Polymorphism of the Tumor Necrosis Factor-α Receptor 2 Gene Is Associated With Obesity, Leptin Levels, and Insulin Resistance in Young Subjects and Diet-Treated Type 2 Diabetic Patients

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OBJECTIVE — Mice lacking the tumor necrosis factor-α receptor 2 (*TNFR2*) gene fed a high-fat diet gain less weight and display reduced leptin and insulin levels. In humans, plasma levels of the soluble fraction of TNFR2 (sTNFR2) circulate in proportion to the degree of insulin resistance. The purpose of this study was to evaluate a polymorphism in the 3′ untranslated region of the *TNFR2* gene on chromosome 1 in relation to BMI, leptin levels, and insulin resistance.

RESEARCH DESIGN AND METHODS — Using single-strand conformation polymorphism, the polymorphism was analyzed in 107 nondiabetic subjects (60 women, 47 men) and in 110 consecutive patients with type 2 diabetes (79 women, 31 men). In a subset of 33 healthy subjects, insulin sensitivity (minimal model analysis) was also evaluated.

RESULTS — Four alleles of the *TNFR2* gene were identified (A1, A2, A3, and A4). BMI and serum leptin levels were significantly increased in young carriers of the A2 allele. Plasma sTNFR2 levels were similar among the different *TNFR2* gene variants. However, in subjects who did not carry the A2 allele, in young subjects, and in women, plasma sTNFR2 levels were proportional to BMI and leptin levels. In the study sample, carriers of the A2 allele (n = 18) showed significantly increased BMI, fat mass, waist-to-hip ratio, serum total and VLDL triglyceride levels, and leptin levels and had a lower insulin sensitivity index than noncarriers of the A2 variant (n = 15). The frequency of the different alleles among diabetic subjects was similar to that in the control population. However, diet-treated diabetic subjects (n = 49) who were carriers of the A2 allele exhibited significantly higher BMI and leptin levels than diet-treated noncarriers of the A2 allele.

CONCLUSIONS — The presence of the A2 allele in the *TNFR2* gene may predispose subjects to obesity and higher leptin levels, which may in turn predispose them to insulin resistance or vice versa. The *TNFR2* gene may be involved in weight-control mechanisms.

Diabetes Care 23:831–837, 2000

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Received for publication 26 October 1999 and accepted in revised form 15 February 2000.

Abbreviations: EASIA, enzyme-amplified sensitivity immunoassay; FSIVGTT, frequently sampled intravenous glucose tolerance test; PCR, polymerase chain reaction; sTNFR2, soluble fraction of tumor necrosis factor-α receptor; TNF-α, tumor necrosis factor-α; TNFR1, tumor necrosis factor-α receptor 1; TNFR2, tumor necrosis factor-α receptor 2; UTR, untranslated region; WHR, waist-to-hip ratio.

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

Increasing evidence exists that tumor necrosis factor-α (TNF-α) plays a key role in mediating insulin resistance as a result of obesity (1–7). In numerous rodent models (1,2,7) of obesity-diabetes syndromes, TNF-α is overexpressed in the adipose and muscle tissues compared with tissues from lean animals. TNF-α blocks the action of insulin in cultured cells and in whole animals (1,2,7). The induction of insulin resistance is mediated through its ability to produce serine phosphorylation of insulin receptor substrate 1 (7), which decreases the tyrosine kinase activity of the insulin receptor (2,7). Neutralization of TNF-α in obese *fa*/*fa* rats by intravenously administering a soluble TNF-α receptor–IgG chimeric protein or by using a replication-incompetent adenovirus 5 vector to endogenously express a TNF-α inhibitor gene substantially improved insulin sensitivity and restored the tyrosine kinase activity in fat and muscle (1,2). Recent studies have shown that mice lacking expression of the TNF-α ligand with a targeted null mutation in the gene encoding TNF-α were spared from obesity-induced deficiencies in insulin receptor signaling in fat and muscle tissues (8,9).

