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Circulating leptin and insulin in obese patients with and without type 2 diabetes mellitus: Relation to ghrelin and oxidative stress

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ABSTRACT

Aim: This case control study aimed to investigate relationship between appetite hormones (ghrelin and leptin) and body mass index (BMI), insulin and oxidative stress in simple obese and type 2 diabetes (T2DM) obese patients.

Methods: Thirty healthy controls; 30 simple obese and 30 T2DM obese patients were enrolled. Demographic and clinical data of all participants were reported. Serum levels of fasting blood glucose (FBG), postprandial blood glucose (PBG), lipid peroxide (LPO) and nitric oxide (NO) were measured by chemical methods while, insulin, leptin and ghrelin by ELISA kits.

Results: Serum levels of insulin, leptin, LPO were significantly higher while, ghrelin was significantly lower in simple obese and obese patients with diabetes versus controls. Insulin resistance was found in 76.67% simple obese and 93.33% obese patients with diabetes. Ghrelin showed a positive correlation with PBG in controls; but negative correlation with BMI in simple obese and with NO in obese patients with diabetes. Positive correlations were found between LPO and FBG, insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and between leptin and FBG in obese patients with diabetes.

Conclusions: Our results suggested that hyperinsulinemia and hyperleptinemia may be most important mechanisms in decreasing ghrelin and inducing oxidative stress in simple obese and T2DM obese patients.

1. Introduction

The increasing availability of energy-dense food and the sedentary lifestyle that is becoming prevalent in both first world and developing nations has led to a worldwide epidemic in type 2 diabetes mellitus (T2DM). Diabetes currently afflicts more than 220 million people and this will increase to more than 400 million by 2030 [1]. Obesity is one of the greatest public health challenges of the 21st century with 1.6 billion adults currently classified as being overweight and 400 million as obese [2]. The simultaneous rise in these two diseases has resulted in a new term, 'diabesity' to describe individuals who have obesity and T2DM. These patients are at increased risk of multiple comorbidities (particularly cardiovascular diseases) and, as such, represent a huge economic burden on health

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services around the world. One of the outstanding problems with current therapy for diabetes is that most conventional treatments (e.g. sulfonylureas, thiazolidinediones and insulin) promote weight gain. The notable exception is metformin [3]. Treatment induced weight gain might promote the development of further insulin resistance and aggravate other comorbidities associated with obesity.

The peptides ghrelin and leptin circulate in blood and participate in energy homeostasis, feeding behavior, and regulation of body weight [4]. Ghrelin is a 28-amino acid peptide derived from pre-progrelin. It undergoes post-translational modification in which the serine-3 residue is covalently linked to octanoic acid. This post-translational acylation is unique to ghrelin and is necessary for ghrelin to bind to the growth hormone secretagogue receptor (GHS-R1a) and to cross the blood–brain barrier and seems to have importance for its endocrine actions, but the unacylated form has also been shown to possess metabolic effects [5]. The X/A-like cells of the gastric oxyntic glands of the stomach are the most abundant source of circulating ghrelin. The small intestine also synthesizes ghrelin to a lesser extent with the amount of ghrelin produced diminishing with increasing distance from the pylorus [6]. Circulating ghrelin levels increase with fasting and fall with nutrient ingestion in rodents and humans [7]. In addition to its effects on short term feeding control several lines of evidence suggest a role for ghrelin in the longer-term regulation of body weight and energy homeostasis. Firstly in rodents, chronic administration of ghrelin induces hyperphagia and weight gain [8]. Secondly, mice lacking ghrelin signaling either due to deletion of ghrelin (*Ghrl−/−*) or GHS-R1a (*GHSR-null*) have lean phenotypes and exhibit marked resistance to high-fat diet induced obesity [7]. Thirdly, in humans circulating ghrelin levels are inversely correlated to the degree of adiposity, with low levels in obese subjects and high levels in conditions such as anorexia nervosa, malignancy and cachexia associated to chronic heart failure [9–11]. Studies have shown that ghrelin also modulates insulin and glucose metabolism [12]. Leptin is a 15-kDa hormone secreted mainly by adipocytes, although leptin expression in placenta, fetal tissue, stomach and other tissues has also been observed from the plasma crossing the blood–brain barrier through a saturable transport system and acting on receptors in the lateral and medial regions of the hypothalamus to suppress food intake and stimulate energy expenditure to regulate appetite and energy balance [13]. Studies have demonstrated that leptin has a direct effect on insulin release through effects on β-cell function [12]. Obesity is generally characterized by increased leptin concentration suggesting that obese subjects are leptin resistant through a chronic low-grade pro-inflammatory state. The β-cell may be adversely affected by chronic increased leptin levels eventually leading to diabetes [14].

