Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 649–655
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Tumor necrosis factor α −238G>A genotype alters postprandial plasma levels of free fatty acids in obese individuals with type 2 diabetes mellitus

Bénédicte Fontaine-Bisson[a], Thomas M.S. Wolever[a,f], Jean-Louis Chiasson[b,d], Rémi Rabasa-Lhoret[c,d], Pierre Maheux[e], Robert G. Josse[a,f], Lawrence A. Leiter[a,i], N. Wilson Rodger[g], Edmond A. Ryan[h], Ahmed El-Sohemy[a,*]

[a]*Department of Nutritional Sciences, University of Toronto, Toronto, Canada H3C 3J7*
[b]*Department of Medicine, Université de Montréal, Montréal, Montréal, Canada H3T 1A8*
[c]*Department of Nutrition, Université de Montréal, Montréal, Montréal, Canada H2W 1T7*
[d]*Research Center, Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Canada H2W 1T7*
[e]*Department of Medicine, Division of Endocrinology and Metabolism, Université de Sherbrooke, Sherbrooke, Canada J1H 5N4*
[f]*Osteoporosis Centre, St Michael's Hospital, Toronto, Canada M5C 2T2*
[g]*Division of Endocrinology, University of Western Ontario, St. Joseph's Hospital, London, Canada N6A 4V2*
[h]*Department of Medicine, University of Alberta, Edmonton, Canada T6G 2S2*
[i]*Division of Endocrinology and Metabolism, St. Michael's Hospital, Toronto, Canada M5C 2T2*

Received 9 March 2006; accepted 11 December 2006

Abstract

Tumor necrosis factor α (TNF-α) is a proinflammatory cytokine that impairs insulin action and alters lipid metabolism. We investigated the effects of genetic polymorphisms of TNF-α on circulating biomarkers of insulin resistance and lipid metabolism during an 8-hour metabolic profile test and a 2-hour oral glucose tolerance test in subjects with type 2 diabetes mellitus. Subjects (N = 123) recruited were type 2 diabetic men (n = 56) and women (n = 67) aged 36 to 75 years with a body mass index of at least 25 kg/m^2. Blood samples were collected to determine postprandial changes in circulating lipid levels and biomarkers of insulin resistance. Subjects were genotyped by polymerase chain reaction-restriction fragment length polymorphism for the TNF-α −238G>A, −308G>A, and −863C>A polymorphisms. Compared with subjects who were homozygous for the −238G allele, carriers of the −238A allele had an altered ability to suppress postprandial free fatty acids as shown by an increased net incremental area under the curve (0.26 ± 2.44 vs −1.33 ± 2.71 mEq h^{-1} L^{-1}, P = .002) during the 8-hour metabolic profile test. This effect was observed in obese (1.04 ± 2.42 vs −1.68 ± 2.70 mEq h^{-1} L^{-1}, P = .0004) but not in non-obese (−0.63 ± 2.20 vs −0.95 ± 2.71 mEq h^{-1} L^{-1}, P = .6) individuals. Among obese subjects, carriers of the −308A allele had greater insulin resistance as estimated by the homeostasis model assessment of insulin resistance index (4.36 ± 2.83 vs 2.85 ± 1.75, P = .01), but no differences were observed among non-obese subjects (2.19 ± 1.24 vs 1.97 ± 0.90, P = .6). Our findings suggest that the −238G>A and −308G>A polymorphisms of TNF-α alter circulating free fatty acids and insulin resistance in obese subjects with type 2 diabetes mellitus.

1. Introduction

The metabolic syndrome is characterized by abdominal obesity, increased fasting blood glucose, dyslipidemia, and hypertension, and is associated with an increased risk of type 2 diabetes mellitus and cardiovascular disease [1]. There is growing evidence that chronic inflammation is also an important feature of the metabolic syndrome [1]. Obese and diabetic individuals have elevated plasma levels of tumor necrosis factor α (TNF-α), a multifunctional regulatory cytokine involved in the inflammatory response [2,3]. TNF-α is highly expressed in adipocytes [4] and inhibits insulin-signaling pathways [5], impairs peripheral

* Corresponding author. Tel.: +1 416 946 5776; fax: +1 416 978 5882.
E-mail address: a.el.sohemy@utoronto.ca (A. El-Sohemy).

0026-0495/$ – see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.metabol.2006.12.013

glucose uptake [6], increases hepatic gluconeogenesis and lipogenesis [6,7], and alters the expression of major genes that control glucose and lipid metabolism [8,9]. These effects have been associated with insulin resistance and an altered lipid profile that commonly occur in both obese and diabetic individuals.

Three common genetic polymorphisms in the promoter region of the TNF-α gene, −238G>A, −308G>A, and −863C>A, have been shown to alter transcriptional activity [10-14], ex vivo TNF-α production in stimulated blood cells [11,15,16], and in vivo circulating levels of the cytokine in humans [14]. Although TNF-α disrupts insulin action and genetic polymorphisms modify the rate of TNF-α transcription, the effects of polymorphisms in TNF-α on insulin resistance and lipid metabolism have been equivocal [17-21]. This might be due to interactions between genotypes and physiological conditions as well as environmental factors such as diet [20]. The objective of this study was to examine the effects of TNF-α genotypes on circulating biomarkers of insulin resistance and lipid metabolism during an 8-hour metabolic profile test after the consumption of mixed meals and during a 2-hour oral glucose tolerance test (OGTT) in subjects with type 2 diabetes mellitus.

