Resistin , Insulin resistance and BMI in type 2 diabetes mellitus and healthy subjects.

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Summary

Background : Obesity and insulin resistance have been quite well recognized as fundamental and leading causes of major health issues such as diabetes, hyperlipidemia, hypertension, and cardiovascular diseases. Abdominal obesity, particularly visceral adiposity is considered to play a major role in causing insulin resistance and type 2 diabetes mellitus , T2DM
The resistin is considered one of the causes of insulin resistance which lead to hyperinsulinemia and a decrease in the quantitative insulin sensitivity check index (Quicki) which has been recently reported to be a useful marker of insulin resistance in patients with T2DM.
Objective : The aim of the present study is to find the relationship between resistin and obesity as modulated by T2DM.
Subjects and methods : The study involved 50 patients with T2DM with age range of 30 -70 years , and 30 healthy subjects (control group) of matching age and sex.
Ten mLs of blood were collected from each patient and normal control subject after an overnight fast . One mL. was kept in an EDTA tube for mesureement of glycated Hb (HbA1c) and the rest was allowed to clot , centrifuged and serum was divided into aliquots . Some was kept at (- 20 ^{o}C) for measurement of resistin and insulin (by enzyme linked immunosorbant assay , ELISA) and the rest for measurement of glucose , urea and creatinine (by the available routine laboratory tests) at the same day of collection.
Results showed a significant rise in serum resistin in the obese diabetic patients as compared to the non obese patients. There are significant correlations between resistin and each of insulin resistance (Quicki) and degree of obesity (BMI) **.**
Conclusion : Resistin & insulin resistance are significantly affected by BMI in diabetic patients only and not in the control group which implies that the obese control subjects didn't have insulin resistances enough to show any change in resistin level. This confirms the synergistic effect of the obesity and diabetes on resistin level, while no effect of the disease per se could be detected from the present study.
Key words : resistin , insulin resistance , obesity , type 2diabetes mellitus

Introduction :

Type 2 diabetes mellitus (T2DM) is a common chronic disease which can be defined as hyperglycemia in the setting of relative insulin deficiency due to increased insulin requirement (insulin resistance), decreased insulin secretion, or both (most common). The incidence of T2DM increases with age and increasing obesity (particularly visceral or abdominal) and is more common in men than in women. The risk of developingT2DM increases with a family history of diabetes or cardiovascular disease (particularly hypertension or dyslipidaemia) and lack of physical activity **(1)**

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Obesity, which results from an imbalance between energy intake and energy expenditure is characterized by a pathologic accumulation of triglycerides (fat molecules) in adipose tissues, thereby promoting insulin resistance in muscles, liver and other tissues. **(2)**
Insulin resistance (IR) is a common pathological state in which target tissues fail to respond properly to normal levels of circulating insulin .Pancreatic cells first compensate for peripheral insulin resistance by increasing insulin secretion to maintain euglycaemia . Thereafter, impaired glucose tolerance can develop, leading to overt clinical T2DM. Chronic elevation in plasma non esterified free fatty acids NEFA levels is commonly associated with impaired insulin-mediated glucose uptake in skeletal muscles and often coexists with obesity and type2diabetes. **(3)**
Despite the very strong linkage between obesity and T2DM (80% of the patients are obese), the

molecular link has remained a mystery .There are many points suspected to link obesity to IR**.(4)**. Few years ago, a novel adipocyte-specific gene was identified which was suppressed by Thiazolidenediones TZD. This gene, called resistin was discovered while screening for genes that are induced during adipocyte differentiation, but down-regulated in mature adipocytes exposed to TZD**.(5)**
Resistin is a 12.5 kD cysteine-rich peptide secreted from adipocytes and present in the circulation**.(6)** It belongs to a protein family known as resistin-like molecules that are probably involved in the inflammatory process, but various studies in rodent models have shown that resistin impairs glucose tolerance and insulin action and inhibits adipogenesis. As this protein is secreted by the adipocytes, it has been suggested that it is involved in the relationship between adipose tissue, insulin resistance and diabetes, especially as the expression of both gene and protein are high in visceral adipose tissue. **(7)**
The level of resistin protein was high in adipocyte in a variety of rodent models of obesity both genetic and diet-induced .Circulating resistin levels in mouse serum decreased with the administration of the antidiabetic drug, roziglitazone and other TZDs. Resistin-like molecules (RELMs) have been identified from different tissues in humans and rodents, which together with resistin form a class of tissue-specific signaling molecules**.(5)**
The aim of the present study was to elucidate the relationship between obesity and insulin resistance IR as modulated by T2DM.

