

Soluble Tumor Necrosis Factor- α Receptors in Young Obese Subjects With Normal and Impaired Glucose Tolerance

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OBJECTIVE — Tumor necrosis factor- α (TNF- α) is a possible link between obesity and impaired glucose tolerance (IGT) and type 2 diabetes. Data about TNF- α and soluble forms of its receptors (sTNFR1 and sTNFR2) in IGT are controversial. The aim of the present study was to assess plasma TNF- α , sTNFR1, and sTNFR2 levels and to evaluate the relationships with insulin resistance in obese subjects with IGT.

RESEARCH DESIGN AND METHODS — A total of 104 subjects participated in the present study: 30 obese subjects with IGT (obese-IGT), 32 obese subjects with normal glucose tolerance (obese-NGT), and 42 lean healthy control subjects (control-NGT). Anthropometry and blood biochemical parameters were measured and euglycemic-hyperinsulinemic clamp was performed.

RESULTS — Obese-IGT subjects were more insulin resistant in comparison with obese-NGT and control-NGT groups; obese-NGT subjects were more insulin resistant than control-NGT. Plasma sTNFR1 and sTNFR2 were markedly higher in both groups of obese subjects in comparison with control-NGT and in the obese-IGT versus obese-NGT group. Plasma sTNFR1 and sTNFR2 were inversely related to insulin sensitivity. Both relationships remained significant after adjustment for age, BMI, waist girth, percent body fat, plasma glucose, insulin, nonesterified fatty acids, cholesterol, and triglycerides. Correlation between sTNFR2 and insulin sensitivity was also present in all the groups analyzed separately, but the correlation between sTNFR1 and insulin sensitivity was present only in the obese-NGT group.

CONCLUSIONS — Our data suggest that TNF- α receptors are increased in obese-IGT subjects and are related to insulin resistance. These findings indicate that the TNF- α system might contribute to the development of insulin resistance in glucose-intolerant subjects.

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Tumor necrosis factor- α (TNF- α) is a cytokine involved in immune response and is also a possible link between obesity and impaired glucose tolerance (IGT) and type 2 diabetes (1). In obese and type 2 diabetic subjects, there is an increased cytokine expression in ad-

ipose tissue and skeletal muscle, where it acts in an auto- and paracrine manner, inducing insulin resistance (2–4). Plasma levels of soluble TNF- α receptors (sTNFR1 and sTNFR2) are supposed to reflect local tissue action of the cytokine (5,6). sTNFR2 seems to be an especially

good marker of TNF- α -induced insulin resistance as both increased adipose tissue receptor expression (5) and plasma soluble form concentrations (6) in obesity were reported. Data about sTNFR1 are less consistent as there are reports about both unchanged (6) and increased (7) levels in obesity.

Plasma sTNFR2 levels are negatively related to insulin sensitivity in subjects with normal glucose tolerance, while for sTNFR1 no such correlation was found (6), although other authors noted an association with homeostasis model assessment-insulin resistance (HOMA-IR), an indirect index of insulin resistance (8). It remains unclear whether the TNF- α system might contribute to the deterioration of insulin sensitivity observed in glucose-intolerant states. Only one study reported that both soluble receptor levels in obese type 2 diabetic patients increased (8). However, hyperglycemia might itself deteriorate insulin sensitivity and influence the immune system. Type 2 diabetes is usually preceded by IGT; studies with individuals with IGT may give a better insight in the pathogenetic mechanisms leading to diabetes. Data about TNF- α receptors in IGT are very limited and controversial, and there is no data about the relationship of those receptors with insulin resistance in IGT subjects so far.

Recently we demonstrated an increase in sTNFR2 concentrations in lean nondiabetic offspring of type 2 diabetic subjects (9). Those data suggest involvement of TNF- α system in early steps of the development of insulin resistance, even before the onset of obesity. To further explore the possible associations between TNF- α system and disturbances of glucose tolerance, in the present study we assess plasma TNF- α , sTNFR1, and sTNFR2 levels and evaluate the relationships of TNF- α system with insulin resistance in obese subjects with IGT.