2. Materials and methods

2.1. Subjects

Subjects were participants in the Canadian trial of dietary Carbohydrate in Diabetes study. The study participants were recruited over 1 year (2002-2003) in five cities across Canada (Edmonton, London, Montreal, Sherbrooke, and Toronto). Subjects (N = 123) recruited were men (n = 56) and women (n = 67) with type 2 diabetes mellitus, aged 36 to 75 years, and with a body mass index (BMI) of at least 25 kg/m^2. The diagnosis of diabetes was made according to the Canadian Diabetes Association criteria, where subjects had to have either fasting plasma glucose of 7.0 mmol/L or higher, or plasma glucose of 11.1 mmol/L or higher at 2 hours after a 75-g OGTT within 2 months before the start of the study. Their glycated hemoglobin (HbA_{1c}) had to be 130% or less of the upper limit of normal of the local hospital laboratory. Subjects were excluded if they were taking antidiabetic medications, had a major cardiovascular event or surgery within the previous 6 months before enrolment into the study, had serum triglycerides (TGs) greater than 10 mmol/L, had a major debilitating disorder such as liver disease, renal failure, cancer, or gastrointestinal disorder, or used a drug that was likely to alter gastrointestinal motility and nutrient absorption or affect insulin resistance. Eligible subjects were first scheduled for an OGTT, and an 8-hour metabolic profile test was performed 2 weeks later. The study protocol was approved by the ethics review committee at each participating institution and informed consent was obtained from all subjects.

2.2. Metabolic tests

2.2.1. Oral glucose tolerance test

After a 12-hour overnight fast, blood samples were taken at time 0, 30, 60, and 120 minutes after oral administration of 75 g of glucose. Plasma insulin and glucose were analyzed at each time point.

2.2.2. Eight-hour metabolic profile test

A metabolic profile test was performed to determine postprandial changes in circulating levels of TGs, free fatty acids (FFAs), glucose, and insulin. The test started at about 8 AM after a 12-hour overnight fast. Blood samples were drawn to measure fasting levels of lipids, glucose, insulin, HbA_{1c}, and high-sensitivity C-reactive protein (hs-CRP). Subjects were then given a standard breakfast meal and 4 hours later a lunch meal. Both meals contained 55% of energy from carbohydrate, 30% from fat, and 15% from protein. Blood samples were obtained at hourly intervals for a total of 8 hours. The energy intake received from breakfast and lunch represented a total of 45% of their daily energy requirements as estimated by the Lipid Research Clinic equation [22].

2.3. Blood measurements

Fasting serum cholesterol and TGs were measured by using the Technicon RA1000 (Bayer, Mississauga, Canada). High-density lipoprotein (HDL) cholesterol was measured in the supernatant after treatment of serum with dextran sulfate magnesium chloride. Circulating FFA concentrations from serum (fasted) and plasma (postprandial) were measured by enzymatic activation by long-chain fatty acid-coenzyme A ligase (Wako Chemical Industries, Dallas, TX). The FFA intra- and interassay coefficients of variation were 1.9% and 3.4%, respectively. Apolipoprotein (apo) A-I and apo B were measured by nephelometry, and serum CRP was determined by using the Behring BN100 hs-CRP reagent (Dade-Behring, Mississauga, ON, Canada). All of the above were measured at the J. Alick Little Lipid Research Laboratory (Toronto, ON, Canada). The hs-CRP cut points for cardiovascular risk are less than 1.0 mg/L for low risk, 1.0 to 3.0 mg/L for average risk, and greater than 3.0 mg/L for high risk [23]. Glycated hemoglobin was assessed by high-performance liquid chromatography (Diamat HPLC, Bio-Rad Laboratories, Mississauga, ON, Canada) at St Michael's Hospital (Toronto, ON, Canada). Plasma insulin was measured by electrochemiluminescence immunoassay and plasma glucose by hexokinase Glucose HK Liquid at the University of Toronto Banting and Best Diabetes Centre Core Laboratory (Toronto, ON, Canada). Low-density lipoprotein (LDL) cholesterol was calculated by using the Friedewald equation for samples with TG values less than 4.52 mmol/L [24].

2.4. Genotyping

DNA was isolated from peripheral white blood cells by using the GenomicPrep Blood DNA Isolation kit (Amer-

Table 1
Clinical and metabolic characteristics of the study population (N = 123)

Variable	
Men/women (n)	56/67
Age (y)	60.6 ± 7.4
BMI (kg/m^2)	30.8 ± 4.3
Fasting glucose (mmol/L)	7.45 ± 1.24
Fasting insulin (pmol/L)	57.7 ± 37.3
Total cholesterol (mmol/L)	4.98 ± 0.97
HDL cholesterol (mmol/L)	1.18 ± 0.27
LDL cholesterol (mmol/L)	2.92 ± 0.84
Total cholesterol/HDL ratio	4.39 ± 1.22
TG (mmol/L)	1.94 ± 1.00
FFA (mEq/L)	0.59 ± 0.23
Apo A-I (g/L)	1.57 ± 0.25
Apo B (g/L)	1.02 ± 0.24
hs-CRP (mg/L)	4.68 ± 6.35
HbA_{1c} (%)	6.18 ± 0.62
HOMA-IR	3.22 ± 2.20
HOMA-B	51.9 ± 33.5
OGTT	
Net iAUC −	
Glucose (mmol min^{-1} L^{-1})	713 ± 181
Insulin (nmol min^{-1} L^{-1})	25.1 ± 18.3
Metabolic profile	
Net iAUC −	
Glucose (mmol^{-1} h^{-1} L^{-1})	10.0 ± 8.9
Insulin (mmol^{-1} h^{-1} L^{-1})	1.52 ± 0.90
TG (mmol^{-1} h^{-1} L^{-1})	1.05 ± 1.96
FFA (mEq h^{-1} L^{-1})	−0.90 ± 2.73