Subjects and methods:
This study included 50 patients with T2DM (32 females and18 males) aged from(30 to 70)years, with disease duration (1-10years) who attended the Diabetic clinic at AL-Kadhymia Teaching Hospital during the period from (September-2007 to February 2008.)..The study also included 30 normal volunteers of matching age and sex. All subjects were non smokers & non alcoholics. Exclusion criteria from the study were type 1 DM, gestational diabetes, renal and thyroid disorders.
All subjects were divided according to their body mass index (BMI) into Obese (≥ 30 n= 38) & non obese (< 30, n= 42).
Ten milliliters (**10ml**) of venous blood were withdrawn from both patients and controls..One milliliter (**1ml)** of the blood was added to EDTA tube for HbA1c measurement. The reaming of the blood sample was kept in a plain tube and centrifuged for 15 minutes at 3000rpm after being allowed to clot at room temperature for 30 minutes. The separated sera were divided into aliquots and stored frozen at (-20^{o} C) until the time of Insulin and resistin measurement. Fasting blood glucose, urea and creatinine were done immediately after separation of the serum.
Serum resistin and insulin were measured by enzyme linked immunosorbent assay. **(**ELISA, Sandwich assay). The quantitative determination of glucose was done by the enzymatic colorimetric method. Urea and creatinine by the available routin laboratory methods. Glycated hemoglobin (HbA1c) was measured by variant HbA1c program which is based on ion-exchange high performance liquid chromatography (HPLC). Assessment of insulin resistance was done by the formula QUICKI (quantitative insulin sensitivity check index) .The equation for measurement is:
Quicki = 1 / log (fasting serum insulin) + log (fasting glucose).(8)

Results:
In table (1) there are no significant differences between mean values of obese patients and those of obese controls in BMI and resistin, ($p>0.05$), but there is highly significant differences in Quicki and Hb1AC ($p<0.001$) and in insulin and FBG, ($p<0.05$).
On the other part of table (1) there are no significant differences between mean values of non obese patients and of non obese controls in BMI, and resistin, ($p>0.05$) ,but there are highly significant differences in Quicki, Hb1AC and FBG ($P<0.001$) and significant differences in insulin ($p<0.05$).
Figs 1 – 4 show the presence of a positive correlation between BMI and resistin , and negative correlations between Quicki and each of resistin and BMI and a positive correlation between resistin and insulin .

TABLE (1):-shows the values of all parameters involved in the study for both patient and control groups (mean ± SD) grouped according to their BMI (obesity).

Characteristic	Obese BMI≥30		Nonobese BMI<30	
	Patients	Controls	Patients	Controls
BMI	33.81± 3.60@@	32.10 ± 2.23	26.21 ± 2.39	25.75 ± 2.30
Resistin (ng/ml)	26.4 ±10.6@	21.7 ± 6.0	17.6 ± 6.1	15.9 ± 8.5
Insulin (mIU/ml)	13.5 ± 6.7* @	7.04 ± 1.60	8.98 ± 2.47*	6.50 ± 2.3
FBG(mg/dl)	160.44 ± 73.4*	92.8 ± 11.18	168.4 ± 45.8**	86.6 ± 12.0
Quicki	0.30 ± 0.04**	0.36 ± 0.02	0.32 ± 0.02**	0.38 ± 0.03
HbA1C%	7.77 ± 2.00	5.10 ± 0.24	8.58 ± 2.12	5.09 ± 0.23

* significant at $p < 0.05$ ** significant at $p < 0.001$when compared to their matching controls.
@ significant at $p < 0.05$ @@ significant at $p < 0.001$ as compared to their matching non obese subjects.