Ghrelin and leptin exert antagonistic effects via their specific receptors in the central nervous system (CNS) and in peripheral tissues. In hepatocytes, ghrelin reduces and leptin augments insulin signal transduction, resulting in increased and decreased glucose production respectively [15]. In pancreatic β-cells, insulin release was stimulated by ghrelin but inhibited by leptin administration [12]. Ghrelin increases appetite and food intake via centrally mediating actions, while peripherally it modulates the pancreatic β-cell function as well as glucose and lipid metabolism [16]. Leptin acts in an opposite direction to ghrelin to decrease appetite and food intake [4]. Insulin was posited to act indirectly via ghrelin and leptin on the suppression of appetite [17]. However, attenuated suppressive action of insulin on ghrelin and strong association between insulin resistance and leptin resistance were shown in T2DM [17,18].

Oxidative stress is thought to be a major risk factor in the onset and progression of diabetes. Many of the common risk factors, such as obesity, increased age, and unhealthy eating habits, all contribute to an oxidative environment that may alter insulin sensitivity either by increasing insulin resistance or impairing glucose tolerance. The mechanisms by which this occurs are often multifactorial and quite complex, involving many cell signaling pathways. A common result of diabetes is hyperglycemia, which in turn contributes to the progression and maintenance of an overall oxidative environment [19]. In obesity, oxidative stress is now recognized to be an important feature that favors atherosclerosis and other adverse metabolic effects throughout the dysregulation of adipokines and inflammation [20].

The purpose of this case control study was to investigate the relationship between appetite related hormones (ghrelin and leptin) with body mass index (BMI), insulin levels, insulin resistance, and oxidative stress in obese patients with and without type 2 diabetes mellitus.

2. Materials and methods

2.1. *Subjects*

This case control study was conducted at Assiut and Sohag University Hospitals, Assiut and Sohag, Egypt from January to December 2010. The following groups were included: 30 healthy controls [14 men and 16 women; mean age ± SD, 41.27 ± 7.88 yr; body mass index (BMI), mean ± SD, 22.62 ± 1.53 kg/m^2]; 30 patients with simple obesity [13 men and 17 women; 43.23 ± 7.48 yr; BMI, 36.12 ± 1.73 kg/m^2]; and 30 obese patients with T2DM [12 men and 18 women; 43.80 ± 4.64 yr; BMI 35.30 ± 1.78 kg/m^2]. The normal weight control group consisted of subjects who came for a routine checkup and from hospital stuff and patients' relatives. Obese patients with and without diabetes mellitus were recruited from Endocrine and Physiotherapy and Rehabilitation outpatients' clinics of Assiut and Sohag University Hospitals. Diabetes mellitus (fasting plasma glucose ≥ 7.8 mmol/L and/or 2-h post-glucose load plasma glucose ≥ 11.1 mmol/L) was diagnosed according to World Health Organization criteria. All patients with diabetes mellitus were newly discovered and were on no medications for diabetes and all were overweight or obese (BMI ≥ 25 kg/m^2). Excluded from the study were patients with: (1) type I diabetes mellitus, (2) patients with complicated T2DM, (3) clinical or laboratory evidence of other hormonal abnormalities or serious systemic diseases such as acute/chronic inflammations or malignancies, (4) history of hospitalization or ketoacidosis in the preceding 6 months; (5) insulin-treated patients because exogenous insulin might lead to a falsely high plasma insulin concentration that was used in the calculation of the insulin resistance