RESEARCH DESIGN AND METHODS

RESEARCH DESIGN AND METHODS — A total of 104 subjects, aged 20–50 years, participated in the present study. Of the 104, 30 were obese

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Abbreviations: CRP, C-reactive protein; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; FM, fat mass; FFM, fat-free mass; HOMA-IR, homeostasis model assessment-insulin resistance; IGT, impaired glucose tolerance; NEFA, nonesterified fatty acid; obese-IGT, obese with IGT; obese-NGT, obese with normal glucose tolerance; TNF- α , tumor necrosis factor- α ; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

with IGT (obese-IGT, BMI >27.8 kg/m², 10 men and 20 women), 32 were obese with normal glucose tolerance (obese-NGT, 14 men and 18 women), and 42 were lean healthy control subjects (control-NGT, BMI <25 kg/m², 17 men and 25 women). Glucose tolerance was assessed during an oral glucose tolerance test according to World Health Organization criteria. Individuals with IGT had no diagnosed disturbances of glucose metabolism before entering the study. All the participants were without ischemic heart disease, unstable hypertension (i.e., above 160/95 mmHg), peripheral vascular disease, infections, or any other serious medical problems. Before participating in the study, physical examination and appropriate laboratory tests were performed. All analyses were performed after an overnight fast. The study protocol was approved by the ethics committee of the Medical Academy, Bialystok. All subjects gave written informed consent before entering the study.

Anthropometry

The BMI was calculated as body weight divided by height squared and expressed in kg/m². The waist-to-hip ratio (WHR) was estimated. The waist circumference was measured at the smallest circumference between the rib cage and the iliac crest, with the subject in the standing position. The hip circumference was measured at the widest circumference between the waist and the thighs. Percent of body fat was assessed by bioelectric impedance analysis using the Tanita TBF-511 Body Fat Analyzer (Tanita, Tokyo, Japan); fat mass (FM) and fat-free mass (FFM) were calculated.

Insulin sensitivity

Insulin sensitivity was evaluated by the euglycemic-hyperinsulinemic clamp technique according to DeFronzo et al. (10). On the morning of the study, two venous catheters were inserted into antecubital veins, one for the infusion of insulin and glucose and the other in the contralateral hand for blood sampling (that hand was heated to $\sim 60^\circ\text{C}$). Insulin (Actrapid HM; Novo Nordisk, Copenhagen, Denmark) was given as a primed-continuous intravenous infusion for 2 h at $50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, resulting in constant hyperinsulinemia of ~ 550 pmol/l. Arterialized blood glucose was obtained every 5 min, and 40% dextrose (2.22 mol/l) in-

fusion was adjusted to maintain plasma glucose levels at 5.0 mmol/l. The glucose infusion rate approached stable values during the final 40 min of the study. The rate of whole-body glucose uptake (M value) was calculated as the mean glucose infusion rate from 80 to 120 min and normalized for FFM (M/FFM).

Other blood analyses

Fasting blood samples were also taken from the antecubital vein before the beginning of the clamp for the determination of HbA_{1c}, plasma lipids, TNF- α , sTNFR1 and sTNFR2, C-reactive protein (CRP), and leptin. For the determination of plasma TNF- α system, samples were frozen at -70°C .

Analytical procedures

Plasma glucose was measured immediately by the enzymatic method using glucose analyzer. Plasma insulin was measured with the Medgenix EASIA test (BioSource Europe, Nivelles, Belgium). The minimum detectable concentration was 1.05 pg/l and the intra- and interassay coefficients of variation (CVs) were <5.5 and $<10\%$, respectively. In that method, human and animal proinsulins present no cross-reaction. HbA_{1c} was measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany) with recommended normal range of the assay 4.1–6.5%. Plasma total cholesterol and triglycerides were assessed by the enzymatic methods (Cormay, Warsaw, Poland). Plasma nonesterified fatty acids (NEFAs) were measured by the colorimetric method (11).

