Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Evaluation of Ghrelin, Insulin and Leptin Levels in Obese Type 2 Diabetic Patients on Metformin or Glimepiride Therapy in Basra, Iraq

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Abstract

Background Recent evidence has demonstrated the complex function of adipose tissue and gastric cells as an endocrine organ through release of hormones into the blood stream and involved in physiological activities of the body; of them is ghrelin. The consequences of insulin resistance manifest at many levels and in many metabolic processes, producing a cluster of homeostatic abnormalities referred to what is called metabolic syndrome.

Objectives To evaluate and compare the possible effects of using metformin or glimepiride on serum concentrations of ghrelin, leptin and insulin resistance in obese type 2 diabetic patients in Basra, Iraq.

Methods Forty type 2 diabetic obese patients and twenty healthy subjects were studied. The patients were divided into 2 groups (each of 20 patients); group 1 on glimepiride therapy while group 2 on metformin treatment. Blood samples were taken after at least 8 hours fasting for measurement of serum glucose, leptin, ghrelin and insulin.

Results Ghrelin levels were significantly lower in the two patient groups with greater significant reduction in metformin group. The highest serum insulin concentration and insulin resistance levels were clearly reported in glimepiride treated group as compared to control and metformin treated group. Leptin levels show no significant differences in all studied groups.

Conclusion Metformin treatment associated significantly with improved insulin sensitivity; insulin resistance associated significantly with decreases ghrelin concentration. Ghrelin is negatively correlated with leptin and obesity while positively correlate with insulin resistance. Our data support the role of body weight as the major determinant of circulating leptin levels.

Keywords Diabetes mellitus, obesity, ghrelin, metformin, leptin.

List of abbreviations: DM = diabetes mellitus, T2DM = type 2 diabetes mellitus, IR= insulin resistance, AMPK = adenosine monophosphate-activated protein kinase, ELIZA = enzyme-linked immune sorbent assay, HOMA-IR = homeostasis model assessment-insulin resistance, CNS = central nervous system.

Introduction

Diabetes mellitus (DM) is an important public health problem with an estimated prevalence of 171 million people worldwide in the year 2000, and this number will almost double by the year of 2030 [(1)]. Type 2 DM (T2DM) is a more complex metabolic disorder characterized by obesity, impaired β-cell function, increased endogenous hepatic glucose output and insulin resistance (IR) in target tissues [(2)]. DM and obesity have a complex relationship where obesity may be a precursor for T2DM following IR [(3)]. Obesity is associated with decreased responsiveness to insulin in muscle, liver and fat. On the other hand, weight

gain associated with insulin therapy need to increase insulin dose and subsequently greater weight gain and obesity [4]. Not all subjects with T2DM are obese and many obese subjects do not have DM, but most of the subjects with T2DM are overweight or obese. These are largely preventable with change in life style and avoidance of sedentary habits and over-consumption of energy [5].

Insulin is a small protein hormone, with a molecular weight of about 6000 Daltons composed of two chains held together by disulfide bonds and synthesized in significant quantities only in β-cells in the pancreas. Binding of insulin to extracellular portion of the receptor activates its kinase activity resulting in autophosphorylation of specific intracellular tyrosine residues [6]. IR and a relative deficiency in insulin secretion contribute to the pathogenesis of T2DM [7].

Glimepiride is a potent sulfonylurea and is associated with a low rate of hypoglycemia (0.9 –1.7%). In addition to effects on pancreatic B-cell function, glimepiride also may enhance tissue sensitivity to insulin and has a favorable safety and efficacy profile with once-daily dosing of 1-8 mg/day [8]. The antidiabetic biguanide Metformin is one of the most prescribed, first-line medications in the treatment of T2DM.

