Increased Plasma-Soluble Tumor Necrosis Factor-α Receptor 2 Level in Lean Nondiabetic Offspring of Type 2 Diabetic Subjects

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OBJECTIVE — Tumor necrosis factor-α (TNF-α) is one of the proposed mediators of insulin resistance, upregulated in human obesity. Insulin resistance, however, might precede the development of obesity, especially in subjects with a family history of type 2 diabetes. Therefore, the aim of the present study was to assess plasma levels of TNF-α and soluble forms of its receptors (soluble TNF-α receptors 1 [sTNFR1] and 2 [sTNFR2]) and to evaluate the relationship of the TNF-α system with insulin resistance in lean, nondiabetic offspring of type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — We compared 20 lean offspring (BMI <25 kg/m^2, 8 men and 12 women) of type 2 diabetic patients with 20 lean subjects with no family history of diabetes, matched for age, sex, and BMI (control group). Anthropometry and blood biochemical parameters were measured, and insulin sensitivity was evaluated with the euglycemic-hyperinsulinemic clamp technique.

RESULTS — Both men and women in the offspring group were markedly more insulin-resistant and had higher plasma levels of sTNFR2 (all $P < 0.05$). TNF-α, sTNFR1, and other examined parameters did not differ between the studied groups. Both TNF-α receptors were related to waist-to-hip ratio (WHR), fat-free mass (FFM), plasma total cholesterol, HDL cholesterol, LDL cholesterol, and nonesterified fatty acids (NEFAs). sTNFR2, but not sTNFR1, was also associated with insulin sensitivity ($r = -0.49$, $P = 0.001$). This relationship remained significant after adjustment for WHR, FFM, plasma insulin, and NEFA.

CONCLUSIONS — TNF-α system might be involved in modulating insulin action before the onset of obesity in subjects at high risk for type 2 diabetes.

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Tumor necrosis factor-α (TNF-α) is one of the proposed mediators of insulin resistance, overexpressed in adipose tissue and skeletal muscle (1) of obese and type 2 diabetic subjects. The cytokine probably induces insulin resistance by acting in an autocrine and paracrine manner. Two cell-surface TNF-α receptors were described in humans: tumor necrosis factor-α receptors 1 [TNFR1 (p60)] and 2 [TNFR2 (p80)]. Soluble forms of both receptors (sTNFR1 and sTNFR2) are present in plasma. In different conditions, sTNFRs might inactivate TNF-α or prolong action of the cytokine by stabilizing its bioactivity (2). Activation of TNF-α results in overexpression of TNFR2 in adipose tissue and the increase in plasma sTNFR2 in obese humans (3). sTNFR2 levels are related to insulin resistance (4). It is suggested that TNFR2 plays a role in inducing insulin resistance by modulating the actions of TNF-α (3,4). Plasma TNF-α values are usually low and do not give the precise information about its action in obesity, whereas sTNFR2 is a much more stable protein. Therefore, it is proposed that sTNFR2 might serve as the best predictor of local TNF-α system activation and as a diagnostic marker for obese individuals with TNF-α–related insulin resistance (3).

Many studies focus on TNF-α actions leading to insulin resistance, mostly in obesity. Insulin resistance, however, might precede the development of obesity, especially in a group of predisposed subjects. Normoglycemic subjects with a family history of type 2 diabetes are, in a large proportion, insulin-resistant and have an increased risk of developing the disease (5). It was found that insulin-stimulated insulin receptor substrate-1 activation is impaired in skeletal muscle of offspring of type 2 diabetic parents (6). Such a defect might be attributable to TNF-α action. Circulating TNF-α levels do not correlate with insulin sensitivity (7), but TNF-α receptors have not been determined in that group of subjects so far.

In the present study, we assess plasma levels of TNF-α and soluble forms of its receptors and evaluate the relationship of the TNF-α system with insulin resistance in lean, nondiabetic offspring of type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

— We compared 20 lean offspring (BMI <25 kg/m^2; 8 men and 12 women) of type 2 diabetic patients with 20 lean subjects with no family history of

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Abbreviations: FFM, fat-free mass; MCR, glucose metabolic clearance rate; NEFA, nonesterified fatty acid; sTNFR1, soluble tumor necrosis factor-α receptor 1; sTNFR2, soluble tumor necrosis factor-α receptor 2; TNF-α, tumor necrosis factor-α; TNFR1, tumor necrosis factor-α receptor 1; TNFR2, tumor necrosis factor-α receptor 2; WHR, waist-to-hip ratio.

