British Journal of Medicine & Medical Research
12(2): 1-12, 2016, Article no.BJMMR.21457
ISSN: 2231-0614, NLM ID: 101570965

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Metabolic and Hormonal Changes in Obese Subjects with and Without Diabetic Mellitus

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAH participated in the design of the protocol of the study, recruitment of the patients, coordination of the research, performance of the clinical part, analyses of the data and writing the draft of the paper. Author RFAE participated in the design of the protocol of the study, recruitment of the patients, analyses of the data and writing the draft of the paper. Author TKS performed the laboratory investigations and participated in the analyses of the results. All authors read and approved the manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/21457
Editor(s):
(1) Kate S. Collison, Department of Cell Biology, King Faisal Specialist Hospital & Research Centre, Saudi Arabia.
Reviewers:
(1) Bhaskar Sharma, Suresh Gyan Vihar University, Rajasthan, India.
(2) Jaspinder Kaur, Punjab Institute of Medical Sciences, Jalandhar, Punjab, India.
(3) Mra Aye, Melaka Manipal Medical College, Malaysia.
(4) Samah Mohamed Ahmed Elattar, Cairo University, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/12033

Original Research Article

Received 18th August 2015
Accepted 6th October 2015
Published 30th October 2015

ABSTRACT

Background: Increasing body weight is a risk factor for development of insulin resistance (IR) and type 2 diabetes mellitus (T2DM).
Aim: We aimed to determine the relationship between insulin, C-peptide, leptin, cortisol, growth hormone (GH) and adiposity in obese and subjects with T2DM as data regarding this issue are still controversial.
Study Design: Cross sectional study.
Methodology: this study included 60 patients with T2DM, 60 obese non-diabetics and 30 healthy controls. Anthropometric parameters, glycemic and lipid profiles, insulin, C-peptide, leptin, cortisol and GH were measured.
Results: Serum C-peptide (P=0.025, P=0.030, P=0.021), insulin (P=0.0001 for all) and leptin

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(P=0.001, P=0.02, P=0.0001) were higher in obese (n=22) and non-obese diabetics (n=38) and obese non-diabetics versus controls. Cortisol was higher in obese non-diabetics versus obese (P=0.017) and non-obese (P=0.007) diabetics and controls (P=0.0001). GH was higher in obese non-diabetics versus obese diabetics (P=0.031). IR was reported in obese (72.70%) and non-obese (71.00%) diabetics and obese non-diabetics (38.30%). Central obesity was reported in obese (59.10%) and non-obese (34.20%) diabetics and obese non-diabetics (45.00%). *In obese diabetics,* a positive correlation was reported between leptin with C-peptide (P=0.001). *In non-obese diabetics*, positive correlations were reported between IR and cortisol (P=0.025) and waist/hip ratio (WHR) with insulin (P=0.029) but a negative correlation was reported between glycosylated hemoglobin (HBAIc) and leptin (P=0.047). *In obese non-diabetics*, positive correlations were reported between leptin with HbA1c (P=0.01) and cortisol (P=0.003), WHR with insulin (P=0.0001) and cortisol with leptin (P=0.003).

Conclusion: The association of insulin and leptin resistances and hypercortisolemia with obesity supports the notion that the regulatory defects of blood glucose and obesity are associated with long-term metabolic complications.

Keywords: Type 2 diabetes; obesity; insulin resistance; leptin; cortisol; growth hormone.

1. INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) has increased worldwide not only in developed nations but also in rapidly industrializing countries in the developing world because of the increasing incidence of obesity and lifestyle changes [1,2]. Regardless to the phenotypic heterogeneity among individuals, T2DM is characterized by abnormalities in carbohydrate and fat metabolism, chronic hyperglycemia, insulin resistance (IR) and a relative insulin secretion defect [3].

General and central (abdominal) obesity are observed in the majority of patients with T2DM and are associated with IR at the level of skeletal muscles, liver and adipose tissues [3]. Also Individuals with obesity are at increased risk for developing IR and T2DM [4]. Therefore, obesity and T2DM may share similar metabolic defects involved in their pathogenesis. It is now apparent that there is an overlap among hormones, neuropeptides, and receptors involved in regulation of blood glucose levels and body adiposity [5-8].