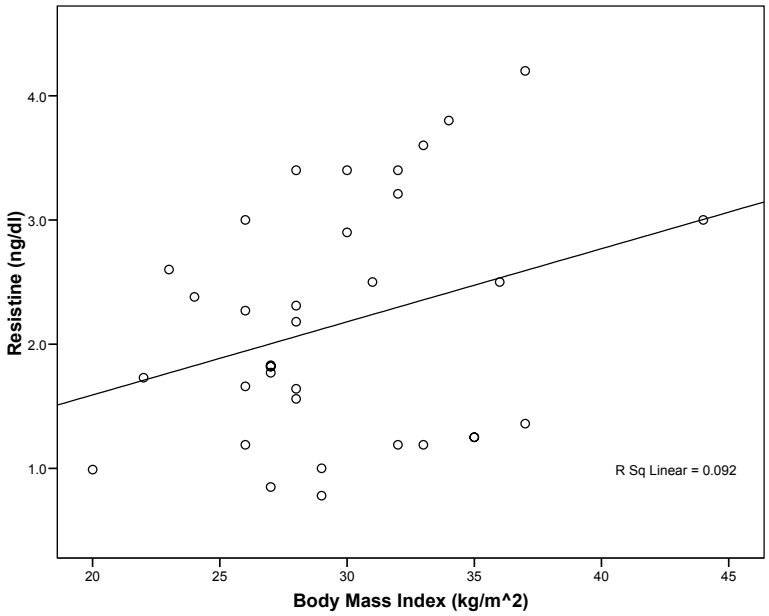

Figure (1) correlation between BMI and serum resistin in patients group(r=0.31, p<0.05)

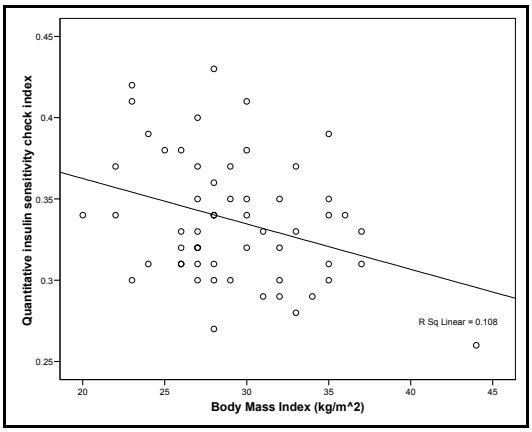

Figure (2) the negative correlation between BMI and quicki in patients group (r = -0.3 , p<0.05).

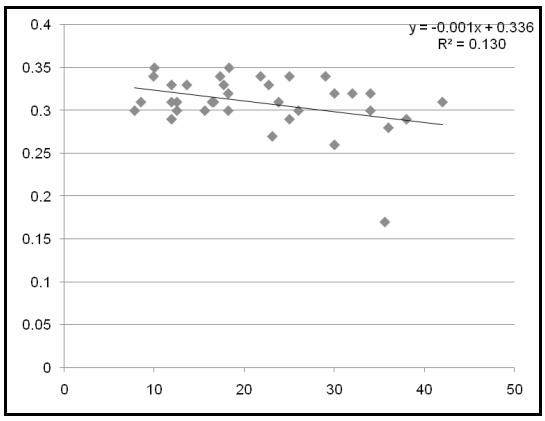

figure (3) The negative correlation between fasting serum resistin and quicki (r = -0.36 , p<0.05) in patient group

