

# THE CORRELATION OF INSULIN RESISTANCE WITH SERUM TNF ALPHA LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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## Abstract

**Objective:** The aim of this study was to assess the occurrence of acute insulin response and insulin sensitivity changes in patients with rheumatoid arthritis, and to evaluate the correlation of serum TNF- $\alpha$  a level changes with glucose metabolism abnormality such as insulin resistance and impaired acute insulin response.

**Methods:** 36 subjects (22 women and 14 men, aged  $51.5 \pm 17.1$  years (range 21-80), BMI  $27.1 \pm 5.0$  kg/m<sup>2</sup>;) with varying degrees of disease activity and 20 healthy controls were studied. After a 12-h overnight fast, all subjects underwent a diagnostic protocol including serum insulin level, HOMA-IR estimated insulin sensitivity, AIR derived from IVGTT data and serum TNF- $\alpha$  a level measurements.

**Results:** The fasting insulin levels, HOMA scores and serum TNF- $\alpha$  a levels were significantly higher in all patients with rheumatoid arthritis than in control subjects ( $14.7 \pm 6.7$  vs.  $8.7 \pm 1.9$  mIU/ml,  $p < 0.001$  and  $3.3 \pm 1.5$  vs.  $1.9 \pm 0.5$ ,  $p < 0.001$  and  $368.4 \pm 649.2$  vs.  $3.6 \pm 5.0$  pg/ml,  $p < 0.001$  respectively). Acute insulin responses were significantly lower in all patients with rheumatoid arthritis than in control subjects ( $35.8 \pm 17$  vs.  $85.2 \pm 17.9$ ,  $p < 0.001$ ). Fasting insulin, HOMA-IR, TNF- $\alpha$ , AIR were correlated in the whole group.

**Conclusions:** Insulin resistance seems to be the main metabolic abnormality which alters glucose metabolism, decreases the sensitivity of peripheral tissues to insulin in patients with rheumatoid arthritis, and complicating rheumatic disease via an increase in atherosclerotic disease risk and prediabetic state.

## Rezumat

### Corelația rezistenței la insulină și nivelul factorului TNF alfa seric la bolnavii cu poliartrită reumatoidă

**Obiectiv:** Obiectivul studiului este să evalueze apariția răspunsului insulinic și a sensibilității la insulină la bolnavii cu poliartrită reumatoidă și să evidențieze corelația dintre modificările TNF alfa seric și anomaliile metabolismului glucidic, cum ar fi rezistența la insulină și modificarea răspunsului la insulină.

**Metode:** 36 de subiecți (22 femei și 14 bărbați, vârsta medie  $51,5 \pm 17,1$  ani, limite 21-80, cu indicele de masă corporală  $27,1 \pm 5,0$  kg/m<sup>2</sup>), cu diferite grade de activitate a bolii, și 20 de martori sănătoși au fost incluși în studiu. După 12 ore de post, aceștia au fost incluși într-un protocol de diagnostic, care a cuprins insulinemia, sensibilitatea la insulină și nivelul seric al TNF alfa.

**Rezultate:** Insulinemia à jeune, scorurile HOMA și nivelurile TNF alfa au fost semnificativ mai mari la pacienții cu poliartrită reumatoidă față de subiecții de control. Răspunsul acut la insulină a fost semnificativ redus la toți bolnavii cu poliartrită reumatoidă față de lotul martor. Cei trei parametri au fost corelați între ei la fiecare grup.

**Concluzii:** Rezistența la insulină pare să fie anomalia principală care alterează metabolismul glucidic, scade răspunsul țesuturilor periferice la insulină la bolnavii cu poliartrită reumatoidă și complică boala reumatologică prin creșterea riscului de ateroscleroză.

## INTRODUCTION

The possibility that there can be defects in the sensitivity of tissues to insulin action in people with diabetes was first reported nearly 50 years ago. Since then many investigators have described, with increasing detail, the pathogenesis and consequences of what has become known as "insulin resistance". Insulin resistance has clearly emerged as an important cause of glucose intolerance leading to type 2 diabetes, and may even play a role in other pathological conditions. More recently,

insulin resistance has been shown to be associated with prevalent atherosclerosis. Thus, the recognition of insulin resistance seems to have investigational and clinical relevance in identifying subjects at high risk of cardiovascular disease.