index; (6) patients who were taking medication that is supposed to influence carbohydrate or lipid metabolisms or oxidative stress or related endocrine functions (e.g., β-blocker, steroids, diuretics, lipid-lowering therapy, vitamins, antioxidants or antihypertensive drugs or antidiabetic drugs). Control subjects had no family history of diabetes mellitus. After informed consent was obtained, all examinations were performed according to the guidelines of the Ethical Committee of Assiut and Sohag University Hospitals, and in accordance to the principles of the Declaration of Helsinki.

Demographic and clinical data of all participants were reported as follow: age, gender, height and weight. BMI was calculated as weight (kg) divided by height squared (m^2). Weight was measured to the nearest 0.10 kg on a calibrated balance beam scale. Height was measured to the nearest 0.50 cm by a tape measure.

2.2. Biochemical measurements

Subjects were asked to overnight fast for 12 h prior to blood draws which were performed in the following morning between 8 and 10 a.m. into plain tubes via venipuncture. The patients received a standard lunch (light balanced diet: 600 kcal, 35% protein, 30% fat, 35% carbohydrates). Two hours after meal, 3 ml blood samples were withdrawn from all participants for estimated of postprandial blood glucose (PBG) level. Blood samples were centrifuged at 1500 × *g* for 10 min and serum was obtained. Samples for further analysis of fasting serum glucose (FBG), PBG, insulin, total ghrelin, leptin, lipid peroxide (LPO) and nitric oxide (NO) concentrations were aliquot and stored at −20 °C. Routine laboratory investigations were done for all participants, e.g., complete blood counts, kidney and liver function tests and fasting lipograms. Radiological examination was also done when indicated. Serum glucose was measured by the glucose oxidase method. The enzyme-linked immunosorbent assay (ELISA) kits were used for determination of serum insulin (Diagnostic Systems Laboratory, Webster, TX, USA) with sensitivity 1.5 μU/ml and coefficient of variations (CVs) of inter-assay and intra-assay 6.29 and 7.67%, respectively; serum leptin (BioVendor Laboratory Medicine, Inc., Modrice, Czech Republic) with sensitivity 0.50 ng/ml and intra-assay and inter-assay CVs 6.70–7.50% and 3.2–9.20%, respectively. Serum total ghrelin levels were measured using human ghrelin (total) ELISA kit that was purchase from Millipore, MS, USA (Cat. # EZGRT-89K), with sensitivity 100 pg/mL, and CVs of inter-assay and intra-assay 1.26 and 7.81%, respectively. Fasting serum ghrelin concentrations were analysed using antibody that recognizes both acylated and des-acylated ghrelin. Although only acylated ghrelin is thought to have endocrine activity, non-endocrine functions have been reported for the non-acylated form of ghrelin [21] and, therefore, the measurement of total ghrelin is reasoned. Another reason for the measurement of total ghrelin concentration is that total ghrelin concentration remains significantly better in all conditions compared to acylated ghrelin concentration. Furthermore, total ghrelin is a good surrogate of acylated ghrelin since they are well correlated, and the ratio of these two remains constant under a wide variety of conditions [6]. The levels of LPO were measured as thiobarbituric acids reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described by Grau et al. [22]. The levels of nitric oxide (NO) were determined as total nitrite after deproteinzation with $ZnSO_4$ (30%), and color developed by reaction with Griess reagent (1% sulfanilamide/0.1% naphthylethylene diamine diHCl (w/v) in 2.5% H_3PO_4) was recorded at 550 nm against reagent blank using sodium nitrite 10–100 μM as standard [23]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 [24]. Patients were considered to have insulin resistance if HOMA-IR ≥2.6 [25].