Values are mean ± SD. BMI indicates body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; FFA, free fatty acids; OGTT, oral glucose tolerance test; net iAUC, net incremental area under the curve; HOMA-(IR or B), homeostasis model assessment of insulin resistance of B-cell function; hs-CRP, high sensitive C-reactive protein; HbA_{1C}, glycated hemoglobin C.

sham Pharmacia Biotech, Piscataway, NJ). Genotyping was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis. Approximately 25 ng of DNA was amplified by thermal cycling using the HotStar DNA polymerase kit (Qiagen, Mississauga, ON, Canada) in 10 μL of PCR buffer containing 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each deoxyribonucleotide triphosphate (dNTP), 0.5 U *Taq*, and 8 pmol of each primer. All primers were synthesized by ACGT (Toronto, ON, Canada). The TNF-α −308G>A polymorphism was genotyped as previously described [18] by using the following primers: F 5′-AGG CAA TAG GTT TTG AGG GCC AT-3′ and R 5′-TCC TCC CTG CTC CGA TTC CG-3′. The A allele eliminates an *Nco*I restriction site. The primers amplify a 107-base-pair (bp) fragment that is cut into 87- and 20-bp fragments with the G allele, but not the A allele. Polymerase chain reaction conditions included an initial denaturation at 95°C for 15 minutes followed by 40 cycles of 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 30 seconds, with a final extension at 72°C for 7 minutes. The TNF-α −863C>A polymorphism was detected under the same PCR conditions using the following primers: F 5′-GGC TCT GAG GAA TGG GTT-3′ and R 5′-CTA CAT GGC CCT GTC TTC GTT ACG-3′ [25]. Samples were digested with the *Bsa*AI restriction enzyme, which cuts the 126-bp fragment into 2 fragments of 103 and 23 bp in the presence of the A allele. The TNF-α −238G>A polymorphism was detected as previously described [26] using the following primers: F 5′-AAA CAG ACC ACA GAC CTG GTC-3′ and R 5′-CTC ACA CTC CCC ATC CTC CCG GAT C-3′. Polymerase chain reaction conditions were the same as those used for the 2 other polymorphisms, except the annealing temperature was 64°C for 30 seconds. The G to A substitution eliminates a *Bam*HI restriction site. The primers amplify a 165-bp fragment that is cut into 123- and 42-bp fragments with the G allele, but not with the A allele. After restriction enzyme digestion (2 U), products were stained with ethidium bromide, resolved by 3% NuSeive (3:1) agarose gel electrophoresis, and visualized by using a FluorChem UV imaging system.

2.5. Statistical analysis

All statistical analyses were performed by using SAS software, version 8.02 (SAS, Cary, NC). Nonparametric analyses were carried out because they do not require transformation of nonnormally distributed variables that could be due to the effect of genotypes. The Wilcoxon rank sum test was used to assess differences in continuous variables between genotypes and the χ^2 test was used to analyze categorical variables. The data were also stratified by age (below/above median), sex (male/female), and BMI (less than/greater than 30 kg/m^2) to test whether they modified the effects of the genotypes on circulating biomarkers of insulin resistance and lipid metabolism. The net estimate of changes over time from baseline in circulating glucose, insulin, TGs, and FFA during the 8-hour metabolic profile test and the 2-hour OGTT was characterized by the net incremental area under the curve (net iAUC). The net iAUC is calculated by subtracting the rectangular area determined by the fasting value (fasting × 8 hours) from the total area under the curve [27] calculated using the trapezoid rule [28]. Because the concentration of FFA often falls after a meal, the net iAUC is usually negative but could be positive when postprandial FFA plasma levels rise above baseline, resulting from either decreased uptake of FFA or increased lipolysis from adipocytes. Estimation of insulin resistance in the fasting state was determined by using the homeostasis model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B). The formulas used are as follows: HOMA-IR = [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5 and HOMA-B = [20 × fasting insulin (μU/mL)]/[fasting glucose (mmol/L) − 3.5] [29]. Insulin values were divided by a factor of 6.0 to convert picomoles per liter to microunits per milliliter [30]. The acute insulin response ($\Delta I_{30\text{-}0}/\Delta G_{30\text{-}0}$) was calculated as follows: $\Delta Insulin_{30\text{-}0\ min}$ (pmol/L)/$\Delta Glucose_{30\text{-}0\ min}$ (mmol/L) [20]. Missing values for the 8-hour metabolic profile test and the OGTT were imputed by using a mathematical model of the least squares estimate method [31]. Linkage disequilibrium between the genotypes was calculated with the Haplo-

