Serum Concentrations of Nitric Oxide, Tumor Necrosis Factor (TNF)-α and TNF Soluble Receptors in Women With Overweight and Obesity

Magdalena Olszanecka-Glinianowicz, Barbara Zahorska-Markiewicz, Joanna Janowska, and Aleksander Zurakowski

The aims of the present study was to examine how overweight and obesity affect serum concentrations nitric oxide (NO) metabolites and to determine whether there is association between serum concentrations tumor necrosis factor (TNF)-α and TNF soluble receptors (sTNF-R) in subjects with overweight and obesity. The study groups involved 154 women: 102 obese (81 obese with body mass index [BMI] 30 to 40 kg/m^2 and 21 obese with BMI >40 kg/m^2), 24 overweight patients, and 28 lean controls. Serum concentrations of NO metabolites and of TNF-α and its soluble receptors (sTNF-R1, sTNFR-2) were measured by enzyme-linked immunosorbent assay (ELISA) kits. Serum concentration of insulin was measured by radioimmunoassay (RIA). Plasma glucose, cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglicerydes were determined by enzymatic procedure. Body composition was determined by impedance analysis using Bodystat (Douglas, British Isles). Serum concentrations of NO in the overweight group (35.1 ± 12.1 μmol/L) and the obese groups with BMI 30 to 40 kg/m^2 (32.8 ± 9.3 μmol/L) and with BMI greater than 40 kg/m^2 (33.3 ± 8.5 μmol/L) were significantly higher when compared to controls (28.2 ± 8.1 μmol/L): $P < .05$; $P < .01$, and $P < .01$, respectively. There was no difference in levels of NO between the overweight group and both obese groups. Serum concentration of TNF-α was also significantly higher in the group with overweight (6.5 ± 3.1 pg/mL), in the obese group with BMI 30 to 40 kg/m^2 (6.8 ± 3.1 pg/mL), and in the obese group with BMI greater than 40 kg/m^2 (7.4 ± 2.6 pg/mL) when compared to controls (2.9 ± 2.2 pg/mL): $P < .00005$; $P < .00005$, and $P < .0000001$, respectively. However, serum concentrations of sTNF-R1 and -R2 did not differ significantly between the overweight group, both obese groups, and controls. In conclusion, we observed increased serum concentrations of TNF-α and NO in overweight and obese women. It seems that there is an association between serum concentrations of TNF-α and NO; however, this relationship depends on the degree of obesity.

NITRIC OXIDE (NO) is a ubiquitous messenger molecule. NO is produced in many different cells through the oxidation of L-arginine. Depending on the cell type, NO formations is catalyzed by one of 3 isoforms of NO synthase (NOS): neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS).

Synthesis and activity of eNOS and nNOS are stimulated by bradykinin, P substance, endothelin 1 and 3, thrombin, acetylocholine, adenosine diphosphate, and thrombocyte-activating factor, and they require both calcium and calmodulin for activity.

eNOS is expressed in endothelial cells, myocardium, thrombocytes, and in the brain, whereas nNOS is found in the central and peripheral nervous systems and skeletal muscles. iNOS activity is increased by proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, and interferon gamma (INF-γ), and is decreased by glucocorticoides.

Initially, iNOS was found in immunoactive cells, but later also in cardiomyocytes, smooth muscle vessels, and neuronal cells.

NO is involved in the regulation of many physiological and pathophysiological processes such as vasodilatation, neurotransmission, inflammation, and metabolism.[1,2]

Recent studies showed the presence of eNOS and iNOS in both rat and human white adipose tissue and increased activity of NOS in human adipose tissue in obesity.

Previous reports demonstrated that inhibition of NOS in adipose tissue leads to increased lipolysis.[3-5]

As described above, TNF-α is one of the factors causing increased activity of iNOS. Many studies showed that TNF-α and TNF receptors are expressed in fat cells and their production is increased in obesity.[6,7] Our[8,9] previous results and other studies[10,11] have shown increased serum levels of TNF-α and TNF soluble receptors (sTNF-R) in obese patients in comparison to lean subjects. One study described elevated serum levels of NO metabolites (nitrate and nitrite) in obese adolescents.[12] However so far, there have been no studies investigating whether these elevated serum levels of TNF-α and sTNF-r in obesity are associated with serum levels of NO. Our preliminary study showed increased serum concentrations of NO and TNF-α in obese patients in comparison to lean subjects.[13]

The aim of the present study was to examine the influence of overweight and obesity on serum concentrations of NO metabolites and to determine whether there is association between serum concentrations of TNF-α and sTNF-r with NO metabolites.