TNF-α signals through at least 2 known cell-surface receptors (TNFRs): TNFR1 (p55) and TNFR2 (p75) (10). In a recent study, mice lacking TNFR2 ($p75^{-/-}$) that were fed a high-fat diet consistently gained less weight and displayed reduced insulin levels as an expression of improved insulin sensitivity than wild-type mice (11). The levels of circulating leptin were modulated by TNFR genotype. Male $p75^{-/-}$ mice that were fed chow had the lowest levels of plasma leptin after 4 and 18 h of fasting. High-fat-fed $p75^{-/-}$ mice were the only mice to display a decrease in leptin levels with increased fasting time (11). In another study in *ob*/*ob* mice, the absence of p55 caused a significant improvement in insulin sensitivity. A p75 deficiency alone did not affect insulin sensitivity but potentiated the effects of p55 deficiency in animals lacking both TNFRs (12).

In humans, TNF-α is also overexpressed in the adipose and muscle tissues of obese subjects in proportion to their degree of insulin resistance (4–6). However, treatment of type 2 diabetic subjects with an antibody specific for TNF-α had no effect on insulin sensitivity (13). The latter study was performed using a single administration of antibody in adults with established diabetes. This approach probably does not affect the autocrine-paracrine effects of TNF-α. A polymorphism in the *TNF-α* gene seems to be linked to body fat and insulin resistance. We recently found that those subjects who were homozygotes for the absence of the restriction site resulting from a guanine-to-adenine substitution at position −308 of the *TNF*-α promoter showed an increased percentage of fat mass and leptin levels and a decreased insulin sensitivity index (14). This substitution leads to higher constitutive and inducible levels of transcription of the *TNF-α* gene than the wild-type allele (15). Subjects with this allele also showed increased BMI in an epidemiological study (16).

Obese women expressed ~2-fold more TNFR2 mRNA in fat tissue and ~6-fold more soluble TNFR2 (sTNFR2) in circulation than lean control subjects (17). The sTNFR2 results from a proteolytic cleavage of the cell-surface forms (18–20) when TNF-α binds to its receptor, and its plasmatic levels are thought to express previous TNF-α action (19). Adipose tissue expression of TNFR2 strongly correlated with BMI and the level of insulinemia (17). Recently, we have found that sTNFR2 is associated with BMI, waist-to-hip ratio (WHR), and insulin resistance independently of BMI and WHR (21). In contrast, no correlation was observed among any of these metabolic variables and TNFR1 expression (17) or sTNFR1 levels (17,21).

Recent works have suggested the existence of a leptin–TNF-α axis in which leptin and TNF-α are mutually interrelated. TNF-α stimulates leptin secretion in cultured adipocytes and obese mice (22,23), and, as a feedback loop, leptin administration to rats decreased TNF-α expression by 40% in young animals. Leptin treatment did not influence TNF-α expression in older rats, which suggests an age-dependent disruption of this axis (24). TNF-α administration increases serum leptin levels in humans (25), and plasma sTNFR1 concentrations circulate in proportion to leptin (26). Disproportionately increased leptin (27,28) and TNF-α levels (27–30) are observed in populations with high rates of type 2 diabetes and in elderly people, and this possibly contributes to insulin resistance and obesity. In our study subjects, we observed that plasma sTNFR2 levels strongly correlated with age.

An abnormality in the *TNFR2* gene may explain the link among obesity, insulin resistance, and increased leptin levels. To test this hypothesis, we studied a polymorphism in the 3′ untranslated region (UTR) of the *TNFR2* gene in nondiabetic subjects and patients with type 2 diabetes and the link of that polymorphism with obesity and insulin sensitivity. Because age probably constitutes an important confounding factor, we arbitrarily divided our study subjects into younger (<54 years [the median age of the group]) and older (≥54 years) subjects.