2.3. Statistical analysis

Statistical Package for the Social Science (SPSS, Chicago, IL, USA) version 12 was used for data analysis. Data are expressed as means ± standard deviation (SD) or number (%) as appropriate. Quantitative data that normally distributed were analysed using one-way analysis of variance [ANOVA] (post hoc test) for parametric variables. Correlations between variables were tested with the Pearson and Spearman tests for parametric and non parametric variables, respectively. For all tests, values of $P < 0.05$ (two-tailed) were considered statistically significant.

3. Results

Table 1 shows the demographic and clinical characteristics of all the studied groups. FBG and PBG were significantly higher in obese patients with diabetes mellitus versus controls and simple obese ($P < 0.0001$ for both). BMI, insulin, insulin resistance, leptin, LPO levels were significantly higher but ghrelin was significantly lower in simple obese and obese patients with diabetes mellitus versus healthy controls ($P < 0.0001$ for all). Insulin resistance (HOMA-IR ≥ 2.6) was found in 76.67% of obese patient and 93.33% of obese patients with diabetes mellitus. Insulin resistance was significantly higher in obese patients with diabetes mellitus versus simple obese ($P < 0.0001$).

In healthy controls, a negative correlation was found between age and ghrelin ($r = -0.362$, $P < 0.049$) but positive correlations were found between BMI and NO ($r = 0.382$, $P < 0.037$), between FBG and insulin and HOMA-IR ($r = 0.403$, $P < 0.027$; $r = 0.635$, $P < 0.0001$); between PBG and insulin, HOMA-IR and ghrelin ($r = 0.403$, $P < 0.027$; $r = 0.635$, $P < 0.0001$; $r = 0.472$, $P < 0.008$) and between insulin and HOMA-IR ($r = 0.961$, $P < 0.0001$) (Table 2).

In simple obese, age was negatively correlated with insulin and HOMA-IR ($r = -0.575$, $P < 0.001$; $r = -0.528$, $P < 0.003$) but positively correlated with NO ($r = 0.433$, $P < 0.017$). BMI was negatively correlated with ghrelin ($r = -0.464$, $P < 0.010$) but positively correlated with LPO ($r = 0.374$, $P < 0.042$). Insulin was positively correlated with HOMA-IR ($r = 0.972$, $P < 0.0001$) (Table 3).

In obese patients with diabetes mellitus, age was positively correlated with PBG ($r = 0.385$, $P < 0.035$) but negatively correlated with NO ($r = -0.579$, $P < 0.001$). BMI was negatively correlated with LPO ($r = -0.368$, $P < 0.045$) but positively correlated with NO ($r = 0.446$, $P < 0.013$). FBG was positively correlated with PBG, insulin, HOMA-IR, leptin, LPO ($r = 0.532$, $P < 0.002$;

Table 1 – Demographic, clinical characteristics and measured biochemical parameters in all the studied groups.