Insulin resistance is an important risk factor for type 2 diabetes and cardiovascular disease (1). There is increasing evidence supporting the fact that by the time glucose tolerance or fasting glucose levels become impaired, appreciable  $\beta$ -cell destruction may have already occurred (2). Predicting insulin sensitivity in normoglycemic individuals is important,

as diabetes intervention programs are more likely to be successful at this stage rather than after the development of impaired glucose tolerance. Thus, it seems likely that attempts to prevent type 2 diabetes will be more successful if intervention is commenced when blood glucose levels are still in the normal range (3).

Several studies demonstrated that insulin resistance is a strong predictor of type 2 diabetes (4,5). More recently, insulin resistance has been shown to be associated with prevalent atherosclerosis (6,7,8). Thus, the recognition of insulin resistance seems to have clinical relevance in identifying subjects at high risk of type 2 diabetes and/or cardiovascular disease (9).

## PATIENTS AND METHODS

Thirtysix patients with rheumatoid arthritis (14 males and 22 females, mean age;  $51.5 \pm 17.1$  years (range 21-80), mean BMI:  $25.8 \pm 2.9$  kg/m<sup>2</sup>) who were recruited among those regularly attending the Internal Medicine Clinic at Gülhane Military Medical Academy, Haydarpasa Training Hospital and 20 age-, sex-, and BMI-matched healthy controls (8 males and 12 females, mean age;  $46.6 \pm 13.9$  years (range 22-71), mean BMI:  $25.2 \pm 3.2$  kg/m<sup>2</sup>) were enrolled in the study. Patients with any other causes of peripheral insulin resistance and hyperlipidemia were excluded. All subjects were willing to participate in the study. All subjects gave their written informed consent to participate in the study. They reported no alterations of body weight for at least three months before the study. All participants underwent a physical examination and routine blood chemistry evaluation. None of them had a history of recent acute illness or clinical evidence of cardiovascular, kidney, liver, or endocrine diseases. Patients with type 2 diabetes and other concomitant severe renal, hepatic or cardiac disease were excluded from the study. Main characteristics of the study and control subjects are shown in table 1.

The diagnosis of rheumatoid arthritis was based on the American Rheumatism Association 1987 Revised Criteria for The Classification of Rheumatoid Arthritis (10). Fasting blood samples were collected via an intravenous catheter in ethylenediamine tetraacetate-coated venepuncture tubes, promptly centrifuged, separated and stored at  $-70$  C until insulin assays were performed. Glucose was measured a glucose oxidase method (*Hitachi 736 Auto Analyzer, Hitachi Co., Japan*). Immunoreactive insulin was determined by double-antibody radioimmunosassay (RIA) (*COAT – A – COUNT, Los-Angeles, USA*). HOMA-estimated insulin sensitivity was calculated with the formula that was defined by Matthews (11), as follows:  $HOMA - IR = [Fasting\ Insulin\ (\mu IU/ml) \times (Fasting\ glucose\ (mmol/l))] / 22.5$ . With this method, high HOMA-IR score shows low insulin sensitivity.

## STATISTICAL ANALYSIS

All data were presented as mean  $\pm$  SD. For comparison of study and control groups data, the Mann-Whitney U test was used. Pearson correlation analysis was used for estimating the relation between test parameters. Probability levels less than .05 were considered significant.

## RESULTS

The fasting insulin levels were significantly higher in all patients with rheumatoid arthritis than in control subjects ( $14.7 \pm 6.7$  vs.  $8.7 \pm 1.9$  IU/ml,  $p < 0.001$ ). HOMA scores and serum TNF- $\alpha$  levels were significantly higher in all patients with rheumatoid arthritis than in control subjects ( $3.3 \pm 1.5$  vs.  $1.9 \pm 0.5$ ,  $p < 0.001$  and  $368.4 \pm 649.2$  vs.  $3.6 \pm 5.0$  pg/ml,  $p < 0.001$  respectively). Acute insulin responses were significantly lower in all patients with rheumatoid arthritis than in control subjects ( $35.8 \pm 17$  vs.  $85.2 \pm 17.9$ ,  $p < 0.001$ ). (Table 2)

Fasting insulin, HOMA-IR, TNF- $\alpha$ , AIR were correlated in the whole group. There was a positive