RESEARCH DESIGN AND METHODS

We studied 110 consecutive subjects with type 2 diabetes (79 women, 31 men) among whom no first-degree relationships existed and 107 similarly unrelated nondiabetic subjects (60 women, 47 men). Inclusion criteria for subjects included BMI (weight in kilograms divided by height in meters squared) <40 kg/m^2, absence of any systemic disease, and absence of any infections during the previous month. No healthy subjects were taking any medication or had any evidence of metabolic disease other than obesity, and all subjects reported that their body weight had been stable for at least 3 months before the study. All nondiabetic subjects were normotensive and normolipemic (data not shown). The protocol was approved by the hospital ethics committee, and informed consent was obtained from each subject.

Study of insulin resistance

A subset of 33 subjects randomly selected from the control group were metabolically characterized to evaluate the components of the insulin resistance syndrome (blood pressure, lipids, abdominal obesity) and insulin sensitivity. Each subject's waist was measured with a soft measuring tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteal region. The percentages of body fat and fat-free mass in these subjects were measured by bioelectric impedance analysis (Holtain BC Analyser, Cambridge, U.K.). Blood pressure was measured in the supine position in the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of 3 readings taken at 5-min intervals. The subjects consumed a weight-maintaining diet containing at least 300 g of carbohydrate/day and refrained from exertion for 3 days before the tests described below. The subjects also abstained from caffeine and alcohol for 72 h before the tests.

An oral glucose tolerance test was performed according to the recommendations of the National Diabetes Data Group (31). After a 12-h overnight fast, 75 g glucose was ingested, and blood samples were collected through a venous catheter from an antecubital vein at 0, 30, 60, 90, and 120 min for measurement of serum glucose and insulin. Insulin sensitivity was analyzed using the frequently sampled intravenous glucose tolerance test (FSIVGTT) with the administration of insulin as a bolus as described elsewhere (14,21).

Analytical methods

The serum glucose level during the FSIVGTT was measured in duplicate by the glucose oxidase method with a glucose analyzer (Beckman, Brea, CA). The coefficient of variation was 1.9%. The serum insulin level during the FSIVGTT was measured in duplicate by monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). The lowest limit of detection was 4.0 mU/l. The intra-assay coefficient of variation was 5.2% at a concentration of 10 mU/l and 3.4% at 130 mU/l. The interassay coefficients of variation were 6.9 and 4.5% at 14 and 89 mU/l, respectively. Data from the FSIVGTT were run through computer programs that calculate the characteristic metabolic parameters by fitting glucose and insulin to the minimal model that describes the time course of glucose and insulin concentrations as described elsewhere (32).

Total serum cholesterol was measured through the reaction of cholesterol esterase, cholesterol oxidase, and peroxidase (33). VLDL cholesterol was measured after ultracentrifugation at 45,000*g*. HDL cholesterol was quantified after precipitation with polyethylene glycol at room temperature (34). Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase (35). VLDL triglycerides were measured after ultracentrifugation at 45,000*g*.

Table 1—*Characterization of variants in 3′ UTR (exon 10) of the* TNFR2 *gene and frequency of these variants in type 2 diabetic patients and in nondiabetic subjects*

Nucleotides	Allele	Variant	Frequency	
			Nondiabetic subjects	Diabetic patients
593-598-620	G-T-T	A1	0.57	0.61
593-598-620	A-T-C	A2	0.34	0.35
593-598-620	A-G-T	A3	0.07	0.04
593-598-620	A-T-T	A4	0.005	0.004

Serum leptin concentrations were measured by radioimmunoassay in samples obtained after an overnight fast (Linco Research, St. Charles, MO). The lower limit of detection is 0.5 ng/ml. Intra- and interassay coefficients of variation were <7 and <8%, respectively. The radioimmunoassay for leptin does not cross-react with human proinsulin, insulin, or glucagon.

Plasma levels of sTNFR2 were determined by a solid-phase enzyme-amplified sensitivity immunoassay (EASIA) performed on a microtiter plate (Medgenix sTNF-R2 EASIA) as described elsewhere (23). The intra- and interassay coefficients of variation were <7 and <9%, respectively. The sTNFR2 EASIA does not cross-react with sTNFR1. TNF-α does not interfere with the assay.

