II– RESISTIN

A)Discovery of Resistin:

Resistin was discovered in 2001 by the group of Dr Mitchell A. Lazar from University of Pennsylvania School of Medicine ***(Wang et al., 2010).*** It was called "resistin" because oftheobserved insulin resistance in mice injected with resistin ***(Garten et al., 2009).***

The discovery of the resistin gene and the fact that it encodes an adipocyte-derived hormone called resistin is consistent with therecognizedroleofadipose tissue as an endocrine organ. Many researches have been made on resistin since its initial description in 2001 ***(Adya et al., 2008)***.

Three groups discovered resistin independently using modern genomic approaches. Firstly, ***Holcomb et al.,2000,***found resistin, which they termed "found in inflammatory zone 3" (FIZZ3), as an expressed sequence tag related to a protein, they found to be induced during lung inflammation. Secondly,***Kim et al., 2001,*** identifiedresistin as an adipose secretory factor (ADSF). The first functional study on resistin revealed that it is an important factor linking obesity to type 2 diabetes. Finally,Steppanandhisgroupidentified resistin in a screen to identify potential targets of the thiazolidinedione (TZD), class of insulin sensitizers in cultureadipocytes ***(Steppan et al., 2001)***.

Several follow-up studies have explored the role ofresistinin obesity and type 2 diabetes and its underlying mechanisms. Other several studies showedthatresistinmay also play a pivotal role in inflammation and inflammation-related diseases ***(Filkova et al., 2009).***

B)The Adipose Tissue as an Endocrine Organ:

In mammals, adipose tissue occurs in two forms: white adipose tissue and brown adipose tissue. While brown adipose tissue is found principally in neonates and regulates body temperature, white adipose tissue, the most abundant in adults, is devoted to storage of excess energy. This energy accumulates into adipocytes in the form of triglycerides and is mobilized when energy intake becomes inadequate. Additionally to adipocytes, adipose tissue also contains other cell types, including endothelial cells, leukocytes, fibroblasts and macrophages. Interestingly, evidence indicates that the percentage of macrophages within adipose tissue is increased in the presence of obesity ***(Lee et al., 2011)***.

Adipose tissue inflammation, which occurs in obese patients, has been suggested to contribute to obesity-related complications such as diabetes and atherosclerosis. The growing interest in the biology of adipose tissue derives from the understanding that fat is not only a passive energy depot, but functions as a hormonally active tissue, capable of producing numerous molecules, including cytokines, chemokines and adipokines ***(Al-Harithyet al.,2010)***.

C)Adipokines:

The term "adipokines" comprises a group of polypeptide hormones which are expressed predominantly, although not exclusively, by adipose tissue in a regulated manner. These molecules are secreted into the circulation and regulate the functions of different tissues through local, central and/or peripheral actions***(Kim et al.,2007)***.

The source ofadipokines within adipose tissue is not only mature fat cells but also cells of the stromal-vascular fraction, including infiltrating macrophages. In particular, in condition of obesity, macrophages may be a major sourceoflocallyproduced pro-inflammatory cytokinesandadipokines ***(Nakajima et al., 2009)***.

Adipokineshavebeen shown to play an importantrole in the physiology of adipose tissue, modulating adipocyte differentiation and regulating lipid accumulation, through autocrine mechanism. Moreover, adipokinesarethought tobe critical mediators in obesity-related diseases, such as type-2 diabetes and hypertension. Indeed, adipokine expression is differentially modulated during conditions of obesity and/or metabolic alterations, as demonstrated by the variations in their blood levels in obese and/or insulin resistance subjects ***(Imai et al., 2009).***

It has become evident that adipose tissue has major integrative physiologic functions and secretes numerous bioactive

low molecular weight proteins, known as adipocytokines such as resistin, adiponectin and leptin. These factors act at both local (autocrine and paracrine) and systemic (endocrine) level with profoundeffects on the function of distant organs, such as muscles, pancreas, liver, and brain. Adipose tissue is integrally involved in coordinating a variety of biological processes including energy metabolism, neuro-endocrine function, and immune function ***(Shackelford et al., 2010).***

D) Resistin Structure:

Resistin, also called adipocytesecretedfactor (ADSF) ; is a cysteine-rich, 108 amino acid peptide hormone with a molecular weight of 12.5 kDa,and its hydrophobic signal peptide is cleaved before its secretion. It is a disulfide-linked homodimer which can be converted easily to a monomer. Cysteineis the most common amino acid in resistin, where it forms approximately 12% of its amino acid sequence ***(Krecki et al., 2011).***

Resistina highsequenceidentity (43% in a mature protein). Crystal structures ofresistinrevealan unusual composition of several subunits that are held together by non-covalent interactions which make up its structure**.** Each protein subunit comprises a carboxy-terminal disulfide-rich Beta-sandwich "head" domain and an amino-terminal alpha-helical "tail" segment. Structure of Resistin(Figure 7)***(Filkova et al., 2009).***