Adipose tissue, particularly visceral fat, is an active metabolic organ which releases different adipocytokines as leptin, adiponectin and visfatin [9]. Body fat store is the main determinant of circulating leptin concentration [10]. There are conjoint relationships between leptin and insulin. This is supported by the following findings: a) Leptin has a direct effect on insulin activity and regulation of total body sensitivity to levels of insulin and triglycerides in lipodystrophic syndromes [11], b) Most obese mammals have elevated plasma concentrations of leptin and insulin, and they appear to be resistant to leptin-induced anorexia [12]. c) In presence of genetic defects of insulin secretion, individuals with leptin resistance were found to be strongly predisposed to both obesity and T2DM [13]. d) Recent studies reported that subjects with IR showed higher values of body mass index (BMI), the homeostasis model assessment for IR (HOMA-IR), serum glucose, insulin, leptin, tumor necrosis factor alpha (TNF-α), C-reactive protein and adipocyte area and altered expression of omental adipose tissue genes and genes variants and reduction in the expression of adiponectin [6]. e) Recent studies reported that IR in adipose tissue (Adipo-IR) is positively correlated with leptin and negatively correlated with plasma adiponectin [7]. f) In obesity, there is increase in cellular uptake of non-esterified fatty acids without β-oxidation. This results in accumulation of intermediate lipid metabolites resulting in the insulin signaling pathway defects [8].

Previous studies investigated the hypothalamic-pituitary-adrenal (HPA) axis secretions in patients with T2DM and obesity, however their results are conflicting or variable [14]. Some suggested that the glucocorticoid secretion in diabetes is one of the possible links between IR and manifestations of the metabolic syndrome (hypertension, obesity, coronary heart disease, hyperlipidemia, and T2DM) [15]. Some suggested that the increase in the secretion of cortisol [16] and the decrease in growth hormone (GH) [17] may contribute to the induction of IR in association with visceral fat accumulation. Recently, it has been observed that extreme

underweight and overweight states may activate the HPA axis, and hypercortisolemia may contribute to increased adiposity in the setting of caloric excess [18].

1.1 Aim of Work

The present study was undertaken to evaluate the association between obesity and T2DM with different hormones which mediate and regulate energy and metabolism which include insulin, C-peptide, leptin, cortisol and GH in obese diabetic and non-diabetic subjects. We also assessed the relationships between these hormones and anthropometric and metabolic variables.

2. SUBJECTS AND METHODS

This cross-sectional study included 60 patients with T2DM (age range: 38-55 years), 60 obese non-diabetic subjects (age range: 39-60 years) and 30 healthy control non-obese subjects matched for age (age range: 37-60 years), sex and socioeconomic state. Control subjects were recruited from the general population. Diabetic and obese subjects were recruited from the out-patients clinics of Internal Medicine and Neurology departments of Assiut University Hospital, Assiut, Egypt over a period of 6 months. Excluded from the study were patients with: 1) type I diabetes mellitus (T1DM), 2) disease duration >5 years, 3) clinical or laboratory evidence of other hormonal abnormalities or serious systemic diseases such as acute/chronic inflammations and malignancies, 4) history of hospitalization or ketoacidosis in the preceding 6 months, 5) regular treatment with any medications other than oral hypoglycemic drugs, 6) insulin treated patients because exogenous insulin might falsely lead to high plasma insulin concentration that was used in the calculation of the IR index.

Each participant was subjected to detailed history taking and clinical examination. Demographic and clinical data were collected as follow: age, gender, systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements. Anthropometric parameters included measurement of height, weight, and waist (minimum circumference between the umbilicus and xiphoid process) and hip (maximum circumference around the buttock and symphysis pubis) circumferences. Body mass index (BMI) was calculated using this formula: BMI = weight (kg) / height $(m)^2$. General obesity was defined if BMI was >30 kg/m^2 and central (abdominal) obesity was defined if a waist/hip ratio (WHR) was >0.95 for men or >0.85 for women [19]. All patients were on diet control and/or oral anti-diabetic medications [gliclazide and/or metformin] at baseline.

All subjects gave their oral informed consent and local ethical committee approved the study in accordance with Helsinki Declaration II.