MATERIALS AND METHODS

The study was performed in a group of 154 women. The study group consisted of 24 overweight women, age 32.9 ± 11.3 years, weight 75.4 ± 6.8 kg, body mass index (BMI) 28.1 ± 1.6 kg/m^2; and 102 obese women: subgroup A—81 obese women with BMI 30 to 40 kg/m^2, age 40.7 ± 11.0 years, weight 90.1 ± 9.9 kg, BMI 34.4 ± 2.8 kg/m^2, and subgroup B—21 obese women with BMI greater than 40 kg/m^2, age 40.9 ± 10.4 years, weight 118.9 ± 13.2 kg, BMI 44.7 ± 4.0

From the Department of Pathophysiology, Silesian University School of Medicine, Katowice, Poland.

Submitted July 11, 2003; accepted May 3, 2004.

Supported by KBN Grant No. C008/P05/2000.

Address reprint requests to Magdalena Olszanecka-Glinianowicz, MD, Department of Pathophysiology, Silesian University School of Medicine, Medyków Street 18, 40-752 Katowice, Poland.

0026-0495/04/5310-0005$30.00/0

doi:10.1016/j.metabol.2004.07.001

Table 1. Patient Characteristics

	Range BMI			
	Obese		Overweight	Controls
	>40	30–40	25–30	<25
N	21	81	24	28
Age (yr)	40.9 ± 10.4	40.7 ± 11.0	32.9 ± 11.3[ce]	29.4 ± 9.4[gh]
Weight (kg)	118.9 ± 13.2	90.1 ± 9.9[b]	75.4 ± 6.8[df]	59.4 ± 7.7[gh]
BMI (kg/m^2)	44.7 ± 4.0	34.4 ± 2.8[b]	28.1 ± 1.6[df]	21.8 ± 2.0[ghj]
Fat-free mass (kg)	57.1 ± 6.2	52.3 ± 6.5[a]	48.8 ± 4.9[cf]	45.8 ± 4.7[ghi]
Fat-free mass (%)	48.2 ± 4.3	58.2 ± 6.0[b]	64.8 ± 3.8[df]	77.5 ± 4.9[ghj]
Body fat (kg)	61.8 ± 10.2	37.8 ± 7.8[b]	26.6 ± 4.0[df]	13.6 ± 4.1[ghj]
Body fat (%)	51.8 ± 4.3	41.8 ± 6.0[b]	35.3 ± 3.8[df]	22.4 ± 4.8[ghj]

[a] $P < .005$, [b] $P < .000$ obese A and B subgroups.
[c] $P < .05$, [d] $P < .000$ obese A subgroup and overweight.
[e] $P < .005$, [f] $P < .000$ obese B subgroup and overweight.
[g] $P < .000$ obese A subgroup and controls.
[h] $P < .000$ obese B subgroup and controls.
[i] $P < .05$, [j] $P < .000$ overweight and controls.

kg/m^2. A control group consisted of 28 lean volunteers, aged 29.4 ± 9.4 years, weight 59.4 ± 7.7 kg, BMI 21.8 ± 2.0 kg/m^2. The characteristics of the study and control groups are listed in Table 1. All obese subjects included in the study were diagnosed as having simple obesity without additional diseases. Patients with evidence of acute or chronic inflammatory diseases and malignant disorders were excluded. The study was conducted after obtaining informed consent from all the subjects. The study was approved by the local ethical committee.

To avoid the effect of diet on serum concentrations of NO metabolites, the subjects were given a list of foods potentially rich in nitrate and were requested to abstain from these foods for 3 days before sample collection. Specifically, herbal or black teas, beer, wine, cured meat, fish, and cheese were excluded from the diet.

Body weights and heights were measured and body mass index (BMI) calculated as weight in kilograms divided by square of the height in meters. The reference interval of BMI is defined as 19 to 24.9 kg/m^2 (controls), overweight as BMI 25 to 29.9 kg/m^2 (group with overweight), and obesity as a BMI greater than 30 kg/m^2 (groups with obesity).

Body composition was determined by impedance analysis using a Bodystat analyzer (Bodystat Ltd, Douglas, British Isles).

Blood samples were collected in the morning after an overnight fast. Plasma glucose, cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglicerydes were determined by enzymatic procedure using the commercially available test kit (Cormay, Great Britain). Low-density lipoprotein (LDL)-cholesterol was calculated using the Friedwald formula. Insulin was determined by radioimmunoassay (RIA; DPC Diagnostic Products Corp, Los Angeles, CA).

