Tumor Necrosis Factor Alpha Gene G-308A Polymorphism, Insulin Resistance, and Fasting Plasma Glucose in Young, Older, and Diabetic Japanese Men

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In obese subjects, the levels of tumor necrosis factor alpha (TNF-α) expression and protein synthesis in adipocytes are increased. A recent study of Caucasians suggested that the TNF-α gene promoter region polymorphism at position −308 influences insulin resistance and percent body fat and increases serum leptin levels, although conflicting data are also reported. The present study was performed to investigate the relationship between this polymorphism and the body mass index (BMI), blood pressure, glucose and lipid profiles, and serum leptin in 122 healthy young men aged 21 to 29, 177 older men aged 45 to 65, and 71 type 2 diabetic male patients aged 42 to 78. The BMI, blood pressure, and fasting plasma glucose (FPG), serum lipids, uric acid, insulin, and leptin concentrations were measured. The TNF-α G-308A polymorphism was assessed by the polymerase chain reaction restriction fragment length polymorphism method. In the young group, 4 subjects (3.3%) were heterozygous for the TNF2 (G-308A–positive) allele, but there were no significant differences between the TNF1 (wild-type) and TNF2 groups in any measured anthropometric or metabolic parameter. In the older group, the frequency of the TNF2 group was 2.8%, and FPG was significantly higher in the TNF2 versus the TNF1 group (108 ± 7 *v* 99 ± 9 mg/dL, *P* = .042 by Mann-Whitney *U* test). Plasma triglycerides and the insulin resistance index tended to be higher and high-density lipoprotein (HDL) cholesterol tended to be lower in the TNF2 group (*P* = .06, .20, and .07, respectively), although these differences were not statistically significant. In type 2 diabetic subjects, the frequency of the TNF2 group was also 2.8%, and there were no significant differences between the TNF1 and TNF2 groups in any parameter. HDL cholesterol tended to be lower (*P* = .10) in the TNF2 group, although it was not statistically significant. In conclusion, no major difference was associated with TNF1 and TNF2 polymorphisms in terms of obesity, blood pressure, lipids, or glucose in young, older, or diabetic Japanese men, although FPG was significantly higher in older men, possibly through increased insulin resistance.

OBESITY, hypertension, diabetes mellitus, and dyslipidemia are important predisposing factors for the development of atherosclerotic cardiovascular disease on the basis of insulin resistance.[1-3] Tumor necrosis factor alpha (TNF-α) is produced by adipocytes in obese subjects, as well as macrophages, and is considered to induce insulin resistance, acting on the neighboring skeletal muscle by a paracrine mechanism or on adipocytes by an autocrine mechanism.[4] Because TNF-α is suggested to be one of the main mediators of insulin resistance in human obesity,[5] recent interest has focused on the relationship between TNF-α gene polymorphisms and insulin resistance.[6-9] Two polymorphisms have been identified in the TNF-α promoter region. One is a G to A substitution at position −308 designated as the TNF2 allele.[6] The other is a G to A substitution at position −238 designated as the TNFA allele.[7] In a study of Caucasian subjects,[8] the TNF-α gene polymorphism at position −308 was reported to induce insulin resistance, increase the percent body fat, and increase serum leptin levels. However, a more recent study of 422 subjects (65% women) has suggested that neither the −238 nor the −308 polymorphism is related to obesity or insulin resistance.[9]

The purpose of this study was to investigate the relationships between this G-308A polymorphism and the body mass index (BMI), blood pressure, glucose and lipid profiles, and serum leptin in young, older, and type 2 diabetic Japanese men.

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SUBJECTS AND METHODS

The present study included 122 young men aged 21 to 29 years and 177 older men aged 45 to 65 years, of which both groups received an annual health checkup. Subjects with diabetes mellitus, endocrine disease, or significant renal or hepatic disease and those receiving antihypertensive agents, systemic corticosteroids, or lipid-lowering medications were excluded. Seventy-one type 2 diabetic men aged 42 to 78 years who were under evaluation by Keio University Hospital were also included. Informed consent was obtained from each subject before the study, and the protocol was approved by the review committees of the Department of Internal Medicine and Health Center at Keio University School of Medicine, Tokyo.