Plasma TNF- α concentrations were measured by the Immunoassay Kit (BioSource International, Camarillo, CA) with the minimum detectable concentration 1.7 pg/ml and with the intra- and inter-assay CVs <5.2 and $<8.5\%$, respectively. Plasma sTNFR1 and sTNFR2 were determined with the EASIA kits. The minimum detectable concentration was 0.05 ng/ml for sTNFR1 and 0.1 ng/ml for sTNFR2. The intra- and interassay CVs for both receptors were <6.5 and $<9\%$, respectively. sTNFR1 EASIA does not cross-react with sTNFR2, and TNF- α does not interfere with the assay.

Serum CRP was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic System Laboratories, Webster, TX) with sensitivity 1.6 ng/ml. Plasma leptin was measured with an

ELISA kit (DRG Instruments, Germany) with sensitivity 1.0 ng/ml.

Statistical analysis

The statistics were performed with the STATISTICA 5.0 program (StatSoft, Krakow, Poland). Differences between the groups were evaluated with the Student's t test. Relationships between variables were estimated with the simple and multiple regression analysis. The level of significance was accepted at $P < 0.05$.

RESULTS

Clinical characteristics of the studied groups are given in Table 1. We observed a marked decrease in insulin sensitivity in obese-IGT group in comparison with obese-NGT and control-NGT groups (both $P < 0.000001$) and in obese-NGT vs. control-NGT groups ($P < 0.01$). All these differences were still present when men and women were analyzed separately; only the difference between obese-NGT and control-NGT men was of borderline significance ($P = 0.07$).

TNF- α concentrations were markedly increased in obese-IGT in comparison with control-NGT women ($P < 0.05$); all other differences were not significant.

Both soluble forms of TNF- α receptors were significantly increased in the obese-IGT group in comparison with other groups (sTNFR1 obese-IGT vs. obese-NGT, $P < 0.05$; obese-IGT vs. control-NGT, $P < 0.000005$; sTNFR2 obese-IGT vs. obese-NGT, $P < 0.02$; obese-IGT vs. control-NGT, $P < 0.00001$) and in obese-NGT compared with control-NGT (sTNFR1 $P < 0.00005$; sTNFR2 $P < 0.02$).

When men and women were analyzed separately, all the differences in TNF- α receptors were present in women (sTNFR1 obese-IGT vs. obese-NGT, $P < 0.05$; obese-IGT vs. control-NGT, $P < 0.00005$; obese-NGT vs. control-NGT, $P < 0.002$; sTNFR2 obese-IGT vs. obese-NGT, $P < 0.05$; obese-IGT vs. control-NGT, $P < 0.0002$; obese-NGT vs. control-NGT, $P < 0.05$). However, in men, both sTNFRs were different between only obese-IGT and control-NGT groups (sTNFR1 $P < 0.01$; sTNFR2 $P < 0.02$). We also observed markedly increased sTNFR1, but not sTNFR2 in the obese-NGT in comparison with the control-NGT group ($P < 0.01$ and $P = 0.24$, respectively). The differences between obese-IGT and obese-NGT men did not

