

## Serum resistin levels and haemostatic changes in experimentally induced diabetic and high fat fed rats

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**Abstract:** Adipose tissue is considered as an active endocrine gland that affects many aspects of body homeostasis. Adipose tissue derived molecules “adipokines” regulate energy homeostasis, dietary behavior, as well as insulin sensitivity and immunity; it refers to leptin, adiponectin, resistin, apelin, visfatin and omentin. Resistin is a cysteine-rich adipokine that is released by adipocytes and macrophages and has been involved in the development of insulin resistance in rodents. Moreover a strong link between diabetes, hypercoagulability and thrombogenesis, had been recognized for decades. Aim: In a trial to identify any possible relationship between resistin levels and some haemostatic changes in streptozotocin-induced diabetic and high fat diet-fed rats (HFD); the present work had been carried out. Design: A total number of 40 adult male albino rats were divided into 2 main groups: Group I (n= 24): To study the effect of streptozotocin-induced type 1 diabetes and was further divided into 3 equal subgroups (n= 8 in each) and survived for 30 days: Ia: (control group), Ib: (experimental diabetic non-treated group (by a single i.p. injection of streptozotocin (65mg/Kg B.W), Ic (experimental diabetic group treated with insulin). Group II (n= 16) : To study the effect of high fat diet and was further divided into 2 equal subgroups (n= 8 in each) and survived for 7 weeks: IIa: (control group), IIb (high fat diet fed (58% fat). In all groups, serum levels of glucose, insulin, resistin, total cholesterol(TC), triglycerides (TG), HDL, LDL, BT, WBCT, PT, aPTT, plasma fibrinogen level, plasma D-dimers level and platelet count were measured. Results: The results of this study showed a significant decrease in serum resistin levels ( $p < 0.001$ ) in streptozotocin-induced diabetic group in comparison with its control group and insulin-treated group. Moreover, no significant correlation could be detected between resistin levels and any of measured parameters in these groups except the significant positive correlation with body weight at the end of experimental period. In addition, our study revealed a significant increase in serum resistin levels ( $p < 0.001$ ) in HFD-fed group in comparison with its controls, which was correlated positively and significantly with body weight, serum glucose levels, insulin levels and HOMA-IR index ( $p < 0.001$ ), atherogenic lipid profile and markers of hypercoagulability (except for platelet count). Conclusion: No role for resistin in metabolic and haemostatic changes in type 1 diabetic rats was detected. Although, hyperresistinemia may represent a link between metabolic signals, atherogenesis, and hypercoagulability in type 2 diabetic rats. However, further studies are needed to clarify this relationship in human cardiovascular diseases. [Journal of American Science 2010; 6(8):557-567]. (ISSN: 1545-1003).

**Keywords:** Resistin, Streptozotocin, high fat, diabetes, haemostasis

### 1. Introduction

Obesity is associated with an array of health problems in adult and pediatric population. Understanding the pathogenesis of obesity and its metabolic sequelae has advanced rapidly over the past decades (American Diabetes Association, 2007).

Adipose tissue represents an active endocrine organ that, in addition to regulating fat mass and nutrient homeostasis, releases a large number of bioactive mediators (adipokines) that signal to organs of metabolic importance including brain, liver, skeletal muscle, and immune system, thereby modulating haemostasis, blood pressure, lipid and glucose metabolism, inflammation, and atherosclerosis (Rabe et al., 2008).

In (2001), Stepan et al. discovered a novel adipocyte-derived hormone called resistin, which was

expressed exclusively in white adipose tissue as a member of a family of cysteine-rich proteins called resistin-like molecules.

Intra-peritoneally administered resistin augments blood glucose and plasma insulin levels and limits the hypoglycemic response to insulin infusion, furthermore, resistin suppresses insulin-stimulated glucose uptake in cultured adipocytes, and this effect is prevented by exposure to anti-resistin antibodies (Stepan et al., 2001).

Hence, these data suggest that resistin could contribute to the insulin resistance observed in obesity by decreasing insulin sensitivity (Rajala et al., 2003, Muse et al., 2007).