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

diabetes, carefully matched for age, sex, and BMI (control group; BMI <25 kg/m^2, 8 men and 12 women). Relatives of type 2 diabetic patients were recruited for the study if both parents had type 2 diabetes or if one parent and one first- or second-degree relative had type 2 diabetes. All participants had no ischemic heart disease, hypertension, peripheral vascular disease, infection, or any other serious medical problems. Before participating in the study, physical examination and appropriate laboratory tests were performed. All subjects underwent an oral glucose tolerance test, and all had normal glucose tolerance according to World Health Organization (WHO) 1999 criteria. The study protocol was approved by the Ethics Committee of Medical Academy, Bialystok. All subjects gave written informed consent before entering the study.

Table 1—*Anthropometric characteristics of the study groups*

	Men		Women	
	Offspring of type 2 diabetic patients (n = 8)	Control subjects (n = 8)	Offspring of type 2 diabetic patients (n = 12)	Control subjects (n = 12)
Age (years)	24.37 ± 4.75	24.75 ± 4.33	24.25 ± 3.22	24.92 ± 3.48
BMI (kg/m^2)	22.69 ± 2.03	22.62 ± 1.44	20.82 ± 1.75	21.07 ± 1.67
WHR	0.84 ± 0.05	0.85 ± 0.02	0.74 ± 0.06	0.75 ± 0.03
Fat mass (kg)	11.45 ± 4.26	11.29 ± 2.47	7.78 ± 2.39	7.75 ± 2.76
FFM (kg)	65.19 ± 6.85	62.88 ± 2.32	47.95 ± 4.27	48.99 ± 4.53

Data are means ± SD.

Anthropometry

All analyses were performed after an overnight fast. The BMI was calculated as body weight × height^{-2} and expressed in kilograms per meter squared. 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 and fat-free mass (FFM) were calculated.

Insulin sensitivity

Insulin sensitivity was evaluated by the euglycemic-hyperinsulinemic clamp technique according to DeFronzo et al. (8), as described previously (9,10). On the morning of the study, two venous catheters were inserted into antecubital veins, one for infusion of insulin and glucose and the other in the contralateral hand for blood sampling; that hand was heated to ~60°C. Insulin (Actrapid HM; Novo Nordisk, Copenhagen, Denmark) was given as a primed, continuous intravenous infusion for 2 h at 50 mU · kg^{-1} · 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) infusion 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 was calculated as the mean glucose infusion rate from 80 to 120 min; glucose metabolic clearance rate (MCR) was used as an index of insulin sensitivity.

Other blood analyses

Fasting blood samples were also collected from the antecubital vein before the beginning of the clamp for the determination of HbA_{1c}, plasma lipids, TNF-α, sTNFR1, and sTNFR2. 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 a 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-assay and interassay 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). Plasma total cholesterol, HDL cholesterol, and triglycerides were assessed by the enzymatic methods (Cormay, Warsaw, Poland); LDL cholesterol was calculated using Friedewald's formula. Plasma nonesterified fatty acids (NEFAs) were measured by the colorimetric method (11).

Plasma TNF-α concentrations were measured using the Immunoassay Kit (BioSource International, Camarillo, CA); the minimum detectable concentration was 1.7 pg/ml, and the intra-assay and interassay CVs were <5.2 and 8.5%, respectively. Plasma sTNFR1 and sTNFR2 were determined with the EASIA kits (BioSource Europe). The minimum detectable concentration was 0.05 ng/ml for sTNFR1 and 0.1 ng/ml for sTNFR2. The intra-assay 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.

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 — The studied groups did not differ in anthropometric measurements (Table 1). Despite that, the offspring of diabetic parents were markedly hyperinsulinemic ($P < 0.05$) and insulin-resistant ($P < 0.005$) and had higher plasma levels of sTNFR2 ($P < 0.005$). TNF-α, sTNFR1, and other examined parameters did not differ between the studied groups (Table 2).

The differences in MCR and sTNFR2 between the studied groups were still present when men and women were analyzed separately. The observed values for MCR were as follows: male offspring of type 2 diabetic patients 6.34 ± 1.59 and control subjects 8.16 ± 1.52 ml · kg^{-1} · min^{-1} ($P < 0.05$), female offspring of type 2 diabetic patients 6.32 ± 1.98 and control subjects 8.58 ± 3.06 ml · kg^{-1} · min^{-1} ($P < 0.05$). The respective values of sTNFR2 were as follows: male offspring of type 2 diabetic patients 5.04 ± 0.83 and control subjects 4.01 ± 0.69 ng/ml