2.1 Biochemical Measurements

No medications were taken on the morning of the study day. Venous blood samples (6 ml) were obtained via venipuncture at 8:00-10:00 a.m. after an overnight fast for at least 12 hours. 2 ml was separated in a vaccutainer tube for preparation of hemolysate which was kept frozen for estimation of HbA_1c, a clinical indicator of blood glucose control. All participants underwent routine laboratory investigations which included: complete blood count (CBC), kidney and liver function tests and lipogram. The remaining 4 ml were immediately centrifuged at 2500 rpm at 4ºC for 15 minutes and the obtained serum was divided into 3 aliquots: one was utilized for immediate assessment of CBC, fasting blood glucose (FBG), kidney and liver functions and lipogram, while the other two were stored at -70℃ for latter assessment of serum leptin, insulin, C-peptide, cortisol and GH. Each participant received a standard lunch (light balanced diet: 600 kcal, 35% protein, 30% fat and 35% carbohydrates). Two hours after meal, 3 ml blood samples were withdrawn for estimation of post-prandial serum glucose (PBG) level. HbA1c was measured by Hitachi 911 autoanalyzer [Hitachi Co. Ltd., Tokyo, Japan]. HbA1c determination is based on turbidimetric inhibition immunoassay for hemolyzed whole blood. HbA1c values were recorded for the previous 12-month period from each participant's clinic record and then averaged. Normal values of HbA1c according to Heap et al. [20] ranged from 4.0-6.0%. Poor metabolic control was considered if HbA1c was >8.0% [20]. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C)] were measured by Hitachi 911 autoanalyzer (Hitachi Co. Ltd., Tokyo, Japan). Insulin was determined using an enzyme-linked immunosorbent assay (ELISA) (Diagnostic Systems Laboratory, Webster, TX. USA). Serum leptin was measured by ELISA (DRG Instruments GmbH, Germany) The intra- and inter-assay coefficients of variation were 8.8% and 12% respectively. C-peptide and GH

were measured by ELISA (GenWay Biotech, San Diego, CA, USA). The intra- and inter-assay coefficients of variation for GH were 3.8% and 5% respectively. Total cortisol was measured with an enzyme immunoassay (EIA; IBL, Hamburg, Germany). IR was calculated using HOMA-IR equation formula as follow: HOMA-IR = Fasting insulin (uU/mL) multiplied by fasting glucose (mmol/L) divided by 22.5. Patients were considered having IR if HOMA-IR was ≥2.6 [21].

2.2 Statistical Analysis

All data were statistically processed with the SPSS program, version 16.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± SD (standard deviation). Quantitative data that were normally distributed were analyzed using one-way analysis of variance test (ANOVA). Mann-Whitney *U*-test was used to compare the mean values between groups that were not normally distributed. Correlations between variables were tested with the Pearson's test and Spearman's correlation coefficient for parametric and non parametric parameters respectively. For all tests, values of $P<0.05$ (two-tailed) were considered statistically significant.

3. RESULTS

Tables 1 and 2 showed the demographic, clinical and laboratory characteristics of the studied groups. Diabetic patients were divided according to their BMI into obese diabetics (BMI ≥30 kg/m^2; n=22) and non-obese diabetics (BMI <30 kg/m^2; n=38). They showed that in obese diabetics, central obesity and poor glycemic control were frequent. TG concentration was significantly higher in obese diabetics compared to non-obese diabetics and obese non-diabetics.

Table 3 showed the hormonal results of the studied groups. Compared to controls, serum leptin, insulin and C-peptide were significantly higher in obese diabetics, non-obese diabetics and obese non-diabetics. Cortisol was significantly higher in obese non-diabetics. Although concentrations of GH were not significantly differed in the studied groups versus controls but obese non-diabetics had significant higher levels compared to obese diabetics.

Tables 4-7 showed the correlations between measured parameters in obese diabetics, non-diabetics and obese non-diabetics. *In obese diabetics,* positive correlations were reported between HBAIc with BMI (P=0.008) and leptin with C-peptide (P=0.001). *In non-obese diabetics*, positive correlations were reported between IR with cortisol (P=0.025) and WHR with insulin (P=0.029) but a negative correlation was reported between HBAIc and leptin (P=0.047). *In obese non-diabetics*, positive correlations were reported between leptin with HbA1c (P=0.01) and cortisol (P=0.003), WHR with insulin (P=0.02) and IR (P=0.045) and cortisol with leptin (P=0.003). *In normal individuals,* positive correlations were reported between HbA1c with cortisol and GH (P=0.01 for both) and WHR with cortisol (P=0.01).