In fact, obese subjects show a reduced insulin-stimulated skeletal muscle glucose uptake as well as an impaired insulin-evoked vasodilatation

(Baron, 1994) and these observations have suggested that the pathophysiological mechanisms linking obesity to the development of cardiovascular diseases could go beyond the classical metabolic derangements, so, much effort has been made to understand the interaction between insulin resistance and vascular function, with particular emphasis on adipocyte-derived hormones and their effects on vascular homeostasis (verma et al., 2003).

Resistin has been shown to selectively impair the effect of insulin on endothelial nitric oxide synthase (eNOS) enzymatic activity and through this mechanism resistin can reduce insulin-evoked vasorelaxation (Gentile et al., 2008). Moreover, in high fat-fed rats, resistin levels correlate negatively with vascular nitric oxide (NO) levels even after correction of insulin measurements, which suggests a direct inhibitory role of resistin on NO secretion (Li et al., 2007).

Also, plasma resistin levels were reported to be associated with many inflammatory markers including C-reactive protein, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6) (Silswal et al., 2005, Stofkova, 2010). Considering the crosstalk between inflammatory pathways and the insulin signaling cascade, resistin may represent a link between metabolic signals, inflammation and atherosclerosis (Lehrke et al., 2004, Daniel et al., 2010).

In general, diabetes is associated with an excessive risk of cardiovascular events (Williams et al., 2002), in that the coagulation system is switched towards a pro-thrombotic state involving, increased blood coagulation, decreased endothelial thrombo-resistance and pro-inflammatory state (Palomo et al., 2006), thereby increasing the risk of micro-vascular disease as well as macro-vascular diseases (Lender and Sysko, 2007).

So, this study was designed in a trial to clarify any possible relationship between resistin levels and some haemostatic changes in streptozotocin-induced diabetic rats and high fat diet-fed rats.

## 2. Material and Methods

This study was conducted on 40 healthy, adult, male albino rats weighing 200–260 gm (animals were obtained from faculty of medicine animal house and the animal experiments were approved by the local ethics committee). The rats had free access to water and chow and are kept at room temperature. All rats received standard chow (25.8 % protein, 62.8 % carbohydrate and 11.4 % fat (Ahren and Scheurink, 1998) except the rats in high fat-fed group, which received high-fat chow (16.4% protein, 25.6% carbohydrate, and 58.0% fat (a total 23.4 kJ/g

in the form of cotton seed oil added to the laboratory chow diet (Cha et al., 2000), (Diets were obtained from faculty of agriculture, Zagazig university).

The animals were divided into 2 main groups:

Group I: To study the effect of streptozotocin-induced type 1 diabetes on the measured parameters. This group was further divided into 3 equal subgroups and survived for 30 days:

Group Ia: "Control group (n=8)". Each rat was intra-peritoneally (i.p.) injected with 0.2 mmol/L Na citrate (0.1 mL).

Group Ib: "Experimental diabetic non-treated group (n=8)". Diabetes was induced by single intra-peritoneal injection of freshly prepared solution of streptozotocin (sigma) 65 mg/kg of body weight dissolved in 0.2 mmol/L sodium citrate, at PH 4.5 (Lutz and Pardridge, 1993) and maintained for 30 days (Toba et al., 2009).

Three days later, diabetes induction was confirmed through measurement of blood glucose level in each animal (from blood sampled from the tail vein) with the One Touch Ultra Glucometer (Yves and Theo, 2007) and rats with blood glucose levels more than 250 mg/dl were selected for experiments (Coskun et al., 2004). The rats were provided with 10% glucose solution after 6 hours of streptozotocin administration for the next 48 hours.

Group Ic: "Experimental diabetic group treated with insulin (n=8)". These animals were treated with regular (R) and NPH (N) insulin (2UR at diagnosis of diabetes and then 1R/3N at 6 P.M and 1R/1N at 9 A.M daily subcutaneously for 30 days after induction of diabetes (Sivitz et al., 1998).

Group II: To study the effect of high fat diet (HFD) on the measured parameters. This group was further divided into 2 equal subgroups:

Group IIa: "control group (n=8)", which was fed a standard chow for 7 weeks.

Group IIb: "High fat diet fed group (n=8)". These rats were fed a high-fat chow for 7 weeks.