In contrast to (sulphonylureas and insulin), metformin does not cause weight gain and can lead to significant weight loss [9]. One of the known targets of metformin action is the intracellular signaling enzyme, adenosine monophosphate-activated protein kinase (AMPK). In the liver and muscle, AMPK activation reduces hepatic gluconeogenesis and promotes fatty acid oxidation, respectively [10]. The consequences of insulin resistance manifest at many levels in metabolic processes, including glucose intolerance, overt hyperglycemia, hyperinsulinemia, and atherogenic dyslipidemia, collectively referred to as metabolic syndrome [11].

The role of visceral or intra-abdominal accumulation of adipose tissue seems to be strongly associated with metabolic syndrome rather than upper body subcutaneous fat [12]. Visceral adipose tissue works as an active endocrine organ able to secrete a wide variety of inflammatory cytokines and hormones with key functions in the development of DM [13].

Recent evidence has demonstrated the complex function of adipose tissue as an endocrine organ through release of hormones into the blood stream involved in physiological activities of the body with potential implication in insulin resistance, obesity and diabetes. One of the most important of these hormones is recently discovered ghrelin, which is a 28 amino acid peptide hormone, primarily produced by the stomach; it has an octanoyl group on the serine at the third position in the amino acid chain which gives the peptide hormone its biological activity [14].

Ghrelin is an appetite-stimulating hormone that increases growth hormone secretion and food intake in animals and humans [15]. The appetite stimulating effects are thought to be mediated via the arcuate nucleus of the hypothalamus and the messenger peptides neuropeptide Y and Agouti-related protein [16].

Leptin, is a single-chain proteohormone with a molecular mass of 16 kDa that is thought to play a key role in the regulation of body weight, it is produced by differentiated adipocytes, although production has been demonstrated in other tissues, such as the fundus of the stomach, skeletal muscle, liver and the placenta [17]. Leptin is a hormone that works as a mediator in the stomach – hypothalamus pathway and provides information about the body's energy storage in adipocytes in addition, its level is associated with obesity [18].

Leptin acts on the central nervous system (CNS), in particular the hypothalamus, suppressing food intake and stimulating energy expenditure [19]. Evidence suggests that circulating ghrelin may work in concert with leptin as an adiposity signal in the CNS [20]. Whether low or high concentration of such hormones is primary event in DM or secondary to anti diabetic drugs is unclear and also its relationship is ambiguous. Therefore, this study was designed to evaluate

and compare the possible effects of using metformin or glimepiride on serum concentrations of ghrelin, leptin and IR in obese T2DM patients in Basra, Iraq and to predict the relationship between above-mentioned parameters in the study groups.

Methods

Forty T2DM obese patients were attended the private medical clinic of Dr. Qais Ali Aljazaari in Basra, Iraq, from Sep. 2013 to Mar. 2014 during their periodic visit seeking for medical advice concerning their diet modification, weight reduction and drug prescription. In addition to 20 healthy subjects with age and sex matched group served as the control group.

We divided the patients into 2 groups (20 patients in each) according to their drug used to treat DM: Group 1 includes T2DM patients on glimepiride therapy while group 2 includes T2DM patients on metformin treatment for at least 4 months in both groups. The inclusion criteria were those with BMI $>$ 30 kg/m^2 and age range between 35-50 years old. The exclusion criteria includes those with BMI $<$ 30 kg/m^2, patients with chronic disease other than DM, pregnant or lactating female patients and any patient with renal or hepatic impairment, and those who are on treatment with drugs, which could interfere with the tested parameters. The study also excluded the patients with any obvious major complications of DM, including heart diseases and patients who were taking other drugs like lipid lowering medications.

Blood samples were taken after at least 8 hours of fasting in all the participants and subjects must refrained from strenuous physical activity for at least 2 hours. Serum glucose was measured by the glucose-peroxidase colorimetric enzymatic assay method. Insulin concentrations were determined by the DRG Insulin ELISA Kit, it is a solid phase ELISA based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the Insulin molecule with range of assay between 1.76-100 µIU/mL.

