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# Plasma Resistin, Adiponectin and leptin levels In Relation To Insulin Resistance

**[1]M. A. Shousha and [2]S. ET.Soliman**

*[1] Biological Application Department, [2]Radioisotopes department, Nuclear Research Center, Atomic Energy Authority*

*_Correspondence to: somayasoliman@yahoo.com*

## ABSTRACT

Adipose tissue regulates insulin sensitivity via the circulating adipocytokines, adiponectin, resistin and leptin. The objective of this study was to compare the levels of resistin, adiponectin and leptin in lean and obese subjects and determine the relationship between circulating adipocytokines and insulin resistance.

We examined plasma levels of resistin, adiponectin and leptin in 20 lean subjects with mean body mass index (BMI) of ~24, and, 36 non-diabetic obese individuals with mean BMI ~34. Insulin resistance was assessed using the homeostasis model assessment ratio (HOMA-R) formula derived from fasting insulin and glucose levels.

Resistin levels were not significantly different between the two groups but were significantly higher in women compared with men, 30.4±6.5 vs. 14.4±2.9 mg/l, P<0.01. Resistin did not correlate with BMI but did significantly correlate with HOMA-R, P < 0.01, and this correlation remained significant after adjustment for gender and BMI. Adiponectin levels were significantly reduced in obese compared with lean subjects, P < 0.005 and higher in women, P< 0.001. Adiponectin levels showed significant correlation with HOMA-R and this correlation remained significant after adjustment for gender and BMI. Leptin levels were significantly higher in obese subjects and women and correlated with resistin, but, didn't correlate with HOMA-R.

In this small group of patients we demonstrated that insulin resistance correlated most strongly and reciprocally with adiponectin levels. Significant correlation between resistin levels and insulin resistance was also observed. Although a similar trend was apparent for leptin, the correlation with insulin resistance did not achieve statistical significance.

***Key Words: adipocytokines, Resistin, Adiponectin, Insulin resistance .***

## INTRODUCTION

The family Acrididae, with the suborder *Caelifera* ( short horned grasshopper**)** contains some of 10,000 different species**.** All locusts, i.e grasshoppers display polymorphism. Belong to this family only about 12 species referred as locusts, many of which are among the most important insect pests[(1)(2)].

When environmental conditions are favourable i.e a few successive warm and humed years, locust populations may grow very fast, eventually leading to high population densities. Under these conditions, locust of the solitary phase will change to the gregarious phase . It usually takes several

generations before the gregarious locusts form the huge migratory swarms that may devastate large area[3,4,5]

The most important organs that involved directly in the polymorphism phenomenon and the transition from solitary phase to gregarious phase are the fat bodies. Locust fat body sustains and assists the formation of the giant locust swarm with perfect flight performance, synthesizes, stores and mobilizes energy-rich substrate that are used to overcome periods of starvation to power flight and / or to fuel egg production[2] .

Investigation the protein patterns of the fat bodies of hoppers and adults of the desert locust *Schistocerca gregaria* may help in the understanding the biochemical and physiological changes occurred in the solitary form of this species, as well as opening new lines in research concerning the the locust phase polymorphism.

## SUBJECTS and METHODS

### Subjects

Both lean and obese male and female Egyptian subjects were studied. Participants in the study, 56 persons, were recruited from healthy volunteers and patients attending obesity clinics in Kasr Elaini hospitals. Informed consent was obtained from all subjects. Subjects were considered lean if their body mass index (BMI) was less than 25(20 persons). Obese subjects (36 patients) were screened for diabetes using an oral glucose tolerance test. The WHO diagnostic criteria were used to interpret the oral glucose tolerance test. Subjects found to have diabetes were excluded.

### Assays

Resistin was measured by an enzyme-linked immunoassay kit obtained from Biovendor Laboratory Medicine Inc. (Brno, Czech Republic). This biotin-labeled antibody sandwich assay measures homodimeric resistin. The intra-assay and inter-assay coefficients of variation were 4.5 and 7.8%.

Adiponectin and leptin were measured by RIA using reagents from Linco Research Inc. (St Charles, MO, USA). Insulin was measured by RIA using Pharmacia- Upjohn Diagnostics (Uppsala, Sweden). The insulin RIA measures immune- reactive insulin since the antibody used shows some cross-reaction with proinsulin and incompletely cleaved proinsulin.Samples were measured in duplicate in the same assay run where the intra-assay coefficients of variation were 5, 4.5 and 6% respectively.

