



Serum concentrations of TNF- α and soluble TNF- α receptors in obesity

B Zahorska-Markiewicz^{1*}, J Janowska¹, M Olszanecka-Glinianowicz¹ and A Zurakowski¹

¹Department of Pathophysiology, Silesian University School of Medicine, 40-752 Katowice, Poland

OBJECTIVE: To study whether weight reducing treatment modulates serum concentration of TNF- α and two soluble TNF- α receptors in obese subjects.

SUBJECTS AND MEASUREMENTS: Serum concentrations of TNF- α and two soluble receptors (sTNF-R1, sTNF-R2), plasma glucose, insulin, total cholesterol, HDL-cholesterol and triglycerides were measured in 27 obese subjects (age 48 ± 12 y, body mass index (BMI): 36 ± 6 kg/m²) before and after 3 months weight reducing treatment consisted of a diet & 1000 kcal/day and physical exercises.

RESULTS: The mean loss of weight during 3 months' treatment was 9.3 ± 3.3 kg. The serum concentration of TNF- α decreased after weight loss and at the same time both of the receptors (sTNF-R1, sTNF-R2) increased significantly.

CONCLUSION: The observed decrease of the serum concentration of TNF- α and the increase in both TNF soluble receptors after weight reducing treatment in obese subjects, may be a counter-regulation preventing further weight loss.

International Journal of Obesity (2000) 24, 1392–1395

Keywords: obesity; TNF α ; soluble TNF receptors

Introduction

Tumor necrosis factor- α (TNF- α) is a cytokine mainly produced by macrophages in response to endotoxemia, inflammation and cancer. Human fat cells are a significant source of endogenous TNF- α production.¹ It remains a question whether adipose tissue is a source of circulating TNF- α .² Some have suggested that the increased TNF- α production is restricted to adipose depots.³

TNF- α plays a role as a mediator of insulin resistance.⁴ The binding of insulin to its receptor initiates a phosphorylation cascade, which starts with autophosphorylation of the insulin receptor, IR activation of the receptor tyrosine kinase and subsequent phosphorylation of insulin receptor substrate 1 (IRS-1). Obesity predisposes to insulin resistance.⁵ Studies in animal models have indicated that TNF- α expression in the adipose tissue is significantly elevated in obesity.⁶

TNF- α has a multiple actions in adipose tissue, which include:

- increased insulin resistance associated with the defects in insulin-stimulated glucose disposal;
- decreased activity of lipoprotein lipase (LPL) and increased hormone-sensitive lipase, preventing lipid accumulation.⁷

TNF- α could be a local regulator of fat cell size, and the overproduction of TNF in adipocytes of obese animals could represent a form of adipostat designed to limit adipocyte size enlargement.⁸

TNF- α exerts its effect on cell function by binding to two specific cell surface receptors:⁹ type I, TNF-R55; and type II, TNF-R75. The extracellular portions of this membrane associated with TNF receptors have been separated from them and have been identified in serum as soluble forms (sTNF-Rs). The endogenous formation of TNF- α leads to the shedding of sTNF-Rs which interfere with the binding of TNF- α to cell surface-bound TNF-Rs. The sTNF-R concentration reflects the activation state of the TNF- α /TNF receptor system.

Recent studies show that TNF- α is overexpressed in adipose tissue in human obesity.^{1,7} Plasma concentrations of two soluble TNF receptors were found to be elevated in obesity.^{2,10}

It is still unclear whether an increased adipose production of TNF- α is reflected by elevated circulating TNF- α levels. Several studies have reported a lack of detectable TNF- α in the serum of obese patients¹ or no differences between obese and lean subjects.² This may be a consequence of the low sensitivity of the tests used. It was also suggested that TNF- α action in the insulin resistance of obesity is mediated through an autocrine-paracrine mechanism. Our previous results¹¹ and other studies¹² have shown increased serum levels of TNF- α in obese patients in comparison with lean subjects.

In the present study we examined whether weight reducing treatment modulates serum concentration of

*Correspondence: B Zahorska-Markiewicz, Department of Pathophysiology, Medykow 18, 40-752 Katowice, Poland.
Received 4 October 1999; revised 11 February 2000; accepted 22 May 2000

TNF- α and two soluble TNF- α receptors in obese subjects.

Patients and methods

The study was carried out on 27 obese subjects, 23 women and four men, aged 48.2 ± 11.7 y, height 1.63 ± 0.07 m, and weight 96.9 ± 14.9 kg. Their body mass index (BMI) was 36.3 ± 5.7 . All persons were diagnosed as having simple obesity without additional diseases. Patients with evidence of acute or chronic inflammatory diseases were excluded. The study was conducted after obtaining informed consent from all the subjects. The study was approved by the local Ethical Committee.

