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Serum Leptin and Adiponectin in Obese Diabetic and Non-Diabetic

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Adiponectin is an adipocyte-secreted protein that circulates in high concentrations in the serum and acts to increase insulin sensitivity. Leptin is an adipocyte-derived hormone that acts to reduce food intake and increase energy expenditure by binding and activating its specific receptor in the hypothalamus. Clinical aspects of diabetes and obesity are somewhat different, even at similar levels of insulin resistance. The purpose of this study was carried out to determine serum leptin, serum adeponectin and to compare leptin to adeponectin ratio in diabetic and non-diabetic obese participants. One hundred patients were enrolled in the study, 40 type 2 diabetic obese, 40 obese persons and 20 non-obese volunteers as a control group. Body mass index and waist-to-hip ratio was calculated. Laboratory investigations were: alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, Creatinine, fasting and post-prandial glucose levels, glycated haemoglobin (HbA1c), cholesterol, triglycerides (TGs), HDL-cholesterol, LDL-cholesterol, leptin and adiponectin. Subjects were categorized into 3 groups, Group I: included 40 obese (mean BMI was 33.6±2.0 kg m^{-2}) patients with type II diabetes. The mean of HbA1c was 6.9±1.5%; serum TGs was 141.6±93 mg %; serum creatinine was 0.86±0.15 mg %; serum leptin was 12.9±0.35 ng mL^{-1}; serum adiponectin was 6.44±0.16 μg mL^{-1}. Group II: included a total number of 40 obese (mean BMI was 34.8±1.2 kg m^{-2}) participants The mean of HbA1c was 5.6±2.7%; serum TGs was 115±54 mg %; serum creatinine was 0.95±0.2 mg %; serum leptin was 8.7±0.5 ng mL^{-1}; serum adiponectin was 7.84±0.25 μg mL^{-1}. Group III: included a total number of 20 non-obese volunteers (age ranged from 28 to 58 with a mean of 44.9±14.5 years; 13 males and 7 females). The mean of HbA1c was 5.2±0.5%; serum TGs was 104±22 mg %; serum creatinine was 0.84±0.5 mg %; serum leptin was 5.2±0.2 ng mL^{-1}; serum adiponectin was 9.2±0.3 μg mL^{-1}. Present results showed that obesity, WHR and DM are inversely associated with adiponectin, directly associated with leptin and leptin/adiponectin ratio.

Key words: Leptin, adiponectin, body mass index, waist to hip ratio

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INTRODUCTION

The association between excess adipose tissue and the development of type 2 diabetes, dyslipidaemia and cardiovascular disease has long been recognized. Aiponectin is the most abundant serum adipokine and it is secreted exclusively by the adipose tissue (Kim *et al*., 2006). Circulating basal adiponectin concentrations are decreased in obesity, type 2 diabetes and insulin resistance. The degree of hypoadiponectinemia is more closely related to the degree of insulin resistance and hyperinsulinemia than to the degree of adiposity. Low baseline adiponectin levels predict the development of subsequent diabetes in humans (Wasim *et al*., 2006) and this reduction is suggested to play a role in the pathogenesis of cardiovascular disease associated with obesity. It is suggested that adiponectin is a potent insulin enhancer linking adipose tissue and whole-body glucose metabolism.

Adiponectin has been implicated in regulating energy homeostasis, functioning in combination with leptin (Matsuzawa, 2005). Insulin resistance in murine models lacking adipose tissue was shown to be completely reversed by a combination of physiological doses of adiponectin and leptin but only partially by either adiponectin or leptin alone (Silha *et al*., 2006). In diabetic and nondiabetic subjects, weight loss resulted in decreased plasma adiponectin concentrations and increased leptin levels (Hotta *et al*., 2000).

In this study, we sought to determine the relationship between serum adeponectin, serum leptin, diabetes mellitus and obesity in Egyptian subjects.

MATERIALS AND METHODS

A total number of 100 participants were enrolled in this study from May 2005 to July 2006, 40 type II diabetic obese patients, 40 obese persons and 20 non-obese individuals were used as a control group. All subjects were nonsmokers and had no evidence of cancer, liver, renal or hematological disease, or other medical disorders. Diabetic obese patients and obese persons were selected from the Endocrinology Department in Ain-Shams University Hospital.