The blood serum concentration of NO metabolites were measured using a commercially available highly sensitive enzyme-linked immunosorbent assay (ELISA) kit (Genzyme Diagnostics, Cambridge, MA; R&D Systems' Total Nitric Oxide Assay, Minneapolis, MN). The transient and volatile nature of NO makes it unsuitable for most convenient detection methods. However, since most of the No is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), the concentrations of these anions have been used as quantitative measures of NO production. After the conversion of NO_3^- to NO_2^-, the spectrophotometric measurement of NO_2^- is accomplished by using the Griess reaction: (1) $NO + O_2^- \rightarrow ONO_2^{-H+} \rightarrow NO_3^- + H^+$; (2) $2NO + O_2 \rightarrow N_2O_4^{H2O} \rightarrow NO_2^- + NO_3^- + 2H^+$; (3) $NO + NO_2^- \rightarrow N_2O_3^{H2O} \rightarrow 2NO_2^- + 2H^+$. The conversion of NO into nitrate and nitrite by these reactions varies in each system. The interaction of NO in a system is measured by the determination of total nitrate and nitrite concentrations in the sample.

R&D Systems' Total Nitric Oxide Assay involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. The detection of total nitrite is then determined as a colored azo-dye product of the Griess reaction that absorbs visible light at 540 nm.

The sensitivity of the Total Nitric Oxide Assay is typically less than 1.35 μmol/L. Mean intra-assay coefficient of variance was 3.1% (range, 1.2% to 5.3%) and mean interassay coefficient of variance was 4.1% (range, 3.3% to 7.0%).

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).

The minimum detectable dose of TNF-α is typically less than 0.18 pg/mL. Mean intra-assay coefficient of variance was 14.4% (range, 8.7% to 14.8%) and mean interassay coefficient of variance was 18.7 % (range, 16.1% to 22.6%).

The minimum detectable dose of sTNF-R1 is typically less than 3.0 pg/mL. Mean intra-assay coefficient of variance was 2.9% (range, 2.7% to 6.9%) and mean inter-assay coefficient of variance was 3.7 % (range, 5.8% to 8.8%).

The minimum detectable dose of sTNF-R2 is typically less than 1.0 pg/mL. Mean intra-assay coefficient of variance was 2.5% (range, 1.6% to 2.5%) and mean interassay coefficient of variance was 3.5% (range, 3.5% to 5.1%).

Data were analyzed using *t* test and Pearson's correlation analysis. *P* values less than .05 were considered to be statistically significant.

RESULTS

Differences in the parameters age, weight, BMI, and body composition between study groups and controls are presented in Table 1.

Serum NO, TNF-α, sTNF-R1, and sTNF-R2 Concentrations

Serum concentrations of NO in the overweight group (35.1 ± 12.1 μmol/L) and obese subgroup A (32.8 ± 9.3 μmol/L) and obese subgroup B (33.3 ± 8.5 μmol/L) were significantly higher when compared to controls (28.2 ± 8.1 μmol/L), $P < .05$, $P < .01$, and $P < .01$, respectively. There were no differences in levels of NO between overweight and both obese subgroups.

Serum concentrations of TNF-α were also significantly higher in group with overweight (6.5 ± 3.1 pg/mL) and obese

Table 2. Serum Concentrations of NO, TNF-α, and STNF Receptors in Obese Group Based on Patient Age

	Age (yr)			
	<30	30–40	40–50	>50
n	22	28	32	20
NO (μmol/L)	32.3 ± 8.9	34.7 ± 11.1	31.1 ± 7.1	33.9 ± 9.1
TNF-α (pg/mL)	6.8 ± 3.1	6.6 ± 2.9	7.2 ± 3.6	6.9 ± 2.2
sTNF-R1 (pg/mL)	1,260.5 ± 211.1	1,294.0 ± 550.0	1,292.2 ± 204.8	1,282.0 ± 195.0
sTNF-R2 (pg/mL)	1,760.0 ± 347.8	1,900.0 ± 678.0	1,927.0 ± 471.8	2,108.0 ± 702.5

subgroup A (6.8 ± 3.1 pg/mL) and obese subgroup B (7.4 ± 2.6 pg/mL) when compared to controls (2.9 ± 2.2 pg/mL), $P < .00005$; $P < .00005$, and $P < .0000001$, respectively. However, serum concentrations of sTNF-R1 and -R2 did not differ significantly between the overweight group, both obese subgroups, and controls.