Detection of the TNFR2 3′ UTR polymorphism by single-strand conformation polymorphism

Polymerase chain reaction and single-strand conformation polymorphism analysis. A total of 250 ng genomic DNA was amplified with specific flanking primers for the 3′ UTR of TNFR2 (191 base pair fragment) (36,37). Primer 1 was as follows: 5′ AGGACTCTGAGGCT CTTTCT 3′. Primer 2 was as follows: 5′ TCACAGAGAGTCAGGGACTT 3′. Polymerase chain reaction (PCR) conditions were 3 min at 93°C followed by 35 cycles consisting of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec with a final extension at 72°C for 9 min. PCR analysis was performed in 25 µl of final volume containing 0.5 µmol/l of each primer, 0.2 µmol/l of each dNTP, 1.2 mmol/l $MgCl_2$, and 1 U Taq DNA polymerase (Life Technologies, Gaithersburg, MD).

A total of 3 µl of each amplificate was mixed with 15 µl of denaturing loading dye (98% formamide, 9.8 mmol/l Tris-Base, 8.9 mmol/l boric acid, 0.2 mmol/l EDTA, 6% glycerol, 0.05% bromophenol blue, 0.05% xylene cyanol) heated to 96°C for 10 min and then placed directly on ice. Samples were then loaded onto a 12% acrylamide:bis (28:1) gel (without glycerol, at room temperature, 100 V constant for 17 h) and subsequent silver staining.

DNA sequencing. PCR analysis was performed under the same conditions as described above. DNA fragments were electrophoresed on 1% agarose gel (Life Technologies) purified with a QUIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany) and sequenced using a Taq Dye Terminator Cycle Sequencing Kit (Perkin Elmer, Norwalk, CT) and a model DRA 373A sequencer (Perkin Elmer).

Variations in the 3′ UTR region (exon 10) of the *TNFR2* gene

A total of 3 electrophoretic patterns were detected in the region of exon 10 that was explored by PCR and single-strand conformation polymorphism analysis. DNA fragments with variable patterns were subsequently analyzed by direct sequencing. Two sequence variants (A3 and A4) were ascribed to 1 electrophoretic pattern, and a total of 4 sequence variants were identified. Characterization of the 4 variants are summarized in Table 1. To avoid a misclassification of the studied population, all subjects with a possible A3 vs. A4 variant according to the electrophoretic pattern were sequenced, and we obtained a very low frequency of the A4 variant (0.004) in the whole population (Table 1).

Statistical analyses

Descriptive results of continuous variables are means ± SD. Non-Gaussian distributed variables were $\log_{10}$ transformed to achieve normality. This applied to serum leptin, insulin sensitivity, serum triglycerides, VLDL triglycerides, and sTNFR2. Comparison of variables across several groups of subjects was performed by 1-way analysis of variance using Fisher's test for multiple comparisons. Relationships between variables were determined by Pearson's correlation coefficient and stepwise multivariate linear regression analysis with forward selection. The regression coefficient generated by this analysis indicates the slope of the association between the dependent variable and the specified independent variable after adjusting for other independent variables in the model. The SEM represents the variability in this association, and the significance is reflected by the *P* value. The model R^2 indicates the percentage of the variance in the dependent variable that is accounted for by the independent variables included in the model. In the subgroup of subjects that were metabolically characterized, a logistical regression analysis was performed as follows: BMI was categorized as 0 or 1 according to the values below (0) or above (1) the median for the group of men and women, separately. TNFR2 variants were considered to be 0 in non-A2 subjects and

Table 2—*Characteristics of the study population*

Characteristic	Nondiabetic subjects			Subjects with type 2 diabetes		
	Non-A2	A2	*P*	Non-A2	A2	*P*
n	47	60	—	45	65	—
Men/women	21/26	26/34	NS	16/29	15/50	NS
Age (years)	42.8 ± 12	44.3 ± 11.5	NS	59.1 ± 8.9	58.9 ± 9.8	NS
Variant						
A1/A1		36			38	
A1/A2		42			51	
A2/A2		14			12	
A1/A3		8			6	
A2/A3		4			2	
A3/A3		2			—	
A1/A4		1			1	

Data are *n* or means ± SD.