The exclusion criteria was: Type I diabetes, elevated serum creatinine, patients with any inflammatory diseases and women on oral contraceptives.

Clinical assessment: This included height, weight, body mass index, waist and hip circumference. Body Mass Index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Obesity was defined as BMI$\geq$25 kg m^{-2} in males and $\geq$22 kg m^{-2} in females. Waist-to-hip ratio (WHR) was calculated as waist circumference at the level of the umbilicus in centimeters divided by the hip circumference at the maximal girth around the buttocks in centimeters (Lohman *et al*., 1988).

Laboratory measurements and analytical procedures: Blood samples were collected in heparinized syringes and placed in prechilled test tubes containing 1.5 mg EDTA/ml of blood and aprotonin (400 KIU mL^{-1}) in a total volume that was 4% of the sample volume. The blood samples were centrifuged at 4°C and a 1 mL aliquot of plasma was rapidly frozen (80°C) for subsequent hormone analysis. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA), (Bergman, 1989).

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), were measured by the enzyme rate method of (Henry *et al*., 1960). Blood urea was determined by using commercially available kit from Randox, Laboratories Ltd, USA (Fawcett and Scotto, 1960) and serum creatinine by Jaff reaction (Husdan and Rapoport, 1968), glycated haemoglobin (HbA1c) by an automated high performance liquid chromatography (Davis *et al*., 1978), total cholesterol was determined by Trinders reaction (Allain *et al*., 1974), HDL-cholesterol was determined using phosphotungestic acid precipitation (Lopes-Virella *et al*., 1977), LDL-cholesterol was determined by (Steinberg, 1981) and Triglycerides (TGs) by using enzymatic hydrolysis of glycerol (Fossati and Prencipe, 1982). Serum leptin and adiponectin were measured using the radio-immunoassay (RIA) commercially available kit by Linco Research (St. Charles MO-63304-USA) (Zhongmin, 1996).

Statistical analysis: All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) statistical program. For comparing categorical data, Chi square test was performed. Yates correction was used instead when the frequency is less than 10. Correlation between various variables was done using Pearson moment correlation coefficient (r). A probability value (p value) less than 0.05 was considered significant.

RESULTS

Patients and control were divided into the following groups (Table 1);

Group I: Included a total number of 40 obese (mean BMI was 33.6±2.0 kg m^{-2}) patients (age ranged from 31-63 years with a mean of 55±8.5 years; 26 males and

Table 1: Comparative study between all groups as regarding demographic and laboratory parameters

Parameters	Group I Obese DM	Group II Obese	Group III Control	p1	p2	p3
Males/Females	26/14	22/18	13/7	<0.5 NS	<0.44 NS	<0.5 NS
Age (years)	55.00±8.50	52.70±8.50	44.90±14.5	<0.45 NS	<0.32 NS	<0.41 NS
BMI (kg m^{-2})	33.60±2.00	34.80±1.20	19.50±0.90	<0.35 NS	<0.001 HS	<0.001 HS
HbA1c (%)	6.90±1.50	5.60±2.70	5.20±0.50	<0.02 S	<0.0001 HS	<0.5 NS
Cholesterol (mg %)	217.00±79.0	190.00±73.0	180.00±45.0	<0.2 NS	<0.35 NS	<0.4 NS
TGs (mg %)	141.60±93.0	115.00±54.0	104.00±22.0	<0.3 NS	<0.2 NS	<0.3 NS
Creatinine (mg %)	0.86±0.15	0.95±0.20	0.84±0.50	<0.2 NS	<0.25 NS	<0.2 NS
Leptin (ng mL^{-1})	12.90±0.35	8.70±0.50	5.20±0.20	<0.001 HS	<0.0001 HS	<0.002 S
Adiponectin (μg mL^{-1})	6.44±0.16	7.84±0.25	9.20±0.30	<0.003 S	<0.001 HS	<0.002 S
Leptin/Adiponectin	2.81±0.17	1.58±0.08	0.79±0.08	<0.001 HS	<0.0001 HS	<0.003 S

p1: Group I vs Group II; p2: Group I vs Group III; p3: Group II vs Group III. S: Significant; NS: Non-Significant and HS: Highly Significant

14 females) with type II diabetes. The mean duration of diabetes was 10±6.2 years. Nineteen patients were treated with insulin (47.5%), with a mean insulin dose of 45±19.5 units/day. The mean of HbA1c was 6.9±1.5%; serum TGs was 141.6±93 mg %; serum creatinine was 0.86±0.15 mg %; serum leptin was 12.9±0.35 ng mL^{-1}; serum adiponectin was 6.44±0.16 μg mL^{-1}.

