساب مؤشر كتلة الجسم لكل مريض وتم قياس نسبة السكر في الدم الصيام ومستوى هرمون الجريلين في مصل الدم لكل مريض.

**النتائج:** تظهر الدراسة انخفاض مستوى هرمون الجريلين في مصل الدم في مجموعة مرضى السكري الضابطة بالمقارنة مع مجموعة السيطرة من الأشخاص الأصحاء. هناك اختلاف معنوي كبير في مستوى هرمون الجريلين بين مجموعة مرضى السكري الضابطة و مجموعة مرضى السكري من الذين يستخدمون الميتفورمين 1000 ملغ يوميا في ( $P < 0.05$ ). ان هرمون الجريلين له علاقة سلبية مع العمر لدى الأشخاص ذوي الأعمار أكثر من 30 سنة و علاقة سلبية أيضا مع مؤشر كتلة الجسم في كل من الأفراد الأصحاء ومرضى السكري.

**الخلاصة:** مستوى الغريلين في المصل لدى مرضى السكري يميل إلى أن يكون أقل بالمقارنة مع الأشخاص الأصحاء. الميتفورمين يسبب زيادة في هرمون الجريلين في مرضى السكري و ان هذا الارتفاع في مستوى الجريلين يزداد مع زيادة جرعة الميتفورمين و مدة تعاطيه.

Diabetes mellitus is a group of diseases characterized by an elevated blood glucose level (hyperglycaemia) resulting from defects in insulin secretion, in insulin action, or both. Diabetes mellitus is not a pathogenic entity but a group of etiologically different metabolic defects. Type 2 diabetes mellitus is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifesting. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance.

Metformin is now established as a first-line antidiabetic therapy for the management of type 2 diabetes. Metformin mechanism of actions includes decreased glucose production in the liver, enhanced insulin-stimulated glucose utilization in peripheral tissues (improved insulin sensitivity), particularly skeletal muscle, reduction of intestinal glucose absorption and apparently, increased insulin-stimulated glycogen synthesis (or reduced glycogenolysis). At the cellular level metformin facilitates glucose transport across membranes; apparently by stimulating glucose transporter activity. Metformin may have special benefits in overweight patients with type 2 diabetes. Unlike sulfonylureas, insulin, and thiazolidinediones, metformin does not affect body mass index (BMI) or decreases body weight in obese patients with and without diabetes.

The peptide hormone ghrelin consisting of 28 amino acids including an O-n-octanoylated Ser 3 residue (acylated ghrelin) which has been identified as an endogenous ligand for the growth hormone secretagogue receptor (GHSR). This O-n-octanoylated Ser 3 residue is essential

for ligand-receptor interaction. GHSR<sub>1a</sub> is the only receptor specific for acylated ghrelin that has been identified. Although ghrelin is synthesized in many tissues, including stomach, pancreas, cardiovascular tissue, hypothalamus, pituitary, kidney, and placenta, the main source of plasma ghrelin is X/A-like cells localized in the oxyntic gland in the mucosa of the gastric fundus. Circulating ghrelin concentration has been shown to increase from early adulthood to middle age in humans and subsequently to decrease in old age.

In addition to its GH-stimulating action, many physiological functions of ghrelin have been revealed during the last decade, including the regulation of food intake, energy metabolism, gastrointestinal coordination, cell proliferation, apoptosis, cardiovascular function, and secretion of insulin and gut hormones.

The aim of this study is to investigate the effect of metformin on ghrelin serum level in type 2 DM.

#### Patients and method:

The study was carried out in Al-Wafaa medical center for diabetic and endocrine disorder patients in Mosul from October 2011 to March 2012. In this study 20 type 2 diabetes mellitus patients and 20 control healthy subjects had been participated. Those patients and subjects had been divided into 4 groups:

**1. Control healthy group:** This group includes 20 healthy subjects (10 males and 10 females) who don't have any disease and do not take any medication. Those subjects are chosen from the medical staff of Al-Wafaa medical center for diabetic and endocrine disorders and college of dentistry and matched with other groups their ages ranges between 20

and 70 years old with mean age of  $39.60 \pm 12.33$  years.

**2. Control diabetic patients group:**

This group was containing 10 type 2 DM (8 males and 2 females matched with other groups) who were newly diagnosed (from one to 4 months) and they were not taking any medication yet and they were not suffering from any other diseases in addition to type 2 DM.

**3. Metformin 500 mg group:** This group includes 20 type 2 DM who were administered only 500mg of metformin daily in different durations.