Measurements

Height, body weight, blood pressure (systolic [SBP] and diastolic [DBP]), and heart rate were measured. Plasma glucose, serum lipid (total cholesterol, triglycerides, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, and free fatty acids), and uric acid levels were measured using peripheral blood samples obtained after an overnight fast. Serum insulin and leptin concentrations were measured by radioimmunoassay as described previously.[10,11] The insulin resistance index was assessed by homeostasis model assessment (HOMA-R).[12,13]

Sample Collection and TNF-α Gene Polymorphism Analysis

A 5-mL sample of venous blood was collected in a vacutainer containing EDTA. Genomic DNA was extracted from the peripheral white blood cell fractions by the standard method. A transition polymorphism of G to A at position −308 of the TNF-α gene was detected as follows. Approximately 500 ng extracted DNA was used as a template. The primers were 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-TCCTCCCTGCTGCTCCGATTCCG-3′. The reaction was performed in a final volume of 20 μL containing 2 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 5 pmol of each primer, and 0.5 U Taq DNA

polymerase (Takara, Tokyo, Japan). DNA was amplified in 35 cycles with 1 minute of denaturation at 94°C, 1 minute of annealing at 60°C, and 1 minute of extension at 72°C. Before that, there was 1 cycle of 2 minutes of denaturation at 94°C, and after the cycles, there was a 4-minute extension at 72°C. Polymerase chain reaction products were digested with a 10-fold excess of *Nco*I restriction enzyme (Takara) at 37°C for 3 hours and electrophoresed on a 3% agarose gel with 0.5X Tris-borate EDTA (TBE) buffer. *Nco*I restriction fragment length polymorphism was detected by ethidium bromide staining, which revealed a 2-allele polymorphism that produced 3 bands of different sizes: a 107–base pair (bp) fragment corresponding to the TNF2 allele (restriction site absent) and a set of 87-bp and 20-bp fragments corresponding to the TNF1 allele (restriction site present).

Statistical Analysis

All of the data are expressed as the mean ± SD. We used the χ^2 test for comparison of proportions and the Mann-Whitney *U* test for comparison of quantitative variables. A *P* value less than .05 was considered statistically significant.

RESULTS

The subjects were divided into 2 groups according to the absence (homozygous for the TNF1 allele) or presence of the TNF2 allele. In the young group, 4 subjects (3.3%) were heterozygous for the TNF2 allele. None were homozygous for the TNF2 allele in this study. There were no significant differences between the TNF1 and TNF2 groups for any anthropometric or metabolic parameters measured (Table 1).

In the older group, the frequency of the TNF2 allele was 2.8%, and the fasting plasma glucose (FPG) concentration was significantly higher in the TNF2 group versus the TNF1 group (108 ± 7 *v* 99 ± 9 mg/dL, $P = .042$). Plasma triglycerides and the HOMA-R tended to be higher ($P = .06$ and .20, respectively) and HDL cholesterol tended to be lower ($P = .07$) in the TNF2 group, but these differences were not significant. There were no differences between the groups for other parameters, including serum leptin (Table 2).

Table 1. Relationship Between TNF-α Genotype and Subject Profile and Metabolic Variables in 122 Men Aged 21 to 29

Parameter	TNF-α Genotype		
	TNF1 (normozygote)	TNF2 (heterozygote)	*P**
No. of subjects	118 (96.7%)	4 (3.3%)	
Age (yr)	23.5 ± 1.5	23.3 ± 0.5	NS
BMI (kg/m²)	21.8 ± 3.2	21.2 ± 2.2	NS
SBP (mm Hg)	125 ± 13	122 ± 16	NS
DBP (mm Hg)	72 ± 9	66 ± 6	.10
Glucose (mg/dL)	89 ± 6	84 ± 7	NS
Insulin (μU/mL)	5.3 ± 3.9	4.8 ± 1.9	NS
HOMA-R	1.2 ± 0.9	1.0 ± 0.4	NS
Leptin (ng/mL)	2.8 ± 2.7	1.7 ± 0.3	NS
Total cholesterol (mg/dL)	171 ± 29	156 ± 14	NS
Triglycerides (mg/dL)	78 ± 36	56 ± 11	NS
HDL cholesterol (mg/dL)	53 ± 11	51 ± 3	NS
LDL cholesterol (mg/dL)	102 ± 25	94 ± 4	NS
Free fatty acids (mEq/L)	0.38 ± 0.14	0.42 ± 0.16	NS
Uric acid (mg/dL)	5.5 ± 1.2	5.3 ± 0.3	NS

