

Serum resistin levels in Syrian obese patients with diabetes mellitus type II

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ABSTRACT

Objective: To determine serum resistin levels in obese patients with diabetes mellitus type II.

Methods: We studied 87 subjects in an sectional study, divided into 3 groups: obese, obese diabetic and normal subjects. Their age, gender and body mass index were recorded. Serum resistin, insulin, glucose, cholesterol, high-density lipoproteins, low-density lipoproteins, triglyceride, urea and creatinine were measured.

Results: The mean \pm SD plasma resistin for the obese diabetic group is 7.32 ± 3.74 $\mu\text{g/ml}$ versus 4.25 ± 1.77 $\mu\text{g/ml}$ in the control group ($p=0.021$). Intra-group comparison of obese subjects (diabetics versus non-diabetics) revealed higher levels of resistin, glucose, triglyceride, cholesterol and low density lipoproteins in diabetic subjects, but no statistically significant difference of high density lipoproteins. Furthermore, resistin correlated significantly and positively with body mass index ($r = 0.375$; $p < 0.05$), resistin correlated significantly and negatively with high-density lipoproteins ($r = -0.363$; $p < 0.05$).

Conclusion: Serum resistin levels are increased in obese patients with type 2 diabetes compared with controls. Resistin appears to be a possible link between obesity and type 2 diabetes in humans.

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It is now recognized that adipose tissue is an endocrine organ that secretes a variety of hormones that have endocrine functions contribute to insulin resistance, the hallmark of type 2 diabetes mellitus (T2DM). Among these hormones is resistin. Resistin belongs to a family of cysteine-rich secretory proteins called resistin-like molecules^{1,2} or FIZZ (found in inflammatory zones) proteins. Human resistin is a cysteine-rich, 108-amino-acid peptide hormone with a molecular weight of 12.5 kDa. It was called "resistin" because of the observed insulin resistance in mice injected with resistin Steppan et al¹ Resistin has been proposed to form a biochemical link between obesity and type 2 diabetes.³ However, the role of resistin in human obesity has also produced conflicting reports, and at present resistin still remains controversial as a potential mediator in the pathogenesis of (T2DM) and the impact on T2DM status.^{4,5} Circulating resistin levels are increased in diet-induced and genetic forms of obesity and decreased by the anti-diabetic drugs thiazolidinediones (TZDs). Thiazolidinediones may work via peroxisome proliferator-activated receptor gamma (PPAR γ) to switch on or off a fat cell specific gene that is involved in the insulin mediated signaling pathways. Administration of resistin was reported to impair glucose tolerance and insulin action, whereas administration of an anti-resistin antibody significantly improved insulin action.^{6,7} In addition, insulin-stimulated glucose uptake by adipocytes was enhanced by neutralization of resistin, and conversely reduced by addition of resistin.² Chronic hyper-resistinemia leads to hepatic insulin resistance as well as whole-body insulin resistance, including skeletal muscle and adipose tissue. The mechanism whereby resistin decreases insulin sensitivity is unclear, however, Satoh et al⁸ revealed that the mechanism whereby resistin decreases insulin sensitivity in rats involves a reduction in AMP-activated protein kinase (AMPK) activity in skeletal muscle, adipose tissue, and liver and of insulin receptor substrate-1 (IRS-1) in adipose tissue and skeletal muscle. These decreases in tissue insulin sensitivity result in glucose intolerance, hyperinsulinemia, elevated free fatty acids (FFA) levels, and hypertriglyceridemia, all of

which are major features of human syndrome X.⁸ In regard to the expression site of human resistin, there has been more argument recently. Although reports from one group showed that resistin messenger RNA (mRNA) is indeed expressed in human adipocytes and that the protein is subsequently secreted, especially in abdominal adipose tissue,^{9,10} other groups indicated that resistin was of monocyte origin.^{11,12} The relationship between serum resistin levels, and T2DM has not been investigated in a Syrian population. The aim of this study was to investigate whether serum resistin levels are increased in obese Syrians with T2DM and to investigate the correlation between clinical and biochemical characteristics and serum resistin levels.