For all groups, body weight was recorded per week, and at the end of the study period.

Haemostatic measurements:

-Determination of bleeding time (BT) according to Martin, (1981).

-Determination of whole blood clotting time (WBCT) according to Quick, (1966).

-Determination of prothrombin time (PT) according to Ansell, (1992).

-Determination of activated partial thromboplastin time (aPTT) according to Ansell, (1992).

-Estimation of plasma fibrinogen levels according to Cooper and Douglas, (1991).

-Estimation of plasma D-dimers levels according to Declerck et al. (1987).

-Determination of platelet count according to Brecher et al. (1953).

## Biochemical and Hormonal measurements:

- Estimation of serum glucose levels according to Trinder, (1969).
  - Estimation of serum insulin levels according to Reaven, (1991).
  - Estimation of serum resistin levels: by enzyme-linked immunoassay kits from Linco Research Inc., (USA) according to Steppan and Lazar, (2004).
  - Estimation of serum total cholesterol (TC) levels according to and Allain et al. (1974).
  - Estimation of serum triglycerides (TG) levels according to Naito, (1989).
  - Estimation of serum high density lipoproteins (HDL) levels according to Warnick et al. (1983)
  - Estimation of serum low density lipoproteins (LDL) levels according to Friedwald et al. (1972).
- Calculations:

The homeostasis model of assessment of insulin resistance (HOMA-IR) = fasting blood glucose (mmol/l) x fasting insulin (uIU/ml)/22.5) was calculated as an index of insulin resistance (Matthews et al., 1985) in group II.

## Statistical analysis:

Data were presented as mean  $\pm$  SD. Statistical significance was determined by unpaired Student's "t" test, P values less than 0.05 were considered to be significant. The correlations between parameters were analyzed using Pearson's correlation.

In statistical analysis, SPSS version 10.0 programs for Windows (SPSS Inc. Chicago, IL, USA) was used.

**3. Results****Table 1:** Body weight and the metabolic parameters of the studied groups:

	Group Ia	Group Ib	Group Ic	Group IIa	Group IIb
Initial BW (gr)	222.5 $\pm$ 16.1	219.4 $\pm$ 18.9	224.6 $\pm$ 21	213.7 $\pm$ 14.8	225.5 $\pm$ 19.3
Final BW (gr)	252.3 $\pm$ 16.5 r=0.73*	192 $\pm$ 14.8*** r=0.86**	251.3 $\pm$ 17.5 r=0.82*	242.5 $\pm$ 15.1 r=0.92**	339 $\pm$ 19.4*** r=0.97***
Glucose (mg/dl)	75.6 $\pm$ 6.6 r=0.31	411.5 $\pm$ 97.6*** r=0.19	79.4 $\pm$ 5.5 r=0.12	86.1 $\pm$ 8.7 r=0.65	262.5 $\pm$ 37.4*** r=0.98***
Insulin (uIU/ml)	19.7 $\pm$ 2.5 r=0.55	1.24 $\pm$ 0.36*** r=0.5	66 $\pm$ 9.2*** r=0.64	18.4 $\pm$ 3.1 r=0.03	46.1 $\pm$ 4.8*** r=0.97***
HOMA-IR				4.13 $\pm$ 0.47 r=0.03	30.28 $\pm$ 7.26*** r=0.99***
Resistin (ng/ml)	7.2 $\pm$ 0.77	3.99 $\pm$ 0.2***	7.4 $\pm$ 0.6	7.6 $\pm$ 0.46	14 $\pm$ 0.78***
TC (mg/dl)	115.25 $\pm$ 6.5 r=0.32	223.6 $\pm$ 24.2*** r=0.45	110.5 $\pm$ 8.5 r=0.39	113.75 $\pm$ 7.8 r=0.04	208.6 $\pm$ 26*** r=0.73*
TG (mg/dl)	49.75 $\pm$ 5.3 r=0.35	82.6 $\pm$ 6.5*** r=0.37	51.5 $\pm$ 7.5 r=0.33	54.25 $\pm$ 7.5 r=0.25	188.25 $\pm$ 12.5*** r=0.9**
HDL (mg/dl)	64.4 $\pm$ 10.2 r= -0.52	35.5 $\pm$ 4.2*** r= -0.25	54.9 $\pm$ 10.2 r= -0.22	40.9 $\pm$ 4.2 r= -0.05	36.1 $\pm$ 4.4* r= -0.91**
LDL (mg/dl)	40.9 $\pm$ 13.3 r=0.53	171.6 $\pm$ 24.3*** r=0.47	47.4 $\pm$ 13.7 r=0.25	62.03 $\pm$ 7.4 r=0.01	134.9 $\pm$ 27.7*** r=0.75*