There were no differences in serum concentrations of NO, TNF-α, and sTNF-R1 and R2 levels in obese subgroups divided by age (Table 2).

Serum Insulin, Glucose, and Lipid Concentrations

Serum concentrations of insulin in the overweight group and obese subgroups A and B (13.0 ± 6.0 μIU/L, 17.1 ± 8.1 μIU/L, and 26.6 ± 17.8 μIU/L, respectively) were significantly higher when compared to controls (9.9 ± 3.0 μIU/L), $P < .05$, $P < .00005$, and $P < .000001$, respectively (Table 3).

Serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, and glucose are shown in Table 3.

Correlations Between All Study Parameters

Significant positive linear correlations were found between BMI and serum NO (Fig 1), TNF-α ($r = 0.40$, $P < .0001$), and insulin ($r = 0.44$, $P < .0001$) levels in the total group of patients. We did not observe such correlations when the study groups were differentiated into subgroups based on BMI (normal, overweight, obese with BMI 30 to 40, and obese with BMI > 40).

In the total group of patients, body fat percent and weight correlated with the serum NO ($r = 0.28$, $P < .000$ and $r = 0.21$, $P < .01$, respectively), TNF-α ($r = 0.43$, $P < .0001$ and $r = 0.37$, $P < .0001$, respectively), and insulin ($r = 0.41$, $P < .0001$ and $r = 0.47$, $P < .0001$, respectively) concentrations. Moreover, serum NO and TNF-α concentrations also correlated with serum insulin concentration ($r = 0.21$, $P = .01$ and $r = 0.19$, $P = .02$, respectively). We did not observe such correlations when the study groups were differentiated into subgroups based on BMI (normal, overweight, obese with BMI 30 to 40, and obese with BMI >40).

No correlations were found between serum NO concentrations and age, lipid levels, and serum TNF-α, sTNF-R1, and sTNF-R2 concentrations in the total group of patients. In addition, no correlations were found between serum TNF-α and age, lipid levels, and serum sTNF-R1 and sTNF-R2 concentrations in this group.

In the total group of patients, serum concentration of sTNF-R1 correlated with serum concentration of sTNF-R2 ($r = 0.42$, $P < .0001$). Moreover, sTNF-R1 and sTNF-R2 correlated with serum insulin concentration ($r = 0.44$, $P < .0001$ and $r = 0.23$, $P = .004$, respectively).

We did not observe correlations between serum sTNF-R1 and sTNF-R2 concentrations and other study parameters.

Significant positive linear correlations were found between serum concentration of sTNF-R2 and fat-free body mass (kg) ($r = 0.43$, $P = .03$) and serum concentration of insulin ($r =$

Table 3. Serum Concentrations of NO, TNF-α, TNF Receptors, Lipids, Glucose, and Insulin

	Range BMI			
	Obese		Overweight	Controls
	>40	30–40	25–30	<25
NO (μmol/L)	33.3 ± 8.5	32.8 ± 9.3	35.1 ± 12.1	28.2 ± 8.1[gil]
TNF-α (pg/mL)	7.4 ± 2.6	6.8 ± 3.1	6.5 ± 3.1	2.9 ± 2.2[ghkm]
sTNF-R1 (pg/mL)	1,257.4 ± 218.5	1,384.6 ± 594.3	1,157.4 ± 156.2	1,158.6 ± 127.4
sTNF-R2 (pg/mL)	1,897.2 ± 519.1	1,995.6 ± 724.1	1,962.1 ± 603.6	1,790.0 ± 462.3
Total cholesterol (mg/dL)	209.2 ± 36.4	213.1 ± 42.0	200.4 ± 25.1	187.6 ± 31.6[hi]
HDL-cholesterol (mg/dL)	52.4 ± 10.9	51.3 ± 8.2	58.0 ± 12.3[cd]	51.3 ± 9.8[l]
LDL-cholesterol (mg/dL)	133.8 ± 32.6	134.5 ± 31.3	125.9 ± 28.5	119.3 ± 29.2[g]
Triglicerides (mg/dL)	110.4 ± 64.3	142.1 ± 88.2	88.3 ± 45.2[e]	91.8 ± 35.0[j]
Glucose (mg/dL)	98.3 ± 31.3	89.0 ± 11.9[a]	84.2 ± 8.8[d]	85.1 ± 9.0[i]
Insulin (μIU/mL)	26.6 ± 17.8	17.1 ± 8.1[b]	13.0 ± 6.0[cf]	9.9 ± 3.9[hkl]

[a] $P < .05$, [b] $P < .0005$ obese A and B subgroups.
[c] $P < .05$ obese A subgroup and overweight.
[d] $P < .05$, [e] $P < .01$, [f] $P < .001$ obese B subgroup and overweight.
[g] $P < .05$, [h] $P < .000$ obese A subgroup and controls.
[i] $P < .05$, [j] $P < .01$, [k] $P < .000$ obese B subgroup and controls.
[l] $P < .05$, [m] $P < .000$ overweight and controls.