Table 1—Clinical characteristics of the studied groups

	Men			Women		
	Control-NGT	Obese-NGT	Obese-IGT	Control-NGT	Obese-NGT	Obese-IGT
n	17	14	10	25	18	20
Age (years) (range)	36.00 ± 7.05 (24.0–48.0)	35.64 ± 10.55 (21.0–47.0)	37.70 ± 6.45 (23.0–45.0)	34.12 ± 8.54 (23.0–50.0)	38.72 ± 9.16 (21.0–49.0)	38.35 ± 5.83 (25.0–49.0)
BMI (kg/m ²) (range)	22.80 ± 2.01 (17.78–24.94)	32.35 ± 3.46* (28.15–41.82)	33.24 ± 4.41* (28.03–41.5)	21.47 ± 2.18 (16.82–24.92)	31.99 ± 4.35* (28.09–46.60)	34.78 ± 4.59* (28.28–43.66)
WHR	0.83 ± 0.05	0.95 ± 0.05*	0.92 ± 0.07*	0.77 ± 0.07	0.84 ± 0.06*	0.87 ± 0.05*
% Body fat	16.97 ± 8.73	35.78 ± 8.73*	35.15 ± 9.62*	15.88 ± 5.67	36.86 ± 9.75*	40.35 ± 8.28*
Fasting glucose (mmol/l)	4.95 ± 0.59	5.50 ± 0.62*	6.17 ± 0.64*†	4.74 ± 0.48	5.25 ± 0.51*	6.10 ± 0.64*†
Postload glucose (mmol/l)	4.54 ± 0.86	5.54 ± 1.58*	9.18 ± 1.18*†	4.66 ± 1.03	5.79 ± 1.25*	9.01 ± 0.90*†
Insulin (pmol/l)	69.43 ± 38.84	128.89 ± 81.32*	133.60 ± 56.34*	67.42 ± 31.02	88.25 ± 25	159.8 ± 115.9*
HbA _{1c} (%) (range)	5.54 ± 0.32 (5.2–6.2)	5.70 ± 0.51 (5.1–6.5)	6.42 ± 0.66*† (5.2–7.0)	5.26 ± 0.41 (4.6–6.0)	5.78 ± 0.52* (4.9–6.8)	6.38 ± 0.60*† (5.4–7.2)
NEFAs (mmol/l)	0.407 ± 0.12	0.492 ± 0.14	0.583 ± 0.16*	0.319 ± 0.11	0.514 ± 0.16*	0.597 ± 0.14*
TC (mmol/l)	5.03 ± 1.29	5.57 ± 1.17	6.34 ± 1.63*	4.15 ± 0.74	5.35 ± 1.04*	5.08 ± 0.98*
TG (mmol/l)	1.41 ± 0.81	2.15 ± 1.05*	2.63 ± 2.69	0.85 ± 0.37	1.40 ± 0.99*	2.07 ± 0.86*†
M/FFM (μmol × kg ⁻¹ × min ⁻¹)	46.41 ± 17.13	36.38 ± 12.57	24.27 ± 8.61*†	48.62 ± 13.72	40.29 ± 12.65*	19.16 ± 8.74*†
TNF-α (pg/ml)	5.33 ± 2.38	5.78 ± 1.31	6.93 ± 3.15	4.59 ± 2.89	5.81 ± 1.45	6.46 ± 2.85
sTNFR1 (ng/ml)	2.08 ± 0.35	2.49 ± 0.48*	2.61 ± 0.59*	1.87 ± 0.38	2.28 ± 0.38*	2.82 ± 0.96*†
sTNFR2 (ng/ml)	4.36 ± 0.77	4.84 ± 1.44	5.86 ± 2.17*	4.13 ± 0.82	4.83 ± 1.12*	5.94 ± 2.01*†
CRP (mg/l)	3.38 ± 1.86	5.17 ± 2.54*	7.29 ± 2.65*	3.07 ± 2.12	4.98 ± 2.36*	5.91 ± 2.06*
Leptin (ng/ml)	3.42 ± 1.96	8.56 ± 3.77*	8.08 ± 4.15*	6.89 ± 4.26	19.31 ± 8.55*	20.90 ± 9.78*

Data are presented as mean ± SD. **P* < 0.05 in obese-NGT or obese-IGT groups in comparison with control-NGT group; †*P* < 0.05 in obese-IGT in comparison with obese-NGT group. TC, total cholesterol; TG, triglycerides.

reach the level of significance (sTNFR1 *P* = 0.58; sTNFR2 *P* = 0.17).

We observed an excellent correlation between sTNFR1 and sTNFR2 (*r* = 0.80, *P* < 0.0000001). Both TNF-α receptors were related to BMI, WHR, percent body fat, FM, FFM, glucose, insulin, HbA_{1c}, NEFAs, triglycerides, CRP, and leptin (all *P* < 0.05) (Table 2). Also, both sTNFR1 and sTNFR2 were negatively related to M/FFM (both *P* < 0.000001) (Table 2). TNF-α was related to sTNFR1 (*r* = 0.41, *P* = 0.00001) and sTNFR2 (*r* = 0.44, *P* = 0.000002). The correlations between TNF-α and other variables mentioned above were much weaker than those for the receptors (correlation with M/FFM, *r* = -0.28, *P* = 0.004).