Group II: Included a total number of 40 obese (mean BMI was 34.8±1.2 kg m^{-2}) persons (age ranged from 36-67 with a mean of 52.7±8.5 years; 22 males and 18 females). The mean of HbA1c was 5.6±2.7%; serum Tgs was 115±54 mg %; serum creatinine was 0.95±0.2 mg %; serum leptin was 8.7±0.5 ng mL^{-1}; serum adiponectin was 7.84±0.25 μg mL^{-1}.

Group III: Included a total number of 20 non-obese (mean BMI was 19.5±0.9 kg m^{-2}) volunteers (age ranged from 28-58 with a mean of 44.9±14.5 years; 13 males and 7 females). The mean of HbA1c was 5.2±0.5%; serum TGs was 104±22 mg %; serum creatinine was 0.84±0.5 mg %; serum leptin was 5.2±0.2 ng mL^{-1}; serum adiponectin was 9.2±0.3 μg mL^{-1}.

All groups were matched as regarding the gender and the age. The BMI was significantly higher in group I (diabetic Obese) and II (non-diabetic Obese) than group III (control); whereas there was no significant difference between groups I and II. The HbA1c was significantly higher in group I in comparison to group II and III.

The serum leptin was significantly higher in groups I and II than group III. A highly significant difference was observed in group I in comparison to groups II and III. The serum adiponectin was significantly lower in groups I and II than group III, a highly significant difference was observed in group I in comparison to group III. The leptin to adiponectin ratio was significantly higher in groups I and II than group III, the difference was highly significant in group I (Fig. 1).

There was no significant difference between all groups as regarding serum cholesterol, TGs and serum creatinine.

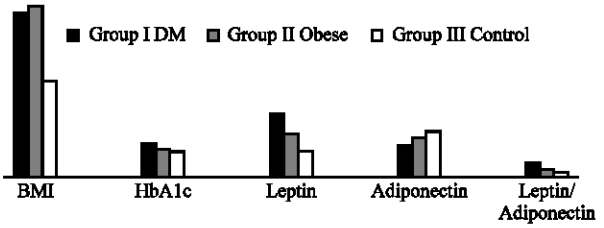

Fig. 1: Comparative study between all groups as regarding BMI and laboratory parameters

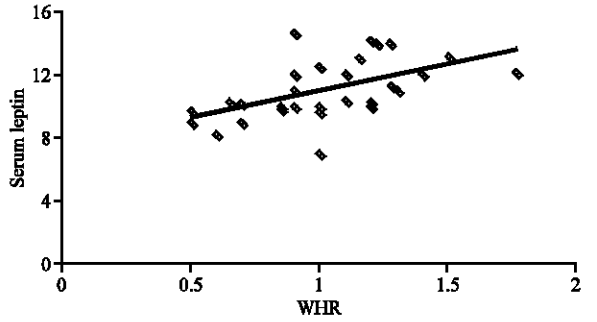

Fig. 2: Comparative study between waist to hip ratio and serum leptin in obese diabetic and non-diabetic participants

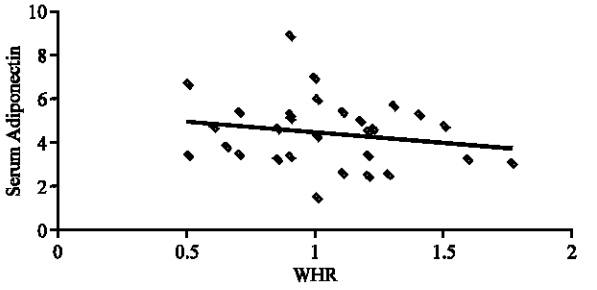

Fig. 3: Comparative study between waist to hip ratio and serum adiponectin in obese diabetic and non-diabetic participants

In the obese diabetic and non-diabetic participants (Groups I and II), there was a significant positive correlation between the waist to hip ratio (WHR) and