Parameters	Control ($n = 30$)	Simple obese ($n = 30$)	Obese with diabetes mellitus ($n = 30$)
Age (years) (mean ± SD)	41.27 ± 7.88	43.23 ± 7.48	43.80 ± 4.64
Range	27.00–52.00	31.00–61.00	35.00–55.00
Significance		*$P < 0.267$	*$P < 0.154$, **$P < 0.748$
Gender (n, %)			
Male	14 (46.67%)	13 (43.33%)	12 (40.00%)
Female	16 (53.33%)	17 (56.67%)	18 (60.00%)
Body mass index (kg/m^2)	22.62 ± 1.53	36.12 ± 1.73	35.30 ± 1.78
	19.60–25.10	32.40–39.10	32.40–39.70
		*$\mathbf{P < 0.0001}$	*$\mathbf{P < 0.0001}$, **$P < 0.063$
Fasting blood glucose (mg/dl)	5.49 ± 0.42	5.74 ± 0.56	16.74 ± 3.92
	4.39–6.11	4.72–6.94	8.06–22.50
		*$P < 0.675$	*$\mathbf{P < 0.0001}$, **$\mathbf{P < 0.0001}$
Postprandial blood glucose (mg/dl)	5.64 ± 0.75	6.06 ± 0.53	19.89 ± 5.31
	4.50–6.90	4.90–7.30	6.80–27.00
		*$P < 0.603$	*$\mathbf{P < 0.0001}$, **$\mathbf{P < 0.0001}$
Insulin (μIU/ml)	6.13 ± 1.35	13.38 ± 5.61	13.42 ± 4.84
	2.00–7.80	7.10–24.50	5.70–20.00
		*$\mathbf{P < 0.0001}$	*$\mathbf{P < 0.0001}$, **$P < 0.967$
HOMA-IR	1.50 ± 0.37	3.38 ± 1.39	10.455.17
	0.42–1.96	1.76–6.65	2.04–19.80
		*$\mathbf{P < 0.021}$	*$\mathbf{P < 0.0001}$, **$\mathbf{P < 0.0001}$
Patients with insulin resistance	–	23 (76.67%)	28 (93.33%)
Ghrelin (ng/ml)	45.13 ± 12.37	11.97 ± 8.08	11.39 ± 2.16
	22.90–76.10	5.07–42.70	5.80–14.90
		*$\mathbf{P < 0.0001}$	*$P < 0.0001$, **$P < 0.795$
Leptin (ng/ml)	7.08 ± 2.38	23.73 ± 5.21	25.48 ± 3.37
	5.01–14.90	12.80–39.00	17.00–30.50
		*$\mathbf{P < 0.0001}$	*$\mathbf{P < 0.0001}$, **$P < 0.081$
Lipid peroxide (μmol/L)	4.11 ± 0.71	6.37 ± 0.92	6.08 ± 0.80
	2.90–5.90	4.20–8.50	4.20–7.20
		*$\mathbf{P < 0.0001}$	*$\mathbf{P < 0.0001}$, **$P < 0.225$
Nitric oxide (μmol/L)	19.56 ± 2.49	19.86 ± 2.23	19.92 ± 3.87
	13.90–23.10	13.20–23.00	12.90–26.10
		*$P < 0.698$	*$P < 0.641$, **$P < 0.937$

HOMA-IR, homeostasis model assessment of insulin resistance.
* P, significance versus controls.
** P, significance versus simple obese.

Table 2 – Correlations between different measured parameters in healthy controls.

Parameters	Age	Body mass index	Fasting blood glucose	Postprandial blood glucose	Insulin	HOMA-IR	Ghrelin	Leptin	Lipid peroxide
Body mass index (correlation, r)	−0.010								
Significance (P)	0.959								
Fasting blood glucose	0.206	0.066							
	0.275	0.727							
Postprandial blood glucose	−0.217	0.215	0.252						
	0.249	0.254	0.180						
Insulin	−0.196	−0.005	**0.403**	**0.637**					
	0.300	0.980	**0.027**	**0.0001**					
HOMA-IR	−0.118	0.037	**0.635**	**0.630**	**0.961**				
	0.534	0.847	**0.0001**	**0.0001**	**0.0001**				
Ghrelin	**−0.362**	0.176	−0.257	**0.472**	0.274	0.157			
	0.049	0.351	0.170	**0.008**	0.142	0.407			
Leptin	0.046	0.000	−0.090	0.050	0.128	0.075	0.003		
	0.811	0.999	0.636	0.792	0.500	0.692	0.986		
Lipid peroxide	0.141	0.315	0.254	0.111	0.148	0.208	0.064	0.035	
	0.458	0.090	0.176	0.560	0.436	0.271	0.736	0.854	
Nitric oxide	−0.217	0.382	0.031	0.000	−0.009	0.022	0.161	0.077	0.139
	0.249	0.037	0.869	0.998	0.964	0.907	0.395	0.684	0.462

HOMA-IR, homeostasis model assessment of insulin resistance.