r=correlation coefficient versus resistin levels.

\*=significant (P<0.05).

\*\*=significant (P<0.01).

\*\*\*=significant (P<0.001).

**Table 2:** The haemostatic parameters of the studied groups:

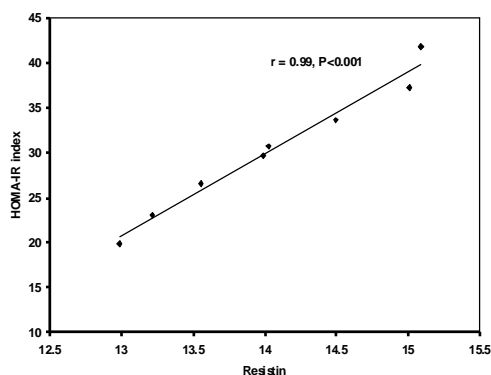
	Group Ia	Group Ib	Group Ic	Group IIa	Group IIb
BT (sec)	214.4±26.5 r= -0.33	150±19.4*** r= -0.52	202.5±35.6 r= -0.38	212.5±25.2 r= -0.31	170.6±14.5*** r= -0.97***
WBCT (sec)	236.1±21.6 r= -0.002	165.1±27*** r= -0.16	220.75±32.4 r= -0.69	228.3±28.7 r= -0.28	166±14.2*** r= -0.88**
PT (sec)	13.3±0.85 r= -0.06	10.25±1.7*** r= -0.17	13.55±0.65 r= -0.25	13.2±1 r= -0.29	10.6±1.28*** r= -0.8*
aPTT (sec)	24.1±2.7 r= -0.2	13.6±4.5*** r= -0.37	23±2.4 r= -0.22	25.6±3.8 r= -0.02	14.7±4.5*** r= -0.93**
Fibrinogen (mg/dl)	272.2±44.6 r=0.52	512.4±89.6*** r=0.19	287.2±46.2 r= -0.59	286.6±56.1 r=0.17	438.9±66.2*** r=0.84**
D-Dimers (mg/dl)	171.7±34.6 r=0.49	255.1±20.6*** r=0.6	170.8±15.9 r=0.28	147.75±27.6 r= 0.02	216.87±27*** r=0.73*
Platelet count (1000/mm <sup>3</sup> )	211.9±17.8 r=0.55	302±21.7*** r=0.05	226.4±19.1 r=0.02	220.4±20.4 r=0.08	216.1±13.8 r= 0.43

r=correlation coefficient versus resistin levels.

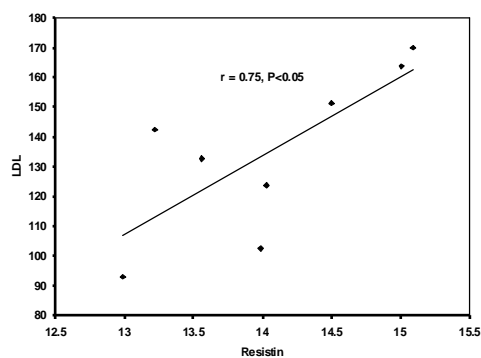
\*=significant (P<0.05).

\*\*=significant (P<0.01).

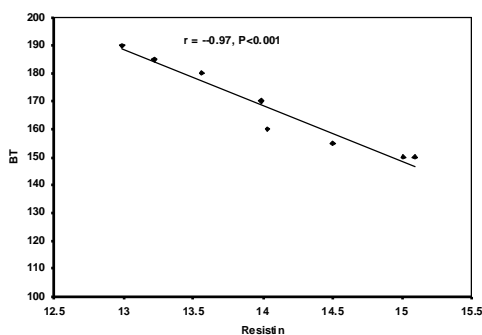
\*\*\* =significant (P<0.001).