Table 3—*Influence of age and sex on the variables*

	BMI (kg/m^2)			Leptin (ng/ml)		
	A2-allele carriers	Non-A2 allele	*P*	A2-allele carriers	Non-A2 allele	*P*
Younger subjects	27.8 ± 5.17	25.7 ± 4.3	0.032	15.6 ± 11	9.5 ± 8.9	<0.05
Older subjects	28.1 ± 4.7	28.1 ± 4.6	NS	21.3 ± 19.2	13.1 ± 9.7	<0.05
Men	26.8 ± 4.6	27 ± 4.07	NS	8 ± 5.3	7.2 ± 7.1	NS
Women	28.5 ± 4.9	26.7 ± 5	0.04	22.1 ± 17	14 ± 9.8	<0.01

Data are means ± SD.

1 in subjects with the A2 allele. Insulin sensitivity index was also categorized as 0 or 1 according to the values below (0) or above (1) the median for the group of men and women separately and was considered the dependent variable. Levels of statistical significance were set at $P < 0.05$. All of these analyses were performed with the BMDP statistical package (Cork, Ireland).

RESULTS — The frequency of the variants in type 2 diabetic patients and nondiabetic subjects is shown on Table 1. Table 2 summarizes the characteristics of the study subjects at the time of entry into the study.

We observed a trend for higher BMI (29.9 ± 5.3 vs. 27.6 ± 4.9 kg/m^2, $P = 0.08$) and significantly higher leptin levels (22.8 ± 8.3 vs. 18.6 ± 15.7 ng/ml, $P = 0.01$) in women homozygous for the A2/A2 variant ($n = 16$). For this reason, we divided our population into carriers of the A2 allele (A2/A2, A1/A2, and A2/A3) and non-A2 carriers (A1/A1, A1/A3, A3/A3, and A1/A4) according to the presence or absence of the A2 variant.

Age and sex significantly influenced the results (Table 3). Younger subjects with the A2 allele ($n = 61$) showed significantly increased BMI and leptin levels versus subjects with the non-A2 allele ($n = 48$). Older A2 subjects showed similar BMI and increased leptin compared with non-A2 carriers. However, the latter differences were ascribed to an increased proportion of women among A2 carriers. When the study was performed separately according to sex, A2 women showed increased BMI and leptin levels compared with non-A2 women. In contrast, A2 men showed BMI and leptin levels similar to non-A2 men.

Similar findings were observed when the analysis was performed with nondiabetic or diabetic subjects. However, in the latter subjects, the differences were mainly observed in diet-treated patients ($n = 49$). A2 diet-treated diabetic subjects exhibited significantly higher BMI (29.2 ± 4.6 vs. 26.4 ± 3.6 kg/m^2, $P = 0.038$) and leptin levels (14.2 ± 9.1 vs. 8.4 ± 6.8 ng/ml, $P < 0.05$) than non-A2 diet-treated diabetic patients (Fig. 1). In patients receiving oral hypoglycemic agents ($n = 24$) or insulin ($n = 37$), these differences were not observed.