Blood glucose was measured using an automated glucose oxidase method. Insulin resistance was assessed using the homeostasis model assessment ratio (HOMA-R) a formula derived from fasting glucose and fasting insulin. [8].

### Statistical analysis

Data are expressed as means ± S.D. Statistical analysis was performed using either two-tailed Student's t-test where single comparisons were made or Dennett t-test where multiple comparison with one control group was required (as in Fig. 2). Linear regression analysis was used to determine correlation coefficients between various parameters. Prior to regression analysis, data were tested for normality of distribution by the Shapiro–Wilk test and consequently BMI, HOMA-R, leptin, insulin,

resistin and adiponectin were log transformed to obtain normal distributions. Partial correlation coefficients were also obtained by controlling for gender, BMI and fasting glucose.

## RESULTS

The mean fasting plasma glucose level was significantly higher in the obese subjects than in the lean subjects but still within the normal non-diabetic range (Table 1). Five obese patients had impaired fasting glucose (6.9-7.5 mmol/l), while no lean subjects had impaired fasting glucose. Insulin levels and insulin resistance, as assessed using theHOMAR formula, were significantly increased in obese subjects compared with lean controls (table1).

**Table 1.Description of study population**

| | Lean subjects | Obese subjects | P value |
|---|---|---|---|
| Males / Females | 10/10 | 18/18 | NS |
| Age (years) | 45.6 ±1.2 | 45.2 ± 1.0 | NS |
| BMI (Kg/m²) | 24.1 ± 0.4 | 33.9 ± 1.3 | <0.001 |
| Insulin (mU/l) | 6.6 ± 0.6 | 11.7 ± 1.0 | <0.001 |
| Glucose (mmol/l) | 4.8 ± 0.2 | 5.1 ± 0.1 | < 0.05 |
| HOMA-R | 1.1 ± 0.2 | 2.8 ± 0.3 | <0.001 |
| Resistin (µg/l) | 20.9± 3.2 | 8.8 ± 5.8 | NS |
| Adiponectin (mg/l) | 8.6 ± 0.8 | 3.3 ±1.8 | <0.005 |
| Leptin (µg/l) | 6.9 ± 0.7 | 27.9 ± 3.9 | <0.001 |

NS: not significant

Plasma resistin levels were similar in lean and obese subjects. However, like adiponectin and leptin, resistin levels were significantly higher in women compared with men; 30.4±6.5 vs. 14.4±2.9 mg/l, P < 0.01. Resistin did not correlate with BMI but did significantly correlate with HOMA-R, P< 0.01 (Fig.1)

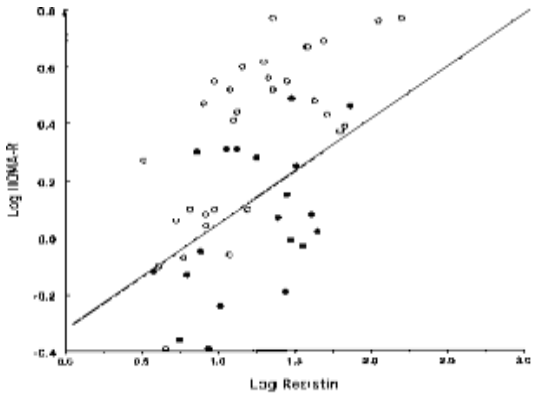


**Figure. 1. Correlation between log-transformed plasma resistin and log HOMA-R. Open circles indicate the obese subjects while closed circles represent the lean subjects. The line of best fit is indicated. P< 0.01**

This correlation remained significant even after adjustment for gender, and for gender and BMI (Table 2). Adiponectin levels were significantly reduced in obese compared with lean subjects

(Table 1). Adiponectin levels were higher in lean women compared with lean men, 18.6±2.0 vs. 7.0±1.7 mg/l, P < 0.005, but no significant gender difference was apparent in obese subjects (8.8±1.1 vs. 7.0±0.7 mg/l for women and men respectively). Adiponectin levels showed significant reciprocal correlation with HOMA-R (Table 2).