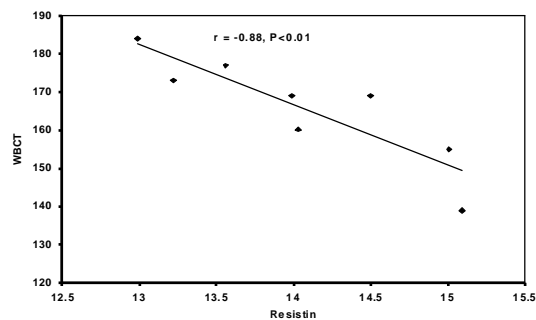
**Figure (1):** Correlation between serum resistin levels and HOMA IR index in group IIb.



**Figure (2):** Correlation between serum resistin levels and serum levels of LDL in group IIb.



**Figure (3):** Correlation between serum resistin levels and bleeding time in group IIb.



**Figure (4):** Correlation between serum resistin levels and WBCT time in group IIb.

The metabolic, hormonal and the haemostatic parameters of the groups are summarized in table 1&2, respectively. There was a significant positive correlation between the final body weight and serum resistin levels in all groups ( $p<0.05$ ,  $p<0.01$ ,  $p<0.05$ ,  $p<0.01$ ,  $p<0.001$ , respectively).

As regards group I, the results of this study showed a significant decrease in serum insulin and resistin ( $p<0.001$ ) levels in group Ib in comparison with that of control group (Ia) in association with increased blood glucose levels ( $p<0.001$ ). In addition, there was a significant increase ( $p<0.001$ ) in the serum levels of TC, TG and LDL while the serum levels of HDL were significantly decreased ( $p<0.001$ ) in the same group. Concerning the haemostatic parameters in group Ib, there was a significant decrease ( $p<0.001$ ) in BT, WBCT, PT, aPTT while plasma fibrinogen levels, D-dimmers and platelets count were significantly increased ( $p<0.001$ ). Moreover, all these metabolic and haemostatic disturbances were normalized in insulin treated group. Finally, no significant correlation could be detected between serum resistin levels and metabolic, hormonal or haemostatic measured parameters in this group.

As regards group II, group IIb showed a significant increase ( $p<0.001$ ) in body weight, serum glucose levels, insulin levels, HOMA-IR, TC levels, TG levels, and LDL levels, in addition to the significant decrease ( $p<0.001$ ) in HDL levels in comparison with that of controls (IIa). Moreover, our study revealed a significant increase ( $p<0.001$ ) in serum resistin levels in this group in comparison with that of controls, which was correlated positively and significantly ( $p<0.001$ ) with body weight, serum glucose levels, insulin levels and HOMA-IR. Also, a significant positive correlation was found between serum resistin levels and serum levels of TC ( $p<0.05$ ), TG ( $p<0.01$ ) and LDL ( $p<0.01$ ) while a significant negative correlation ( $p<0.05$ ) between its levels and serum levels of HDL was reported. Moreover, BT, WBCT, PT and aPPT were found to be significantly decreased ( $p<0.001$ ), while, plasma fibrinogen and D-dimmers levels were found to be significantly increased ( $p<0.001$ ). However, no significant difference in the platelets count was found. Furthermore, serum resistin levels were found to be correlated negatively and significantly with BT ( $p<0.001$ ), WBCT ( $p<0.01$ ), PT ( $p<0.05$ ), aPTT ( $p<0.01$ ) and positively with plasma fibrinogen ( $p<0.01$ ) and D-dimmers ( $p<0.05$ ) levels.

#### 4. Discussions

The results of this study showed a significant decrease in serum insulin and resistin

levels in streptozotocin-induced diabetic group in comparison with that of control group and insulin-treated group, in association with increased blood glucose levels. This decrease in serum resistin levels could be attributed to the weight loss that occurs in type 1 diabetes, as resistin levels were positively and significantly correlated with the body weight in this study, which is in agreement with that of Stroubini et al. (2009).