4. DISCUSSION

In this study, obese non-diabetic as well as diabetic patients (both obese and non-obese) showed higher serum levels insulin and proinsulin connecting peptide (C-peptide), IR and significant positive correlations between WHR (a marker of central or abdominal obesity) and concentrations of insulin and C-peptide particularly in non-obese diabetics. Many investigators use C-peptide levels as a biomarker of endogenous production of insulin by pancreatic β-cells [22]. IR was observed in more than one third of obese non-diabetic subjects. It has been reported that in experimental studies, IR increases when a non-diabetic individual consumes excessive calories and gains weight and this is followed by increased insulin secretion to offset IR [23]. In animal studies, it was reported that chronic overfeeding is associated with hypersecretion of insulin [24].

In this study, obese non-diabetics as well as diabetic patients (both obese and non-obese) showed higher serum levels of leptin. Obese diabetics had higher levels of leptin than obese non-diabetics. Leptin, a hormonal product of lipocytes, is responsible for regulation of body fat [25]. Earlier reports for patients with T2DM showed that circulating leptin was unchanged [26], reduced [27] or increased [28]. Even, others reported lower leptin levels in obese diabetics than obese non-diabetic subjects [29]. Leptin levels were found to be positively correlated to body fat and adipocyte size. Hyperleptinemia in obese diabetics might be due to the stimulatory effect of insulin on adipose tissues resulting in over expression of obese gene and more leptin secretion [30]. Some studies suggest that IR could raise plasma leptin levels [31]. However, hyperleptinemia and hyperinsulinemia might be due to the effect of oral hypoglycemic drugs (as sulphonyl urea and glibenclamide). These drugs

are reported to increase the circadian leptin and insulin concentrations [32]. Hyperleptinemia with obesity imply a state of leptin resistance which may result from a defect in leptin receptors [29], decrease in leptin transport through the blood-brain barrier [33], reduction in signaling distal to the leptin receptor or as a functional leptin defect or due to lesions in the hypothalamus [34]. We also reported a positive correlation between leptin and C-peptide in obese diabetics. It is known that basal insulin secretion is strongly associated with circulating leptin concentrations [28], and insulin plays a crucial role in the maintenance of circulating leptin levels [35]. Kellerer and co-workers [29] have assessed the effects of insulin concentration on the leptin signaling pathway in rat-1 and HEK293 cells. Their results suggest that the insulin receptor signaling pathway interferes with leptin signaling at the level of JAK-2, indicating that hyperinsulinaemia contributes to the pathogenesis of leptin resistance. Although the exact molecular mechanisms for the relationship between insulin and leptin are not fully understood, however, it is clear that obesity constitutes a major risk for the development of IR which predisposes pancreatic-β cell failure and clinical diabetes mellitus.

Table 1. Demographic and clinical characteristics of the studied groups

Variables	Diabetics (n=60)		Obese non-diabetics (n = 60)	Controls (n = 30)
	Obese diabetics (n =22)	Non-obese diabetics (n =38)		
Age; years	43.00-55.00	38.00-55.00	39.00-60.00	37.00-60.00
	47.73±3.04	47.53±3.39	47.08±4.60	46.30±4.92
P1	0.227	0.233	0.818	
P2	0.118	0.099		
P3	0.858			
Gender (male/female)	2/20	6/32	6/54	10/20
Disease duration; years	0.50-4.00	0.08-5.00		
	2.05±1.08	2.08±1.31		
P1	-	-		
P2	-	-		
P3	0.368	-		
Blood pressure; mmHg				
Systolic blood pressure	120.00-150.00	110.00-150.00	110.00-150.00	110.0-130.00
	127.72±8.69	126.32±9.13	127.33±8.41	119.33±5.53
P1	**0.045**	**0.043**	**0.045**	
P2	0.847	0.549		
P3	0.520	-		
Diastolic blood pressure	80.00-90.00	70.00-90.00	70.00-100.00	70.00-80.00
	83.64±4.92	81.84±5.50	84.60±6.23	78.33±3.30
P1	**0.01**	**0.01**	**0.001**	
P2	0.474	**0.034**	**-**	
P3	0.216	**-**	**-**	
Body mass index; kg/m^2	30.10-37.50	22.20-29.80	34.10-49.50	22.20-25.30
	33.15±1.96	26.53±1.70	41.37±3.24	24.32±0.78
P1	**0.0001**	**0.068**	**0.0001**	
P2	**0.0001**	**0.0001**	**-**	
P3	**0.0001**	**-**	**-**	
General obesity	22 (100%)	-	60 (100%)	-
Waist/ hip ratio	0.78-0.95	0.71-1.12	0.08-0.96	0.82-0.91
	0.88±0.05	0.86±0.08	0.85±0.11	0.76±0.03
P1	**0.01**	**0.01**	**0.01**	
P2	0.116	0.628	-	
P3	0.276	-	-	
Central obesity	13 (59.10%)	13 (34.20%)	27 (45.00%)	-