Table 2—*Metabolic characteristics of the study groups*

	Offspring of type 2 diabetic patients ($n = 20$)	Control subjects ($n = 20$)
Plasma glucose (mmol/l)	4.84 ± 0.49	4.67 ± 0.51
HbA_{1c} (%)	5.41 ± 0.29	5.37 ± 0.41
Plasma insulin (pmol/l)	74.26 ± 32.21	53.74 ± 31.35*
NEFA (mmol/l)	0.521 ± 0.14	0.451 ± 0.16
Plasma cholesterol (mmol/l)	4.40 ± 0.79	4.32 ± 0.86
Plasma triglycerides (mmol/l)	1.11 ± 0.56	0.92 ± 0.42
HDL cholesterol (mmol/l)	1.44 ± 0.32	1.42 ± 0.31
LDL cholesterol (mmol/l)	2.45 ± 0.85	2.48 ± 0.90
MCR ($ml \cdot kg^{-1} \cdot min^{-1}$)	6.33 ± 1.79	8.42 ± 2.52*
TNF-α (pg/ml)	5.95 ± 1.98	5.93 ± 0.84
sTNFR1 (ng/ml)	1.96 ± 0.39	1.84 ± 0.33
sTNFR2 (ng/ml)	4.56 ± 0.87	3.81 ± 0.58*

Data are means ± SD. *$P < 0.05$ between the offspring and control subjects.

($P < 0.02$), female offspring of type 2 diabetic patients 4.24 ± 0.77 and control subjects 3.68 ± 0.49 ng/ml ($P < 0.05$).

Both TNF-α receptors were related to WHR, FFM, plasma total cholesterol, HDL cholesterol, LDL cholesterol, and NEFA (Table 3). sTNFR2, but not TNF-α or sTNFR1, was also associated with insulin sensitivity ($r = -0.49$, $P = 0.001$) (Fig. 1). When two groups were analyzed separately, the correlation between sTNFR2 and MCR was significant in the offspring ($r = -0.52$, $P = 0.018$) but not in the control group ($r = -0.26$, $P = 0.26$).

Multiple regression analysis showed that sTNFR2 was associated with MCR independently of WHR, FFM, plasma insulin, and NEFA ($\beta = -0.41$, $P = 0.002$).

CONCLUSIONS — In the present study, no significant difference in plasma TNF-α between the studied groups was found. As mentioned, circulating TNF-α is a rather poor indicator of its autocrine and paracrine action. Plasma TNF-α did not differ between insulin-sensitive and insulin-resistant subjects in other reports (12). We demonstrated an increase in sTNFR2 in lean normoglycemic, insulin-resistant offspring of type 2 diabetic parents, although there was no difference in sTNFR1. Similar data concerning two TNF-α receptors, i.e., unchanged sTNFR1 and increased sTNFR2, were obtained when lean and obese insulin-resistant subjects were compared (4).

Both receptors were markedly related to FFM. Therefore, it is possible that skeletal muscle is the important source of sTNFR1 and sTNFR2, especially in lean subjects, in whom body fat content is relatively small. It is well known that human skeletal muscle expresses TNF-α (1). Association between sTNFR2 and FFM was reported by Fernandez-Real et al. (4), and higher levels of sTNFRs in patients with myotonic dystrophy in comparison to weight-matched control subjects were found (13). We were unable to detect a correlation between sTNFRs and FFM in our previous studies, but that was probably due to inclusion in the analysis of obese subjects with various degrees of glucose tolerance (9).

The important finding of the present study is the relationship between sTNFR2 and insulin sensitivity in lean offspring of type 2 diabetic parents. Studies with this group of subjects might allow determination of which metabolic abnormalities are primary to the development of obesity and which are secondary to the adipose tissue accumulation and/or hyperglycemia. In the present study, the only difference between the offspring and the control subjects, except hyperinsulinemia and insulin resistance, is increased sTNFR2. These data suggest that TNF-α is involved in early stages of the development of insulin resistance and that it might deteriorate insulin action even before the onset of obesity. In conditions of low circulating TNF-α levels, sTNFR2 might prolong its activity and/or reflect local action of the cytokine, probably in skeletal muscle, which is the most important tissue responsible for glucose uptake during an insulin clamp. Our results support the hypothesis that sTNFR2 might serve as a marker of TNF-α–related insulin resistance.

Both sTNFR1 and sTNFR2 were also positively related to total and LDL cholesterol and negatively to HDL cholesterol. The lipid parameters remained within the normal range and did not differ between the studied groups. Similar correlations, except for sTNFR1 and HDL cholesterol, were reported previously (14). In that study, lean and obese subjects were analyzed together. In the study of Chu et al. (15), conducted on men with a wide range of BMI, the relationships between