In addition, our results indicated that there was a significant increase in the serum levels of total cholesterol, triglycerides and LDL while the serum levels of HDL were significantly decreased in streptozotocin-induced type 1 diabetes which is defined as an atherogenic lipid profile (Vergè, 2009).

Concerning the haemostatic parameters in group Ib, there was a significant decrease in BT, WBCT, PT, aPTT while D-dimmers, platelets count and plasma fibrinogen levels were significantly increased, denoting a hyper-coagulable state (Khatun et al., 1999). Moreover, all these metabolic (Sivitz et al., 1998) and haemostatic (Sobel, 2003, Nishikawa et al., 2008) disturbances were normalized in insulin treated group.

In addition, no significant correlation could be detected between serum resistin levels and either metabolic or haemostatic measured parameters in this group.

So, it can be concluded that resistin has no role in type 1 diabetes as regards haemostatic changes, however, these changes could be attributed to the presence of high levels of LDL particles, as these particles have atherogenic properties (Skyrme-Jones et al., 2000). Also, lowered HDL may play a role as HDL has antioxidative, anti-inflammatory, anti-thrombotic and vasorelaxant properties, all of which are potentially anti-atherogenic (Link et al., 2007). Therefore, it can be concluded that this dyslipidemia is associated with an increased cardiovascular risk in type 1 diabetes (Vergè, 2009).

As regards group HFD group, it was found to show marked increase in body weight, insulin resistance and dyslipidemia in rats. These results are in accordance with those of Willett, (2002) and Schaalán et al. (2009).

Moreover, our study revealed a significant increase in serum resistin levels in this group in comparison with that of controls, which correlate positively and significantly with body weight, serum glucose levels, insulin levels and HOMA-IR index, while no significant correlation could be found between its levels and those measured parameters in controls except a significant positive correlation with body weight.

These results are supported by that of Azuma et al. (2003) and Silha et al. (2003) who



reported that the mean circulating resistin levels in obese subjects is increased about four folds compared with lean subjects and by Stroubini et al. (2009), who reported that resistin levels were elevated in many experimental models of obesity and decreased after weight loss.

Moreover, de Luis et al. (2009) demonstrated that resistin concentrations were related to the total fat mass in patients with metabolic syndrome.

Concerning resistin-insulin relationship, our results are supported by those who concluded that, administration of antiresistin antibody improved insulin action and glucose metabolism in mice with diet-induced obesity (Steppan et al., 2001). While, infusion of recombinant resistin to rats rapidly induces hepatic IR and increases hepatic glucose production (Rajala et al., 2003). Also, ablation of the resistin gene in mice decreases fasting glucose through reducing gluconeogenesis, while resistin administration in these resistin-deficient mice increases hepatic glucose production (Banerjee et al., 2004).

Resistin primarily exerts its glucoregulatory effect by stimulating hepatic glucose output (Rangwala et al., 2004). As elevation of circulating resistin in rodents, either acutely (Muse et al., 2007) or chronically (as following diet-induced obesity) (Muse et al., 2004), leads to marked decreases in hepatic insulin sensitivity.

Moreover, the findings of this study are in line with that of Kushiyaama et al. (2005), who found that transgenic mice with hepatic resistin over-expression exhibit significant hyperglycemia, hyperlipidemia, fatty liver, and pancreatic islet enlargement, when fed an HFD. These effects may be due to resistin-induced impairment of glucose homeostasis and insulin action, thus modulating one or more steps in the insulin signaling pathway and possibly playing a role in the pathogenesis of insulin resistance (Muse et al., 2004).

The majority of in vivo studies showed that resistin has a negative effect on insulin signaling in the liver (Qi et al., 2006).

In contrast with our results, no significant correlation was found between resistin levels and glucose levels in high fat-fed rats (Li et al., 2007), and also in patients with type 2 diabetes mellitus (T2DM) (Mojiminiyi and Abdella, 2007). Also, some studies have observed significant low resistin mRNA levels in adipose tissue in different obese mouse models, such as db/db, or high-fat-diet-induced obesity, and in rat models characterized by IR (Way et al., 2001).