Data are expressed as range, mean ± SD (standard deviation) and number (%)

P1: Significance versus control; P2: Significance versus obese non-diabetics; P3: Significance versus non-obese diabetics

Table 2. Laboratory characteristics of the studied groups

Variables	Diabetics (n=60)		Obese non-diabetics (n = 60)	Controls (n = 30)
	Obese diabetics (n =22)	Non-obese diabetics (n =38)		
HbA1c (%)	6.40-12.90	5.90-10.90	4.20-6.10	4.00-5.70
	9.34±2.40	8.11±1.22	5.09±0.44	4.86±0.48
P1	**0.0001**	**0.0001**	0.367	
P2	**0.0001**	**0.0001**	-	
P3	**0.01**	-	-	
Poor metabolic control	14 (63.6%)	13 (34.2%)	-	-
FBG; mmol/L	6.80-15.80	4.50-15.20	3.10-5.90	3.10-5.40
	11.14±3.36	9.35±2.55	4.56±0.67	4.30±0.71
P1	**0.0001**	**0.0001**	0.538	
P2	**0.0001**	**0.0001**	-	
P3	**0.01**	-	-	
PBG; mmol/L	9.30-22.50	7.00-16.00	4.90-7.30	4.50-6.30
	15.15±4.97	12.69±2.39	5.84±0.57	5.37±0.59
P1	**0.0001**	**0.0001**	0.355	
P2	**0.0001**	**0.0001**	-	
P3	**0.01**	-	-	
TC; mg/dl	137.00-272.00	71.00-272.00	115.00-262.00	124.00-195.00
	185.14±42.49	186.89±48.09	170.53±38.32	168.13±27.16
P1	0.130	0.056	0.788	
P2	**0.043**	**0.040**	-	
P3	0.869	-	-	
TG; mg/dl	70.00-488.00	75.00-282.00	49.00-253.00	69.00-152.00
	182.18±94.93	144.18±56.49	131.73±59.25	123.20±30.93
P1	**0.001**	0.161	0.532	
P2	**0.001**	0.326	-	
P3	**0.01**	-	-	
HDL-C; mg/dl	23.00-81.00	11.00-83.00	27.00-65.00	35.00-48.00
	46.68±18.70	46.87±14.55	43.87±10.47	43.87±4.50
P1	0.418	0.321	1.000	
P2	0.362	0.243		
P3	0.955	-		
LDL-C; mg/dl	47.00-156.00	36.00-189.00	49.00-166.00	49.00-128.00
	101.27±32.82	105.58±36.95	100.82±32.44	92.07±31.12
P1	0.328	0.100	0.244	
P2	0.493	0.493	-	
P3	0.632	-	-	

Data are expressed as range, mean ± SD (standard deviation) and number (%)
HbA1c, glycosylated hemoglobin; FBS, fasting blood sugar; PBG, post-prandial glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol
P1: Significance versus control; P2: Significance versus obese non-diabetics; P3: Significance versus non-obese diabetics

In this study, we reported a positive correlation between leptin and HA1c in obese non-diabetics but a negative correlation between leptin and HbA1c in non-obese diabetics. This negative correlation can be explained by improvement in glycemic control which may cause reduction of plasma leptin. It has been reported that glucosamine is one of the key factors that upregulate the obese gene expression [36], and that diabetes mellitus itself is associated with increased glucosamine accumulation. This may suggest that blood glucose is involved in decreasing the circulating leptin levels by energy restriction in non-obese diabetics but leptin resistance in obese non-diabetics.

