Turkish Journal of Endocrinology and Metabolism, (1999) 2 : 77-80 **ORIGINAL ARTICLE**

The Relationship Between Serum Tumour Necrosis Factor- α Levels and Insulin Sensitivity in Obese Women

Mithat Bahçeci* Alpaslan Tuzcu* Melikşah Ertem Leyla Colpan*****
Suzan Kandiya Çakmak** Murat Çelik******

Dicle University School of Medicine, Diyarbakır
** Department of Endocrinology*
*** Department of Public Health*
**** Department of Biochemistry*
***** Department of Internal Medicine*

In this study, we aimed to determine the relationship between tumour necrosis factor-alpha (TNF- α) and insulin levels in obese women. A total of 40 subjects, twenty obese (BMI>26.4 kg/m ^{2}) and 20 nonobese, were studied. The obese group was constituted from the female patients who applied to our clinic with obesity. The nonobese group was consituted from healthy female medical students and staff whose BMIs were less than 25 kg/m ^{2}. Body weight, BMI, waist circumference, hip circumference, waist/hip ratio, fat weight and lean weight of the obese patients and non-obese control group were recorded. Mean fasting insulin of the obese patients was 23.7±31.9 mIU/ml while it was 8.5±7.4 in nonobese people (p<0.05). Serum TNF- α levels were 14.8±3.66 ng/ml and 9.86±2.74 in obese and nonobese subjects respectively. TNF- α levels of the obese group was higher than that of the nonobese group (p<0.001). There was no significant difference between obese and nonobese groups from the point of view of fasting glucose/insulin (G)I) ratio. However we found a significant negative correlation between TNF-alpha and fasting G/I ratio in obese women (r=-0.54 and p=0.01). We concluded that the development of hyperinsulinemia and insulin resistance in obese subjects may be related to an increase in TNF- α levels .

KEY WORDS Tumour necrosis factor-alpha, fasting insulin, fasting glucose/insulin ratio and obesity

Introduction

Obesity is a very important health problem especially in the developed countries. The prevalence of obesity has increased related to industrialisation. The proportion of adult people with a weight problems is well over 50 percent, perhaps over 75% (1). Obesity being defined as BMI>28.6 kg/m^2, the prevalence of obesity has risen to 24.7% from 15% in 30 years (From 1960 to 1991) in USA. In Western Samoa, obesity prevalence in the 25-69 age group in 1991 reached 58.4% in men and 76.8% in women respectively (2,3). In the determination of obesity lots of methods have been used such as body mass index (BMI), circumferences (waist/hip ratio; WHR), skinfolds, ultrasound and computed tomography. Prospective studies in Gothenburg, Sweden, have shown that both men and women who have a high ratio of waist-to-hip circumferences have an increased risk of death,

Correspondence address:

Mithat Bahçeci, M.D.
Dicle Üniversitesi Tıp Fak. Endokrinoloji Bilim Dalı
21280 Diyarbakır
Phone: +90 412 223 08 24
Fax: +90 412 248 84 40
E-mail: mbahceci@dicle.edu.tr

stroke, ischaemic heart disease, hypertension, glucose intolerance, and higher serum lipid levels (4-6). Obesity can cause a lot of diseases such as type-2 diabetes, hypertension and atherosclerosis. Obesity is associated with an increase in TNF-α expression in animals and humans (7). The role of TNF-α in the development of insulin resistance has repeatedly been emphasised in the past few years (8). Obesity may induce insulin resistance through increased secretion by fat of TNF-α, which inhibits insulin-receptor activity. The ways in which an increased fat mass may reduce sensitivity to insulin action in other tissues (primarily skeletal muscle and liver) are unknown at present. It was claimed that, increased secretion of cytokine TNF-α may be involved in the development of insulin resistance in obese insulin-resistant Zucker rats (9). TNF-α is secreted by fat tissue and acts to inhibit intracellular signalling after insulin binds to its receptor (9). Richard S et al. have reported that fasting glucose: insulin (G:I) ratio may show insulin resistance in non-Hispanic obese white women (10). In this study, we aimed to determine the relationship between tumour necrosis factor-alpha (TNF-α) and insulin levels in obese women.