The mechanism whereby resistin decreases insulin sensitivity involves several impacts. First,

resistin reduces adenosine 5'-monophosphate-activated protein kinase activity in skeletal muscle, adipose tissue, and liver. In addition, insulin receptor substrate-1 (IRS-1) and IRS-2 protein levels and phosphorylation states, as well as protein kinase B activity, were decreased in hyperresistinemic animal tissues. These alterations decrease tissue insulin sensitivity that results in glucose intolerance, hyperinsulinemia, elevated free fatty acid (FFA) levels, and hypertriglyceridemia (Rajala et al., 2003).

Secondly, the resistin-induced reduction in IRS-1 and IRS-2 elevates mRNA levels of gluconeogenic enzymes, such as glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase, thus suggesting a direct resistin induction of insulin resistance in the liver (Moon et al., 2003).

Thirdly, it was found that resistin decreased glycogen synthase (GS) activity both in the presence or absence of insulin, this suggests that resistin directly down-regulates GS activity (Ferrer et al., 2003).

Insulin signaling in pancreatic islets plays an important role in the maintenance of  $\beta$ -cell functions and glucose-induced insulin secretion in islets of pancreas (Otani et al., 2004). Therefore, the inhibition of insulin signaling could underlie the impairment of glucose-induced insulin secretion by resistin (Nakata et al., 2007).

Moreover, it was observed that there is a positive correlation between resistin levels and C-reactive protein (CRP) (Kunnari et al., 2006). Accordingly, the correlation between fasting glucose and resistin levels might be explained by this inflammatory state (Bo et al., 2005).

As regards the lipid profile in the high fat diet-fed group, our results revealed a significant increase in serum levels of total cholesterol, triglycerides and LDL, while serum levels of HDL were significantly decreased (atherogenic lipid profile). Also, a significant positive correlation was found between serum resistin levels and serum levels of total cholesterol, triglycerides and LDL while a significant negative correlation between its levels and serum levels of HDL was reported.

These results are supported by those of Mojiminiyi and Abdella (2007), who concluded that resistin was correlated positively and significantly with atherogenic lipid profile in type 2 diabetic patients, and in patients with metabolic syndrome (de Luis et al., 2009).

On the contrary to our results, Qi et al. (2008), found no significant correlation between resistin levels and lipid profile parameters except a negative correlation with HDL levels only in patients with metabolic syndrome.

Moreover, it was reported that resistin directly increases the endothelial expression of adhesion molecules, vascular wall adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) that play central roles in the early stage of the atherogenic processes (Burnett et al., 2005), it also promotes lipid accumulation in human macrophages by up-regulating CD36 cell surface expression, which is one of the scavenger receptors in macrophages involved in the uptake of modified LDL (Xu et al., 2006). Based on these data, resistin is supposed to induce atherosclerosis by mediating endothelial hyperactivity in response to the systematic inflammatory condition in human (Tsukahara et al., 2009).

At the cellular level, resistin has also been shown to exert potent pro-inflammatory properties by up-regulating pro-inflammatory cytokines, probably via the nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway, and resistin can induce inflammation in animal models (Stofkova, 2010). Besides this inflammatory induction, resistin also promotes proliferation and activation of human smooth muscle cells and endothelial cells, (Calabro et al., 2004) and induces angiogenic responses in endothelial cells, in part via phosphorylation and activation of different phosphate-kinase pathways (Mu et al., 2006).

Accordingly, it was reported that resistin may influence angiogenesis, not only in adipose tissue but also at other sites, so that systemic concentrations of resistin may contribute to the development of vascular disease (Robertson et al., 2009).

Hence, considering the expression of resistin by mononuclear cells and that obesity and T2DM are states of low-grade inflammation with activated inflammatory cascades, resistin may indeed present a molecular link between metabolic signals, inflammation and atherosclerosis (Kadoglou et al., 2007).

As insulin resistance is associated not only with hyperinsulinemia and hyperglycemia but also with other disorders such as abnormal lipid profile (Ding et al., 2005). These findings indicate a link between lipid profile and insulin sensitivity, since systemic excess of FFAs impairs the ability of insulin to stimulate glucose metabolism, contributing to whole-body insulin resistance (Schaalan et al., 2009).