Table 3 – Correlations between different measured parameters in simple obese patients.

Parameters	Age	Body mass index	Fasting blood glucose	Postprandial blood glucose	Insulin	HOMA-IR	Ghrelin	Leptin	Lipid peroxide
Body mass index (correlation, r)	−0.233								
Significance (P)	0.216								
Fasting blood glucose	0.227	0.133							
	0.228	0.485							
Postprandial blood glucose	−0.013	−0.052	0.255						
	0.945	0.783	0.174						
Insulin	**−0.575**	0.066	−0.203	−0.012					
	0.001	0.728	0.282	0.951					
HOMA-IR	**−0.528**	0.115	0.018	0.020	**0.972**				
	0.003	0.545	0.925	0.917	**0.0001**				
Ghrelin	0.230	**−0.464**	0.045	0.012	−0.091	−0.077			
	0.222	**0.010**	0.814	0.951	0.633	0.685			
Leptin	−0.0019	0.229	0.043	0.154	0.100	0.126	−0.160		
	0.922	0.223	0.823	0.415	0.600	0.506	0.399		
Lipid peroxide	−0.026	**0.374**	0.151	0.146	0.090	0.119	−0.059	−0.112	
	0.890	**0.042**	0.426	0.440	0.635	0.531	0.757	0.555	
Nitric oxide	**0.433**	−0.044	0.201	0.162	−0.355	−0.318	0.061	0.020	0.129
	0.017	0.818	0.287	0.391	0.054	0.087	0.749	0.915	0.498

HOMA-IR, homeostasis model assessment of insulin resistance.

$r = 0.570$, $P < 0.001$; $r = 0.830$, $P < 0.0001$; $r = 0.368$, $P < 0.046$; $r = 0.418$, $P < 0.022$). PBG was positively correlated with insulin and HOMA-IR ($r = 0.380$, $P < 0.038$; $r = 0.516$, $P < 0.004$). Insulin was positively correlated with HOMA-IR and LPO ($r = 0.909$, $P < 0.0001$; $r = 0.426$, $P < 0.019$). HOMA-IR was positively correlated with LPO ($r = 0.474$, $P < 0.008$). Ghrelin was negatively correlated with NO ($r = -0.520$, $P < 0.003$) (Table 4).

4. Discussion

Ghrelin is the first orexigenic protein to be identified that is derived from the stomach that stimulates appetite. Decreased plasma ghrelin levels may have a protective role against the development of obesity and T2DM. However, scientific evidence that supports this speculation is lacking. In this study, the fasting serum level of ghrelin was significantly decreased while insulin, insulin resistance (HOMA-IR) and leptin levels were significantly increased in simple obese and obese patients with diabetes mellitus compared to healthy controls. One possibility to explain lower ghrelin levels in simple obese and obese patients with diabetes mellitus reported in this study is the elevation of insulin, which is supported by some [26] but not all [27] insulin infusion studies. In vitro in the isolated stomach, insulin is a potent inhibitor of ghrelin secretion [28]. Thus, increased insulin levels in obese

Table 4 – Correlation between different measured parameters in obese patients with diabetes mellitus.