Regarding leptin levels were measured by using The DRG Leptin ELISA Kit. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule. An aliquot of specimen sample containing endogenous leptin is incubated in the coated well with a specific biotinylated monoclonal anti Leptin antibody.

Serum ghrelin levels were measured by enzyme Immunoassay DRG- kit were designed to detect a specific peptide (ghrelin) based on the principle of "competitive" enzyme immunoassay. The immunoplate in this kit was pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in sample.

IR was assessed using the (HOMA-IR) according to the formula: fasting insulin (µIU/ml) x fasting glucose (mmol/l)/22.5 [21].

Statistical analysis

Values were expressed as mean ± SD; this values were statistically tested using unpaired Student's t-test and one way analysis of variance (ANOVA), supported by Bonferroni's post hoc analysis. Values with $P < 0.05$ considered significantly different. Analysis was performed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

Results

Fasting serum glucose concentrations were significantly increased in both meformin and glimepiride treated groups as compared with control but these concentrations with in upper limit of normal fasting serum glucose range (Fig. 1).

The highest serum insulin concentration and IR levels were clearly reported in glimepiride treated group as compared to control and metformin treated group this result clearly summarized in fig. 2 and 3.

Regarding leptin our finding show no significant differences in all studied groups, Obese T2DM patients on metformin therapy had higher leptin levels compared to obese control, but not significantly so as shown in fig. 4.

Ghrelin levels were significantly lower in obese T2DM patients on both metformin and glimepiride therapies as compared with control group. Ghrelin levels were significantly lower in metformin group as compared to glimepiride treated group (Fig. 5).

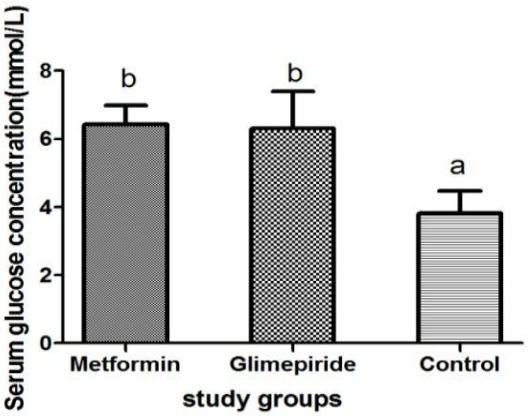

Fig. 1. Serum glucose concentration in obese type 2 diabetic patients on metformin and glimepiride therapy.

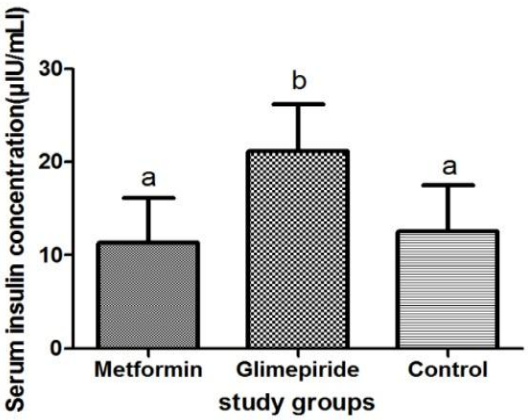

Fig. 2. Concentration of insulin in obese type 2 diabetic patients on metformin and glimepiride therapy.

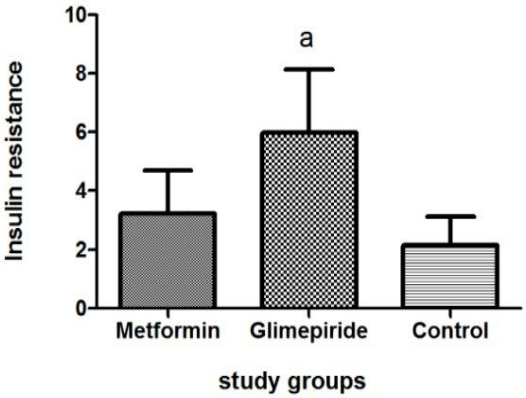

Fig. 3. Insulin resistance values in obese type 2 diabetic patients on metformin and glimepiride therapy.