Table 2
Comparison of clinical and metabolic characteristics among TNF-α −238G>A, −308G>A, and −863C>A genotypes (N = 123)

	−238G>A			−308G>A			−863C>A		
	GG	AA + GA	*P*	GG	AA + GA	*P*	CC	AA + CA	*P*
Men/women (n)	38/53	18/14	.22	37/43	19/24	.85	35/55	21/12	.01
Age (y)	60.5 ± 7.3	60.4 ± 7.9	.96	60.2 ± 7.2	60.9 ± 7.9	.72	60.5 ± 7.5	60.3 ± 7.2	.84
Fasting glucose (mmol/L)	7.56 ± 1.32	7.13 ± 0.96	.06	7.40 ± 1.29	7.55 ± 1.15	.58	7.48 ± 1.32	7.35 ± 1.00	.90
Fasting insulin (pmol/L)	60.0 ± 40.3	51.3 ± 26.7	.42	53.1 ± 28.3	66.2 ± 49.1	.22	55.8 ± 31.3	63.2 ± 50.7	.97
BMI (kg/m^2)	31.0 ± 4.5	30.3 ± 3.6	.72	31.2 ± 4.2	30.1 ± 4.3	.13	31.1 ± 4.4	30.0 ± 4.0	.23
Total cholesterol (mmol/L)	5.01 ± 1.03	4.91 ± 0.78	.70	5.00 ± 0.93	4.94 ± 1.05	.95	4.87 ± 0.93	5.28 ± 1.01	.03
HDL cholesterol (mmol/L)	1.19 ± 0.26	1.15 ± 0.30	.42	1.16 ± 0.24	1.23 ± 0.31	.32	1.19 ± 0.27	1.17 ± 0.26	.79
Total cholesterol/HDL	4.35 ± 1.24	4.50 ± 1.18	.41	4.43 ± 1.02	4.30 ± 1.55	.20	4.28 ± 1.14	4.69 ± 1.39	.10
LDL cholesterol (mmol/L)	2.93 ± 0.88	2.89 ± 0.72	.93	2.95 ± 0.85	2.86 ± 0.83	.86	2.85 ± 0.81	3.11 ± 0.89	.19
TG (mmol/L)	1.94 ± 0.97	1.94 ± 1.07	.57	1.97 ± 0.83	1.87 ± 1.26	.19	1.85 ± 0.82	2.18 ± 1.34	.10
FFA (mEq/L)	0.61 ± 0.22	0.52 ± 0.24	.05	0.58 ± 0.22	0.59 ± 0.24	.88	0.60 ± 0.24	0.56 ± 0.19	.44
Apo A-I (g/L)	1.58 ± 0.25	1.55 ± 0.26	.51	1.55 ± 0.24	1.61 ± 0.27	.22	1.56 ± 0.23	1.58 ± 0.30	.77
Apo B (g/L)	1.02 ± 0.25	1.02 ± 0.21	.93	1.03 ± 0.22	1.00 ± 0.28	.66	0.99 ± 0.24	1.10 ± 0.23	.01
hs-CRP (mg/L)	4.32 ± 4.90	5.76 ± 9.49	.93	4.85 ± 6.82	4.39 ± 5.47	.84	4.49 ± 5.09	5.20 ± 8.93	.58
HbA_{1c} (%)	6.23 ± 0.61	6.01 ± 0.61	.10	6.11 ± 0.58	6.30 ± 0.66	.19	6.18 ± 0.64	6.15 ± 0.56	.90
HOMA-IR	3.38 ± 2.34	2.77 ± 1.67	.18	2.96 ± 1.80	3.70 ± 2.75	.15	3.12 ± 1.89	3.49 ± 2.92	.98
HOMA-B	53.1 ± 36.2	48.5 ± 24.6	.88	48.7 ± 26.0	57.8 ± 43.9	.60	50.4 ± 29.6	56.2 ± 43.8	.89
OGTT									
Net iAUC −									
Glucose ($mmol\ min^{-1}\ L^{-1}$)	711 ± 188	716 ± 163	.82	722 ± 190	694 ± 164	.40	717 ± 193	700 ± 146	.77
Insulin ($nmol\ min^{-1}\ L^{-1}$)	25.2 ± 19.5	24.7 ± 14.3	.94	24.1 ± 14.3	26.8 ± 24.2	.95	24.9 ± 16.1	25.6 ± 23.6	.51
Metabolic profile									
Net iAUC −									
Glucose ($mmol^{-1}\ h^{-1}\ L^{-1}$)	10.5 ± 9.3	8.5 ± 7.5	.34	10.8 ± 8.2	8.6 ± 10.1	.08	10.1 ± 9.3	9.7 ± 8.1	.98
Insulin ($nmol^{-1}\ h^{-1}\ L^{-1}$)	1.58 ± 0.92	1.35 ± 0.86	.21	1.49 ± 0.81	1.58 ± 1.07	.76	1.53 ± 0.85	1.52 ± 1.06	.52
TG ($mmol^{-1}\ h^{-1}\ L^{-1}$)	1.11 ± 1.86	0.91 ± 2.23	.69	0.93 ± 2.12	1.29 ± 1.59	.57	0.94 ± 1.80	1.38 ± 2.33	.24
FFA ($mEq\ h^{-1}\ L^{-1}$)	−1.33 ± 2.71	0.26 ± 2.44	.002	−0.96 ± 2.55	−0.78 ± 3.07	.93	−1.09 ± 2.65	−0.40 ± 2.91	.33