**4. Metformin 1000 mg group:** This group includes 20 type 2 DM who were administered only 1000mg of metformin daily in different durations.

**Inclusion Criteria:**

Type 2 diabetic patients who treated with metformin or newly diagnosed diabetic patients who didn't take any hypoglycemic medication yet.

**Exclusion Criteria:**

1. Patients with type 1 diabetes mellitus.
2. Patients taking insulin or oral hypoglycemic drugs other than glibenclamide, metformin.
3. Patients with renal or hepatic diseases.
4. Patients with myocardial infarction, angina, heart failure or high uncontrolled hypertension.
5. Pregnant and lactating women.

Blood samples had been collected from all subjects participated in this study in fasting condition and allowed to clot at room temperature

**Results:**

Table 1 shows the demographic characteristic of subjects enrolled in this study.

and then serum was separated by centrifugation for 10 minutes at 3000 rpm and the serum was divided into 2 parts of 0.5 ml each, placed in a sterile eppendroff tubes of 1.0 ml size and stored at (-20 °C) in a deep freeze until the time of analysis<sup>11</sup>. BMI had been calculated for each patient according to the equation<sup>12</sup>: **BMI = weight (kg)/height (m<sup>2</sup>)** and then the patients were classified into 4 groups:

**Table 1: NIH/NHLBI classification of BMI<sup>12</sup>**

BMI	Weight category
Less than 18.5	Underweight
18.5 – 24.9	Normal weight
25 – 29.9	Overweight
30 and above	Obese

Fasting blood glucose was estimated by enzymatic method<sup>13</sup> and also ghrelin serum level was estimated for each patient by enzyme linked immunosorbent assay using a technique called a quantitative sandwich immunoassay<sup>14</sup>.

Statistical analyses of data were conducted using SPSS 17 for windows software. A p-value of  $\leq 0.05$  was considered statistically significant. One-way ANOVA and Duncan's test were used to analyze the difference between the tested groups and also to analyze the difference within groups after dividing them into subgroups according to the dose and the duration of the treatment with metformin. Pearson correlations were used to evaluate correlations between parametric variables<sup>15</sup>.

**Table 2: Demographic characteristic of subjects enrolled in this study**

Parameters		Control healthy n=20		Control diabetic patients n=10		Metformin 500 mg n=20		Metformin 1000 mg n=20	
		n	%	n	%	n	%	n	%
sex	Male	10	50%	14	30%	12	60%	9	45%
	Female	10	50%	6	30%	8	40%	11	55%
Age years		44.60 ± 9.87		44.40 ± 11.84		40.90 ± 8.08		48.90 ± 8.33	
BMI kg/m <sup>2</sup>		20.34 ± 2.37		20.80 ± 0.00		20.90 ± 2.00		22.80 ± 4.30	

\* Age and BMI were presented as mean ± SD.

**Table 3: Comparison among parameters in all tested groups:**

Parameters	Control healthy n=20	Control diabetic patients n=10	Metformin 500 mg n=20	Metformin 1000 mg n=20	p-value
FBS mg/dl	96 ± 6 A	200 ± 77 B	183 ± 76 B	182 ± 09 B	0.00
Ghrelin serum level ng/ml	13.08 ± 0.20 AB	8.74 ± 2.22 A	13.73 ± 8.80 AB	17.60 ± 12.81 B	0.207
BMI kg/m <sup>2</sup>	20.34 ± 2.37 A	20.80 ± 0.00 A	20.91 ± 2.00 A	22.80 ± 4.28 A	0.226
Age years	44.60 ± 9.87 A	44.40 ± 11.84 A	40.90 ± 8.08 A	48.90 ± 8.33 A	0.434

\*Different letters horizontally means significant difference at  $P < 0.05$  between tested groups by Duncan's test.

\* FBS= Fasting blood sugar.

\* Age, BMI and ghrelin serum level were presented as mean ± SD.

Table 3 shows significant lower level in FBS in control healthy group when compared with the other three diabetic patients groups with or without metformin therapy at  $P < 0.05$ .