Table 1

Variable n	Study subjects 36	Control subjects 20
Sex (M/F)	14/22	8/12
Age (years)	$51.5 \pm 17.1$	$46.6 \pm 13.9$
BMI (kg/m <sup>2</sup> )	$25.8 \pm 2.9$	$25.2 \pm 3.2$
Fasting glucose (mmol/L)	$5.1 \pm 0.5$	$5.0 \pm 0.4$

Tabel 2

Variable n	Study subjects 36	Control subjects 20	P values
Sex (M/F)	14/22	8/12	---
Age (years)	51.5±17.1	46.6±13.9	NS
BMI (kg/m <sup>2</sup> )	25.8±2.9	25.2±3.2	NS
Fasting glucose (mmol/L)	5.1±0.5	5.0±0.4	NS
Fasting insulin (μIU/ml)	14,7±6,7	8,7±1,9	p<0.001
TNF-α (pg/ml)	368,40±649,15	3,55±4,96	p<0.05
HOMA-IR	3,3±1,5	1,93±0,5	p<0.001
AIR (μIU/ml)	35,8±17	85,2±17,9	p<0.001

(NS= non-significant, p&lt;0.05= significant)

Tabel 3

TNF-α	0,311*		
AIR	-0,446**	-0,305*	
Insulin	0,981**	0,355**	-0,408**

\* p&lt;0,05; \*\* p&lt;0,01

significant correlation between TNF-α and fasting insulin, HOMA-IR (r=0.98, p<0.01 and r=0.31, p<0.05 respectively). There was a negative significant correlation between TNF-α and AIR (r= 0.31, p<0.05). (Table 3)

## DISCUSSION

Insulin resistance plays a major role in the development of type 2 diabetes (4, 5, 13) and may also be involved in the atherogenesis (6-8). Thus, the assesment of insulin sensitivity has become a frequent need for clinical investigators and epidemiologists to identify subjects at high risk of type 2 diabetes and/or cardiovascular disease (9).

Insulin signaling at the target tissue results in a large array of biological outcomes. These events are essential for normal growth and development and for normal homeostasis of glucose, fat, and protein metabolism. Elucidating the intracellular events after activation of the IR has been the primary focus of a large number of investigators for decades. Understanding the signalling pathways involved in insulin action could lead to a better understanding of the pathophysiology of insulin resistance associated with obesity and type 2 diabetes, and identifying key molecules and processes could lead to newer and more effective therapeutic agents for treating these common disorders (14). Insulin resistance is a common pathologic state in which target cells fail to respond to ordinary levels of circulating insulin. It is frequently associated with a number of diseases, including chronic infection, human obesity, and type 2 diabetes (15).

The vast and growing array of cytokines is the subject of intense research for these potential to ameliorate a range of diseases that extends from autoimmune disorders to cancer and beyond. Among the cytokines, tumor necrosis factor-α (TNF-α) has proven to be a key ligand in triggering intracellular processes, both physiological and pathological rheumatoid arthritis and Chron's disease etc). TNF is a principal mediator of natural immunity. In the 1980s, TNF was cloned, purified, and crystallized, and its tertiary and quaternary structure described. Biological studies showed that TNF increases synoviocyte proliferation, and triggers the release of secondary mediators involved in the recruitment of inflammatory cells in neo-angiogenesis, and in the process of joint destruction. Those actions contribute in a major way to the establishment of rheumatoid synovites, the formation of pannus tissue, and the process of joint destruction. Transgenic mice overexpressing TNF-α develop a rheumatoid – like destructive arthritis (16).

TNF originates as a 26 kDa transmembrane precursor molecule in a variety of cells throughout the body. In rheumatoid arthritis, the principal site of origin appears to be activated macrophages, from which it is cleared by an enzyme called TACE (TNF-α converting enzyme) to a soluble 17 kDa active fragment by a process known as protein ectodomain shedding. It then aggregates into trimolecular complexes travels to fibroblasts or endothelial or inflammatory cells and binds to one of their two TNF receptor sites on the cellular membrane, designated p55 (also called p60) or p75 (also

called p80). Crosslinking two of these receptors initiates its biological action. In the case of rheumatoid arthritis, this action results in joint inflammation, cartilage destruction, and many of the other clinical manifestations of the disease (17).