NOTE. Values are the mean ± SD.
Abbreviation: NS, not significant ($P > .1$).
*Mann-Whitney *U* test.

Table 2. Relationship Between TNF-α Genotype and Subject Profile and Metabolic Variables in 177 Men Aged 45 to 65

Parameter	TNF-α Genotype		
	TNF1 (Normozygote)	TNF2 (Heterozygote)	*P**
No. of subjects	172 (97.2%)	5 (2.8%)	
Age (yr)	54.7 ± 5.8	54.6 ± 3.8	NS
BMI (kg/m²)	23.4 ± 2.7	24.0 ± 1.5	NS
SBP (mm Hg)	122 ± 19	117 ± 12	NS
DBP (mm Hg)	77 ± 13	78 ± 6	NS
Glucose (mg/dL)	99 ± 9	108 ± 7	.04
Insulin (μU/mL)	8.0 ± 5.1	9.0 ± 2.6	NS
HOMA-R	2.0 ± 1.3	2.3 ± 0.6	NS
Leptin (ng/mL)	3.9 ± 1.9	4.0 ± 0.5	NS
Total cholesterol (mg/dL)	206 ± 29	208 ± 10	NS
Triglycerides (mg/dL)	143 ± 113	221 ± 112	.06
HDL cholesterol (mg/dL)	55 ± 15	43 ± 7	.07
LDL cholesterol (mg/dL)	123 ± 30	121 ± 25	NS
Uric acid (mg/dL)	6.3 ± 1.4	6.8 ± 1.3	NS

NOTE. Values are the mean ± SD.
Abbreviation: NS, not significant ($P > .1$).
*Mann-Whitney *U* test.

In the type 2 diabetic patients, the frequency of the TNF2 allele was also 2.8%, and there was no significant difference in the genotype frequency even if compared with normal subjects (FPG < 110 mg/dL) in the older group. There were no significant differences between the TNF1 and TNF2 groups in any anthropometric or metabolic parameter. HDL cholesterol tended to be lower ($P = .10$) in the TNF2 group, but it was not statistically significant (Table 3).

DISCUSSION

In the adipocytes of obese subjects, the amount of TNF-α expression and protein synthesis is increased,[4] so recent interest has focused on the role of TNF-α as a key component in the link between obesity and type 2 diabetes.[5] The mechanism by which

Table 3. Relationship Between TNF-α Genotype and Subject Profile and Metabolic Variables in 71 Male Type 2 Diabetic Patients Aged 42 to 78

Parameter	TNF-α Genotype		
	TNF1 (Normozygote)	TNF2 (Heterozygote)	*P**
No. of subjects	69 (97.2%)	2 (2.8%)	
Age (yr)	58.8 ± 7.0	57.5 ± 2.1	NS
Duration of diabetes (yr)	7.8 ± 7.2	3.0 ± 2.8	NS
BMI (kg/m²)	23.8 ± 3.2	26.0 ± 3.2	NS
SBP (mm Hg)	133 ± 21	121 ± 24	NS
DBP (mm Hg)	76 ± 11	75 ± 18	NS
Glucose (mg/dL)	155 ± 45	144 ± 25	NS
Hemoglobin A_{1c} (%)	7.8 ± 7.2	8.1 ± 2.6	NS
Total cholesterol (mg/dL)	193 ± 32	228 ± 64	NS
Triglycerides (mg/dL)	163 ± 103	116 ± 10	NS
HDL cholesterol (mg/dL)	45 ± 11	34 ± 1	.10
LDL cholesterol (mg/dL)	127 ± 29	152 ± 20	NS
Free fatty acids (mEq/L)	0.51 ± 0.23	0.38 ± 0.35	NS
Uric acid (mg/dL)	5.7 ± 1.6	5.3 ± 2.1	NS

NOTE. Values are the mean ± SD.
Abbreviation: NS, not significant ($P > .1$).
*Mann-Whitney *U* test.