Values are mean ± SD. *P* values from Wilcoxon rank sum test.

view software package (http://www.broad.mit.edu/mpg/haploview), but no common haplotypes were found. Analysis of covariance was also performed to ensure that potential confounders that differed between genotypes were accounted for and did not change the effect of the genotype on the outcome. Multiple regression models that accounted for problems of colinearity were also used to assess whether the genotype had a direct effect on postprandial FFA or whether it was indirectly driven by insulin resistance (assessed by fasting and postprandial [net iAUC] glucose and insulin, HbA_{1c}, HOMA-IR, and HOMA-B). For parametric analyses, the bootstrap technique of sampling with replacement was applied to non-Gaussian distribution of the residuals of the models to generate confidence intervals of the betas [32]. Departure of genotype distributions from Hardy-Weinberg equilibrium was assessed by using the χ^2 test with 1 degree of freedom.

3. Results

Characteristics of the subjects are summarized in Table 1. The variant allele frequencies were 14.6% for the −238A allele, 19.6% for the −308A allele, and 15.0% for the −863A allele. The distributions of the 3 genotypes for each of the 3 single nucleotide polymorphisms (SNPs) were in Hardy-Weinberg equilibrium. Table 2 shows the mean ± SD of clinical and metabolic characteristics of subjects by genotype for the 3 SNPs in the promoter region of the TNF-α gene. Carriers of the −238A allele had marginally lower fasting circulating levels of FFA when compared with the GG genotype ($P = .05$; Table 2). However, during the 8-hour metabolic profile test, carriers of the −238A allele had an altered ability to suppress plasma FFA as shown by an increased net iAUC ($P = .002$; Table 2). Multiple regression analyses revealed that the contribution of the TNF-α −238G>A polymorphism to postprandial FFA is independent of different measures of insulin resistance (fasting and postprandial [net iAUC] glucose and insulin, HbA_{1c}, HOMA-IR, and HOMA-B). For the −308G>A polymorphism, no differences in blood measurements were observed between genotypes. The −863C>A polymorphism increased serum levels of total cholesterol ($P = .03$) and apo B ($P = .01$) in carriers of the A allele compared with those with the CC genotype (Table 2). Although a greater proportion of carriers of the −863A allele were men, adjusting for sex in an analysis of covariance did not materially alter the results (data not shown). No differences in plasma insulin or glucose were observed between genotypes for the 3 polymorphisms during the OGTT (Table 2).

Stratified analyses were performed to determine whether any associations were modified by age, sex, or BMI. Only BMI appeared to modify the association between TNF-α genotypes and certain biomarkers. Among obese individuals

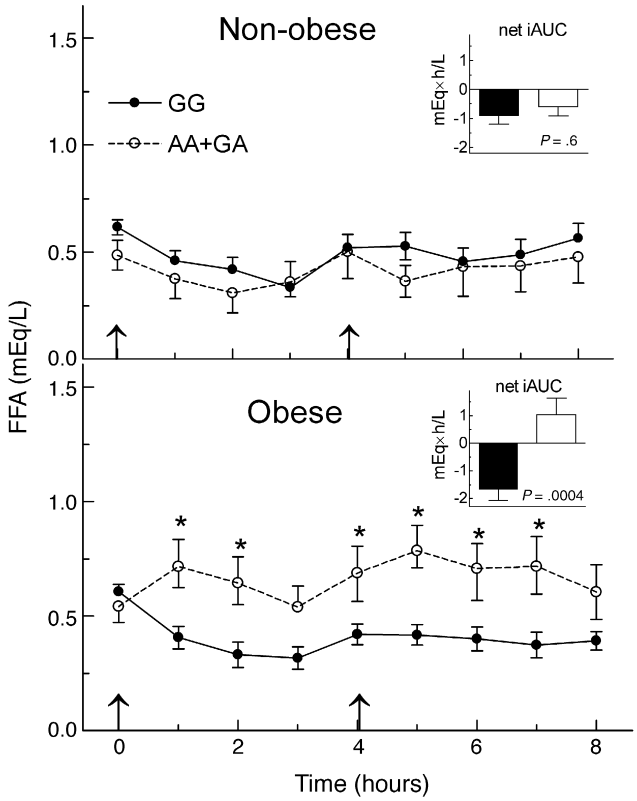

Fig. 1. Effect of the TNF-α −238G>A polymorphism among non-obese (BMI <30, n = 60) (top), and obese (BMI ≥30, n = 63) (bottom) individuals on postprandial plasma FFA concentrations (mEq/L) and net incremental AUC (net iAUC) (mEq h^{-1} L^{-1}) during an 8-hour metabolic profile test. The black arrows (↑) indicate the mealtime. Values are mean ± SEM. *Means between genotypes are significantly different, $P < .05$ from the Wilcoxon rank sum test.