**Table 2 Correlations between adipocytokines and HOMA-R**.

| | Resistin | Adiponectin | Leptin |
|---|---|---|---|
| Unadjusted | R = 0.564<br>P < 0.001 | R =- 0.841<br>P < 0.001 | R = 0.055<br>P= 0.702 |
| Adjusted for Gender | R = 0.490<br>P < 0.001 | R = - 0.806<br>P< 0.001 | R = 0.140<br>P = 0.331 |
| Adjusted for gender and BMl | R = 0.513<br>P < 0.001 | R = - 0.608<br>P < 0.00 | R = 0.105<br>P = 0.478 |

Leptin levels were significantly higher in obese subjects (Table 1) and women. Leptin levels were correlated significantly with BMI, R = 0.732, P< 0.001, and, although seems to be correlated with HOMA-R, the correlation between leptin and HOMA-R was not statistically significant even after adjusting for gender, and gender and BMI (Table 2). Significant correlation was observed between leptin and resistin, whereas there was no significant correlation between adiponectin and either leptin or resistin. Since HOMA-R is not a particularly precise measure of insulin resistance we also analyzed the correlation between the adipocytokine levels, fasting glucose and insulin separately.

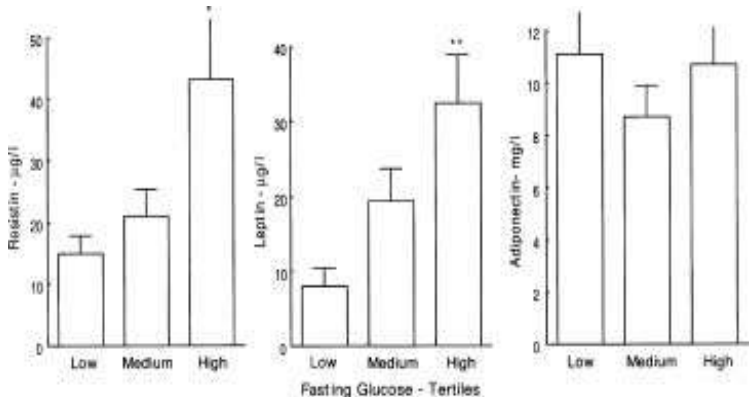


**Fig. (2) shows the relationship between fasting glucose and adipocytokine levels. Subjects with fasting glucose in the lowest and highest tertiles had similar adiponectin levels, whereas subjects who were in the highest tertile group for fasting glucose had significantly higher resistin and leptin levels than those in the lowest tertile for fasting glucose.**

Figure. 2. Distribution of adipocytokines level according to fasting glucose. Lean and obese subjects were divided into tertiles based upon fasting glucose levels. *P< 0.05, **P < 0.01 for the

significance of the difference between the highest and lowest tertile of fasting glucose. The glucose ranges for the tertiles were 3.1–4.7, 4.70–5.2 and 5.2–6.4 mmol/l respectively

Resistin and adiponectin significantly correlated with fasting insulin levels even after adjusting for fasting glucose, gender, and gender and BMI (Table 3).

**Table 3 Correlations between adipocytokines and fasting insulin.**

| | Resistin | Adiponectin | Leptin |
|---|---|---|---|
| Unadjusted | R = 0.547<br>P < 0.001 | R = 0.880<br>P < 0.001 | R = 0.059<br>P = 0.683 |
| Adjusted for Fasting glucose | R = 0.364<br>P = 0.009 | R = 0.873<br>P < 0.001 | R = 0.132<br>P= 0.309 |
| Adjusted for gender | R = 0.501<br>P < 0.001 | R= 0.681<br>P < 0.001 | R= 0.181<br>P = 0.201 |
| Adjusted for gender and BMI | R = 0.456<br>P < 0.001 | R = 0.66<br>P < 0.01I | R = 0.160<br>P = 0.272 |

## DISCUSSION

In this study we report for the first time resistin levels in normal men and women. There was significant gender difference, with women having higher values than men. Resistin levels were similar in lean and obese subjects and no significant correlation was observed between resistin levels and BMI. Despite these observations there was significant correlation between resistin level and insulin resistance as measured by the HOMA-R technique. This correlation was observed even after correcting for gender and BMI. HOMA-R is relatively an imprecise method for measuring insulin resistance compared with the glucose clamp technique. The RIA used to measure insulin may measure proinsulin and partially cleaved proinsulin molecules that may be more abundant in insulin-resistant subjects. However, despite these potential limitations, significant correlation was observed between fasting insulin levels and resistin and between fasting insulin and adiponectin levels. In contrast, leptin levels did not correlate with HOMA-R or fasting insulin. Resistin is a peptide hormone produced by adipocytes [9, 10] and regulates insulin sensitivity [2]. It is more highly expressed in omental and abdominal subcutaneous white fat than in adipose tissue from the thigh or breast [9]. It was initially isolated as an mRNA whose expression is suppressed in response to rosiglitazone [2]. Rosiglitazone, a PPAR-γ receptor agonist, enhances insulin sensitivity and this effect may be due in part to suppression of resistin expression. Furthermore, non-coding single-nucleotide polymorphisms in the resistin gene have been associated with both type 2 diabetes and obesity in Caucasian populations [11, 12]. As previously reported by others, adiponectin levels were reduced in obese subjects and increased in women compared with men [13–16].Other investigators have reported significant correlations between adiponectin levels and insulin resistance in larger studies [13, 16], a similar finding was demonstrated in our subjects. Unlike resistin and leptin, where there was an obvious relationship between fasting glucose levels and these adipocytokines, no such relationship was observed with adiponectin. The limited role of adiponectin in enhancement of insulin action would be consistent with the reported studies with adiponectin null mutant mice, which demonstrate that adiponectin deficiency itself results in no insulin resistance or glucose intolerance [18] or only moderate insulin resistance and mild glucose intolerance rather than type 2 diabetes [19, 20]. However, our data clearly demonstrate that insulin resistance is more tightly correlated with circulating resistin and adiponectin than leptin levels. Hulver et al. [17] demonstrated that exercise training-induced enhancement of insulin sensitivity was not