Subjects and Methods

Subjects: As according to Richard S's report, 20 obese (BMI>26.4 kg/m^2) and 20 nonobese, in total 40, women were studied. The subjects were evaluated by age, blood pressure, BMI, WHR, fasting blood glucose, fasting insulin levels, fasting glucose/insulin ratio and tumour necrosis factor-α. Obese persons were selected from patients hospitalised due to obesity in teh endocrinology department. Body mass index was estimated as weight (kg)/height (meter)^2 and subjects whose BMI>26.4 kg/m^2 were accepted as obese. Waist/hip ratio was determined by waist circumference/hip circumference, and values which exceeded 0.85 for females and 1 for males were accepted as abnormal. Subjects with diabetes were excluded from the study by using the diabetes criteria of the World Health Organisation. Subjects did not have any drug or medication that could affect their insulin level or insulin sensitivity. All subjects were informed about the study.

Study protocol and methods: Blood samples were drawn from all subjects at 08:00 am, after an overnight fasting and resting. All of the blood samples were centrifuged and stored in deep freeze at -20°C. TNF-α level of serum samples was measured using a Pelikine human TNF-α ELISA kit (Central Laboratory of the Netherlands Red Cross Blood transfusion Service PO Box 9110 1006 AD Amsterdam Batch Nr: 1920-14-00). The principle of the test briefly: The Pelikine human TNF-α kit is a "sandwich-type" of enzyme immunoassay in which a monoclonal anti human TNF-α antibody is bound onto polstrene microtiter wells. Human TNF-α, present in a measured volume of sample or standard was captured by the antibody on the microtiter plate, and non-bound material was removed by washing. Subsequently, a biotinylated second monoclonal antibody to human TNF-α was added. This antibody bound to the TNF-α-antibody complex present in the microtiter well. Excess biotinylated antibody was removed by washing, followed by addition of horseradish peroqidase (HRP) conjugated streptavidin, which bound onto the biotinylated side of the TNF-α sandwich. After removal of non-bound HRP conjugate by washing, a substrate solution was added to the wells. A coloured product was formed in proportion to the amount of TNF-α present in the sample or standard. After the reaction had been terminated by the addition of a stop solution, absorption was measured in a microtiter plate reader. From the absorption of samples and those of a standard curve, the concentration of TNF-α could be determined by interpolation with the standard curve. TNF-α values in fresh serum and plasma samples of healthy individuals were below 10 pg./ml. Serum insulin levels of subjects were measured by using accecs immunoassay system with access ultrasensitive insulin kits Beckman (USA). Fasting blood glucose was obtained with a Beckman Cx-5 Delta autoanalyzer. Blood pressure was measured by using an Erkameter 3000 syphigmomanometer.

Statistical methods: The results of measurements were presented as the mean ± standard deviation (m ± sd). Comparison of means was made by using independent-t test for independent groups. The Pearson correlation analyses were carried out

between TNF-alpha and fasting glucose/insulin ratios in obese women.

Results

Mean fasting insulin levels were 23.7±31.9 mIU/ml in obese patients and 8.5±7.4 in the nonobese control group. The mean fasting insulin level of obese subjects was higher than that of nonobese ones ($p<0.05$). The mean fasting G:I ratio of obese patients was 11.08±8.75 whereas this ratio was 15.2±9.46 in nonobese subjects. Although mean fasting G:I ratio was higher in the nonobese group than in obese patients the difference is not meaningful. Serum tumour necrosis factor-α levels were 14.8±3.66 ng/ml and 9.86±2.74 in obese and nonobese women respectively. Mean serum TNF-α level of obese women was higher than that of non-obese women ($p<0.001$). All of the physical and metabolical parameters of subjects are shown in Table 1. There was significant correlation between TNF-alpha and fasting glucose/insulin ratio in the obese group ($r=-0.54$ and $p=0.014$). The scatter plot of TNF-alpha and fasting glucose/insulin ratio is shown as Figure 1.

Table 1. The Physical and metabolical findings of the obese and nonobese subjects.