In the subgroup analysis, the relationship between sTNFR2 and M/FFM was present in the obese-IGT (*r* = -0.42, *P* = 0.02), obese-NGT (*r* = -0.54, *P* = 0.001), and control-NGT (*r* = -0.31, *P* = 0.048) groups (Fig. 1). The correlation between sTNFR1 and M/FFM was present in the obese-NGT (*r* = -0.56, *P* = 0.001), but not in the obese-IGT (*r* = -0.25, *P* = 0.18) and control-NGT (*r* = -0.25, *P* = 0.10) groups. Correlation be-

tween NEFA and M/FFM, which was significant in the whole group (*r* = -0.35, *P* < 0.0001), was no longer present in the subgroup analysis (obese-IGT *r* = -0.03, *P* = 0.87; obese-NGT *r* = 0.04, *P* = 0.81; control-NGT *r* = 0.06, *P* = 0.71).

In the control-NGT group, receptors were related to FFM (sTNFR1 *r* = 0.43,

P = 0.005; sTNFR2 *r* = 0.33, *P* = 0.03), while the relationship with FM was significant only for sTNFR1 (*r* = 0.31, *P* = 0.049). TNF-α receptors were related to FM in the obese-NGT (sTNFR1 *r* = 0.35, *P* = 0.048; sTNFR2 *r* = 0.44, *P* = 0.012) and obese-IGT (sTNFR1 *r* = 0.61, *P* = 0.00049; sTNFR2 *r* = 0.44, *P* = 0.015)

Table 2—Correlations between sTNFR1 and sTNFR2 and other examined parameters in the whole population (N = 104)

	sTNFR1	sTNFR2
Age	0.08	0.03
BMI	0.62*	0.52*
WHR	0.41*	0.33*
% Body fat	0.57*	0.47*
Glucose	0.40*	0.40*
Insulin	0.65*	0.57*
HbA _{1c}	0.39*	0.35*
NEFAs	0.45*	0.43*
TC	0.16	0.10
TG	0.36*	0.44*
M/FFM	-0.51* <i>P</i> < 0.000001	-0.54* <i>P</i> < 0.000001
CRP	0.54*	0.51*
Leptin	0.36*	0.36*

**P* < 0.05. TC, total cholesterol; TG, triglycerides.

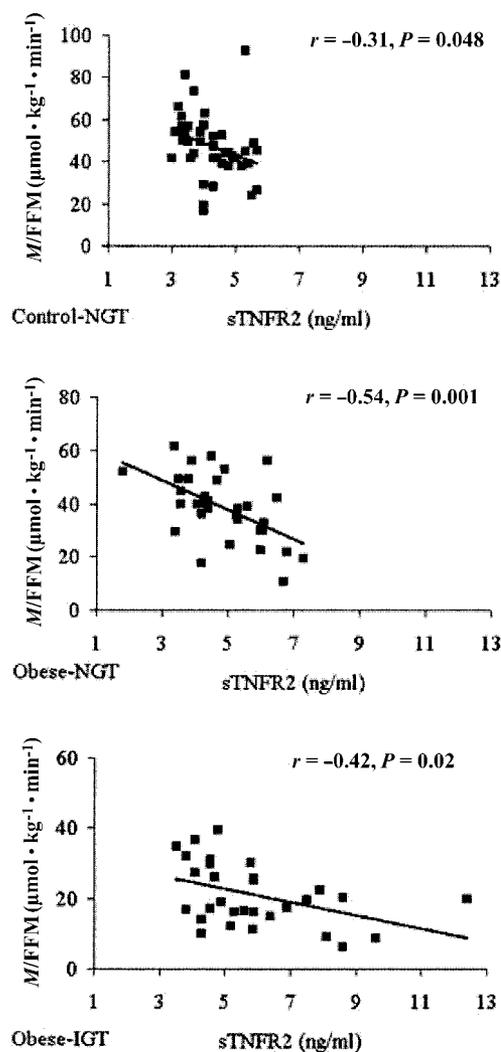


Figure 1—Correlation between M/FFM and sTNFR2 in control-NGT, obese-NGT, and obese-IGT.

groups. In the obese-NGT group, there was also a marked correlation between sTNFR1 and FFM ($r = 0.46$, $P = 0.008$); all other correlations of sTNFRs with FFM in obese groups were not significant.