After measurement of the baseline values, the subjects participated in a 3 month program that included two-weekly group instruction in behavioral and dietary methods of weight control. All participants were submitted to a 1000 kcal/day balanced diet.

Each obese subject was tested twice: before and after 3 months of weight reducing treatment. The body weight and height were measured and body mass index (BMI) was calculated. The waist-to-hip ratio (WHR) was used as a measure of the pattern of fat distribution. Body composition was determined by impedance analysis using Bodystat analyzer.

Blood samples were collected in the morning, after an overnight fast. Plasma glucose, cholesterol, HDL cholesterol and triglycerides were determined by enzymatic procedure using a commercially available test kit (Cormay). LDL cholesterol was calculated using the Friedwald formula. Insulin was determined by radioimmunoassay (DPC Diagnostic Products Corporation, Los Angeles, USA).

The concentrations in blood serum of TNF- α and soluble forms of both TNF- α receptors (sTNF-R1, and sTNF-R2) were measured using a commercially available highly sensitive ELISA kits (Genzyme Diagnostics, Cambridge, USA). The detection limits were: for the TNF- α , 3.0 pg/ml; sTNF-R1, less than 3.0 pg/ml; and for sTNF-R2, 1.0 pg/ml. The inter-assay and the intra-assay coefficients of variation were less than 8.8%.

Data were analyzed using paired *t*-test. *P*-values < 0.05 were considered to be statistically significant. Values are presented as mean \pm s.d. Multiple regression analyses were performed.

Results

The main characteristics of the patients and the effect of treatment are presented in Table 1. The mean loss of weight during treatment was 9.3 ± 3.3 kg, and BMI decreased from 36.3 ± 5.7 at the baseline to 33.3 ± 4.9 after treatment. Plasma concentration of cholesterol,

LDL-cholesterol and triglycerides decreased ($P < 0.01$, $P < 0.05$, $P < 0.01$ respectively); no significant changes of glucose and insulin were observed (Table 2). The serum concentration of TNF- α significantly decreased after weight loss (Table 3).

The most pronounced differences during weight loss were observed in the soluble TNF receptors. Both receptors sTNF-R1 and sTNF-R2 increased significantly ($P < 0.0001$), by approximately 40%.

There was a positive correlation (Table 4) between the serum concentration of sTNF-R1 and body weight; the sTNF-R2 correlated with body fat (% and kg). We performed a multiple regression analysis using Δ TNF- α , Δ receptor TNF-1 and Δ receptor TNF-2 as dependent variables. Models were fitted to estimate the role of gender, age, WHR, loss of weight (Table 5) and plasma glucose, insulin and

Table 1 Patient characteristics and the effect of weight reducing treatment

	Before	After
Weight (kg)	96.9 \pm 14.9	87.6 \pm 14.9***
BMI	36.3 \pm 5.7	33.3 \pm 4.9**
Waist	99.0 \pm 21.2	97.7 \pm 16.4
Hip	120.3 \pm 9.7	113.9 \pm 9.3***
WHR	0.89 \pm 0.24	0.83 \pm 0.08
Body fat (kg)	40.3 \pm 9.2	34.2 \pm 9.7***
Body fat (%)	42.5 \pm 6.1	38.7 \pm 6.6***
Fat-free mass (kg)	54.2 \pm 9.4	53.5 \pm 9.5*
Fat-free mass (%)	57.3 \pm 6.0	61.7 \pm 7.4***

BMI = body mass index; WHR = waist-to-hip ratio.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.

Table 2 Plasma lipids, glucose and insulin

	Before	After weight loss
Total cholesterol (mg/dl)	208 \pm 45	188 \pm 40**
HDL cholesterol (mg/dl)	43 \pm 11	43 \pm 12
LDL cholesterol (mg/dl)	142 \pm 46	126 \pm 35*
Triglycerides (mg/dl)	118 \pm 39	97 \pm 33**
Glucose (mg/dl)	94 \pm 14	93 \pm 9
Insulin (μ U/dl)	13 \pm 8	12 \pm 5

* $P < 0.05$; ** $P < 0.01$.

Table 3 Serum concentrations of TNF- α and soluble receptors (pg/ml)

	Before	After weight loss
TNF- α	7.79 \pm 4.65	6.09 \pm 2.96**
sTNF-R1	1719.48 \pm 688.32	2389.81 \pm 313.88***
sTNF-R2	1643.70 \pm 584.94	2414.30 \pm 211.27***

** $P < 0.01$; *** $P < 0.0001$.

TNF = tumor necrosis factor; sTNF-R1, R2 = soluble TNF receptors.