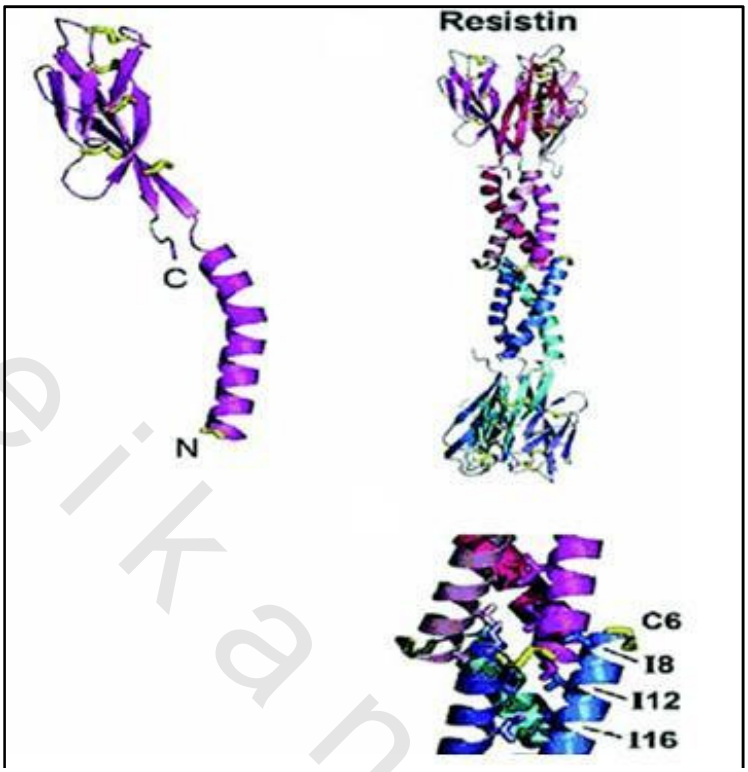

Figure (7): Structure of Resistin ***(Filkova et al., 2009).***

Resistin belongs to a class of cysteine-rich secreted proteins termed the resistin like molecules (RELMs).They are polypeptides of 105 - 114 amino acids with three domains (N-terminal signal sequence, a variable middle portion and a highly constant C-terminal sequence that forms about half of the molecule). The C-terminal ofRELMappears to determine the signature of the molecule. Three types ofRELMs have been described, RELM-α, RELM-β and RELM-γ**.** Resistin-like molecules-beta (RELM-β) is the only form of the resistin like molecules present in human ***(Peleg et al., 2008).***

E)Physiological Role of Resistin:

Resistinincreases blood glucose and insulin concentrations in mice andimpairs hypoglycemic response to insulin infusion. In addition,anti-resistin antibodies decrease blood glucose and improveinsulin sensitivity inobesemice ***(Toruner et al., 2008)***. Resistin suppressesinsulin-stimulated glucose uptake in cultured adipocytes,and this effect is prevented by anti-resistin antibodies. Thesedata suggest that resistin induces insulin resistance and thathyperresistinemia contributes to impaired insulin sensitivityin obese rodents ***(Adya et al., 2008).*** The suppressive effect ofthiazolidinedioneson resistin secretion found in some studies may contribute tothe insulin-sensitizing effect of this class of drugs***(Peleg et al., 2008).***

Rajala et al.,2003,provided clarification ofthe biologicalfunctions of the FIZZ/RELM family.

Administration of recombinantresistin and RELMß to rats led to acutely impairedhepatic insulin sensitivity and glucose metabolism. The primarypathway underlying changes in hepatic glucose metabolism appearsto be increased glucose production ***(Sharma et al., 2011).***

In humans resistin antagonizes the effects of insulin on glucosemetabolism in liver and skeletal muscle, interacts with andreinforces inflammatory pathwaysandmay promoteendothelialcell activation.Increased resistin levels have beenassociated with obesity, insulin resistance, metabolic

syndrome,type 2 diabetes and increased cardiovascular risk***(Pantsulaia et al., 2007).***Plasmaresistin levels have been associatedwithmarkers ofchronickidney disease and it is speculated that inflammatory,metabolic and vascular abnormalities associated with increasedcirculatingresistinlevels may have a pathogenic role in chronickidney disease ***(Gartenet al., 2009)***.

F)TissueDistributionofResistin:

Resistinis producedintheadipose tissue and also has been identified in several other tissues, and there is a species differences in cellular resistin distribution. The presence ofresistin in many tissues may indicate an ubiquitousnatureofresistin and global role in the control of body homeostasis ***(Gartenet al., 2009).***

1-White Adipose Tissue:

Janke et al., 2002,observed that resistin was barely detected in mature human adipocytes but was highly expressed in cultured preadipocytes.