In multiple regression analysis, performed in the whole studied population ($n = 104$), both sTNFR1 ($\beta = -0.21$, $P = 0.04$) and sTNFR2 ($\beta = -0.25$, $P = 0.01$) were associated with M/FFM independently of age ($\beta = -0.07$, $P = 0.33$), BMI ($\beta = -0.59$, $P = 0.02$), waist girth ($\beta = 0.11$, $P = 0.54$), percent body fat ($\beta = 0.28$, $P = 0.17$), plasma glucose ($\beta = -0.41$, $P = 0.00005$), insulin ($\beta = -0.02$, $P = 0.82$), NEFAs ($\beta = 0.17$, $P = 0.11$), total cholesterol ($\beta = 0.01$, $P = 0.99$), and triglycerides ($\beta = -0.15$, $P = 0.10$).

When we performed multiple regres-

sion analysis within subgroups, in the obese-IGT group both sTNFR1 and sTNFR2 were not independent factors determining insulin sensitivity ($\beta = -0.05$, $P = 0.85$ and $\beta = -0.04$, $P = 0.89$, respectively). However, when we performed multiple regression analysis in a stepwise manner, sTNFR2, but not sTNFR1, independently predicted M/FFM and was responsible for 9% of its variability ($P < 0.0001$).

In the obese-NGT group, the relationship between sTNFR2 and M/FFM was independent of other tested variables ($\beta = -0.40$, $P = 0.035$), while for sTNFR1 coefficient was of borderline significance ($\beta = -0.36$, $P = 0.068$). In contrast, in the control-NGT group the regression coefficient reached the level of significance for sTNFR1 ($\beta = -0.35$, $P =$

0.032), but not for sTNFR2 ($\beta = -0.21$, $P = 0.13$).

Serum CRP was markedly higher in both obese groups in comparison with control-NGT within subgroups of men and women (obese-IGT versus control-NGT, both $P < 0.001$; obese-NGT versus control-NGT, both $P < 0.05$). It was also higher in the obese-IGT than in the obese-NGT group when we analyzed all subjects together ($P < 0.05$); however, the difference was not significant within subgroups of men and women ($P = 0.06$ and $P = 0.20$, respectively). Plasma leptin was higher in both obese groups in comparison with control-NGT in men (both $P < 0.01$) and women (both $P < 0.000001$). As expected, in all subgroups it was higher in women (all $P < 0.001$). It did not differ between obese-IGT and obese-NGT individuals. We observed marked correlations between M/FFM and CRP ($r = -0.52$, $P < 0.000001$) and leptin ($r = -0.28$, $P = 0.004$).

CONCLUSIONS— In the present study, despite a similar degree of adiposity, obese-IGT subjects were more insulin resistant in comparison with weight-matched obese-NGT individuals.

We found the differences in TNF- α system parameters between the studied groups; both receptors were independent predictors of insulin sensitivity. As mentioned, correlation for TNF- α itself was much weaker than for its receptors. It is supposed that circulating TNF- α levels do not reflect auto- and paracrine action of the cytokine. Bluher et al. (12) found no differences in TNF- α concentrations between insulin-sensitive control subjects and insulin-resistant IGT subjects.

Fernandez-Real et al. (6) observed a correlation between sTNFR2, but not sTNFR1, and insulin resistance. In contrast, other studies reported a relationship of both receptors with HOMA-IR (8). Probably soluble receptor concentrations increase with increasing local TNF- α action in adipose tissue and skeletal muscle (5). In our previous studies, we were unable to find an increase in sTNFR1 in obesity; however, this was probably due to a limited number of subjects (13,14). In the present study we demonstrated an association of both receptors with insulin sensitivity; only correlations observed for sTNFR2 were significant within all three subgroups. Our data indicate that soluble forms of TNF- α receptors might be a

better marker of TNF- α action in insulin-resistant states than the circulating cytokine level itself, and sTNFR2 might be especially important in glucose-intolerant individuals. We also observed association of both receptors with triglycerides. Similar relationships were previously reported by Fernandez-Real et al. in healthy women (15) and in a population of healthy subjects and myotonic dystrophy patients (16). TNF- α is thought to increase triglyceride levels through increase in VLDL secretion; however, contribution of TNF- α associated insulin resistance is also possible.