Plasma sTNFR2 levels were measured in 76 subjects randomly selected (50% diabetic subjects, 50% control subjects). Plasma sTNFR2 levels positively correlated with age ($r = 0.66$, $P < 0.0001$, $n = 76$) (Fig. 2). The sTNFR2 levels were higher in younger men than in younger women (3.28 ± 0.63 vs. 2.82 ± 0.67 ng/ml, $P = 0.026$), and these differences were lost in older subjects. Plasma sTNFR2 levels were similar among A2 and non-A2 carriers. However, in non-A2 carriers, plasma sTNFR2 concentration was proportional to BMI (Pearson's $r = 0.48$, $P < 0.01$) and leptin levels ($r = 0.48$, $P < 0.01$). These associations were even stronger in non-A2 women ($r = 0.55$, $P = 0.015$, and $r = 0.65$, $P < 0.01$, for BMI and leptin, respectively). On the contrary, these associations were lost among A2 carriers.

In a multiple linear regression analysis conducted in a stepwise manner to predict leptin levels with BMI, age, sex, diabetes status, and *TNFR2* gene variants as independent variables, the latter predicted leptin levels ($P = 0.04$) independently of BMI ($P < 0.0001$), age ($P = 0.0001$), and sex ($P = 0.0003$), which contributed to 49% of its variance.

Table 4 summarizes the characteristics of this subgroup of subjects (9 A2 women and 9 non-A2 women). A2 subjects showed significantly increased BMI, fat mass, WHR, serum triglycerides, VLDL triglycerides, and leptin levels and a lower insulin sensitivity index (Table 3) than non-A2 carriers. The differences were more marked among women. Again, plasma sTNFR2 levels correlated with BMI in non-A2 subjects ($r = 0.73$, $P < 0.01$) but not in A2 carriers ($r = 0.36$, P = NS). In a logistical regression analysis, both BMI ($P = 0.04$) and TNFR2 polymorphism ($P = 0.02$) independently predicted insulin sensitivity.

CONCLUSIONS — We have identified a polymorphism in the *TNFR2* gene on chromosome 1 that was associated with higher BMI and leptin levels in nondiabetic subjects and diet-treated type 2 diabetic patients. In contrast with the nondiabetic subjects, the diabetic patients treated with oral hypoglycemic agents or insulin were not found to differ in BMI according to the polymorphism of the *TNFR2* gene. Potential differences among these patients may possibly have been masked by their concomitant hyperglycemia and its treatment.

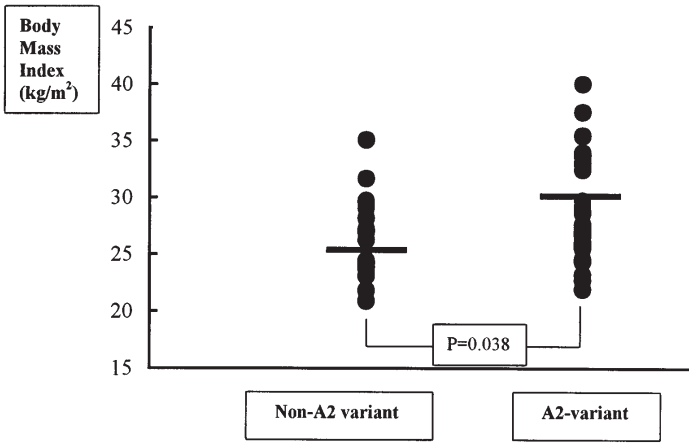

Figure 1—*Differences in BMI according to the* TNFR2 *gene variants among diet-treated type 2 diabetic subjects.*