	Obese	nonobese	P
Age (year)	32±10.3	31±11.7	NS
Hegiht (cm)	166±7.3	167±9.5	NS
Weight (kg)	90.8±9.4	58.6±8.7	<0.0001
BMI (kg/m^2)	33.3±3.6	21.5±2.9	<0.0001
Waist (cm)	104.3±810.3	68.5±5.1	<0.0001
Hip (cm)	116.4±8.9	90.8±4.5	<0.0001
WHR	0.89±0.007	0.74±0.06	<0.0001
Fat weight (kg)	34.2±10.9	11.5±4.4	<0.0001
Lean weight (kg)	56.3±8.4	45.7±10.3	<0.0001
Blood pressure (sys)	121±19	109±17	NS
Blood Press (dias)	79.5±14.4	71±14.4	NS
Fasting glucose (mg/dl)	93.1±18.6	86.6±15.1	NS
G:I ratio	11.08±8.75	15.2±9.46	NS
Insulin (mIU/ml)	23.7±31.9	8.5±7.4	<0.05
TNF-α (pg/ml)	14.8±3.66	9.86±2.74	<0.001

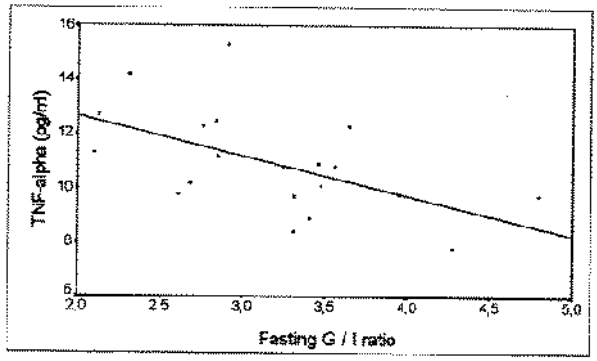

Figure 1. The scatter plot of serum TNF-α and fasting glucose/insulin ratio of obese women.

Discussion

We found that mean serum fasting insulin levels of obese patients were higher than those of nonobese subjects. It has been suggested that the simplest way to derive an index of whole-body insulin sensitivity is to measure the fasting plasma insulin concentration. In individuals who are resistant to insulin, the fasting plasma insulin concentration is elevated and the level of hyperinsulinemia has been shown to correlate with the severity of insulin resistance, as determined with the euglycemic insulin clamp technique (13). Therefore we can say that fasting serum insulin concentration may increase related to obesity. However, use of the fasting plasma insulin concentration suffers from a number of interpretive problems such as the fact that its correlation with insulin action in vivo is weak, and it may crossreact with proinsulin. Richard S et al. (10) claimed that fasting G:I ratio is an easily obtainable, safe, highly sensitive, and specific measure of insulin sensitivity in obese non-Hispanic white women. Thus we decided to study obese women and to use G:I ratio for the determination of insulin sensitivity. We have found no significant difference from the point of view of fasting G/I ratio between obese and nonobese women. This result indicated that fasting G/I ratio is more effective than fasting insulin concentration for showing insulin sensitivity in obese women. Obesity is characterised by insulin resistance in skeletal muscle, which may be induced by nonesterified fatty acids liberated by lipolysis, acting through the randle cycle to decrease glucose utilisation. Insulin extraction by the liver is also reduced, contributing to hyperinsulinemia. Insulin resistance stimulates insulin secretion; hyperinsulinemia may be able to maintain