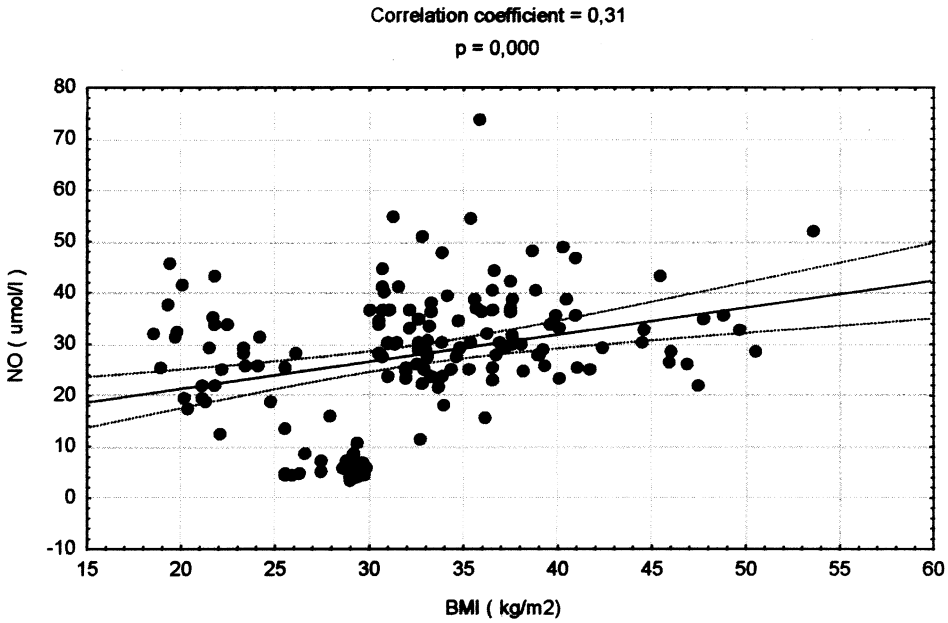

Fig 1. Correlation between NO concentration and BMI in the total group (N = 154).

0.47, $P = .02$) in the overweight group. We did not observe an association between serum concentrations of NO and TNF-α and sTNF-R1 and -R2 and insulin. No correlations were found between serum concentration of NO and age and serum lipids and BMI and body fat (percent and kilograms) in the overweight subjects.

A significant positive linear correlation was found between serum concentrations of sTNF-R1 and sTNF-R2 ($r = 0.35$, $P = .002$) in the obese subgroup A. We also observed a significantly positive linear correlation between serum concentration of insulin and body fat (kg) ($r = 0.27$, $P = .01$), body mass ($r = 0.31$, $P = .005$), and BMI ($r = 0.23$, $P = .04$) in the obese subgroup A. No correlations were found between NO and serum TNF-α, sTNF-R1, sTNF-R2, insulin, lipids, and age and BMI and body fat (percent and kilograms) in this subgroup.

There were significant positive linear correlations between the serum concentration of insulin and serum concentrations of sTNF-R1 ($r = 0.77$, $P = .0001$) and sTNF-R2 ($r = 0.55$, $P = .01$) in the obese subgroup B.

Significant positive linear correlations were found between the following parameters in obese subgroup B: plasma concentrations of TNF-α and NO ($r = 0.47$, $P = .03$) (Fig 2); plasma