Another study carried outshowed that resistin mRNA expression has been shown in preadipocytes and in white adipose tissue (which represent the vast majority ofadipose tissue), andalso stated thatresistinmRNA expressionismoreprominentin the abdominal subcutaneous andomentalfat compared to thigh and mammary fat***(Al-Harithy et al., 2010).***

2-Placenta:

ResistinmRNA expressionhasbeen identified in human placenta where it is localized in trophoblastic cells. Resistin expression was more obvious in term placenta compared to placenta of first trimester. Placental resistinmaybetransportedto thefetus inpregnancy ***(Nakajima et al., 2009).***

Resistin release from placental and adipose tissue was up-regulatedby phorbolmyristateacetate (PMA), which is a potent activator of protein kinase C (PKC). Protein kinase C increasesthe release of several cytokines and other cellular mediatorssuch as TNF-α and IL-6. Itis therefore possible that phorbolmyristate acetate induces resistinreleaseinplacentaand adipose tissue via one of these pathways***(Imai et al., 2009).***

3-White blood cells:

Resistin mRNA expression is present in human peripheral blood monocytes and in macrophages.Resistin mRNA is also abundant in primary acute leukaemia and myeloid cell lines. The significance of the expression of resistin mRNA in blood cells is unknown; however, resistin may play a role in the function ofthese cells ***(Csaba et al., 2003; Patel et al., 2003and Yang et al., 2003).***

4- Pancreas:

Resistin protein and mRNA are present in pancreatic islets. Thismay imply a roleforresistinin the aetiology of insulin resistance ***(Lee et al., 2011).***

5-Synovial Fluid:

Resistin is present in synovial fluid of patients with both rheumatoid arthritis (RA) and osteoarthritis (OA). In patients with inflammatory joint effusion as in rheumatoid arthritis, however, synovial resistin levels were approximately 10 times higher than in those with primarily noninflammatory joint disease such as osteoarthritis or joint trauma. Furthermore, the synovial fluid levels of resistin are positively correlated with systemic markers of inflammation such as erythrocyte sedimentation rate and C-reactive protein. This observation supports the notion that resistin is involved in inflammatory and metabolic pathways in human rheumatological disease ***(Shackelford et al., 2010).***

6-Plasma:

Azuma et al., 2003,reported that, the mean plasma level of resistin in human is 14.3 ng/ml, ranged from (7.3-21.30). The plasma levels of resistinweresignificantly higher in women (3.60 +/- 2.53 ng/ml) than in men (3.15 +/- 2.48 ng/ml), and varied independently of age in either sex. Statistical-genetic analysis revealed significant familial correlations for resistin. Large portion of theresistin variation was attributable to putative genetic factors, also relatively small portion of the resistin variation was attributable to sharing a common household environment and the remaining variation, was due to random environmental (i.e., unmeasured non-additive genetic) effects ***(Pantsulaia et al., 2007).***

G)Inducersofresistinexpression:

1)Hyperglycaemia:

Hyperglycaemia increases resistin expression in the culture adipocyte cell line. Hyperglycaemia is a known cause ofrelease of reactive oxygen (ROS) and nitrogen (RNS) species.

The release of ROS and RNS induces oxidative stress, leading to abnormal gene expression and faulty signal transduction ***(Pantsulaia et al., 2007).***

Chung et al.,2005,suggested that stimulatory protein-1 (Sp1) is an important factor regulating transcription of human resistin gene. A common polymorphism ofthehumanresistinpromoter is critical for the binding of Sp1 and modulates the transcriptional activity of the resistin gene by changing thebindingability ofSp1. In addition, stimulatory protein-1 may be involved in the increase of resistin expression by hyperglycaemia.

2-Steroid hormones:

Both in vitro and in vivo studies have demonstratedthat steroid hormone treatment results in stimulation of resistin secretion, mRNA and protein expression ***(Wang et al., 2010).***

Since resistin has been demonstrated to antagonize insulin's action, it could be a link between steroid hormones and altered glucose metabolism. Steroid hormones have been implicated in the pathogenesis of obesity and diabetes. For

example, the rise of morning cortisol values was positively associated with high body mass index, waist/hip ratio, abdominal sagittal diameter and glucose in men. Moreover, steroid hormones can induce insulin resistance through physiological modulation ofadipocyokines,andthusinsulin resistance***(Garten et al., 2009).***

3- Gonadal Hormones:

Steppan et al.,2001,found thatresistinmRNA levels increase during the secondweek of gestation. Thus, it is possible that the stateof insulin-resistance presentin the second week of gestationcould be mediated by resistin.

It is well known that pharmacologicalligands for the nuclear receptor peroxisomeproliferator–activated receptor γ (PPARγ) suppress resistingene expression. Interestingly, the levels of PPARγ markedlydecrease during pregnancy, suggestingthatPPARγdownregulationcould be responsible, at least to some extent, for increasedresistin mRNA levels during pregnancy ***(Gartenet al., 2009).***

4-Proinflammatory Cytokines:

Lipopolysaccharide increases resistin gene expression in human peripheral bloodmonocytes, also tumour necrosisfactor alpha, and Interleukin-6 (IL-6)significantly increased resistin mRNA expression in human peripheral blood mononuclear cells ***(Kim et al., 2007).***

5-Age:

Wilson and Kannel,2002,showed that the prevalenceof type 2 diabetesincreasesprogressively with age, peaking at 16.5% inmen and 12.8%in women at age 75-84. Sinceresistinhasbeendemonstrated to have a role in diabetes, resistin might be increased with increasing age.It is well known thattherisk ofhavingobesityanddiabetesincreases with age. Thereisan age-related increase in total body fat and visceral adiposity until age 65 years which may be associatedwith diabetes and or impaired glucose tolerance ***(Aeghate, 2004).***

6-Gender:

Silha et al.,2003,found higher resistin levels in girls compared with boysof corresponding age and pubertal stage in a large cohort ofchildren and adolescents.