Methods. We investigated 87 objects attending the out-patient clinic of Al-Asad University Hospital and Diabetes Center, Damascus, Syrian Arab Republic, from June 2006 to September 2006. We divided the patients into 3 groups: obese diabetic group (n=35 [21 females, 14 males]), age: 51.7 ± 9.9 years, body mass index [BMI] 36.74 ± 5.46 kg/m²) taking at least one of oral anti-diabetic drugs, they were diagnosed with diabetes by an experienced endocrinologists since more than 6 months; fasting blood glucose >140 mg/dl; obese non-diabetic group (n=31 [18 females, 13 males], age: 40 ± 10.9 years, BMI 36.33 ± 4.2 kg/m²) healthy objects with no symptoms of any disease, none was taking any medication, fasting blood glucose <110 mg/dl, and control group (n=21 [7 females, 14 males], age: 40.8 ± 12 years, BMI 23.35 ± 3.5 kg/m²). healthy objects with no symptoms of any disease. Patients with cardiovascular disease (CVD), nephritic syndrome and BMI of <30 kg/m² were excluded. Patients taking insulin along with oral anti-diabetic drugs were also excluded. Informed consent was obtained from the hospital administration, diabetes center, and all subjects after a full explanation of the study. Our study was conducted to identify serum resistin levels in obese patients with T2DM to determine the possibility of using this hormone as a parameter in the diagnosis of diabetes mellitus type II.

Biochemical analysis. Fasting blood samples were collected into plain tubes (5 ml). Serum was harvested for 5 minutes by high-speed centrifugation (4000 rpm). Aliquots of serum were stored immediately at -80°C until analysis. Resistin concentrations, in serum samples, were measured with a commercially available Enzyme Linked Immunosorbant Assay (ELISA) (Biovendor Laboratory Medicine, Brno, Czech Republic). The assay Sensitivity is 0.1 ng/ml. The antibodies in human resistin ELISA kit are highly specific for human resistin with no detectable cross-reactivities to human leptin, leptin receptor, adiponectin, TNF-alfa. Insulin was

determined by radioimmunoassay using Linco's Ultra Sensitive Human Insulin Radioimmunoassay (RIA) Kit (LINCO Research, St. Charles, Missouri, United States). The assay sensitivity, expressed as the lowest level of sensitive human insulin that can be detected by this assay, is 0.2 µU/ml when using a 100 µl sample size. The specificity is very high in the presence of other like components in the sample matrix. The assay procedures were performed according to the supplier's instructions. Analysis of glucose, cholesterol, high-density lipoproteins, triglyceride, urea and creatinine was performed on the 911 Hitachi auto analyzer (Hoffmann-La Roche-BM, Germany). Using glucose oxidase method for glucose, glycerol 3-phosphate PAP (GPO/PAP) for triglyceride, cholesterol oxidase for cholesterol, direct method for high-density lipoprotein (HDL)- cholesterol, urease for urea and creatininase for creatinine. Low density lipoprotein (LDL)- cholesterol was calculated using the Friedwald et al¹³ formula for samples with triglyceride levels not exceeding 4.5 mmol/L.

Statistical analysis. For the statistical evaluation of the results, we used Statistical Pacakage for Social Sciences Version 10 for windows. Results were expressed as mean ± SD. Correlations between variables were based on Person's correlation coefficient using regression modeling. T-test was performed to determine differences between variables. A p-value of <0.05 was considered statistically significant.

Results. The concentrations of fasting serum resistin increased from control group (4.25 ± 1.77 µg/ml) to obese non-diabetic group (5.73 ± 2.61 µg/ml) to obese diabetic group (7.32 ± 3.74 µg/ml), (Table 1 shows median ± SD values for characteristics and biochemical parameters of study groups). Serum resistin concentrations showed significant differences among the 3 groups; the difference was significant between obese diabetics and obese non-diabetics ($p=0.037$), there was also significant difference between obese diabetics and control group ($p=0.021$) (Table 1). Insulin levels in obese non-diabetics were significantly higher than the levels in obese diabetics ($p=0.023$), there was no significant difference between obese diabetics and control group ($p=0.92$) (Table 1). Compared with controls, obese subjects showed higher values of homeostasis model assessment of insulin resistance (HOMA-IR), intro-group comparison of obese subjects (diabetics versus non-diabetics) revealed higher values of HOMA-IR in diabetic subjects ($p=0.0007$) (Table 1) In regard to the other biochemical parameters: serum glucose ($p<0.00001$), cholesterol ($p=0.0024$), LDL-C and triglyceride in obese diabetics were significantly higher than in obese non-diabetics ($p<0.00001$, $p=0.0024$, $p=0.0067$, $p=0.00028$) there was also significant

difference between obese diabetics and control group ($p < 0.0001$, $p < 0.00001$, $p = 0.008$, $p = 0.004$). We found no significant differences in HDL-c values among the 3 groups, (Table 1). Associations between biochemical parameters within obese diabetic group were studied. We found a statically significant positive correlation between body mass index and resistin values ($r = 0.375$; $p < 0.05$) (Figure 1), (Table 2). We also found a statically significant negative correlation between resistin values and HDL-c, ($r = -0.363$; $p < 0.05$), (Figure 2) (Table 2).