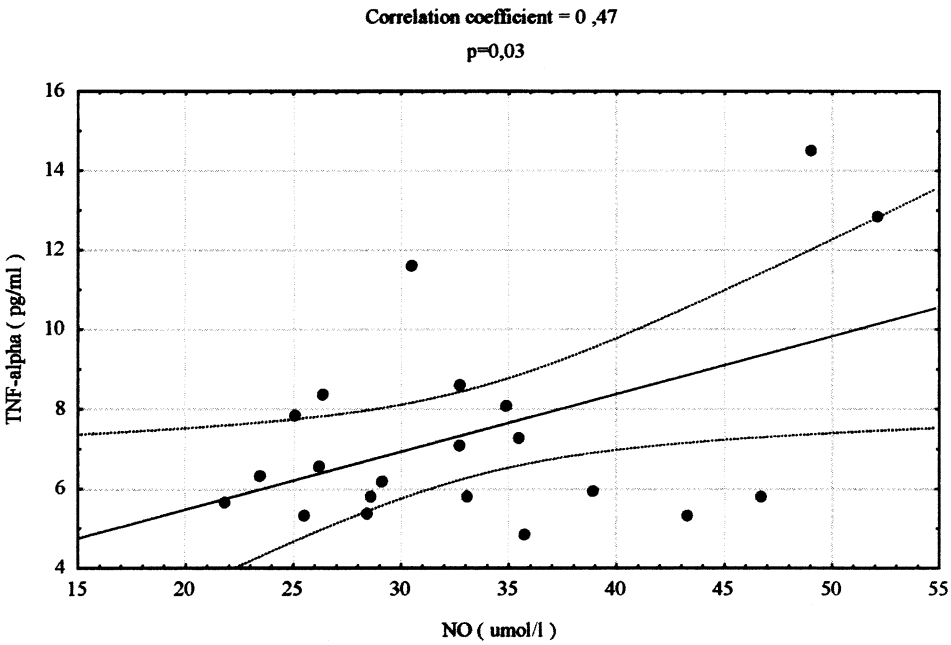

Fig 2. Correlation between TNF-α concentration and NO concentration in obese subgroup B (n = 21).

concentrations of TNF-α and sTNF-R1 ($r = 0.47$, $P = .03$) and sTNF-R2 ($r = 0.47$, $P = .03$); and plasma concentrations of sTNF-R1 and -R2 ($r = 0.64$, $P = .002$).

No correlations between serum concentrations of NO and age, serum lipids, serum insulin body fat (percent and kilograms) and BMI were detected in the obese subgroup B.

DISCUSSION

Our results are in accordance with previous reports which showed that inducible NOS is present in human adipose tissue; therefore, adipose tissue may be a source of NO. Other studies also reported increased expression of the TNF-α mRNA in the adipose tissue of obese rodents and human.[4,5,14]

Our results showing increased serum concentrations of NO in overweight and obese women when compared to controls and significant positive linear correlations between serum NO concentration and BMI are also in accordance with the study of Choi et al.[12] These investigators showed that obesity leads to increased NO production in humans and that this increase begins in subjects with a BMI greater than 25 kg/m^2 in both males and females. Despite the facts that Choi et al investigated adolescents (males and females) aged between 14 and 19 years and we evaluated overweight and obese women aged 17 to 64 years, both studies found increased serum concentrations of NO in subjects with a BMI greater than 25 kg/m^2 and correlations between serum NO concentration and BMI. We did not observe an association between serum concentrations of NO and age. Our study found also a significant correlation between serum NO and body fat expressed as percentage and kilograms in the total group of patients. Therefore, it seems that increased serum concentrations of NO may be a result of its production in adipose tissue, but further studies are required to examine if, and to what extent, fat cells are able to release NO.

Ferlito and Gallina[15] reported results that differed from ours. They observed that in overweight and hypertensive diabetics there is no significant increase of NO production in comparison to healthy controls. However, Ferlito and Gallina measured plasma nitrite in patients with type 1 and 2 diabetes and we measured NO in overweight and obese women with serum concentrations of glucose and insulin with references range. Our study found a significant correlation between serum NO and insulin concentrations in the total group of patients.

It is known from previous reports that TNF-α is produced by adipose tissue and that its production is increased in obese subjects.[14] In our previous studies, we also observed increased serum levels of TNF-α in obese women in comparison to lean controls.[9,10]

In the present study, we found increased serum concentrations of TNF-α in women with obesity. An interesting and novel finding is the increased serum concentrations of TNF-α in overweight subjects. We observed an association between serum TNF-α concentration and BMI and body fat (percent and kilograms) in the total group of patients. These findings are in accordance with the study by Winkler et al,[16] which revealed expression of the TNF-α protein in both fat depots and the correlation of serum TNF-α with the adipocyte cell volume.

In the total group of patients, serum TNF-α concentration correlated positively with serum insulin concentration. No such correlation was found when the study group was divided into subgroups according to BMI. Similar data concerning serum TNF-α concentrations and insulin sensitivity were obtained in our previous study[17] when obese insulin-sensitive and insulin-resistant subjects were compared.