Although the role of TNF- $\alpha$  in inducing or perpetuating insulin resistance has yet to be confirmed, it represents a paradigm from which investigators have slowly accumulated a large amount of information that has enabled them to formulate more exactly the mechanisms involved in the insulin resistance seen in obesity and type 2 diabetes (14). Since its initial isolation in 1984, tumor necrosis factor has continued to be a major topic of scientific investigation as indicated by over 27 000 citations published within the past 15 years. These studies have indicated that TNF is a pleiotropic cytokine that mediates apoptosis, cell proliferation, immunomodulation, inflammation, viral replication, allergy, arthritis, septic shock, insulin-resistance, autoimmune diseases, and other pathological conditions (18).

Agents that enhance Ser/Thr phosphorylation of insulin receptor substrate (IRS) proteins or other down – stream effectors of the insulin signaling cascade play negative – regulatory roles in insulin action. Ser/Thr phosphorylation impairs insulin – stimulated Tyr phosphorylation of IRS proteins, uncouples insulin signal transduction, and has been implicated in the development of insulin resistance (19,20,21). TNF- $\alpha$  has direct effects on the insulin signaling cascade in cultured cells. TNF- $\alpha$  increases Ser phosphorylation of IRS-1 and IRS-2. Serine phosphorylation of these substrates results in a reduction in both insulin-receptor Tyr outophosphorylation and Tyr kinase activity of the receptor and markedly reduces the ability of the IRS molecules to dock with the receptor and interact with downstream pathways such as PI 3-K and glucose transport (19,22,23). Activation of the peroxisome proliferator-activated receptor-gamma (PPAR-gamma) by thiazolidinediones (TZDs) reduces the expression of TNF- $\alpha$  and hinders TNF- $\alpha$ 's inhibition of insulin action (24).

Circulating levels of TNF- $\alpha$  are elevated in obese subjects and decrease with weight reduction (25). Further support for its role in affecting insulin sensitivity resulted from studies using a soluble TNF- $\alpha$  receptor Ig G fusion protein, which neutralized TNF- $\alpha$  when administered to animals with insulin resistance: this neutralization was associated with

improvement in insulin action (26). Whether the effects of TNF- $\alpha$  are direct or indirect has not yet been determined, because it has been shown that TNF- $\alpha$  stimulates leptin secretion from adipocytes, and free fatty acid (FFA) levels are correlated with TNF- $\alpha$  levels. Both leptin and FFAs play a role in insulin resistance (27).

Although the effects of tumor necrosis factor- $\alpha$  on whole body glucose and lipid metabolism have been known for many years (28,29), the potential relevance to insulin signaling was not described until recently (30). A 2.6-fold increase in insulin-stimulated glucose disposal rates after TNF neutralization in obese rats was reported by Hotamisligil et al. (31), and this has been followed by observations of increased rates of TNF production in adipose tissue and muscle of insulin-resistant humans (32). Halse et al. have investigated the effects of tumor necrosis factor- $\alpha$  in modulating insulin sensitivity of cultured human muscle cells. They reported that TNF decreases insulin-stimulated rates of storage of glucose as glycogen and total glycogen synthase activity (33). Del Aguila et al. have planned an experimental study to determine whether TNF- $\alpha$  decreases insulin responsiveness at the cellular level. Their study data suggested that increases in TNF- $\alpha$  may cause insulin resistance in skeletal muscle by inhibiting IRS-1 and IRS-2-mediated PI3-kinase activation, leading to impaired insulin-stimulated glucose uptake (34).

The present study clearly demonstrated that fasting insulin levels and HOMA scores were significantly higher in all patients with rheumatoid arthritis than in controls. As a marker of insulin resistance, acute insulin responses were lower in all patient with rheumatoid arthritis than in controls. TNF- $\alpha$ , fasting insulin, HOMA-IR, AIR were correlated in whole group. The present study was also the first one that evaluates the importance of serum TNF- $\alpha$  a levels for developing insulin resistance in patients with rheumatoid arthritis. The study data showed a strong correlation among serum insulin levels, HOMA-IR score, AIR and serum TNF- $\alpha$  a levels in patients with rheumatoid arthritis. Such a relationship may prove the pathogenetic role of TNF- $\alpha$  for insulin resistance.

## CONCLUSION

In summary, insulin resistance seems to be the main metabolic abnormality which alters glucose

metabolism, decreases the sensitivity of peripheral tissues to insulin in patients with rheumatoid arthritis, and complicating rheumatic disease via an increase in atherosclerotic disease risk and prediabetic state. Work upon the mechanism of TNF

interference with insulin signaling is therefore required in cells from physiological target organs for insulin action in patients with rheumatoid arthritis.

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