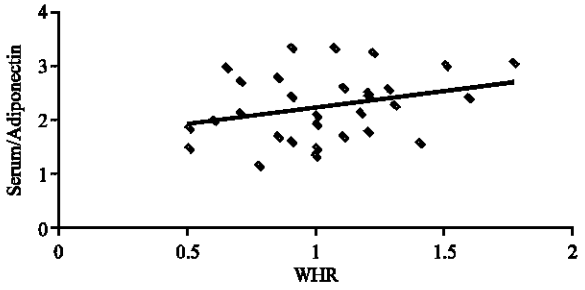

Fig. 4: Comparative study between waist to hip ratio and leptin to adiponectin ratio in obese diabetic and non-diabetic participants

serum leptin; $p<0.002$ $r = 0.4$. Also, a significant negative correlation was observed between WHR and serum adiponectin; $p<0.003$ $r = -0.33$. Consequently, the leptin to adiponectin ratio has a significant positive correlation with WHR; $p<0.0035$ $r = 0.35$ (Fig. 2, 3 and 4).

DISCUSSION

Serum leptin concentrations reflect closely the amount of fat stored in adipose tissue and leptin plays an important role in energy homeostasis and metabolism by acting either directly on specific target tissues or indirectly through leptin-induced alterations of neuroendocrine function (Matsuzawa, 2005). Adiponectin and resistin are another two identified adipokines that have also been suggested to play a role in regulating energy homeostasis and insulin resistance (Wasim *et al*., 2006).

Adiponectin-Leptin ratio was more efficacious as a parameter of insulin resistance than adeponectin or leptin alone and more sensitive and reliable marker of insulin resistance (Inoue *et al*., 2006).

Subjects with type 2 diabetes and impaired glucose tolerance test showed significantly decreased serum adiponectin concentrations. Although serum adiponectin levels were negatively correlated with BMI, diabetic subjects had lower values of serum adiponectin than did non-diabetic subjects, independent of the BMI (Cruz *et al*., 2004; Yoshida *et al*., 2005). Serum adiponectin concentrations were more closely related to fasting insulinemia and to the rate of insulin-stimulated glucose disposal, a direct measure of insulin sensitivity, than to percent body fat and the 2 h glucose concentration suggesting that hyperinsulinaemia and/or insulin resistance might be a major determinants of the hypoadiponectinemia in obesity and type 2 diabetes. One of possible mechanisms for this that has been suggested is, overproduction of TNF-α by adipose tissue (Hotamisligil *et al*., 1994). Also in the recent study showed AdipoR1/R2 appears to be inversely regulated by insulin in physiological and pathophysiological states such as fasting/refeeding, insulin deficiency and hyper-insulinemia models via the insulin/phosphoinositide 3-kinase/Foxo1 pathway and is correlated with adiponectin sensitivity (Tsuchida *et al*., 2004). Adiponectin interferes with TNF-α signalling in endothelial cells (Ouchi *et al*., 1999). Decreased serum adiponectin may play a causative role in the development of insulin resistance.

In the present study, we addressed the question of whether serum leptin, adiponectin and leptin to adiponectin ratio are related to obesity and diabetes mellitus. The relation to central body fat was also observed. We used WHR as a parameter for central body fat. We had been reported that adiposity (BMI) is inversely associated with adiponectin, directly associated with leptin and leptin to adiponectin ratio. The presence of DM significantly increases the association of obesity and leptin, adiponectin and leptin to adiponectin ratio. Our results were in agreement with others (Kim *et al*., 2006; Matsubara *et al*., 2002; Weyer *et al*., 2001).

Present data shows that, in addition to overall obesity and DM, central fat distribution (WHR) is an independent negative predictor of serum adiponectin and positive predictor of serum leptin. The relationship of leptin and adiponectin with WHR appears to be stronger than that with BMI, indicating that central fat distribution is a better determinant of circulating leptin and adiponectin than total fat mass. Similar to our findings, (Cnop *et al*., 2003) had reported that intra-abdominal fat (but not BMI) was significantly and independently associated with decreased adiponectin level. Also (Yang *et al*., 2002) had reported an inverse association between adiponectin and WHR in morbidly obese patients. Adiponectin was negatively and leptin was positively correlated with waist-to-hip ratio reported by (Inoue *et al*., 2006, Park *et al*., 2004; Staiger *et al*., 2003).

CONCLUSIONS

We concluded that obesity, WHR and DM are inversely associated with adiponectin, directly associated with leptin and leptin/adiponectin ratio.

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