The endogenous formation of TNF leads to the shedding of sTNF–R, which interferes with the binding of TNF to cell surface–bound TNF-R. Increase of sTNF-R in serum parallels or exceeds TNF-α production.[18] It is interesting that in our study despite the increased serum concentrations of TNF-α in overweight and obese subjects, we did not observe differences of serum concentrations of sTNF-R between overweight, obese, and normal-weight subjects. These findings are in accordance with our previously studies,[12] which did not find differences in serum concentrations of both sTNF-R1 and sTNF-R2 between lean controls and obese women with normal and impaired glucose tolerance. In contrast, Dzienis-Strączkowską et al[19] showed increased serum concentrations of both sTNF receptors in obese subjects with normal and impaired glucose tolerance in comparison to lean controls. However, we observed a positive correlation between serum concentrations of both sTNF receptors and serum concentration of insulin in our total group and in the subgroup with a BMI greater than 40 kg/m^2. These findings are with accordance with results obtained by Dzienis–Strączkowską et al.

Our results are also contradictory to findings described by Hauner et al,[11] who found elevated plasma concentrations of both sTNF receptors in obesity but did not find differences in serum concentrations of TNF-α between obese and lean subjects. However, about half of Hauner et al's patients had type 2 diabetes and we included only patients with a BMI between 25 and 30 kg/m^{2}or with a BMI greater than 30 kg/m^2 without additional diseases.

Experimental studies[1,2] have shown that iNOS is activated by cytokines such as TNF-α in skeletal muscle and fat. Therefore, considering the facts mentioned above, as well as the observed increase in both serum concentrations of NO and TNF-α, we evaluated the association between serum concentrations of NO and TNF-α. In the total group, overweight group, and obese subgroup A, we did not find an association between serum concentrations of NO and TNF-α or between NO and sTNF receptors and between serum concentrations of TNF-α and its soluble receptors. However, when we considered the obese group with BMI greater than 40 kg/m^2, we found positive correlations between plasma concentrations of NO and TNF-α but not between NO and sTNF receptors. In this subgroup we also found a positive association between serum concentrations of TNF-α and its sTNF-R1 and -R2.

Recent studies[5] demonstrated that NO inhibits activity of lipolysis stimulated by catecholamines. On the other hand, studies[20] performed on cultures of human adipose cells showed that TNF-α may prevent the development of adipose tissue. One mechanism that inhibits lipogenesis is probably the strong inhibition of insulin activity in human adipocytes by TNF-α and inhibition of lipoprotein lipase induced by TNF-α. It seems that the association between increased serum level of TNF-α and NO in severe obesity may reflect its production in fat cells and that the opposite effects of these substances on the devel-

opment of adipose tissue may constitute a mechanism regulating further body mass gain.

Perreault and Marette[21] showed that iNOS expression increased in muscle and fat of genetic and dietary models of obesity. Moreover, they observed that mice in which the gene encoding iNOS was disrupted (NOS 2−/− mice) are protected from high-fat–induced insulin resistance. Whereas both wild-type and NOS 2−/− mice developed obesity on the high-fat diet, obese NOS 2−/− mice exhibited improved glucose tolerance, normal insulin sensitivity in vivo, and normal insulin-stimulated glucose uptake in muscles.

We observed positive correlations between serum concentrations of NO and TNF-α and serum concentration of insulin in the total group. These findings, together with the correlation between serum concentrations of NO and serum concentrations of TNF-α in subgroup with BMI greater than 40 kg/m^2 described above, support the hypothesis that higher serum concentrations of NO in overweight and obese subjects may be the result of increased synthesis in adipose tissue.

Nicoletti et al[22] and Ziccardi et al[23] described an association between higher serum concentrations of inflammatory cytokine (TNF-α and IL-6) and impairment endothelial functions (vascular responses to L-arginine, the natural precursor of NO) in obese subjects. Both of these groups of investigators showed that after weight loss serum concentrations of TNF-α and IL-6 decreased and vascular response to L-arginine improved. These findings combined with our results also support the hypothesis that higher serum concentrations of NO in overweight and obese subjects may be a result of increased NO production. It seems that increased production of TNF-α in adipose tissue may induce iNOS, and thus increased production of NO in adipose tissue and skeletal muscle. This increased production of NO may be reflected by higher serum concentrations of NO in overweight and obese subjects. Because this is the first report to show increased serum concentrations of NO in overweight and obese women, further studies are necessary to clarify which tissue is the main source of its production.