Our study does not provide evidence about the source of circulating TNF- α receptors. In the present study, sTNFRs were related to FFM in lean subjects, but in the obese individuals correlations with FM were stronger. Therefore, one may hypothesize that in lean subjects, skeletal muscle might be an important source of circulating TNF- α receptors, while in the obese the effect of adipose tissue accumulation might be more pronounced. Fernandez-Real et al. (6) observed correlation between sTNFR2 and FFM, while Bullo et al. (8) reported association of both receptors with FM in obese and morbidly obese subjects.

Although there are many clinical studies about associations of TNF- α and insulin resistance, only a few of them analyzed individuals with disturbances of glucose tolerance. In the study of Bluher et al. (12), cited above, TNF- α receptors were not measured. Bullo et al. (8) observed an impact of type 2 diabetes on sTNFR1 and sTNFR2 in obese and morbidly obese women. Very recently, Muller et al. (17) reported unchanged sTNFRs concentrations in glucose-intolerant subjects. Those data are in contrast with the results reported in our study. The difference might come from the selection of study subjects. Individuals recruited in the cited study were much older (55–74 years, mean age \sim 65 years) than subjects reported here. IGT and type 2 diabetes in the elderly might have different pathogenetic mechanisms, and also, the TNF- α system changes with age (18)—it is possible that it can mask differences present in younger subjects. Also, in the IGT group we recruited only individuals with marked overweight or obesity, while in the study of Muller et al. (17), the BMI range in the IGT group was 19.9–40.4 (median 29.4). IGT and type 2 diabetes

are heterogeneous disorders. It is probable that the contribution of TNF- α -associated insulin resistance to the deterioration of glucose tolerance is more important in the obese. In the cited study (17) insulin sensitivity was not measured, while we can define our obese-IGT subjects as insulin resistant. Therefore, we provide the first (to our knowledge) data that show that soluble forms of TNF- α receptors are increased in obese insulin-resistant IGT individuals and that they are negatively related to insulin sensitivity in that group.

The reason for the upregulation of TNF- α system in IGT remains unclear. It might be genetic predisposition or acquired (at present unknown) factors. As mentioned, we recently demonstrated higher sTNFR2 levels in lean nondiabetic, but insulin-resistant, normoglycemic offspring of type 2 diabetic subjects (9). Our findings might suggest some genetic predisposition; however, there might be other factors primary to the observed increase in sTNFR2.

There are some studies reporting association of TNFR2 gene polymorphism and features of insulin resistance syndrome (19–21), but the relationship with insulin action and deterioration of glucose tolerance deserves further investigation. Polymorphisms of TNFR1 gene have not been extensively studied so far.

Both obese groups also differed in NEFA levels. Although NEFA level was negatively related to insulin sensitivity, this association was not present in subgroup correlations and it was not significant after adjustment for other parameters. Those data suggest that NEFA are probably not the main reason for the differences in insulin action observed between groups. Studies with clamp and lipid infusion revealed that NEFAs might account for a maximum of 50% muscle insulin resistance in type 2 diabetes (22).

In our study, leptin was not different between either of the obese groups. It was reported previously that BMI is a more important determinant of plasma leptin than glucose tolerance status (23). Also, associations with the TNF- α system are similar to those observed by other investigators (23), and it was observed that TNF- α might increase leptin gene expression and circulating leptin levels.

Increased CRP levels in obese groups and the negative relationship between CRP and insulin sensitivity give addi-

tional support to the hypothesis about the role of chronic inflammation in the pathogenesis of insulin resistance syndrome. When we analyzed men and women together, obese-IGT individuals also had higher CRP levels than obese-NGT group. This is in agreement with the data of Muller et al. (17). Correlations between CRP and insulin resistance and other features of metabolic syndrome (24) were also found previously.

We conclude that TNF- α receptors are increased in obese-IGT subjects and related to insulin resistance. These findings indicate that the TNF- α system might contribute to the development of insulin resistance in glucose-intolerant individuals. Plasma sTNFR2 concentration seems to be a marker of TNF- α -related insulin resistance, which is especially important in obese-IGT subjects.

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