Discussion. Type 2 diabetes (T2DM), characterized by target-tissue resistance to insulin, is strongly associated with obesity. However, the mechanism by which increased adiposity causes insulin resistance is unclear.² Resistin has emerged as a novel secreted protein with links to both insulin resistance (IR) and obesity in rodents.^{6,14} There are conflicting reports about the role of serum resistin in obesity mediated T2DM. In this study, we found that serum resistin levels were increased in obese non-diabetic and

Table 1 - Median \pm SD values for characteristics and biochemical parameters of study groups.

| Variables | Obese objects | | P1 | Control n=21 | P2 |
|--------------------------------------|-------------------|------------------|----------|-----------------|----------|
| | DM n=35 | Non-DM n=31 | | | |
| Age (years) | 51.7 \pm 9.9 | 40 \pm 10.9 | <0.0001 | 40.8 \pm 12 | 0.0004 |
| Body mass index (kg/m ²) | 36.74 \pm 5.46 | 36.33 \pm 4.2 | 0.73 | 23.35 \pm 3.5 | <0.00001 |
| Resistin (μ g/ml) | 7.32 \pm 3.74 | 5.73 \pm 2.61 | 0.037 | 4.25 \pm 1.77 | 0.021 |
| Glucose (mg/dl) | 262.4 \pm 61.4 | 94.3 \pm 9.9 | <0.00001 | 89.7 \pm 8.5 | <0.00001 |
| Insulin (μ U/ml) | 7.4 \pm 3.91 | 10.6 \pm 6.4 | 0.023 | 6.4 \pm 2.5 | 0.92 |
| Cholesterol (mg/dl) | 225.1 \pm 28.6 | 193.8 \pm 40 | 0.0024 | 165.7 \pm 22 | <0.00001 |
| HDL-C (mg/dl) | 43.95 \pm 10.6 | 44.58 \pm 12.2 | 0.408 | 42.23 \pm 6.1 | 0.14 |
| LDL-C (mg/dl) | 117.5 \pm 33.3 | 107.9 \pm 30.6 | 0.0067 | 94.8 \pm 21.6 | 0.008 |
| Triglyceride (mg/dl) | 226.3 \pm 106.5 | 180 \pm 81 | 0.00028 | 157.6 \pm 38 | 0.004 |
| HOMA-IR | 4.55 \pm 2.56 | 2.63 \pm 1.9 | 0.0007 | 1.7 \pm 1.2 | 0.0017 |

DM- diabetes mellitus, HDL-c - high density lipoprotein cholesterol, LDL-c - low density lipoprotein cholesterol, HOMA-IR - Homeostasis Model Assessment of Insulin Resistance, P1 - comparison between obese diabetes mellitus versus obese non-diabetes mellitus, P2 - comparison between obese diabetes mellitus versus control.

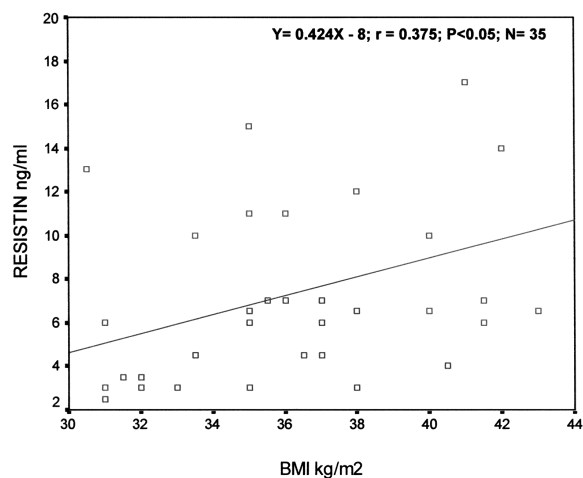


Figure 1 - Correlation between resistin and body mass index (BMI) in obese diabetic patients (n=35).

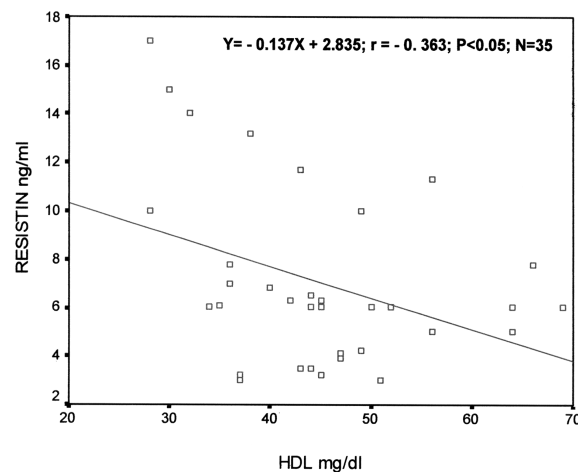


Figure 2 - Correlation between resistin and high density lipoprotein (HDL) in obese diabetic patients (n=35).