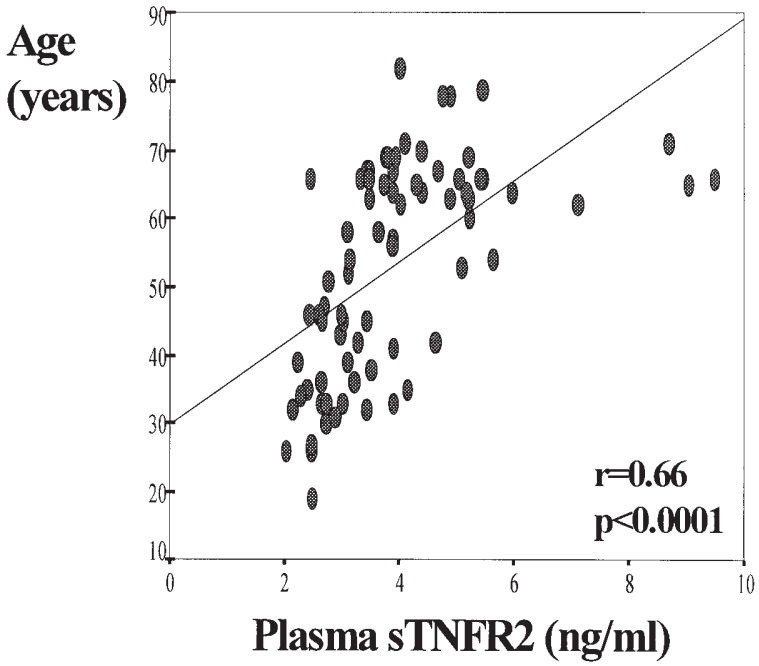

Figure 2—*Linear association between plasma sTNFR2 and age in all subjects.*

The *TNFR2* A2 allele could have been expected to be more frequent in subjects with type 2 diabetes than in nondiabetic subjects. However, heterogeneity of type 2 diabetes and the transversal design of our study constitute important confounders in interpreting these results. Our findings are somewhat limited by the relatively small study size, but they are consistent with experimental studies and human physiology. At this time, we can only speculate about the different activity of TNFR2 according to the different *TNFR2* gene variants that leads to differences in weight-control mechanisms.

The polymorphism in the 3′ UTR segment of the *TNFR2* gene may encompass mRNA destabilizing signals, thus affecting mRNA stability and leading to a reduction in the abundance of TNFR2 mRNA in adipose or skeletal muscle. Nevertheless, the polymorphisms in the *TNFR2* gene may possibly represent markers for yet another susceptibility gene in this region that could be the basis for the observed associations. In this sense, a single-gene rodent mutation (diabetes) and a quantitative trait locus (dietary obese 1) mapped to the midportion of mouse chromosome 4 have been related to obesity and/or insulin levels. Synteny relationships place their putative human homologs on 1p31 and on 1p35 through p31, respectively, which are very close to the *TNFR2* gene location (on chromosome 1p36.2) (36–38). In this respect, our findings agree with those of Chagnon et al. in the Quebec Family Study (39). In that report, suggestive linkages between several microsatellite markers from 1p32 to p22 and for BMI, fat mass, and fasting insulin were found. In fact, chromosome 1 is one of the few chromosomes showing at least 3 putative loci related to obesity in both arms (40).

Of interest is the relationship between sTNFR2 levels and BMI and between sTNFR2 and leptin in subjects in whom the *TNFR2* gene variants determined differences in BMI and leptin. Plasma sTNFR2 concentration is believed to be a surrogate of previous TNF-α action because TNF-α selectively upregulates the 75-kDa TNFR (41). The sTNFR2 levels were proportional to BMI and leptin in young subjects but not in older subjects. The sTNFR2 levels were proportional to BMI and leptin in women, who were the subjects in whom the *TNFR2* gene variants marked more differences in BMI and leptin. Interestingly, the attenuation of body weight in $p75^{-/-}$ mice was sex specific (11). Finally, sTNFR2 levels were proportional to BMI and leptin among non-A2 subjects. One may be tempted to speculate that, when the leptin–TNF-α axis feedback is functioning (i.e., in non-A2 subjects), the result is a lower BMI and concomitantly lower leptin levels. In our hypothesis, subjects with a disrupted leptin–TNF-α axis (A2 subjects and older individuals) would be prone to higher BMI and leptin levels with disproportionally higher sTNFR2 levels. In our study sample, both BMI (r = 0.19, P = 0.007) and leptin levels (r = 0.25, $P < 0.01$) correlated with age as previously reported (27). Researchers have previously noted that the relationship between fat content and leptin is disrupted in elderly subjects and thus possibly contributes to the obesity that occurs with age (27).