normoglycemia, but individuals who have defetive B-cell function will develop hyperglycaemia, with impaired glucose tolerance and ultimately type-2 diabetes (II). We carried out the Pearson correlation analyses between TNF-alpha and fasting *G/l* ratio in obese women. There was no statistically significant difference between obese and nonobese women from the point of view of *G/l* ratio. But we found a significant negative correlation between TNF-alpha and fasting *G/l* ratio in obese women. As a result, we can say that there was significant negative correlation between serum TNF-a and fasting *G/l* ratio in obese women. This result may reflect that the development of insulin insensitivity may be related lo TNF-a in obese women. Mean serum TNF-a level of obese women is also hjgher than that of nonobese women statistically in our study. Circulating levels of TNF-a are increased in obese, insulin-resistant rats and in obese humans, and a convincing case has been made that it is important in exacerbating the insulin resistance of the fatty Zucker rat (9-12). The role of TNF-a in the development of insulin resistance has repeatedly been emphasised in the past few years (8). Obesity may also induce insulin resistance through increased secretion by fat of tumour necrosis factor-alpha (TNF-a), which inhibits insulin-receptor activity. The ways in which an increased fat mass may reduce sensitivity to insulin action İn other tissues (prinıarily skeletal muscle and liver) are unknown at present, although studies of obese insulin-resistant Zucker rats suggest that increased secretion of cytokine, TNF-a, may be involved. TNF-a is secreted by fat and acts to inhibit intracellular signalling after insulin binds to its receptor. TNF-a induces insulin resistance by inhibiting the tyrosine kinase activity of the insulin receptor (9). in our study we found that the TNF-a level of obese subjects was higher than in nonobese people. For this reason we decided that an increase in TNF-a in obese subjects may induce the development of insulin resistance, perhaps overt diabetes mellitus. it is accepted that the WHR value of patients with upper body obesity is higher than 0.85 for women. The WHR ratios of ali obese patients were higher than 0.85 in our study. Therefore, it can be said that an increase in fasting insulin concentration may also be related to abdominal obesity particularly. We concluded that fasting insulin levels of obese women were high, and these high insulin levels may be related to TNF-a which is secreted by fat tissue. Obesity may induce insulin resistance through increased secretion by fat of TNF-a, because there was a significant negative correlation between TNF-a and fasting G/I ratio in obese women.

References

1. Lcan M. Clinical handbook *of* vveight management. Martin Dınitz Blackwell Science Inc. Martin Dünüz Ltd the Livery Housc 7-9 Pralt street Landon NW10AE 1998, pp3-5.
2. Seidell JC, Hautvast JG, Deurenbcrg P. Ovcrweight: fat distribution and health risks. Epidcmioİogical obser-valions. A review. *hfusionrherapîe* **16:** 276-81. İ 989.
3. Scidell JG, Fiegal KM. Assesing obesity: Classificatiorı and epidemiology. *Br Med Buil* 53: 2.38-52, i 997.
4. Larsson B, Svardsudd K, Welin L et al. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year fbllow-up of participants in the study of men born in 1913. *B r Med J* 288: 1401-1404, 1984.
5. Kissebah AH, VydelİngumN, Murray R et al. Rclation of body fat distribution to metabolic complications of obesity.,/ *C/in Enâncrmol Metub* 54: 254-260, 1982.
6. Krotkiewski M, Bjorntorp C, Larsson B et al. Impact of obesity on metabolism in men and wonıen: Importance of rcgional adipose tissue distribulion. ./ *Clirı Invest 72:* 1150-1162, 1983.
7. Dandona P, Weinslock R, Thusu et al. Tümör necrosis factor-alpha in sera of obese patients; f u 11 wilh vveight *\oss.JClinEndocrinolMetahS3(S):* 2907-10, 1998.
8. Winkler G, Salamon, Simon K and Cseh K. Tumour necrosis factor-alpha; a possible pathogenctic factor in obesity in insulin resistant and non-insulin depcndent diabetes mellitus. ***Orv*** *Hetil 5:* 139(27): 1627-32, 1998.
9. Holamisligil GS, Shargill NS, Spicgelman BS. Tumour Necrosis Factor-a direct role in obcsity-linkcd insulin resistance. *Science* **259:** 87-91, 1993.
10. Richard S. Legro, Diane Finegood, Andrca Dunaif. A fasting glucose to insulin ratio is a useful mcasurc of insulin sensitivity in women vvilh polycysiic ovary syndrome. ,/ *Clin Endocrinol Metah* 83: 2694-2698, 1998.
11. Rol and TJ. Obesity and nutritional facîors in the pathogcnesis of non-insulin-dependent diabetes mellitus. Texîbook of diabetes. Vol. l Seç. ed. Edited by John Pickup and Gareth Williams. *Blackwcll Science* 1997, pp 19.1.
12. Hotamisligil **GS,** Arner P, Aro **JF,** Atkinson **RL,** Spiegelman BM. increased adipose tissue expression of lumour necrosis factor-a in human obesity and insulin resistance.,/*Clin Invest* **95:** 2409-15, 1995.
13. Matsuda M, DeFronzo RA. in vivo mcasurcnıent of insulin sensitivity in humans. in Clinical researclı in diabetes and obesity. Ed boris DraznİIn and Robert Rizza. Vol. i Humana press, Totowa, NevvJerscy, 1997, p 24.