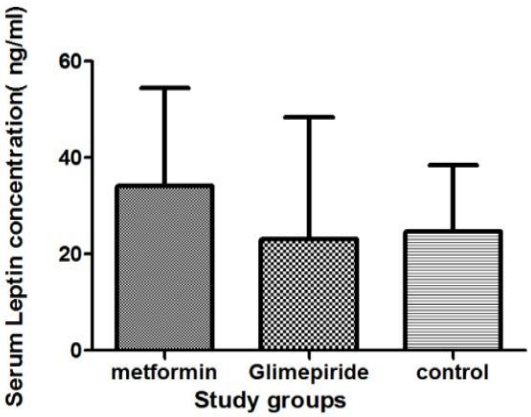

Fig. 4. Concentration of Leptin in obese type 2 diabetic patients on metformin and glimepiride therapy

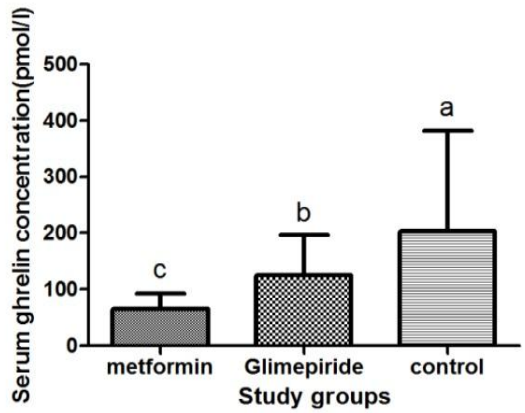

Fig. 5. Concentration of ghrelin in obese type 2 diabetic patients on metformin and glimepiride therapy.

Table 1 demonstrate the relationship between BMI, IR and studied parameters (ghrelin, leptin and insulin) the result showed there were significant correlation between IR and ghrelin, leptin and insulin in metformin treated group while the correlation observed with insulin only in glimepiride and control groups.

Table 1. Correlation between serum ghrelin, leptin and insulin levels and body mass index

Parameter	Group	Pearson Correlation Coefficient (P Value)					
		Serum ghrelin		Serum leptin		Serum insulin	
		r	*P*	*r*	*P*	*r*	*P*
BMI	**Metformin**	-0.2682	0.281	-0.1883	0.4543	-0.2895	0.2439
	glimepiride	-0.05709	0.8220	-0.1352	0.5926	0.1432	0.5709
	Control	-0.4368	0.0699	0.08646	0.7330	0.3434	0.1630
IR	**Metformin**	0.7438	0.0004*	-0.5161	0.0283*	0.9842	< 0.0001**
	Glimepiride	-0.03754	0.8824	-0.002752	0.9914	0.8782	< 0.0001**
	Control	-0.4040	0.0963	0.3080	0.2137	0.9086	< 0.0001**

IR = Insulin resistance, * = $P < 0.05$, ** = $P < 0.0001$.

In all subjects, no correlation observed between BMI and tested parameters was observed. The relationship between ghrelin, leptin and insulin among three groups are summarized in fig. 6-8. Positive correlation was observed between ghrelin and insulin in metformin treated group in other hand our finding showed negative correlation between ghrelin and leptin in control group.