Parameters	Age	Body mass index	Fasting blood glucose	Postprandial blood glucose	Insulin	HOMA-IR	Ghrelin	Leptin	Lipid peroxide
Body mass index (correlation, r)	−0.019								
Significance (P)									
	0.922								
Fasting blood glucose	0.281	−0.114							
	0.133	0.547							
Postprandial blood glucose	**0.385**	0.049	**0.532**						
	0.035	0.797	**0.002**						
Insulin	0.058	−0.025	**0.570**	**0.380**					
	0.762	0.894	**0.001**	**0.038**					
HOMA-IR	0.161	−0.071	**0.830**	**0.516**	**0.909**				
	0.394	0.708	**0.0001**	**0.004**	**0.0001**				
Ghrelin	0.165	−0.227	−0.077	−0.092	0.034	0.011			
	0.385	0.227	0.685	0.628	0.859	0.956			
Leptin	−0.018	0.016	**0.368**	0.117	0.255	0.317	−0.006		
	0.926	0.932	**0.046**	0.539	0.174	0.088	0.974		
Lipid peroxide	−0.225	**−0.368**	**0.418**	−0.039	**0.426**	**0.474**	0.223	0.351	
	0.232	**0.045**	**0.022**	0.836	**0.019**	**0.008**	0.237	0.057	
Nitric oxide	**−0.579**	**0.446**	−0.292	−0.134	−0.265	−0.326	**−0.520**	−0.0003	−0.176
	0.001	**0.013**	0.118	0.480	0.157	0.079	**0.003**	0.990	0.352

HOMA-IR, homeostasis model assessment of insulin resistance.

subjects are one potential pathway for a cross talk between the energy reserve of the organism and the gastric neuroendocrine control system of short-term feeding regulation. A second pathway could be generated by a direct effect of fat cell secretory products on ghrelin secretion. A major candidate in this context is leptin, which is elevated in the serum of obese subjects. An inverse relationship between leptin and ghrelin has been reported [29], which would support a contribution of leptin to obesity related low ghrelin levels. The experimental evidence, however, for such a negative feedback control is still a matter of debate. In mice, intraperitoneal leptin inhibits ghrelin release [30], and in rats, leptin can prevent the rise of ghrelin during moderate food restriction [31]. In line with this observation are the inverse amplitude changes of leptin and ghrelin pulse discharge in rats [4]. Furthermore, in the isolated rat stomach, leptin is a potent inhibitor of ghrelin secretion [32]. Chan et al. [33] reported that the reduction of basal ghrelin levels is not only associated with elevated insulin and leptin but also with higher BMI levels. This as well as age and sex are potential confounding factors. When patients were matched for age, sex, BMI, and also leptin, the difference in insulin was still associated with a significant reduction of ghrelin, whereas subgroups with comparable high insulin levels but progressively increasing BMI and leptin concentrations did not show any alteration of basal ghrelin. This supports the concept that in obese subjects with associated hyperinsulinemia, ghrelin suppression is due to insulin, whereas leptin is of only minor or no importance. Molecular signals that regulate ghrelin secretion are not known. Further investigation is needed to define the receptors, transporters, and/or channels expressed in ghrelin-producing cells.

In this study, ghrelin was positively correlated with postprandial blood glucose but negatively correlated with age in healthy controls. In this respect, it had been reported that ghrelin is secreted in the early phases of eating, acting to increase food intake and to lower energy expenditure in rodent models and to decrease glucose-stimulated insulin secretion [34]. A negative correlation between ghrelin with BMI in simple obese patients was observed in this study. Similarly, Ritland et al. [35] reported negative correlation between ghrelin concentration with body fat and BMI in healthy postmenopausal women. Peterson et al. [36] reported that ghrelin concentrations were inversely correlated to body weight, BMI, and deposition of fat in the waist and hips, as well as to truncal fat and insulin concentrations in Hispanic patients with type 2 diabetes mellitus. In some [34] but not all [37] human studies, fasting plasma ghrelin negatively correlated with BMI. In obese patients with diabetes mellitus, a negative correlation was found between ghrelin and nitric oxide in this study. Since decreased plasma levels of ghrelin and increased oxidative stress have been reported in obese subjects it is not surprising that there is an inverse relationship between them [38].