(BMI ≥30 kg/m^2, n = 63), carriers of the −238A allele had increased FFA net iAUC during the 8-hour metabolic profile test compared with those with the GG genotype (1.04 ± 2.42 vs −1.68 ± 2.70 mEq h^{-1} L^{-1}, $P = .0004$) (Fig. 1). In non-obese individuals (BMI <30 kg/m^2, n = 60), no effect of the −238A allele was observed on postprandial circulating FFA net iAUC (−0.63 ± 2.20 vs −0.95 ± 2.71 mEq h^{-1} L^{-1}, $P = .6$). Although no association was found between the −308G>A polymorphism and HOMA-IR in the entire population ($P = .15$; Table 2), obese individuals who were carriers of the A allele had an increased HOMA-IR index when compared with obese subjects with the GG genotype (4.36 ± 2.83 vs 2.85 ± 1.75, $P = .01$). No differences in the HOMA-IR index between genotypes were observed among nonobese individuals (2.19 ± 1.24 vs 1.97 ± 0.90, $P = .6$). The $\Delta I_{30\text{-}0}/\Delta G_{30\text{-}0}$, which is an indicator of the acute insulin response, was also significantly increased in obese carriers of the −308A allele compared with those with the GG genotype (42.1 ± 33.0 vs 24.4 ± 15.1, $P = .04$). No difference was observed between the −308 genotypes in the non-obese subjects ($P = .32$). Regardless of the genotype, obese individuals had increased insulin resistance when compared with nonobese individuals, as they had a significantly higher HOMA-IR, as well as plasma levels of fasting and postprandial insulin (data not shown).

The 3 polymorphisms were not in linkage disequilibrium because the r^2 values were all close to zero. However, analyses with combined genotypes were performed and revealed that the difference in the postprandial plasma FFA in obese individuals was due to the presence of the −238A allele, independent of the other 2 SNPs (data not shown). Thus, it appears that the genotypic effect on the postprandial FFA response is explained solely by the −238 polymorphism. This effect remained significant after applying a Bonferroni correction for multiple comparisons.

4. Discussion

The purpose of this study was to investigate the effects of TNF-α polymorphisms on postprandial changes in circulating biomarkers of insulin resistance and lipid metabolism in subjects with type 2 diabetes mellitus. Our results demonstrate that among obese individuals, carriers of the −238A allele have a decreased ability to suppress postprandial levels of circulating FFA. It is possible that the overexpression of TNF-α in adipocytes of obese individuals [4] may have enhanced the difference in the rate of TNF-α transcription between the different alleles, which could explain why the effects were observed only among obese individuals. Suganami et al [33] recently showed that a paracrine loop involving FFAs and TNF-α between adipocytes and macrophages creates a cycle that aggravates inflammatory changes in the adipose tissue. Furthermore, the production of TNF-α was even greater with larger adipocytes [33]. This finding provides further support for the observed effect among obese, but not non-obese, individuals. Elevated levels of TNF-α have also been shown to increase circulating levels of FFA in mice and humans [34,35] and to have adverse effects on insulin action [5,6]. Using multiple regression analyses, we found that the effect of TNF-α on postprandial FFA was independent of insulin resistance, but this hypothesis remains to be tested.

Two previous studies have investigated the postprandial effects of only one of the TNF-α polymorphisms at position −308G>A on biomarkers of insulin resistance or lipid metabolism after a single high-fat meal [20,21]. Wybranska et al [20] found no clear effects of the −308G>A polymorphism on postprandial blood insulin, TGs, or FFA. Nicaud et al [21] only measured changes in postprandial serum TG and observed no differences between genotypes. Our data are consistent with those observations because we found no effects of the TNF-α −308G>A polymorphism on circulating levels of glucose, insulin, TGs, or FFA in the postprandial state after the consumption of mixed meals. The effect we observed with the TNF-α −238G>A polymorphism on postprandial circulating levels

of FFA may involve transcription factors that require nutrients as activators and bind uniquely to this region of the promoter to increase the levels of TNF-α.

The effects of TNF-α on glucose and lipid metabolism have been shown to vary under different experimental conditions [34,36]. For example, Green et al [36] found that neither TNF-α nor glucose alone induced lipolysis in cultured adipocytes, but a combination of both had a marked lipolytic effect. Memon et al [34] demonstrated that in fasted mice, the injection of TNF-α did not exert a stimulatory effect on lipolysis or alter serum FFA levels, but increased serum FFA by more than 50% in fed mice. Evidence from human studies has also demonstrated an interaction between TNF-α genotypes and various dietary factors on TNF-α production and the risk of developing type 2 diabetes mellitus [37,38]. Thus, it appears that the effects of TNF-α on various metabolic pathways may depend on certain environmental factors such as diet.