Also **Yannakoulia** found that resistin concentrations were significantly higher in women compared to men***(Krassas et al., 2006)***. This observations show thatthe significance ofgender onthedegreeofresistin expression in adipocytes is far from understood ***(Adya et al., 2008).***

H) Inhibtors ofresistin expression:

1-Insulin:

Insulincansignificantly suppress resistin expression in culture adipocyte cell lines, asinsulintreatmentcauseda reduction ofresistin mRNAintime-dependentanddose-dependentmanners ***(Stepien et al., 2012).***

Pre-treatment with an inhibitor of extracellular signal-regulated kinasepathway, oraninhibitorofp38mitogen-activatedprotein-kinase(p38 MAP-kinase) pathway, didnot influence insulin-induced reduction of resistin mRNA. Inhibition ofPI 3-kinasealso failed to block insulin-induced reduction of resistin mRNA. Cycloheximide, a protein synthesis inhibitor, completely blocked insulin-induced reduction ofresistinmRNA.Actinomycin D, an RNA synthesis inhibitor, also blocked insulin-induced reduction of resistin mRNA, and the decreasing rate of resistin mRNA in cells treated with insulin alone was faster than that with actinomycin D. Thus insulin downregulates resistinmRNA via PI 3-kinase, extracellular signal-regulated kinase (ERK) or p38 MAP-kinase independent pathways in culture adipocytes cell line***(Nannipieri et al., 2009).***

In the study of***Lappas et al., 2004***, they found that insulin stimulated the release of resistin from humanplacenta. Normal pregnancy is considered a state of hyperinsulinemiaand insulin resistance, and this study demonstrates that increased resistinsecretion by insulinis consistent witharole for resistinin the induction of insulin resistance during pregnancy.

2-Endothelin-1:

Endothelin-1 (ET-1) is an endothelium-derived peptide with potent vasoconstrictor and proliferative properties. Endothelin-1 is produced in a variety of tissues. It acts as a modulator of vasomotor tone, cell proliferation, and hormone production. Endothelin-1 has vasoconstrictor, positive inotropic,

mitogenic and metabolic properties and inhibits resistin expression in culture adipocytes cell line***(Kaplan et al., 2012).***

Increased levels of plasma ET-1 have been observed in numerous diseases, including human congestive heart failure, obesity and diabetes. In all of these disease conditions, a strong correlation between ET-1 levels and the severity of the disorder has been observed. Interestingly, human recombinant resistin can induce an increase in ET-1 release and ET-1 mRNA expression in endothelial cells. This effect was specific to resistin because resistin neutralizing antibodies completely inhibited resistin-induced ET-1 release ***(Verma et al., 2003).***

3-Neurotransmitters:

Isoproterenol, which is a sympathomimetic synthetic derivative of noradrenaline, inhibitsresistingene expression in the culture adipocyte cell line by 20% when compared to non-treated controls. The inhibition of resistin gene expression by isoproterenol is mediated via a G-protein-coupled pathway and adenylyl cyclase. The β-adrenergic receptorantagonist propranolol reverses theinhibitory effect of isoproterenol, whereas the β-adrenergic receptor antagonist phentolamine has no effect ***(Bossowski et al., 2010).***

Epinephrine, which is one of the small molecular neurotransmitters,is able to moderately inhibit resistin expression of both the transcript and protein forms of by 30-50% in the culture adipocyte cell line ***(Luvizotto et al., 2011).***

4-Peroxisome Proliferator-Activated Receptorγ:

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor with an important role in the regulation of adipocyte differentiation, andlipidandglucosemetabolism.AlthoughPPARγ is found in several typesoftissues,itis most abundant in adipose tissue. Peroxisome proliferator-activatedreceptor γtogetherwiththeretinoid X receptor binds to DNA as a heterodimer, acting as a transcription factor to regulate the productionofproteinsinvolvedinlipidand glucose metabolism. Peroxisomeproliferator-activated receptor γ plays a key role in adipogenesis because it regulates the differentiation of preadipocytes to adipocytes in cooperation with other transcription factors. Moreover, it is involved in the modulationofinsulinsensitivityandregulationofthe endocrine functions of fat tissue ***(Krassas et al., 2006).***

Overexpression of PPARγreduces resistin expression. The PPARγ-inducedreductioninresistin expression reached 80% after exposure to 100 nM rosiglitazonefor 96 h. One group of PPARγ agonists, the thiazolidinediones (TZDs), decreases insulin resistance, and because of this effect the three currently available TZDs (troglitazone, rosiglitazone and pioglitazone) have been approved for the treatment of type 2 diabetes. Thus thesedatamatchedwiththeroleof resistin in insulin resistance ***(Chiamolera et al., 2009).***