Conclusions

We observed increased serum concentrations of TNF-α and NO in overweight and obese women. It seems that there is a relationship between serum concentrations of TNF-α and NO; however, this association depends on the degree of obesity.

REFERENCES

1. Michel T, Feron O: Nitric oxide synthases: Which, where, how, and why ? J Clin Invest 9:2146-2152, 1997
2. Vallance P, Collier J: Fortnightly review biology and clinical relevance of nitric oxide. BMJ 309:453-457, 1994
3. Elizalde M, Ryden M, van Harmelen V, et al: Expresion of nitric oxide synthases in subcutaneous adipose tissue of nonobese and obese humans. J Lipid Res 41:1244-1251, 2000
4. Ribiere C, Jaubert AM, Gaudiot N, et al: White adipose tissue nitric oxide synthase: A potential source for NO production. Biochem Biophys Res Commun 222:706-712, 1996
5. Ryden M, Elizalde M, van Harmelen V, et al: Increased expression of eNOS protein in omental versus subcutaneous adipose tissue in obese human subjects. Int J Obes 25:811-815, 2001
6. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alfa: Direct role in obesity-linked insulin resistance. Science 5091:87-91, 1993
7. Mohamed-Ali V, Goodrick S, Rawesh A, et al: Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo. J Clin Endocrinol Metab 82:4196-4200, 1997
8. Zahorska-Markiewicz B, Janowska J, Olszanecka-Glinianowicz M, et al: Serum concentrations of tumor necrosis factor in obese women. J Endocrinol Invest 22:66, 1999 (suppl 7, abstr)
9. Zahorska-Markiewicz B, Janowska J, Olszanecka-Glinianowicz M, et al: Serum concentrations of TNF-α and soluble TNF-α receptors in obesity. Int J Obes 24:1392-1395, 2000
10. Mohamed-Ali V, Goodrick S, Bulmer K, et al: Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo. Am J Physiol Endocrinol Metab 82:971-975, 1999
11. Hauner H, Bender M, Haastert B, et al: Plasma concentrations of soluble TNF-α receptors in obese subjects. Int J Obes 22:1239-1243, 1998
12. Choi JW, Pai SH, Kim SK, et al: Increases in nitric oxide concentrations correlate strongly with body fat in obese humans. Clin Chem 47:1106-1109, 2001
13. Zahorska-Markiewicz B, Olszanecka-Glinianowicz M, Janowska J, et al: Serum NO (nitric oxide) and TNF-α in obesity. Int J Obes 25:S129, 2001 (suppl 2, abstr)
14. Kern PA, Saghizadeh M, Ong JM, et al: The expression of tumor necrosis factor in human adipose tissue regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 5:2111-2119, 1995
15. Ferlito S, Gallina M: Nitrite plasma levels in acute and chronic heart disease. Minerva Cardioangiol 11:553-558, 1997
16. Winkler G, Kiss S, Keszthelyi L, et al: Expression of tumor necrosis factor (TNF)-α protein in the subcutaneous and visceral adipose tissue in correlation with adipoctyte cell volume, serum TNF-α, soluble serum TNF-receptor-2 concentrations and C-peptide level. Eur J Endocrinol 149:129-135, 2003
17. Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Janowska J, et al: Serum concentrations of tumor necrosis factor TNF-α, and its soluble receptors in obese women with insulin resistance. Pol J Endocrinol 54: 414-420, 2003
18. Diez-Ruiz A, Tilz GP, Zangerle R, et al: Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. Eur J Hematol 54:1-8, 1995
19. Dzienis-Strączkowską S, Strączkowski M, Stępień Z, et al: Soluble tumor necrosis factors in young obese subjects with normal and impaired glucose tolerance. Diabetes Care 26:875-880, 2003
20. Holm C, Osterlund T, Laurell H, et al: Molecular mechanism regulating hormone-sensitive lipase and lipolysis. Annu Rev Nutr 20: 365-393, 2000
21. Perreault M, Marette A: Targeted disruption of inducible nitric oxide synthase protection against obesity-linked insulin resistance in muscle. Nat Med 7:1138-1143, 2001
22. Nicoletti G, Giugliano G, Pontillo A, et al: Effect of multidisciplinary program of weight reduction on endothelial functions in obese women. J Endocrinol Invest 26:RC5-8, 2003
23. Ziccardi P, Nappo F, Giugliano G, et al: Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation 105:804-809, 2001