accompanied by changes in adiponectin levels. Of interest was the observation that leptin levels didn't correlate with insulin resistance as measured by HOMA-R, although, correlated strongly with fasting glucose levels even after correcting for gender and BMI. While previous reports have indicated a relationship between leptin levels and insulin resistance, this relationship has been considered by some to be due to increased fat mass [21]. Other investigators have demonstrated both in experimental animals and in human subjects that the association between insulin resistance and leptin levels may be independent of body fat mass [22, 23]. Leptin can also inhibit insulin-stimulated glucose uptake in cultured rat skeletal muscle cells [24]. Since our study had a relatively small number of subjects it is possible that with larger number of subjects the correlation between leptin and insulin resistance may have achieved statistical significance.

## CONCLUSION

In summary, we have provided evidence that resistin levels correlate with insulin resistance in non-diabetic subjects. This association is less marked than that seen for adiponectin and insulin resistance but stronger than the association between leptin and insulin resistance.

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# العلاقة بين مستوى الريزستين، الأديبونكتين و الليبتين فى بلازما الدم و عدم الأستجابة للأنسيولين

**مصطفى عبد الله منصور شوشة[1] و سمية التابعى محمد سليمان[2]**

*قسم التطبيقات البيولوجية [1] و قسم النظائر المشعة[2] –مركز البحوث النووية – هيئة الطاقة الذرية*

النسيج الدهنى بالأضافة إلى كونه مخزن للطاقة؛إلا أنه يتحكم فى الأيض و البناء من خلال مجموعة البروتينات شبيهة الهرمونات التى يفرزها ، و من أهم هذه المجموعة الرزستين ، الأديبونكتين و اللبتين حيث تتحكم فى حرق الدهون و الكربوهيدرات و تنظم حساسية خلايا الجسم للأنسيولين .و لهذا السبب تعد السمنة من أهم عوامل الأصابة بمرض السكرى من النوع الثانى و كذلك أمراض القلب و الأوعية الدموية.هدف هذه الدراسة هومقارنة مستوى هرمونات الريزستين ،الأديبونكتين و اللبتين فى بلازما الدم لدى الأشخاص الطبيعيين و مرضى السمنه،و كذلك دراسة العلاقة بين هذه الهرمونات و عدم إستجابة خلايا الجسم للأنسيولين.

إشتملت الدراسة56 شخصا (36 رجلا و 36 سيدة) ؛ 36 منهم مرضى بالسمنة و غير مصابين بالسكرى حيث كان متوسط معامل الجسم الكتلى تقريبا 34 و عشرون من الأصحاء الطبيعيين -متوسط معامل الجسم الكتلى تقريبا 24 وهذه أخذت كمجموعة ضابطة.

تم تقدير مستوى الرزستين ،الأديبونكتين و اللبتين فى بلازما الدم بإستخدام طرق المناعة الأشعاعية- وتقدير معامل عدم الأستجابة للأنسيولين بمعادلة حسابية مستخرجة من مستوى السكر الصائم و الأنسيولين فى الدم كما وردت فى المراجع العلمية –ثم دراسة العلاقة الأحصائية بينهم.كانت النتائج كالأتى:

لم يختلف مستوى الرزستين فى المجموعتين و لكنه كان أعلى لدى السيدات من الرجال بفارق إحصائى ذو قيمة معنوية ؛ وتعد هذه الدراسة أول تقرير عن مستوى الرزيستين فى الأشخاص الطبيعيين..