Ghrelin serum level in control diabetic patients is lower when compared to that of control healthy group while it is higher in diabetic

patients' group using metformin 500 mg daily when compare with control diabetic patients group but there is no significant difference. There is significant difference in ghrelin serum level between control diabetic patients group and diabetic patients' group using metformin 1000 mg daily at  $P < 0.05$ .(Table 3)

**Table 4: The effect of metformin duration of usage on ghrelin serum level in diabetic patients groups using metformin**

Parameters	0 - 6 months (1)	6 - 12 months (2)	> 12 months (3)	p-value
FBS mg/dl	169 ± 64	183 ± 64	198 ± 76	0.044
Ghrelin serum level ng/ml	12.68 ± 6.28	17.37 ± 7.66	17.01 ± 9.76	0.703
BMI kg/m <sup>2</sup>	31.82 ± 4.00	31.02 ± 3.93	32.72 ± 4.07	0.062

Table 4 shows that ghrelin serum level is higher in the 2<sup>nd</sup> 6 months and after 12 months of treatment in comparison to the 1<sup>st</sup> 6 months of treatment.

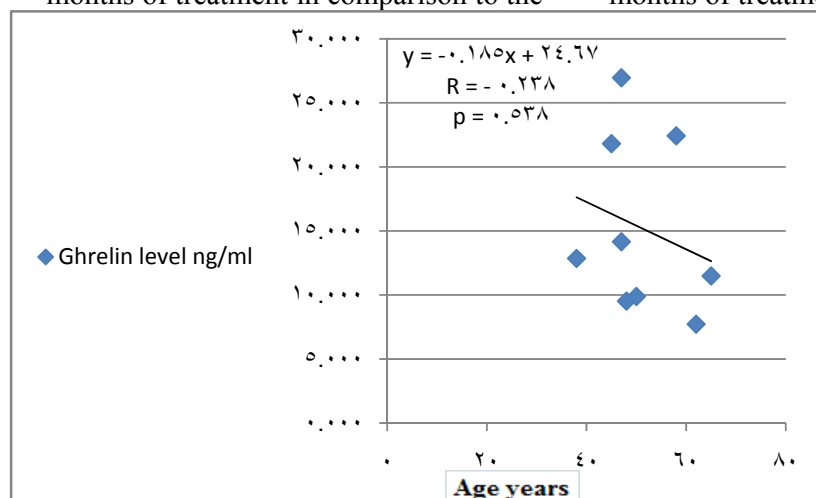


Figure 1: Correlation between ghrelin serum level and age in healthy subjects with ages more than 30 years old.

Figure 1 shows a negative correlation between ghrelin serum level and age in healthy subjects with ages more than 30 years old.

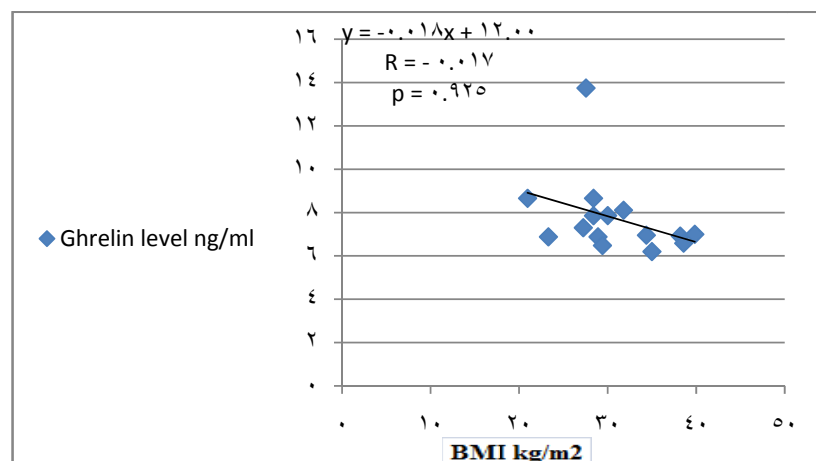


Figure 2: Correlation between ghrelin serum level and BMI in healthy subjects.

Figure 2 shows negative correlation between ghrelin serum level and BMI in healthy subjects.

## Discussion:

Ghrelin stimulates insulin release in the presence of high levels of glucose (8.5 mM) that could independently cause insulin release and increase insulin sensitivity<sup>15</sup>. In contrast, ghrelin had no effect on insulin release in the context of a basal level of glucose (5.5 mM). On the other hand, ghrelin reduces insulin secretion and induces hyperglycaemia in humans in the presence of low levels of glucose<sup>16</sup>. Administration of ghrelin intravenously at low concentrations was shown to decrease glucose induced insulin secretion of isolated pancreatic islets *in vitro*, while supraphysiological ghrelin concentrations had an opposite effect on insulin secretion<sup>17</sup>.