Thefact that several factors (table 7) can induce or reduce resistin mRNA expressioninhuman tissuessuggeststhatresistin can be controlled by therapeutics (insulin, PPARγ agonists) as well as life style (fasting/dieting). This could imply that obesity-induced type 2 diabetes, involving a possible overexpression of resistin, can be managed ***(Unek et al., 2010).***

I)Clinical Significance of Resistin:

1-Resistin and Obesity:

The role of resistininobesityiscontroversial. Some Studies suggested that elevated resistin expression is a result of adipocyte differentiation. Moreover, the increase in adipocyte number causing a rise in localresistinproduction, inhibitinginsulin action on glucose uptake in adiposetissueand thus, preventing further adipocyte differentiation.

Therefore, at least in rodents, a regulatory feedback mechanism forresistin in adipogenesis may occur, acting as an adipose sensor for nutritional status***(Wanget al., 2010)***.

2-Resistin, insulin resistance and type 2 diabetes mellitus:

It is currently established that central obesity is a contributing factor to the pathogenesis of insulin resistance and consequently to type 2 diabetes mellitus.

Although it is apparent that inconsistenciesremain in the dataforarole of resistin in obesity, there is a growing body of

evidence suggesting a role for resistin in the etiology of insulin resistance and type 2 diabetes mellitus ***(Luvizottoetal., 2011)***.

Early rodent studies determined that reduced serum resistin levels in mice were associated with decreased adiposity and improved insulin sensitivity **(*El Gawadet al., 2012)***.

***Rajala et al.,2003*,**demonstrated that circulating resistin levels were significantly elevated and positively concordant with rising levels of insulin, glucose and lipids in mice.

Other study also highlighted the potential interplay between resistin and leptin, withleptinsuppressingresistinmRNAand protein levels, concomitant with the reduction in glucose and insulin***(Kaplan et al., 2012).***

In evaluating resistin and its association with insulin sensitivity in humans, several studies have identified positive correlations between resistin levels and insulin resistance in vivo and in vitro. Additionally, serum resistin levels were increased by approximately 20% in type 2 diabetes mellitus subjects ***(Bossowskiet al.,2010).*** In contrast other studies have reportedno associations between serum resistin levels and markers of insulin resistance in type 2 diabetes mellitus patients or insulin-resistant patients ***(Kaplan et al., 2012).*** Moreover, serum and plasma resistin levels were either reduced or increased in type 2 diabetes mellitus patients with no significant correlation with HOMA-IR (homoeostasis model assessment

for insulin resistance), waist circumference, BMI or total cholesterol. Consequently, these studies suggest resistin is unlikely to play a critical endocrine role in insulin resistance or energy homoeostasis in humans **(*Nannipieriet al., 2009)***.

Resistin expression is reduced by the anti diabetic drug (TZDs), this suppression may contribute to the insulin-sensitizing and glucose-lowering actions of the TZDs. Furthermore, the potential anti-inflammatory effects of TZDs onadipocytokine mediation may be of equal importance in the prevention of type 2 diabetes mellitus***(Schulzeet al., 2011)***.

3-Resistin and Inflammation:

Thefact that resistin is abundantly expressed in bone marrow cells, in particularin leukocytes and macrophagesand thatmolecules of the RELM family are foundin inflamed tissues suggeststhat resistin can play a role in the inflammatory process ***(Toruner et al.,2008).***

A significant association between resistin concentration andmarkers of inflammation has been demonstrated in subjects withsevere inflammation. Proinflammatory cytokines, such asIL-1, IL-6, TNF αand also lipopolysaccharides (LPS)increaseresistin mRNA expression in human peripheral blood mononuclearcells in vitro. LPS treatment also increases resistinproteinsecretion from primary human macrophages. In addition,resistinhasbeen reported to increase the expression of adhesionmolecules, such as vascular cell adhesion molecule-1, monocytechemoattractant chemokine-1 and antiintercellular

adhesionmolecule-1 in endothelial cells in vitro. Therefore,resistin could mediate the inflammatory effects onarterialwalland contribute to the development of atherosclerosis ***(Sharma et al., 2011)***.

Several studies indicate that resistin may promotetheinitiation or perpetuation of the atherosclerotic state by activating vascular endothelial cells***(Devaraj et al., 2009).***

Other evidence linking resistin to inflammation is that plasma resistin levels were found associated with many inflammatory markers in some pathophysiologicalconditions ***(Sharma et al., 2011)***.

It was found that persons with clinical signs of severe inflammation showed significantly higher concentrations ofresistinthan healthy individuals. Also a significant positive correlation between resistin and inflammatory markers as C-reactive protein was showed***(Wang et al., 2010)***.