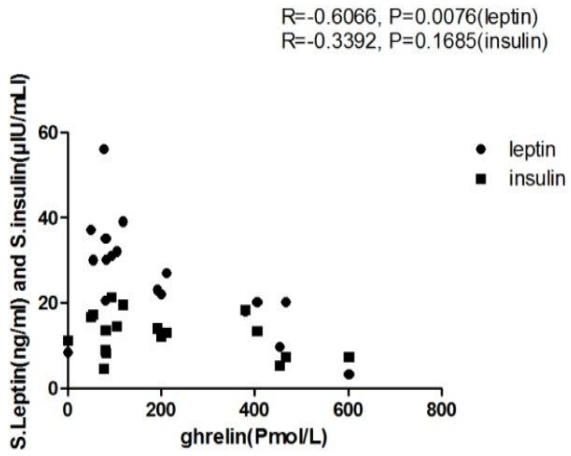

Fig. 6. Relation of ghrelin level to leptin and insulin levels in control group

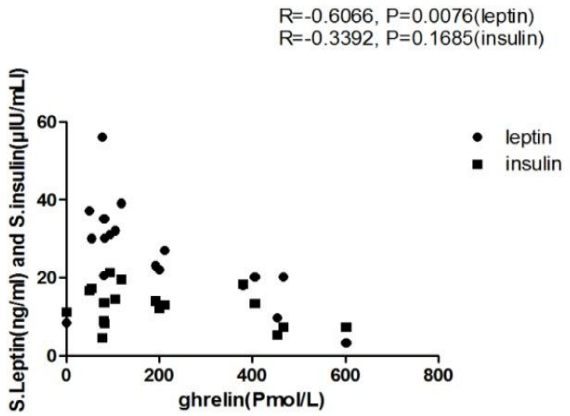

Fig. 7. Relation of ghrelin level to leptin and insulin levels in glimepiride treated group

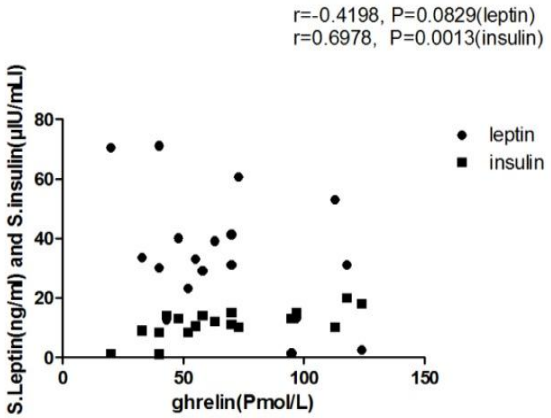

Fig. 8. Relation of ghrelin level to leptin and insulin levels in metformin treated group

Discussion

The present study demonstrated that both metformin and glimepiride treatment have beneficial effects on serum glucose concentrations in T2DM as compared with it is normal range. This result supported by Yoon *et al*. (2011), they found that glimepiride comparable to metformin in treating T2DM patients including those who are not responding well to non-glimepiride sulfonylureas [22]. Insulin resistance, their levels were significantly high in diabetic patients treated with glimepiride as compared with control and metformin treated group. This came in line with those of previous research that support the idea of greater improvement in insulin sensitivity reported in diabetes patients treated with metformin [23].

Metformin appears to have promise in obese diabetic subjects as an effective weight-loss agent with a good safety profile and has the added bonus of reducing the chronic risk factors for heart disease including hyperinsulinaemia and high low density lipoprotein cholesterol levels that are present in obese subjects [24]. In general, different mechanisms support metformin action as antidiabetic drug, the principle action is to reduce hepatic gluconeogenesis by inhibiting hepatocyte mitochondrial respiratory chain oxidation and interfere with mitochondrial energy production [25,26], possibly via an activation of the AMP-activated protein kinase [27].

The present study revealed a significant decrease in serum ghrelin concentration in all obese diabetic patients as compared with control group with significant differences was found between the three studied groups for their mean serum concentrations of ghrelin with lowest concentration observed in metformin treated group. The explanation for such finding seems to be a little bit difficult, since conflicting reports are available regarding the influence of obesity, T2DM and even antidiabetic agents on serum ghrelin concentration. Increase body weight and hyperglycemia may explain these finding. In study of Sharifi *et al*. (2013) concluded that ghrelin concentrations decreases prior to the onset of hyperglycemia and are more related to the fat pad of the body that was came in agreement of our study results [28]. Other study reported an inverse relationship between ghrelin levels, BMI and waist circumference [29]. Furthermore, plasma concentrations of ghrelin inversely associated with food intake (acute effect) and obesity (chronic effect) [30].