We reported normal cortisol levels in diabetics (both non-obese and obese) [37]. In contrast, several previous studies showed that type 2 diabetic patients have increased HPA axis activity measured as basal adrenocorticotrophic hormone (ACTH) levels [14] and basal cortisol levels or cortisol levels after dexamethasone suppression [38,39] and between salivary cortisol levels and levels of fasting, postprandial, and urinary glucose as well as with HbA1c [40]. However, we reported significant high serum cortisol levels and a positive correlation between cortisol and leptin in obese non-diabetics. The observed increase in serum cortisol in obese subjects may be explained by the fact that

cortisol has a potent effect on lipid metabolism. It stimulates hepatic triglyceride synthesis, increases the number of adipocytes in the visceral depots and stimulates appetite and hence obesity [41]. It also induces IR probably by antagonizing the anti-lipolytic effect of insulin or increasing triglyceride production [42]. It is known that glucocorticoids have orexigenic and adipogenic effects or counter-regulatory effects against insulin such as gluconeogenesis and impaired glucose intake, thus are considered important in body weight regulation and pathogenesis of obesity. It has been suggested that increased cortisol secretion, altered cortisol metabolism and increased tissue sensitivity to cortisol might link IR, hypertension and obesity [43]. In support: 1) Glucocorticoids induce obese gene expression in rat adipose tissue both in vivo and in vitro [44], 2) Glucocorticoids increase leptin synthesis and secretion in human adipose tissue both in vivo and in vitro [45], 3) Glucocorticoids may also interfere with leptin's interaction with its receptor and produce central leptin resistance [45], 4) Recent studies indicate that stress induces increased food intake only when stress is followed by a neuroendocrine reaction with increased cortisol and leptin concentrations [46]. 5) Increased adipose tissue in obesity is associated with hypothalamic-pituitary-adrenal axis overactivation, increased cortisol production at the local tissue level, and probably higher mineralocorticoid receptor activation in certain tissues [47]. 6) Clinical-overt and experimental cortisol excess has been found to be associated with profound metabolic disturbances of intermediate metabolism resulting in abdominal obesity, IR, and low HDL-cholesterol levels, which can lead to diabetes. Thus it was therefore suggested that subtle abnormalities in cortisol secretion and action are one of the missing links between insulin resistance and other features of the metabolic syndrome [48].

Table 3. Hormonal results of the studied groups

Variables	Diabetics (n=60)		Obese non-diabetics (n = 60)	Controls (n = 30)
	Obese diabetics (n =22)	Non-obese diabetics (n =38)		
C-peptide; ng/ml	2.00-19.30	2.00-35.10	1.00-31.00	2.00-7.20
	10.81±6.07	14.17±10.83	11.79±6.95	5.56±1.93
P1	**0.025**	**0.030**	**0.021**	
P2	0.599	0.125	**-**	
P3	0.094	-	**-**	
Insulin; µIU/ml	2.00-19.30	2.00-35.10	1.00-31.00	2.00-7.20
	10.81±6.07	14.17±5.83	11.79±6.95	5.56±1.93
P1	**0.0001**	**0.0001**	**0.0001**	
P2	0.599	0.125	**-**	
P3	0.094	-	**-**	
Leptin; ng/ml	3.50-112.30	1.00-106.50	1.00-119.50	1.00-22.10
	35.53±29.10	25.32±27.53	42.78±36.59	11.49±0.62
P1	**0.001**	**0.020**	**0.0001**	
P2	0.324	**0.005**	**-**	
P3	0.196	-	**-**	
IR	16 (72.70%)	27 (71.10%)	23 (38.30%)	-
Cortisol; µg/ml	2.70-34.40	4.70-37.00	6.80-50.00	6.40-30.00
	14.23±7.09	14.52±6.14	19.12±9.96	11.77±7.02
P1	0.285	0.170	**0.0001**	
P2	**0.017**	**0.007**	**-**	
P3	0.895	-	**-**	
GH; ng/ml	0.05-3.50	0.05-5.80	0.04-8.20	0.06-4.70
	0.85±0.83	1.18±1.44	1.81±2.32	1.26±1.31
P1	0.500	0.084	0.168	
P2	**0.031**	0.837	-	
P3	0.500	-	-	