In addition, several studies reported that resistin is implicated in the control of lipolysis (Rae et al., 2007). Also, in the humanized resistin mice, resistin was found to increase hormone sensitive lipase (HSL) activity by inducing white adipose tissue (WAT) inflammation and enhance the phosphorylation of HSL at its activating protein kinase-A (PKA) site (Qatanani et al., 2009).

Taken together, it could be postulated that the significant relationship between serum resistin levels and atherogenic lipid profile may be due to a direct effect in addition to induction of insulin resistance.

In relation to the haemostatic changes in the same group (group IIb), BT, WBCT, PT and aPTT were found to be significantly decreased, while, plasma fibrinogen and D-dimers levels were found to be significantly increased (indicating hypercoagulable state). However, no significant difference in the platelets count was found. Furthermore, serum resistin levels were found to be correlated negatively and significantly with BT, WBCT, PT and aPTT and positively with plasma fibrinogen and D-dimers levels.

Our findings are in line with those of other investigators who concluded that, resistin is an emerging cardiovascular risk factor implicated in T2DM (Kershaw and Flier, 2004). Furthermore, the patients with myocardial infarction showed higher plasma resistin levels especially those with coronary heart disease (CHD) when compared with the controls (Burnett et al., 2006).

Hyperfibrinogenaemia is associated with an increased prevalence and incidence of primary and recurrent CHD and thrombosis (Mc Dermott et al., 2003) and correlates with measures of obesity in several studies (Woodward et al., 1997).

Also, Menzaghi et al. (2006) reported a significant positive correlation between resistin levels and fibrinogen levels in insulin resistant patients.

Furthermore, it was found that, resistin has been shown to selectively impair the effect of insulin on endothelial nitric oxide synthetase (eNOS) enzymatic activity and indicate a mechanism through which resistin can reduce insulin-evoked vasorelaxation (Gentile et al., 2008).

Moreover, resistin induces serine protease (Akt)-dependent endothelial NO dysfunction through the inhibition of IRS-1 signaling pathway and IRS-1 itself is present in a lower amount in cells challenged with insulin and pretreated with resistin suggesting that resistin interferes with the insulin-stimulated IRS-1-dependent signaling pathway, acting both on the IRS-1 protein and on its ability to activate phosphatidyl inositol tri-phosphate kinase (PI3K) (Palanivel et al., 2006).

Moreover, in high fat diet-fed rats, resistin levels correlate negatively with vascular NO levels even after correction of insulin levels, which suggests a direct inhibitory role of resistin on NO secretion (Li et al., 2007). And since endothelial NO has a crucial role not only in modulating vascular tone but also in anti-atherogenic protection (Myazaky et al., 2003) by inhibiting inflammation, oxidation, vascular smooth

muscle cell proliferation and migration, it can be speculated that endothelial NO dysfunction induced by resistin could also participate to the enhanced atherosclerotic process that occurs in obese subjects (Gentile et al., 2008).

In vitro studies of Takahashi et al. (2006), who described that resistin activates endothelial cell directly by promoting endothelin-1 (ET-1) release and expression of ET. Jung et al. (2006). In addition, Li et al. (2007) reported a significant positive correlation between resistin levels and ET levels in high fat-fed rats.

Furthermore, Li et al. (2007) concluded that chronic administration of resistin in rats produced a significant increase in plasminogen activator inhibitor-1 (PAI-1) and Von Willebrand factor (VWF). Moreover, they also reported that, diet-induced hyperresistinemia in rats correlated positively with levels of PAI-1 and VWF even after correction of insulin levels. In addition, Qi et al. (2008) reported that, resistin was correlated positively with PAI-1 levels in women with metabolic syndrome. In fact, the increased levels of PAI-1 found in obesity may predispose to micro- and macro-vascular, arterial and venous thrombosis (Lundgren et al., 1996).

## 5. Conclusion:

No role for resistin in metabolic and haemostatic changes in type 1 diabetic rats was detected. Although, hyperresistinemia may represent a novel link between metabolic signals, atherosclerosis, and hypercoagulability in type 2 diabetic rats. However, further studies are needed to clarify this relationship in human cardiovascular diseases.

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