According to previous studies, ghrelin is one of the factors that are involved in appetite regulation [31] and acts as an appetite stimulating factor to pass starvation messages to brain. So its reduction in obesity can be considered as a defense mechanism of body to decrease appetite. Another explanation we speculate that reduction of serum ghrelin concentration in metformin treated group due to direct effect of metformin treatment on synthesis and release of ghrelin from the stomach, i.e., lead to greater reduction of serum ghrelin in metformin treated group.

A recent study by Gagnon *et al*. (2013) reported that metformin inhibits stomach proghrelin mRNA production and ghrelin secretion an effect mediated through AMPK phosphorylation [32]. The results of the present study are relatively comparable with this finding and this could explain loss of weight in people treated with metformin. One of the possible pathways by which Metformin inhibits ghrelin secretion is through AKT phosphorylation, as AMPK activation has been shown to increase AKT activity [33].

Whereas the results of this study are consistent with those of Gagnon *et al*. (2013) and another observational study [34], they contrast with the findings of Doogue *et al*. (2008), they conclude that treatment of T2DM with metformin was associated with increased plasma ghrelin concentrations, without associated changes in hunger and satiety [35].

The present study reveals no significant difference between the three study groups for their mean serum concentrations of leptin. This in line of many studies demonstrated no effect of antidiabetic drugs on serum leptin was found [36,37]. However, in other studies a decrease in

leptin levels in metformin-treated individuals has been found [38]. Several researches have been done to analyze the molecular mechanism behind the effect of metformin on leptin levels. An *in-vitro* study reports that metformin inhibits leptin secretion by inhibiting MAPK signaling pathway in adipocytes**.** Possible explanations for these discrepancies in different studies may be the length of treatment, fasting hours, BMI of studies subjects and the study population as in obese people showing a decrease in leptin levels after long-term treatment [39].

The correlation analysis of the present study showed no significant relationship between BMI and studied parameters. IR was positively correlated with serum ghrelin and insulin while negatively correlated with serum leptin with greater r-values in metformin treated group. Actually IR correlates positively with serum insulin concentrations in all study groups. These correlations can be explained, in part by the failure of beta cells to respond to the changes in glucose levels and the inability of insulin receptors to work properly to respond to insulin and this may give an indication to the roles of ghrelin and leptin levels on insulin secretion and sensitivity [40,41]. Furthermore, a significant negative correlation between ghrelin and leptin seen only in control group; this explain the effect of antidiabetic drugs on ghrelin and leptin in the present study [42]. In contrast to this idea study done by Chan *et al* reported no correlation observed between ghrelin and leptin [43].

While the treatment of hyperglycemia, has historically taken center stage in the treatment of DM, therapies directed at other coincident features, such as dyslipidemia, hypertension, hypercoagulability, obesity, ghrelin, leptin and insulin resistance, have also been a major focus of research and therapy.

In conclusion, metformin treatment was associated with significantly improved insulin sensitivity, insulin concentration and insulin resistance using (HOMA-IR) model it also reduces ghrelin concentration significantly as compared to control and glimepiride treated groups. Furthermore, the findings of the present study support that ghrelin is negatively correlated with leptin and obesity while positively correlate with insulin resistance. Since leptin levels were affected neither by metformin nor by glimepiride, our data support the role of body weight as the major determinant of circulating leptin levels.

Acknowledgment

We would like to express our grateful thanks to the Pharmacy Collage, University of Basra for endless support, encouragement, and help in providing the research materials and all facilities will never be forgotten.

Author contribution

All authors contributed extensively to the work presented in this paper according to their order.

Conflict of interest

Nothing declared

Funding

None

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Received 18th Jun. 2014: Accepted 3rd Sep. 2014.