Inhuman study, synovialfluidfrompatients with rheumatoid arthritis (RA) showed significantly higher level of resistin compared with control samples. Moreover, resistin level in RA synovial fluid positively correlated with synovial leukocyte count and IL-6 level. However, plasma resistin concentrations were not different between RA patients and healthy counterparts. Thus, the role of resistin in RA is apparent, but the underlying mechanism needs further investigations***(Bokarewa et al., 2005)***.

Also injection of resistin into mice joints induces an arthritis-like condition, with leukocyte infiltration of synovial tissues, hypertrophy of the synovial layer and pannus formation ***(Unek et al.,2010)***.

4-Resistin and Liver Diseases:

Resistin is regarded as an important factor to promote the development of obesity and insulin resistance. Obesity and insulin resistance are part of the alterations knownas the metabolic syndrome, which also includes hypertensionand dyslipidemia.

Non-alcoholic fattyliver disease isconsideredthehepaticmanifestation of the metabolic syndrome and ina subset of patients, non-alcoholic steatohepatitis may leadto progressive fibrosis and end-stage liver disease***(Unek et al.,2010)***.

In additionobesity and insulin resistance have been shown to acceleratethe fibrogenic progression of different types of chronic liverdisease, including those caused by hepatitis C virus (HCV) infection,alcoholor iron overload ***(Powell et al., 2005)***.

Adipokinesrepresent a class of molecules possibly connecting these phenomenavia a direct action on the biology of hepatic stellate cells(HSCs). HSCs are key cellular elements involved in liverwound healing and the development of hepatic fibrosis ***(El Gawad et al., 2012)***.

In human study, quantitative RT-PCR(reverse transcriptase PCR) analysisdemonstratedthatresistin mRNA was barely detectable in normalliver. However, when tissue obtained from patientswith end-stage liver disease was analyzed, a marked up-regulationofresistinexpression was observed, indicating increased intrahepaticexpression of this adipokine in conditions of chronic damageand repair ***(El Gawad et al., 2012)***.

Also, using immunohistochemistry, in normal human liver, lowlevels of specific signal for resistin were observed in theportal tract and in surrounding hepatocytes. Incontrast, in patients with HCV-induced chronic hepatitis, resistinexpression was increased particularly in areas of inflammationand ongoing fibrogenesis **(Bertolani et al., 2006)**.

5-Resistin, Atherosclerosis and Acute Coronary Syndrome (ACS):

Inflammatory process has been connected with the pathogenesis of atherosclerosis.

Several studies indicate that resistin may promote the initiation or perpetuation of the atherosclerotic state by activating vascular endothelial cells***(Bertolani et al., 2006)***.***Verma et al.,2003,***found that resistin promoted endothelial cell activation by promoting endothelin-1 release, partly byinducing endothelin-1 promoter activity. Furthermore, resistin upregulated vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemotactic protein-1 (MCP-1), and downregulated TNF-receptor associated

factor-3, an inhibitor of CD40 ligand signaling which can induce MCP-1 production. Other investigators observedthatresistincouldinduceadhesionmolecules VCAM-1 and ICAM-1, and long pentraxin 3 (a marker of inflammation) in vascular endothelial cell ***(Chiamolera et al., 2009).***

Reillyet al.,2005,reported that, in non-diabetic and diabetic subjects, plasma resistin levels were associated with metabolic and inflammatory markers, includingsolubletumournecrosisfactor-receptor 2 (sTNF-R2), IL-6 and lipoprotein-associated phospholipase A_2 (LpPLA2).Therefore,resistinis considered an inflammatory marker of atherosclerosis in humans and may represent a link between metabolic signals, inflammation and atherosclerosis ***(Krassas et al., 2006).***

ACS results from a rupture of vulnerable plaques in coronary atherosclerosis and leads to severe coronary ischemia***(Luvizottoet al., 2011)***.

Resistin is concentrated in atherosclerotic plaques and its expression is increased with the development of atherosclerosis. Acute rupture of atherosclerotic plaques could be an important origin of circulating resistin. Myocardial injury induces inflammation reactions as well. Macrophages and multiple active factors in ruptured plaques are released into the circulation in ACS. Activated inflammatory cells release multiple cytokines, andinflammatory cytokines and

endotoxemia lead to a cascade of inflammationand stimulate macrophages to secreteresistin, which could be another pathway to increasing resistin levels in ACS. Furthermore, increasing levels of glucocorticoid in a stressed state such as ACS could stimulate elevatedlevels of plasma resistin as well ***(Krassas et al., 2006).***

The increasinglevel of plasma resistin could influence the cardiovascular system via several mechanisms. Resistin could induce insulin resistanceand leadtometabolicdisordersofglucose, lipid and free fatty acid, which further accelerate metabolic dysfunction in ACS. Resistin could activate endothelial cells and promote expression of inflammatory cytokines, chemokinesandadhesion cytokines, thusaccelerating endothelial dysfunction. It could also suppress the expression of nitric oxides and induce superoxide anion production in endothelial cells, which weaken the endothelial-dependent vascular relaxation and impair normal contraction relaxation regulation in coronary vessels. Furthermore, it could stimulate migration and proliferation of smooth muscle cells to accelerate the progression of coronary atherosclerosis.Thus, increasing levels of resistin in patients with ACS may not favor rehabilitation from an acute coronary event through several mechanisms***(Luvizottoet al., 2011)***.