Table 2 - Resistin in relation with body mass index (BMI) and high density lipoprotein-cholesterol (HDL-c) in the obese group.

| Variables | r | P-value |
|--------------------|--------|---------|
| Resistin and BMI | 0.375 | <0.05 |
| Resistin and HDL-c | -0.363 | <0.05 |

r - correlation coefficient

obese diabetic patients when compared with control group. Moreover, serum resistin levels were increased in obese diabetic patients when compared with obese non-diabetics. These data suggest a potential role for resistin in T2DM. Other studies have examined the relationship between body mass index (BMI) and serum resistin levels. While some studies failed to determine such an effect,^{15,16} others showed such results as correlation.^{5,17-20} Degawa-Yamauchi et al⁴ revealed that BMI is a significant predictor of insulin resistance. Our study revealed a positive correlation between BMI and serum resistin levels, so it is possible to use BMI as a predictor of insulin resistance. Another interesting observation of our study is the relationship between resistin concentrations and high-density lipoprotein cholesterol (HDL). A recent study has shown that HDL cholesterol levels were significant predictors of serum resistin levels in patients with T2DM,²¹ while other study showed that serum resistin concentrations correlated with HDL cholesterol in obese women.²¹ In our study, serum resistin correlated with HDL cholesterol in obese diabetic group, which means that HDL may serve as a predictor of resistin levels. Conflicting reports of the relationship between serum resistin and glucose were reported.^{15-18,23} Al-Daghri et al¹⁶ observed a significant correlation between fasting glucose and serum resistin¹⁹ while Heilbronn et al²³ found no correlations in serum resistin and glucose among non-obese, obese, and obese T2DM subjects by examining the glucose disposal rate during a hyperinsulinaemic glucose clamp across groups. We found no correlation of circulating resistin and blood glucose in all groups even though this needs more investigation. Banerjee et al²⁴ reported that mice lacking the adipocyte hormone resistin exhibit low blood glucose levels after fasting due to reduced hepatic glucose production. Their data supported a physiological function for resistin in the maintenance of blood glucose during fasting. Remarkably, lack of resistin diminished the increase in post-fast blood glucose normally associated with increased weight suggesting a role for resistin in mediating hyperglycemia associated with

obesity. Resistin deficiency improved glucose tolerance and insulin sensitivity in severely obese mice, largely by enhancing insulin-mediated glucose disposal in muscle and adipose tissue.²⁵ Additional in vitro studies in human adipose cells support the role of resistin in reducing glucose uptake.¹⁵ Insulin levels in obese non-diabetics were higher than the levels in obese diabetics, this may be explained by the fact that the majority of obese diabetics are on insulin sensitizers such as thiazolidinediones and metformin. Some studies found that resistin correlated significantly and positively with insulin in diabetic women²⁶ and in patients with T2DM.¹⁹ However, Janowska et al²² found no correlation between resistin and fasting plasma insulin, this is in agreement with our study. In evaluating resistin and its association with insulin resistance in humans, several studies have identified positive correlations between resistin levels and insulin resistance in vivo^{26,27} and in vitro.²⁸ In contrast, other studies have reported no associations between serum resistin levels and markers of insulin resistance in T2DM patients^{4,29,30} and in obese subjects.³¹ Insulin resistance was evaluated by HOMA-IR. We found no correlation of circulating resistin and insulin resistance in all groups. In our study, we found no correlation of circulating resistin and LDL-c levels in all groups while other study¹⁷ found that serum resistin correlated with LDL-c in obese subjects, this needs more investigation.

Finally, although other studies revealed that plasma resistin correlated positively with triglyceride,^{32,17} our study revealed no correlation between triglycerides and serum resistin levels; this may be due to the little number of our study subjects. Although a sectional study may be biased by many factors affecting resistin levels, we had to carry out this as a sectional study and not longitudinal prospective one because it was more convenient than following the same group. This study, however, shows the need for long-term prospective trials of large numbers of patients

In conclusion, our findings in this Syrian cohort suggest that resistin may have a role in altering glucose metabolism and insulin sensitivity leading to the development of T2DM.

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