Table 4 The significant correlation coefficients

	TNF- α	sTNF-R1	sTNF-R2
Weight	—	0.27	—
Body fat (%)	—	—	0.25
Body fat (kg)	—	—	0.26

TNF = tumor necrosis factor; sTNF-R1, R2 = soluble TNF receptors.

Table 5 Regression analysis with Δ TNF- α and Δ receptors TNF-1 and TNF-2 as dependent variables

	Sex	Age	WHR	Δ weight
Δ TNF- α	- 641.4*	- 4.9	576.1	- 61.4*
Δ receptor, TNF-1	- 806.4*	- 2.3	1303.9*	- 56.8
Δ receptor, TNF-2	576.1	- 4.9	- 641.4*	- 61.4*

* $P < 0.05$.

Table 6 Regression analysis with Δ TNF- α and Δ receptors TNF-1 and TNF-2 as dependent variables

	Sex	Age	Glucose	Insulin	Δ cholesterol
Δ TNF- α	3.0*	0.03	- 0.004	- 0.1	0.6***
Δ receptor, TNF-1	- 295.9	- 7.2	- 1.4	- 1.4	7.5
Δ receptor, TNF-2	- 473.0*	- 4.3	2.6	4.4	6.8

* $P < 0.05$; *** $P < 0.0001$.

Δ cholesterol concentrations (Table 6). Δ TNF- α and Δ receptor TNF-2 concentrations were significantly associated with loss of weight; Δ TNF- α showed a significant association with Δ cholesterol concentration. Analysis of the remaining regression coefficients did not reveal any significant associations.

Discussion

Previous studies reported the increased expression of the TNF- α mRNA in the adipose tissue of obese rodents¹³ and in obese human subjects.^{1,7} Adipose tissues of obese subjects secrete increased amounts of the corresponding protein.¹

The determination of circulating cytokines is complicated by its relative instability in biological fluids. The presence of soluble inhibitors may interfere with their measurement. TNF is rapidly cleared from the circulation, being frequently extremely low or undetectable. We observed¹¹ increased serum levels of TNF α in obese women in comparison with lean women. TNF α function depends of the relative concentration and clearance rate. The endogenous formation of TNF leads to the shedding of sTNF-Rs, which interfere with the binding of TNF to cell surface-bound TNF-Rs. Increase of sTNF-Rs in serum parallels or exceeds TNF α production.

Body weight reduction in obese subjects was associated with a decrease in TNF α mRNA expression in fat tissue¹ and serum.^{14,15} Bastard¹⁶ observed an increase in TNF- α mRNA levels during a very low calorie diet in subcutaneous adipose tissue in obese women. No alteration of plasma TNF- α concentrations in nine subjects was also shown after weight loss.² In the present study the question has been posed as to whether alterations in body mass and weight loss might lead to change in the concentrations in blood serum TNF α and both TNF soluble receptors.

In our study serum concentrations of TNF α were significantly lower after weight reduction. An inter-

esting and novel finding is the association of the decrease of TNF α during weight-reducing treatment with an increase in both TNF soluble receptor.

It was shown that obese subjects express more TNFR2 mRNA in fat tissue and more soluble TNFR2 in circulation relative to lean control subjects.^{17,18} TNFR1 expression and protein levels were similar in these subjects. We observed increased plasma concentrations of two soluble TNF α receptors; this discrepancy is difficult to explain and requires further study.

It was suggested that TNF- α could represent a form of local adipostat.⁸ TNF- α participates in obesity-related insulin resistance. Adipose tissue insulin resistance could be explained as a mechanism preventing further adipocyte lipid accumulation. Besides insulin resistance TNF α could limit adiposity through decreasing fat storage, connected with diminished expression of lipoprotein lipase (LPL).⁷

The assessment of the TNF α system activity on the basis of plasma TNF receptor concentrations seems to be more reliable, because these proteins are easily detectable in plasma and remain elevated for longer periods of time. Altered expression of TNF α and its receptors after weight loss^{14,15} is probably the key to regulation of weight loss during slimming treatment. TNF α was rapidly cleared from the circulation and levels were significantly lowered; the biologically stable soluble receptors, which bind and neutralize TNF α increased. The lower TNF α / sTNFRs ratio after weight reducing treatment plays a role by modulating the biological effects of TNF α and serves as a mechanism that prevents further weight loss.

Conclusion

Our results are consistent with the hypothesis that decrease of the serum concentration of TNF α and the increase in both TNF soluble receptors after weight reducing treatment in obese women may be a contra-regulation preventing further weight loss.

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