Table 4—*Anthropometric and biochemical variables of nondiabetic subjects (selected subgroup)*

Variable	Non-A2 subjects	A2 subjects	*P*
n	15	18	NS
Age (years)	36.6 ± 6.5	34.7 ± 7	NS
BMI (kg/m^2)	24.6 ± 5.3	30.7 ± 4.8	0.002
WHR	0.94 ± 0.05	1.00 ± 0.06	0.014
Fat mass (kg)	18.42 ± 10.7	27.3 ± 12.7	0.04
Systolic blood pressure (mmHg)	118.7 ± 13.9	125.3 ± 7.2	NS
Diastolic blood pressure (mmHg)	69 ± 12.3	76.8 ± 9.8	NS
AUC glucose during OGTT (mmol/l)	8.1 ± 3.6	8.37 ± 2.5	NS
AUC insulin after OGTT (mU/l)	58.5 ± 26.5	89.4 ± 48	0.04
Insulin sensitivity (min · mU^{-1} · l^{-1})	3.7 ± 1.7	1.9 ± 1.3	0.003
Cholesterol (mmol/l)	4.85 ± 0.8	5.3 ± 1	NS
LDL cholesterol (mmol/l)	3.3 ± 0.93	3.8 ± 0.9	NS
HDL cholesterol (mmol/l)	1.29 ± 0.42	1.07 ± 0.21	NS (0.07)
Triglycerides (mmol/l)	0.95 ± 0.42	1.52 ± 0.75	0.012
VLDL triglycerides (mmol/l)	0.52 ± 0.37	0.94 ± 0.6	0.02
Serum leptin (ng/ml)	10.1 ± 8	18 ± 12.3	0.03
Plasma sTNFR2 (ng/ml)	2.91 ± 0.58	2.96 ± 0.68	NS

Data are *n* or means ± SD. AUC, area under the curve; OGTT, oral glucose tolerance test.

An important finding of our study was that the *TNFR2* gene variants were associated with insulin sensitivity independently of BMI. Subjects with the A2 allele showed several characteristics of the insulin resistance syndrome: increased WHR, postglucose load insulin levels, and hypertriglyceridemia in association with a lower insulin sensitivity index. The A2 allele would constitute one of the few genotype markers for insulin resistance. Whether the latter is a cause or consequence of an impaired leptin–TNF-α axis is only a matter for speculation. In different animal models, leptin administration increased insulin sensitivity both under fasting conditions and in the presence of hyperinsulinemia at clamped glucose and also increased glucose utilization by 30% (42–44). In humans, leptin is involved in sex-related differences in insulin sensitivity: women are more insulin sensitive than men despite increased fat mass (45). The increased insulin sensitivity of women is perhaps the result of downregulation of TNF-α induced by leptin.

Plasma levels of sTNFR2 have been described to circulate in proportion to the degree of insulin resistance in young nondiabetic subjects (21). However, we did not detect differences in sTNFR2 levels among the different *TNFR2* gene variants. We cannot exclude that age, diabetes status, or diabetes complications affect metabolism or clearance of sTNFR2 and thus lead to disproportionate plasma sTNFR2 levels. Moreover, we cannot exclude that other factors may have influenced our results. For example, in very obese people, TNF-α production tends to decrease (46), and the insulin resistance state is maintained in all likelihood by hyperglycemia and high levels of free fatty acids. For this reason, we considered morbid obesity to be an exclusion criterion.

In conclusion, the *TNFR2* gene polymorphism is independently associated with obesity in healthy subjects and diet-treated type 2 diabetic subjects, with higher leptin levels and with insulin resistance. Determining the molecular mechanisms by which a change in the *TNFR2* gene leads to phenotypic differences will provide important insights into the genetic basis of insulin resistance.

Acknowledgments — This work was partially supported by Grant CICYT SAF 98-0130 and Grant 98/0808 from the Fondo de Investigaciones Sanitarias, National Health Institute.

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