TNF-α induces insulin resistance is considered as follows. After binding to the TNF-α receptor, TNF-α phosphorylates the serine residues of insulin receptor substrate-1, and subsequently, the activity of insulin receptor kinases is inhibited.[14] This causes an inhibition of the cascade of the insulin-signaling pathway distal to the insulin receptor and decreases GLUT4 translocation and final glucose uptake.

The TNF-α polymorphism at position −308 reportedly leads to a higher rate of transcription of the TNF-α gene.[15] In the present study, FPG in older men was higher in the TNF2 group. Our speculation is that insulin resistance increased gradually in these subjects together with metabolic changes, such as hyperglycemia and dyslipidemia, on the basis of primary genetic defects. In type 2 diabetic patients, on the other hand, there were no significant differences between the TNF1 and TNF2 groups in any of the parameters measured. We speculate that in type 2 diabetic subjects, insulin resistance had already increased enough together with metabolic changes, regardless of the presence of the TNF-α gene polymorphism.

The TNF2 allele frequency was low in the Japanese population (~1.4%) compared with the rate observed in Caucasians (~17.8%).[8,9] Concerning the effects of this G-308A polymorphism on obesity or insulin resistance, the results were negative in a larger study in Caucasians.[9] Our data showed only weak significance ($P = .04$) in a small number of the TNF2 group ($n = 5$). There was also a trend for triglycerides to be higher and HDL cholesterol to be lower in this group. However, we think that even if this polymorphism does have an effect, it may not be very strong.

In conclusion, no major difference was associated with TNF1 and TNF2 polymorphisms in terms of obesity, blood pressure, lipids, or glucose in young, older, or diabetic Japanese men, although FPG was significantly higher in older men, possibly through increased insulin resistance. Further studies will be needed to clarify the significance of the TNF-α gene polymorphism in different age, sex, and ethnic groups in combination with other variant alleles.

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REFERENCES

1. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

2. Kaplan NM: The deadly quartet: Upper-body obesity, glucose intolerance, hypertriglyceridemia and hypertension. Arch Intern Med 149:1514-1520, 1989

3. DeFronzo RA, Ferrannini E: Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

4. Hotamisligil GS, Arner P, Caro JF, et al: Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95:2409-2415, 1995

5. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: A key component of the obesity-diabetes link. Diabetes 43:1271-1278, 1994

6. Wilson AG, de Vries N, Pociot F, et al: An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. J Exp Med 177:557-560, 1993

7. D'Alfonso S, Richiardi PM: A polymorphic variation in a putative regulation box of the *TNFA* promoter region. Immunogenetics 39:150-154, 1994

8. Fernandez-Real JM, Gutierrez C, Ricart W, et al: The TNF-α gene *Nco*I polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. Diabetes 46:1468-1472, 1997

9. Walston J, Seibert M, Yen CJ, et al: Tumor necrosis factor-α −238 and −308 polymorphisms do not associate with traits related to obesity and insulin resistance. Diabetes 48:2096-2098, 1999

10. Hirose H, Saito I, Kawai T, et al: Serum leptin level, possible association with haematopoiesis in adolescents, independent of body mass index and serum insulin. Clin Sci (Colch) 94:633-636, 1998

11. Hirose H, Saito I, Tsujioka M, et al: The *obese* gene product, leptin: Possible role in obesity-related hypertension in adolescents. J Hypertens 16:2007-2012, 1998

12. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419, 1985

13. Rudenski AS, Matthews DR, Levy JC, et al: Understanding ''insulin resistance'': Both glucose resistance and insulin resistance are required to model human diabetes. Metabolism 40:908-917, 1991

14. Hotamisligil GS, Peraldi P, Budavari A, et al: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. Science 271:665-668, 1996

15. Wilson AG, Symons JA, McDowell TL, et al: Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 94:3195-3199, 1997