Data are expressed as range, mean ± SD (standard deviation) and number (%)
IR, insulin resistance; GH, growth hormone
P1: Significance versus control; P2: Significance versus obese non-diabetics; P3: Significance versus non-obese diabetics

Table 4. Correlations between measured parameters in obese diabetic patients

Variables	BMI	Duration of disease	HBAIc	HOMA-IR	WHR	Leptin	Insulin	C-peptide	Cortisol
Age	0.250								
	0.086								
Duration of disease	0.100								
	0.658								
HBAIc	**0.547**	0.387							
	0.008	0.075							
HOMA-IR	0.259	0.082	**0.647**						
	0.245	0.717	**0.001**						
WHR	0.218	0.256	0.123	0.288					
	0.329	0.250	0.585	0.194					
Leptin	0.132	0.260	0.139	0.178	0.080				
	0.559	0.242	0.538	0.428	0.722				
Insulin	0.003	0.204	0.233	**0.855**	0.359	0.166			
	0.989	0.363	0.297	**0.0001**	0.101	0.460			
C-peptide	0.030	0.214	0.212	0.125	0.180	**0.613**	0.097		
	0.896	0.338	0.342	0.581	0.423	**0.001**	0.666		
Cortisol	0.069	0.168	0.236	-0.032	-0.094	0.044	-0.170	0.115	
	0.759	0.454	0.291	0.888	0.677	0.847	0.450	0.610	
GH	-0.019	0.050	0.003	0.060	0.150	0.144	-0.028	-0.185	-0.033
	0.933	0.825	0.989	0.792	0.505	0.521	0.903	0.409	0.886

BMI, Body Mass Index; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment equation; IR, insulin resistance; WHR, waist/hip ratio; GH, growth hormone

Table 5. Correlations between measured parameters in non-obese diabetic patients

Variables	BMI	Duration of disease	HBAIc	HOMA-IR	WHR	Leptin	Insulin	C-peptide	Cortisol
Age	0.157								
	0.312								
Duration of disease	0.056								
	0.998								
HBAIc	**0.372**	0.130							
	0.021	0.435							
HOMA-IR	0.254	-0.128							
	0.124	0.443							
WHR	0.137	-0.193	-0.080						
	0.413	0.245	0.632						
Leptin	0.102	-0.032	**-0.324**	0.054	-0.153				
	0.544	0.848	**0.047**	0.749	0.360				
Insulin	0.167	-0.273	0.105	**0.914**	**0.355**	0.234			
	0.315	0.097	0.532	**0.0001**	**0.029**	0.157			
C-peptide	0.035	**0.323**	-0.042	**0.605**	0.120	0.140	**0.712**		
	0.835	**0.048**	0.801	**0.001**	0.472	0.401	**0.001**		
Cortisol	0.193	0.193	0.341	**0.364**	-0.153	-0.199	0.197	0.208	
	0.246	0.245	0.036	**0.025**	0.359	0.231	0.235	0.210	
GH	-0.050	0.037	0.001	0.023	0.134	-0.085	0.023	-0.073	-0.018
	0.766	0.825	0.996	0.891	0.422	0.610	0.890	0.663	0.915

BMI, Body Mass Index; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment equation; IR, insulin resistance; WHR, waist/hip ratio; GH, growth hormone

We reported higher serum GH in obese non-diabetics versus obese diabetics. In contrast, GH deficiency in adults is associated with elevated BMI, increase WHR and fat mass and others reported reduced GH secretion [49], presence of excess fuels including glucose and lipid intermediates [50], hyperinsulinemia [51], and hypercortisolemia [52] with obesity. Lee et al. [53] reported lower GH and higher cortisol serum concentrations in young T2DM versus control subjects. Kjeldsen et al. [54] reported reduced basal GH concentrations in T2DM. Lewitt et al. [55] reported that in obesity, hyperinsulinemia that accompanies peripheral IR leads to reduced GH secretion. A recent finding that tissue specific deletion of GH receptors results in hepatic

steatosis due to GH role in triglycerides export from the liver, so when GH receptors are deleted in the liver, hepatic steatosis results and these effects may explain the findings in obesity [56]. The conflicting results could be explained by the fact that the secretion of GH and cortisol follows a well-defined circadian rhythm [57]. GH secretion is periodic and occurs in a pulsatile fashion where plasma cortisol has a peak in the early day [58]. Hence, ideally, mean 24-h hormone concentrations, based on measurements taken at different intervals, should be used. However, such an assessment is labor intensive, expensive and difficult to perform in a relatively large sample size like that in the present study. Despite the inherent limitations of a single blood measurement, Lee et al. [53] were able to demonstrate the intimate relationships between GH, cortisol, and central obesity in young type 2 diabetic patients despite the inherent limitations of a single blood measurement. One would therefore expect stronger associations if multiple measurements in longer adequately powered studies has been involved.