In obese carriers of the −308A allele, we found an increased HOMA-IR index, which is a measure of insulin resistance in the fasted state, and an increased $\Delta I_{30\text{-}0}/\Delta G_{30\text{-}0}$, which is an indicator of early insulin secretion. Wybranska et al [20] also found an increase in some, but not all, indices of insulin resistance in obese individuals who were carriers of the −308A allele. Other studies that investigated the effect of the −308G>A polymorphism on HOMA-IR found no differences between genotypes [19,39], but analyses among obese individuals alone were not performed. In the present study, obese individuals were more insulin resistant than nonobese individuals. Insulin resistance or obesity could potentiate the effects of TNF-α polymorphisms, and failure to account for these factors could explain some of the inconsistencies in results observed between different studies. For example, Pihlajamäki et al [40] studied the effect of the −308 polymorphism on glucose and lipid oxidation during a euglycemic clamp in normal-weight and overweight healthy subjects. They found that normal-weight (BMI <26 kg/m^2) carriers of the −308A allele had a higher rate of glucose oxidation, but no change in lipid metabolism. Overweight (BMI ≥26 kg/m^2) carriers of the −308A allele, however, had no difference in the rate of glucose oxidation, but a greater rate of lipid synthesis and an enhanced ability to suppress FFA [40]. Although we did not measure the rate of lipid synthesis or glucose oxidation during the OGTT or the 8-hour metabolic profile test, we found no association between the TNF-α −308G>A genotypes and the postprandial glucose or FFA response. During an OGTT, the −308G>A polymorphism has been shown to be associated with insulin resistance in some studies [18,20,21], but we and others found no such associations [17,19]. Although it has been reported that the polymorphism at position −308G>A increases transcriptional activity in vitro [10], the functional significance of this polymorphism has been shown to vary depending on the experimental conditions used [41]. These observations suggest that a variety of conditions could affect the functional significance of the polymorphism and may also explain some of the inconsistent findings of this SNP on insulin resistance.

Studies investigating the functional significance of the −238G>A and −863C>A polymorphisms have also shown that the variant allele could either increase or decrease transcriptional activity [11-14]. This may explain, in part, why carriers of the −238A allele in the fasted state had lower circulating levels of FFA, but higher plasma levels of FFA, in the postprandial state. The −863A allele resulted in greater fasting serum levels of total cholesterol and apo B, which is consistent with in vitro studies showing that the A allele increases gene expression under certain conditions [13]. Moreover, rats that were injected with TNF-α had increased hepatic production and greater serum concentrations of cholesterol [7,42]. No effects of the −238G>A or −863C>A polymorphisms were observed on biomarkers of insulin resistance during the OGTT, which is consistent with findings from previous studies [17,25]. Because the effects of the polymorphisms of TNF-α have not been examined under similar experimental conditions in nondiabetic subjects, it is not clear whether our findings are specific to diabetic individuals.

In summary, our findings suggest that the −238A allele impairs postprandial suppression of circulating FFA and the −308A allele increases insulin resistance, but only in obese individuals. These findings provide further evidence for a role of genotypes affecting proinflammatory cytokines in metabolic disturbances related to the metabolic syndrome and type 2 diabetes mellitus.

Acknowledgments

This study was supported by a grant from the Canada Institutes of Health Research (CIHR) MCT-44205. Bénédicte Fontaine-Bisson is a recipient of a Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship. Ahmed El-Sohemy holds a Canada Research Chair in Nutrigenomics.

The authors thank Vasanti Malik, the research coordinator of the Carbohydrate in Diabetes study, and the Institute for Social Research at York University for data management.

References

[1] Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005;111:1448-54.

[2] Pickup J, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. Life Sci 2000;67:291-300.

[3] Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P. Circulating mononuclear cells in the obese are in a proinflammatory state. Circulation 2004;110:1564-71.

[4] Hotamisligil G, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995;95:2409-15.

[5] Hotamisligil G, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1–mediated inhibition of insulin receptor tyrosine kinase