According to this ghrelin is important to achieve a good glycemic control in both hypo and hyperglycemia. In this study ghrelin serum level in diabetic patients is tends to be lower (8.5 ± 2.22 ng/ml) when compared with healthy subjects (13.08 ± 0.20 ng/ml). Also low ghrelin serum level is usually associated with insulin resistance<sup>18</sup>. Leena et al. (2010)<sup>19</sup> indicated that circulating ghrelin concentrations are reduced in healthy offspring of type 2 diabetic patients indicating the presence of possible genetic component in the regulation of ghrelin plasma levels. Pöykkö et al. (2003)<sup>20</sup> showed that fasting plasma concentrations of total ghrelin were lower among subjects with type 2 diabetes compared to those without the disease. The difference remained significant even when age, sex and BMI were taken into account in multivariable models. In addition, Adelino and Joaõ-Bruno, (2007)<sup>21</sup> indicated that low ghrelin levels are associated with insulin resistance, hypertension and the prevalence of type 2 diabetes; polymorphisms of the ghrelin gene are associated with the

risk of diabetes because ghrelin promotes regeneration of  $\beta$ -cells preventing the development of diabetes in disease-prone animals after  $\beta$ -cell destruction. Furthermore, ghrelin is shown to prevent cell death and apoptosis of HIT-T10 pancreatic  $\beta$ -cell line<sup>22</sup>. This low ghrelin serum level could be one of the pathophysiological reasons of DM.

Patients using metformin 500 mg daily in this study have higher ghrelin serum level (13.63 ± 8.80 ng/ml) when compared with diabetic patients group who did not take any medication yet (8.5 ± 2.22 ng/ml) but there is no significant difference while there is significant difference in ghrelin serum level between patients using metformin 1000 mg daily (17.6 ± 12.81) and diabetic patients group who did not take any medication yet (8.5 ± 2.22 ng/ml) as appeared in table 3. According to this; elevation of ghrelin level could be one of the mechanisms by which metformin increase insulin sensitivity and produce a better glycemic control.

Ghrelin serum level tend to be higher in the patients using metformin in the 2nd six months (17.37 ± 7.66) group and in patients using metformin for more than one year (17.01 ± 9.76) when compared with ghrelin serum level in the patients using metformin in the 1st six months (12.68 ± 6.28) as appeared in table 3. So, ghrelin serum level elevating action of metformin increased with time in the 1st year of treatment. Bray et al (2012)<sup>23</sup> mentioned that metformin induced weight loss caused by anorexia is usually happened in the 1st 3 months of treatment while Generali and Cada (2002)<sup>24</sup> mentioned that it is usually happened in the 1st 6 months of treatment. As appeared in table 4

ghrelin serum level tend to be lower in the 1<sup>st</sup> 6 months group than the other 2 groups which could explain the reason behind the less appetite and weight loss that accompanied metformin usage even though it is still higher than that of control disease group so, how metformin can cause weight loss while it increases the hunger hormone (ghrelin). English et al (2007)<sup>71</sup> mentioned that metformin therapy was found to prolong the postprandial fall in total plasma ghrelin levels, and thus had concomitant effects on appetite in type 2 DM and decreasing the number of meals during the day contributing to its actions in promoting weight loss and attenuating weight gain in these patients.

Figure 1 shows a negative correlation between ghrelin serum level and age in healthy subjects with ages more than 30 years old and this is mentioned in Kozakowski J et al (2008)<sup>72</sup>. So, ghrelin tend to decreased with age, therefore; elderly subjects usually have lower ghrelin serum level when compared with young age subjects which could explain why type 2 DM usually appear in older individuals<sup>73</sup>.

Figure 2 shows negative correlation between ghrelin serum level and BMI in healthy subjects so, obese subjects have lower ghrelin serum level than in normal subjects<sup>74</sup> and this could explain the reason behind insulin resistance in obese patients with type 2 DM<sup>75</sup>.

### Conclusion:

Ghrelin is important to achieve a good glycemic control in both hypo and hyper glycemia. Ghrelin serum level in diabetic patients is tends to be lower when compared with healthy subjects. Metformin increase ghrelin serum level in diabetic patients and this could be one of the mechanisms by which metformin increase insulin sensitivity and produce a better

glycemic control. Elevation of ghrelin serum level in diabetic patients by metformin increased with dose and also increased with duration. Higher age and BMI negatively affect ghrelin serum level which in turn could explain the higher prevalence of DM in older subjects with higher BMI.

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