Insulin resistance, a state of diminished response to insulin, is the key pathological feature of T2DM and is accompanied by compensatory increases in pancreatic β-cell insulin secretion for the maintenance of euglycemia. If left unchecked, insulin resistance culminates in β-cell exhaustion and diminished insulin secretion, resulting in hyperglycemia and the development of overt diabetes [39]. In this study, insulin resistance (HOMA-IR $\geq$ 2.6) was found in 76.67% simple obese and 93.33% obese patients with diabetes mellitus. Fasting leptin concentration in simple obese and type 2 diabetes mellitus patients was significantly elevated than healthy controls in this study. Increased leptin levels, probably reflecting leptin resistance was shown to be strongly related to insulin resistance [18]. Resistance to insulin and leptin may be involved in increasing food intake in type 2 diabetes. In this study, in obese patients with diabetes mellitus, serum leptin was positively correlated with fasting blood glucose. Leptin has to be considered as a tonic regulator of the gastrohypothalamic axis involved in short-term feeding regulation [40]. Leptin regulates appetite control and energy metabolism, is strongly correlated with obesity, and may play a major role in islet cell growth and insulin secretion [41]. Although mechanisms for leptin resistance in obese subjects are not well established, the suppressor of cytokine signaling (SOCS)-3, increased by cytokine activation of the janus kinase (JAK) – signal transducer and activator of transcription (STAT) pathway, appears to play a major role in inflammation and obesity-associated resistance to the actions of both leptin and insulin [42].

In this study, simple obese and obese patients with diabetes mellitus showed significant increased in LPO serum levels meanwhile; NO levels did not show any changes versus healthy controls. There are extensive associations between obesity and T2DM and markers of oxidative stress in humans [43]. In contrary to our results, Gomes et al. [44] have described that adult patients with metabolic syndrome had lower concentrations of markers of NO formation. Elevated levels of LPO have been linked to injurious effects such as loss of fluidity, inactivation of membrane enzymes, and increases in the permeability of ions, which may lead to disruption of cell membrane potential [45]. Peroxidation products can also damage DNA [46]. In this study, a positive correlation was found between serum levels of LPO with insulin and HOMA-IR in obese patients with diabetes mellitus. Strong experimental evidence from various animal models of obesity and T2DM links ROS and insulin resistance in vivo [43]. Hyperglycemia has been hypothesized to contribute to oxidative stress either by the direct generation of ROS or by altering the redox balance. This is thought to occur via several well-studied mechanisms, including increased polyol pathway flux, increased intracellular formation of advanced glycation end products, activation of protein kinase C, or overproduction of superoxide by the mitochondrial electron transport chain, or glycation of antioxidative enzymes [19]. It has also been suggested that leptin might be associated with oxidative stress in patients with impaired glucose tolerance or diabetes [47]. In 1999, Bouloumıe et al. [48] had reported that leptin, at (patho)-physiologically relevant concentrations (1–100 ng/mL), led to ROS generation in cultured human umbilical vein endothelial cells. In that study, leptin triggered the generation of H_2O_2 rather than superoxide. Stimulation of ROS resulted in increased activity of c-jun N-terminal kinase, increased DNA binding activity of two proinflammatory transcription factors, activator protein-1 and nuclear factor kappa B, and overexpression of monocyte chemoattractant protein-1—a chemokine involved in atherogenesis.

5. Limitation of the study

The major limitation of this study is that it includes only newly discovered untreated obese patients with diabetes mellitus. The effect of different antidiabetic drugs on ghrelin levels must be a target of new research which may explain resistance to some of them. The levels of ghrelin in lean patients with diabetes mellitus and in patients with diabetic complications is another limitation of this study and is obviously needed to validate our exciting findings in obese patients with diabetes mellitus.

6. Conclusion

In summary, the present study suggests that hyperinsulinemia and hyperleptinemia may be the most important mechanisms in decreasing circulating total ghrelin and inducing oxidative stress in simple obese and T2DM obese patients. These findings shed new light upon that ghrelin antagonist may be ineffective in reducing weight in obese patients with and without diabetes mellitus with hyperinsulinemia and hyperleptinemia. Drugs that combine carefully balanced activities of gut and adipose tissue hormones will become the 'new wave' of diabesity therapies.

Conflicts of interest

There are no conflicts of interest.

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