Table 6. Correlations between measured parameters in obese non-diabetic subjects

Variables	BMI	HBAIc	HOMA-IR	WHR	Leptin	Insulin	C-peptide	Cortisol
Age	0.158							
	0.207							
HBAIc	0.014							
	0.916							
HOMA-IR	0.074	0.182						
	0.573	0.164						
WHR	0.132	0.007	**0.310**					
	0.314	0.956	**0.045**					
Leptin	0.169	**0.448**	0.073	0.084				
	0.197	**0.01**	0.581	0.525				
Insulin	-0.062	**0.271**	**0.971**	**0.299**	0.097			
	0.639	**0.036**	**0.0001**	**0.020**	0.461			
C-peptide	0.160	0.236	**0.461**	0.204	0.149	**0.480**		
	0.223	0.069	**0.01**	0.118	0.256	**0.01**		
Cortisol	0.047	0.073	0.189	0.048	**0.378**	0.209	-0.047	
	0.721	0.581	0.149	0.713	**0.003**	0.109	0.720	
GH	0.029	0.113	-0.035	0.018	-0.018	0.004	0.109	-0.232
	0.829	0.389	0.793	0.894	0.894	0.978	0.407	0.075

BMI, Body Mass Index; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment equation: insulin resistance; GH, growth hormone

Table 7. Correlations between measured parameters in healthy controls

Variables	BMI	HBAIc	HOMA-IR	WHR	Leptin	Insulin	C-peptide	Cortisol
Age	0.180							
	0.443							
HBAIc	0.249							
	0.184							
HOMA-IR	0.199	0.080						
	0.291	0.674						
WHR	0.060	0.147	0.153					
	0.753	0.437	0.419					
Leptin	0.088	0.252	0.135	0.155				
	0.643	0.180	0.477	0.412				
Insulin	0.313	0.052	**0.900**	0.268	-0.009			
	0.092	0.786	**0.0001**	0.152	0.962			
C-peptide	0.214	**0.456**	0.223	0.165	0.018	-0.201		
	0.256	**0.01**	0.236	0.382	0.924	0.286		
Cortisol	0.064	**0.549**	0.120	**0.450**	0.351	0.274	0.205	
	0.737	**0.01**	0.529	**0.01**	0.057	0.143	0.278	
GH	-0.137	**0.506**	-0.045	0.224	-0.251	0.076	0.344	0.219
	0.469	**0.01**	0.813	0.234	0.181	0.692	0.063	0.244

BMI, Body Mass Index; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment equation; IR, insulin resistance; WHR, waist/hip ratio; GH, growth hormone

Some limitations of this study need to be considered, the most important limitation is the cross-sectional design of this study. Cross-sectional analyses provide information at a single point in time, however progressive and reciprocal mechanisms are likely to be involved in the association between leptin, insulin, GH and cortisol in obesity and T2DM patients. Thus serial analysis of cortisol and GH are needed to evaluate their changes in different times of the day.

5. CONCLUSION

In this cross-sectional study, both obese and non-obese T2DM patients had high levels of insulin and leptin and high frequencies of IR. There were intimate relationships between GH, cortisol, insulin, leptin and IR in obese diabetics and non-diabetics. This article provides additional evidence about the importance of obesity in T2DM. We postulate that maladaptive hormonal responses to rapid changes in lifestyle characterized by over nutrition and physical inactivity may lead to obesity and T2DM. Further clinical and experimental studies are required to test these hypotheses. Understanding the underlying mechanisms of IR and the transition from normal glucose tolerance to T2DM in individuals at risk will help to develop new therapeutic strategies for the treatment and eventually prevention of T2DM.

ACKNOWLEDGEMENT

This work received no financial or institutional support.

COMPETING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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