activity in TNF-alpha– and obesity–induced insulin resistance. Science 1996;271:665-8.
[6] Lang C, Dobrescu C, Bagby GJ. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology 1992;130:43-52.
[7] Feingold K, Grunfeld C. Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat in vivo. J Clin Invest 1987;80:184-90.
[8] Stephens J, Pekala PH. Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. J Biol Chem 1991;266:21839-45.
[9] Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM, et al. Negative regulation of peroxisome proliferator–activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha. Mol Endocrinol 1996;10:1457-66.
[10] Kroeger K, Carville KS, Abraham LJ. The −308 tumor necrosis factor-alpha promoter polymorphism affects transcription. Mol Immunol 1997;5:391-9.
[11] Kaluza W, Reuss E, Grossmann S, Hug R, Schopf RE, Galle PR, et al. Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. J Invest Dermatol 2000;114:1180-3.
[12] Bayley J, de Rooij H, van den Elsen PJ, Huizinga TW, Verweij CL. Functional analysis of linker-scan mutants spanning the −376, −308, −244, and −238 polymorphic sites of the TNF-alpha promoter. Cytokine 2001;14:316-23.
[13] Higuchi T, Seki N, Kamizono S, Yamada A, Kimura A, Kato H, et al. Polymorphism of the 5′-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. Tissue Antigens 1998;51:605-12.
[14] Skoog T, van't Hooft FM, Kallin B, Jovinge S, Boquist S, Nilsson J, et al. A common functional polymorphism (C→A substitution at position −863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. Hum Mol Genet 1999;8:1443-9.
[15] Heesen M, Kunz D, Wessiepe M, van der Poll T, Zwinderman AH, Blomeke B. Rapid genotyping for tumor necrosis factor-alpha (TNF-alpha)−863C/A promoter polymorphism that determines TNF-alpha response. Clin Chem 2004;50:226-8.
[16] Bouma G, Crusius JB, Oudkerk Pool M, Kolkman JJ, von Blomberg BM, Kostense PJ, et al. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. Scand J Immunol 1996;43:456-63.
[17] Koch M, Rett K, Volk A, Maerker E, Haist K, Weisser M, et al. The tumour necrosis factor alpha −238 G → A and −308 G → A promoter polymorphisms are not associated with insulin sensitivity and insulin secretion in young healthy relatives of Type II diabetic patients. Diabetologia 2000;43:181-4.
[18] Fernandez-Real J, Gutierrez C, Ricart W, Casamitjana R, Fernandez-Castaner M, Vendrell J, et al. The TNF-alpha gene NcoI polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. Diabetes 1997;46:1468-72.
[19] Day C, Grove J, Daly AK, Stewart MW, Avery PJ, Walker M. Tumour necrosis factor-alpha gene promoter polymorphism and decreased insulin resistance. Diabetologia 1998;41:430-4.
[20] Wybranska I, Malczewska-Malec M, Niedbal S, Naskalski JW, Dembinska-Kiec A. The TNF-alpha gene NcoI polymorphism at position −308 of the promoter influences insulin resistance, and increases serum triglycerides after postprandial lipaemia in familiar obesity. Clin Chem Lab Med 2003;41:501-10.
[21] Nicaud V, Raoux S, Poirier O, Cambien F, O'Reilly DS, Tiret L. The TNF alpha/G-308A polymorphism influences insulin sensitivity in offspring of patients with coronary heart disease: the European Atherosclerosis Research Study II. Atherosclerosis 2002;161:317-25.
[22] The Lipid Research Clinics Population Studies Data Book. Vol. 2. The prevalence study—nutrient intake. Washington (DC): Government Printing Office; 1982 (NIH publication no. 82-2014).
[23] Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. Am J Med 2004;116(Suppl 6A): 9S-16S.
[24] Sniderman A, Blank D, Zakarian R, Bergeron J, Frohlich J. Triglycerides and small dense LDL: the twin Achilles heels of the Friedewald formula. Clin Biochem 2003;36:499-504.
[25] Costa A, Fernandez-Real JM, Vendrell J, Broch M, Casamitjana R, Ricart W, et al. Lower rate of tumor necrosis factor-alpha −863A allele and higher concentration of tumor necrosis factor-alpha receptor 2 in first-degree relatives of subjects with type 2 diabetes. Metabolism 2003;52:1068-71.
[26] Ozen S, Alikasifoglu M, Bakkaloglu A, Duzova A, Jarosova K, Nemcova D, et al. Tumour necrosis factor alpha G→A −238 and G→A −308 polymorphisms in juvenile idiopathic arthritis. Rheumatology 2002;41:223-7.
[27] Matthews J, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. BMJ 1990;300:230-5.
[28] Wolever T. Effect of blood sampling schedule and method of calculating the area under the curve on validity and precision of glycaemic index values. Br J Nutr 2004;91:295-301.
[29] Haffner S, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. Diabetes Care 1997;20:1087-92.
[30] Système International (SI) units for plasma, serum, or blood concentrations. Diabetes Care 1993;16:556.
[31] Cochran W, Snedecor GW. Statistical methods. Iowa: Iowa State University Press; 1980.
[32] Henderson A. The bootstrap: a technique for data-driven statistics. Using computer-intensive analyses to explore experimental data. Clin Chim Acta 2005;359:1-26.
[33] Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol 2005;25:2062-8.
[34] Memon R, Feingold KR, Moser AH, Doerrler W, Adi S, Dinarello CA, et al. Differential effects of interleukin-1 and tumor necrosis factor on ketogenesis. Am J Physiol 1992;263:E301-9.
[35] Van Der Poll T, Romijn JA, Endert E, Borm JJJ, Buller HR, Sauerwein HP. Tumor necrosis factor mimics the metabolic response to acute infection in healthy humans. Am J Physiol 1991;261: E457-65.
[36] Green A, Rumberger JM, Stuart CA, Ruhoff MS. Stimulation of lipolysis by tumor necrosis factor-α in 3T3-L1 adipocytes is glucose dependent. Diabetes 2004;53:74-81.
[37] Kubaszek A, Pihlajamaki J, Komarovski V, Lindi V, Lindstrom J, Eriksson J, et al. Promoter polymorphisms of the TNF-alpha (G-308A) and IL-6 (C-174G) genes predict the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study. Diabetes 2003;52:1872-6.
[38] Grimble R, Howell WM, O'Reilly G, Turner SJ, Markovic O, Hirrell S, et al. The ability of fish oil to suppress tumor necrosis factor alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alpha production. Am J Clin Nutr 2002;76:454-9.
[39] Romeo S, Sentinelli F, Capici F, Arca M, Berni A, Vecci E, et al. The G-308A variant of the tumor necrosis factor-α (TNF-α) gene is not associated with obesity, insulin resistance and body fat distribution. BMC Med Genet 2001;2:10.
[40] Pihlajamaki J, Ylinen M, Karhapaa P, Vauhkonen I, Laakso M. The effect of the −308A allele of the TNF-α gene on insulin action is dependent on obesity. Obes Res 2003;11:912-7.
[41] Kroeger K, James HS, Joyce DA, Abraham LJ. Effects of stimulus and cell type on the expression of the −308 tumour necrosis factor promoter polymorphism. Cytokine 2000;12:2000.
[42] Krauss R, Grundfeld C, Doerrler WT, Feingold KR. Tumor necrosis factor acutely increases plasma levels of very low density lipoproteins of normal size and composition. Endocrinology 1990;127: 1016-21.