Subjects with pre-clinical atherosclerosis, with a family history of premature coronary artery disease, showed increased plasma resistin level.

Resistin is also associated with coronary artery calcification (CAC), a measure of coronary atherosclerosis, even in patients without established risk factors, metabolic syndrome or increased CRP level***(Reillyet al., 2005)***.Itwas proposed that serum resistin might be a biological marker forCADandre-stenosis after percutaneous coronary intervention (PCI) in type 2 diabetes mellitus patients.

Circulating resistin level is further increased during an acute event in patients with unstable angina(UA),ST segment elevation myocardial infarction (STEMI)or non-STEMI as compared with patients with SA. Thus, resistin level intends to be increasingly elevated with atherosclerosis progression. Plasma resistin level increased markedly to 2.8 fold the control level at 24 h in patients with ACS and remained at a high level for 1 week after symptoms onset. Plasma resistin level in patients with AMIwas higher than that in patients with UA. It wasalso found that plasma resistin level was correlated with levels of cardiac injury biomarkers and inversely correlatedwith left ventricular ejection fraction (LVEF), thus suggesting that resistin level increased with severity of ischemic injury to the myocardium***(Luvizottoet al., 2011)***.

6- Resistin and Pre-eclampsia:

Preeclampsia shares cardiovascular risk factors with the metabolic syndrome such as subclinical inflammation, insulin resistance, and obesity. Since resistin levels are enhanced in all these pathological conditions, several studies have aimed to determine if resistin may contribute to preeclampsia***(Krassas et al., 2006).***

J) Methods of Assay of resistin:

1)Enzyme-Linked Immunosorbent Assay (ELISA):

Two site-enzyme-linked immunosorbent assay is one of the most commonly used techniques for measuring antigens in serum. The technique involves immobilizing antibody to the protein of interest within the plastic wells of a microtiter plate. Unbound binding sites within the plate are then blocked by incubation with an irrelevant protein. Samples are added to the antibody-coated wells and incubated for a couple of hours to allow capture of the antigen by antibody. Following washing to remove non binding material, the bound antigen is then detected by adding a second antibody which is directed against a different binding-site on the antigen to the one recognized by the capture antibody. The antigen is now sandwiched between the two antibodies giving rise to the terms sandwich ELISA or antigen-capture assay. The detection antibody is conjugated to an enzyme which, upon addition of the enzyme substrate, produces a colored quantity of standard or unknown in the reaction increases, the amount of enzyme labeled peptide (after addition of a chromogenic substrate) able to bind to the antibody is decreased. By measuring the amount of enzyme labeled peptide, it is possible to construct a standard curve from which the concentration of peptide in unknown samples can be determined (figure 7) ***(Fujinami et al., 2006).***

Measurement of resistinby ELISA has advantages of that it is quick and convenient, antigens of very low or unknown concentration can be detected since capture antibody only grabs specific antigen, moreover it is generally safe (do not require radioactive substances, contains diluted sulfuric acid).

a- Principle of the assay:

A sandwich ELISA for human resistin has been developed, using two polyclonal antibodies. The first antibody is directed to the N(21-40)-terminal region of resistin, it is designated as the capture antibody and is coated on the wells of a microtitre plate. The second antibody is specific for the C(79-91)-terminus and is conjugated to horseradish peroxidase. It is designated the tracer antibody. Enzyme-linked immunosorbentassayis based on the sandwiching of antigen between the capture antibody coated on the plates and the tracer antibody. The addition of a substrate produces a colour. The reaction is then stopped by HCl, and the solution turns to yellow. The absorbance is measured at 492nm ***(Fujinami et al., 2006).***Principle of Sandwich Enzyme Linked Immunosorbant AssayFigure (8)***(Krassas et al., 2006).***

b-Sample preparation and storage:

The assay can be performed on either serum or plasma. Blood samples are collected on plain test tubes and blood is allowed to clot for 30 minutes before centrifugation at 4°C for 15 minutes at approximately 1000 x g then serum should be separated.

Plasma samples are collected using heparin or EDTA as an anticoagulant and blood is centrifuged at 4°C for 15 minutes at 1000 x g within 30 minutes of collection and plasma is separated.