Table 3—*Correlations between sTNFR1 and sTNFR2 and anthropometric and metabolic parameters in the whole study population (n = 40)*

	sTNFR1		sTNFR2	
	r	*P*	*r*	*P*
Age	0.05	0.74	−0.09	0.57
BMI	0.27	0.09	0.22	0.17
WHR	0.35	0.029	0.33	0.036
Fat mass	0.30	0.056	0.17	0.29
FFM	0.52	0.001	0.39	0.013
Plasma glucose	0.14	0.40	0.02	0.92
HbA_{1c}	−0.01	0.94	−0.03	0.83
Plasma insulin	0.15	0.37	0.23	0.16
Plasma NEFA	0.32	0.04	0.34	0.034
Total cholesterol	0.44	0.004	0.33	0.037
Plasma triglycerides	0.26	0.09	0.29	0.069
HDL cholesterol	−0.45	0.004	−0.31	0.049
LDL cholesterol	0.52	0.001	0.35	0.026
MCR	−0.24	0.12	−0.49	0.001

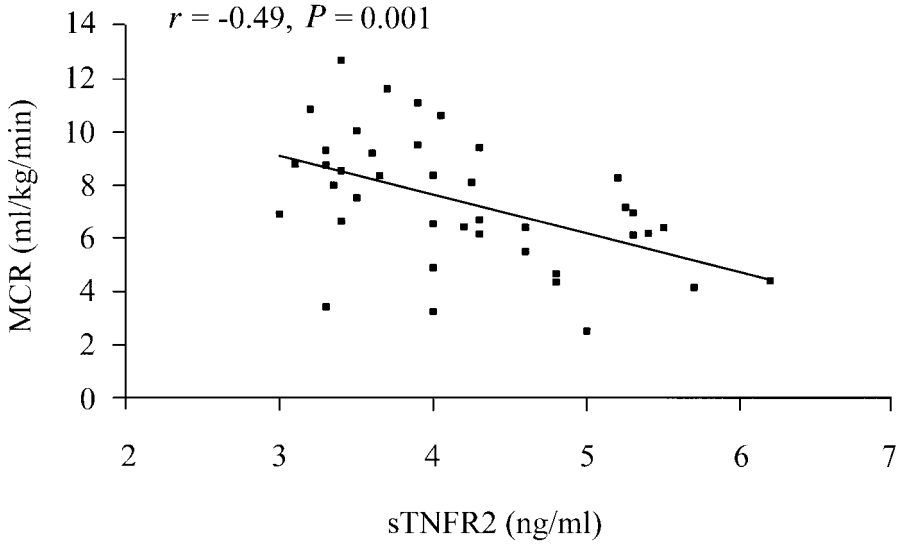

Figure 1—*Correlation between sTNFR2 and insulin sensitivity.*

both sTNFRs and total and HDL cholesterol were significant; however, they did not markedly influence an association between BMI and lipid parameters in a multiple-regression analysis. Therefore, we suppose that the role of TNF-α in determining plasma cholesterol levels might be significant, especially in lean persons, whereas in obese subjects, other factors are more important. The explanation of such an association is unclear; however, it was demonstrated that TNF-α produced an increase in serum cholesterol and in hepatic hydro-3-methyl-glutaryl coenzyme A reductase activity in mice (16).

The reason for the increase in sTNFR2 in the offspring group remains unknown. Probably, it reflects increased TNF-α action in muscle, caused by genetic factors or secondary upregulation of the TNF-α system by other, as yet unknown, activators. Large studies on TNF-α −238 and −308 G→A gene polymorphism in healthy relatives of type 2 diabetic subjects did not find, as previously suggested, an association with insulin sensitivity (17) or other features of metabolic syndrome (18).

Data obtained from identical twins discordant for obesity showed that sTNFR2 is determined by both genetic factors and adiposity; no relationship with indirect index of insulin action derived from an oral glucose tolerance test was found (19). Polymorphism in the 3′-untranslated region (exon 10) of the *TNFR2* gene was identified, and its association with obesity and insulin sensitivity was found. However, it is unlikely that this polymorphism might explain findings of the present study, as carriers of different *TNFR2* gene alleles did not differ in circulating concentrations of sTNFR2 (20). Other authors reported a microsatellite marker with five alleles in intron 4 of the *TNFR2* gene, which was associated with hypertension, hypercholesterolemia (21), and coronary artery disease (22). The genotypic effect on plasma sTNFR2 levels was also observed (21,22). It should be noted that the above studies were performed on different groups of subjects than the present study. The impact of *TNFR2* gene polymorphisms on impairing insulin action in predisposed subjects requires further investigations. Insulin resistance and type 2 diabetes have multifactorial etiology, and we suggest that an observed increase in sTNFR2 should be interpreted in the context of other multiple metabolic abnormalities present in offspring of type 2 diabetic subjects, which together may result in insulin resistance.

We conclude that the TNF-α system might be involved in modulating insulin action before the onset of obesity in subjects at high risk for type 2 diabetes.

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