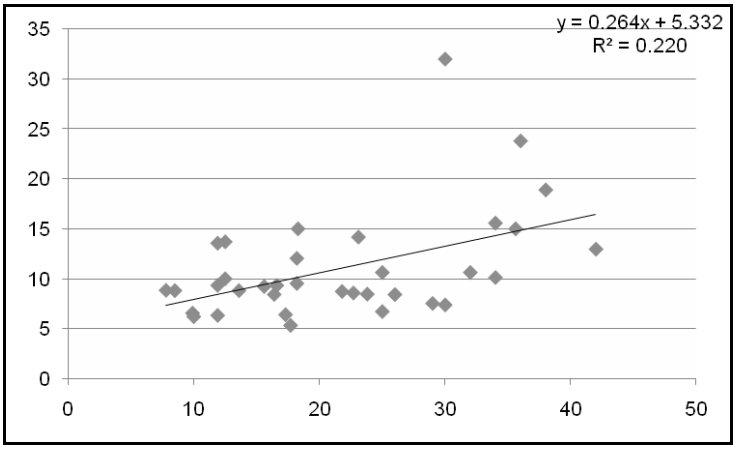

Figure (4) Correlation between serum resistin level and serum insulin level (r=0.46, p<0.05) in patients group.

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Discussion:

In this study neither the difference in BMI nor T2DM could cause any significant change in serum resistin level, but still the difference between obese patients and obese controls (p=0.162) is higher than that of non obese patients and non obese controls (p=0.56), this may imply the synergistic effect of the obesity and diabetes on resistin level, while there was no effect of disease per se in this respect. This is confirmed by the presence of a positive correlation between resistin and BMI in the patient group but not in the control group of the same BMI (Fig.1).This agrees with Mojiminiyi OA, Abdulla NA study who showed a significant association of resistin with BMI –dependent insulin resistance and factors linked diabetes with obesity **(9).**Human studies on the other hand , have highlighted increased resistin expression in adipose tissue, particularly abdominal depots. **(10&11)** and positive correlations between serum resistin and body fat content have also been reported**.(12)**

Lazar and coworkers have recently shown resistin to induce the expression of SOCS (suppressor of cytokine signaling-3), a known inhibitor of insulin signaling. Moreover, the loss of SOCS-3 function was shown to impair resistin from antagonizing insulin action in adipocytes. This has suggested that the insulin-independent action of resistin on adipocytes could partly be mediated by SOCS-3, which could have an impact on normal glucose homoeostasis**.(13)**

On other hand, the negative correlation of BMI with Quiki test (fig 2) confirms the belief that excess abdominal distribution of fat is more closely associated with the development of unfavorable metabolic abnormalities **(14).** .The possible mechanism is hypothesized to be mediated by the intra-abdominal fat depot. A preponderance of enlarged fat cells in this type of adipose tissue was seen to increase the risk of glucose intolerance, hyperinsulinemia and hypertriglyceridemiaThese hypertrophied adipocytes are more responsive to lipolytic hormones than smaller fat cells leading to increased delivery of NEFA into the portal circulation. Elevated levels of these fatty acids may induce insulin resistance in peripheral tissues and liver as well as leading to increased rates of hepatic glucose production. Therefore, poor glycemic status may be observed with abdominal adiposity. **(15&16).** However we could not find , in the present study ,a significant difference in HbA1c levels between the obese and non obese patients although it was obviously lower in the non obese patients implying a better glycemic status in such patients.

Accordingly serum resistin would be expected to show a negative correlation with Quicki (figs.3) and positively correlated with fasting serum insulin as shown in figure (4), a finding which confirms previous reports on T2DM patients **.(17)**

At last the absence of correlations between serum resistin and each of FBG and HbA1C may be due to that neither glucose or HbA1c had reached a certain level to exert an effect on resistin , however the present data are in accord with others**.(9)**

In conclusive remarks it could be stated that the work on resistin is still in the beginning and extensive genetic studies are needed to elucidate the exact role of this hormone in obesity, insulin resistance or diabetes mellitus and their metabolic consequences.

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