Samples can be stored at -20° C until the assay time. Repeated freeze-thaw cycles should be avoided. Citrated plasma has not been validated for use in assay. Serum and plasma samples require a 5-fold dilution with a calibrator diluent ***(Krassas et al., 2006).***

c- Linearity:

This method is linear up to 1.5 ng /mL ***(Azuma, 2003).***

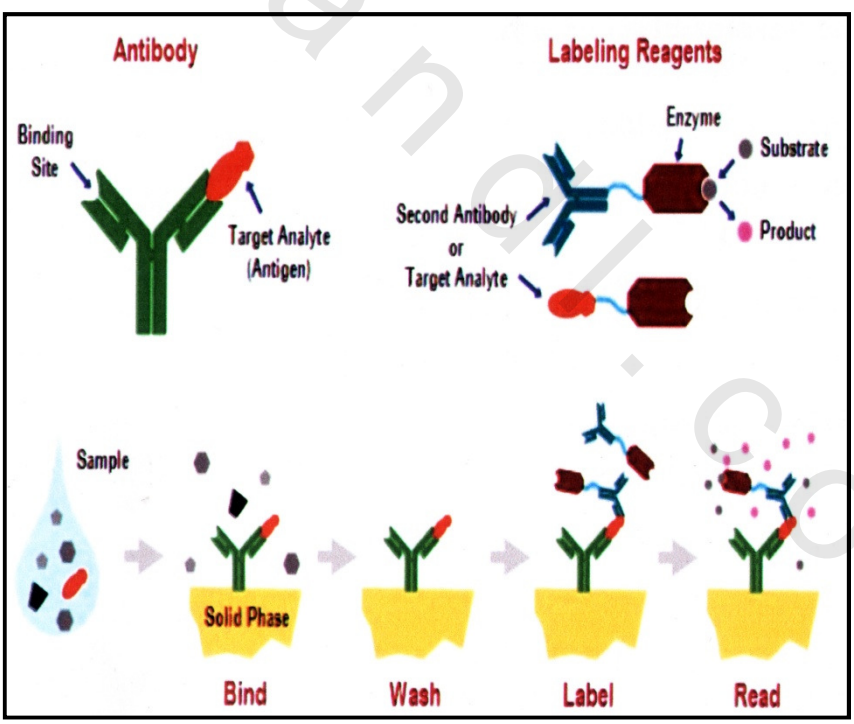

Figure (8): Principle of Sandwich Enzyme Linked Immunosorbant Assay ***(Krassas et al., 2006).***

d-Sensitivity:

Forty assays were evaluated and the minimum detectable dose (MDD) of resistin by ELISA ranged from 0.010-0.055 ng/mL. The mean MDD was 0.026 ng/mL ***(Azuma, 2003).***

e-Specificity:

This assay recognizes recombinant and natural human resistin. Factors like leptin, leptin R and RELM- β were prepared at 100 ng/mL in Calibrator Diluent RD5K and assayed for cross-reactivity. No significant cross-reactivity or interference was observed ***(Azuma, 2003).***

2)- Reverse Transcriptase Polymerase Chain Reaction (RT-PCR):

This technique is used for detection of mRNA expression of resistin gene in human tissues (adipose tissues) by using primers based in exons 1 and 2 of the human gene. Reverse transcriptase polymerase chain reaction (RT-PCR) is a laboratory technique for amplifying a defined sequence of ribonucleic acid (RNA) molecule. The RNA strand is first reverse transcribed into its complementary DNA, followed by amplification of the resulting DNA using polymerase chain reaction. This can either be a 1 or 2 step process. Polymerase chain reaction itself is the process used to amplify specific sequence of a DNA molecule via the temperature-mediated enzyme DNA polymerase ***(Fujinami et al., 2006).***

In the first step of RT-PCR, called "the first strand reaction "complementary DNA is synthesized from a messenger RNA template using a Reverse transcriptase through the process of reverse transcription. After the reverse transcription reaction is completeandcomplementary DNA has been generated from the original single-strandedmRNA stranded polymerase chain reaction, termed " the second strand reaction " is initiated***(Fujinami et al., 2006).***

RT-PCR is the most sensitive method of mRNA detection available, but it does have drawbacks. It can be the most technically challenging method of detection and quantitation, often requiring substantial pre-experimental planning and design. Additionally, because of its extreme sensitivity, even minute amounts of contamination by genomic DNA or previously amplified PCR products can lead to aberrant results, so steps must be taken to avoid this pitfall ***(Fujinami et al., 2006).***

a- Principle of the assay:

The assay is based upon isolation of the total RNA using the single step guanidinium thiocyanate-phenol-chloroform extraction method. First strandcDNA is synthesized. The resulting cDNA is subjected to PCR amplification in a flurescent thermocycler. PCR cycling condition include an initial denaturation, annealing and extension. PCR products are separated by electrophoresis and visualized by ultraviolet light ***(Seow et al., 2004).***

The PCR primers specific for resistin are 5'-TGC AGG ATG AAA GCT CTC TG-3' for sense and 5'-CCA GGT TTA TTT CCA GCT CC-3' for antisense.

First strand cDNA is synthesized. It is amplified by PCR using a DNA thermal cycler. It is found that a minimum of 30 PCR cycles are required to produce an optimal amount of nucleic acids for measuring on an agarose gel ***(Seow et al., 2004).***

b-Sample preparation and storage:

In this assay, total RNA is extracted from the adipocytes isolated from omental fat tissue using a Tri reagent kit. The integrity of a total RNA is examined by 1% agarose gel electrophoresis, and the RNA concentration is determined by UV absorbance at 260 nm. Samples can be stored at – 70 °C until the assay time ***(Seow et al., 2004).***