Genetic polymorphisms of tumor necrosis factor-α modify the association between dietary polyunsaturated fatty acids and fasting HDL-cholesterol and apo A-I concentrations[1–3]

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ABSTRACT

Background: Heterogeneity in circulating lipid concentrations in response to dietary polyunsaturated fatty acids (PUFAs) may be due, in part, to genetic variations. Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that can induce hyperlipidemia and is known to be modulated by dietary PUFAs.

Objective: The objective was to determine whether TNF-α genotypes modify the association between dietary PUFA intake and serum lipid concentrations.

Design: The study involved 53 men and 56 women aged 42–75 y with type 2 diabetes. Dietary intakes were assessed with the use of a 3-d food record, and blood samples were collected to determine fasting serum lipids. DNA was isolated from blood for genotyping by polymerase chain reaction–restriction fragment length polymorphism for the *TNF*-α −238G→A and −308G→A polymorphisms.

Results: PUFA intake was positively associated with serum HDL cholesterol in carriers of the *−238A* allele ($\beta = 0.06 \pm 0.03$ mmol/L per 1% of energy from PUFAs; $P = 0.03$), but negatively associated in those with the *−238GG* genotype ($\beta = -0.03 \pm 0.01$, $P = 0.03$) ($P = 0.004$ for interaction). PUFA intake was inversely associated with HDL cholesterol in carriers of the *−308A* allele ($\beta = -0.07 \pm 0.02$, $P = 0.002$), but not in those with the *−308GG* genotype ($\beta = 0.02 \pm 0.02$, $P = 0.13$) ($P = 0.001$ for interaction). A stronger gene × diet interaction was observed when the polymorphisms at the 2 positions (−238/−308) were combined ($P = 0.0003$). Similar effects were observed for apolipoprotein A-I, but not with other dietary fatty acids and serum lipids.

Conclusion: TNF-α genotypes modify the relation between dietary PUFA intake and HDL-cholesterol concentrations. These findings suggest that genetic variations affecting inflammation may explain some of the inconsistencies between previous studies relating PUFA intake and circulating HDL. *Am J Clin Nutr* 2007;86:768–74.

KEY WORDS Tumor necrosis factor-α, genotype, polyunsaturated fatty acids, HDL cholesterol, apolipoprotein A-I, type 2 diabetes

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) have been shown to have some protective cardiovascular effects by influencing many risk factors including serum lipids (1). The n−3 PUFAs that are derived mainly from fish oils (EPA and DHA) are known to lower plasma triacylglycerols, whereas n−6 PUFAs have been shown to reduce total and LDL cholesterol (1). The effects of these 2 classes of PUFAs on other lipids and lipoproteins are not consistent, and the reasons for these inconsistencies are unclear. Chronic inflammation has been associated with dyslipidemia (2) and may mediate the effects of PUFAs on lipid metabolism (3).

PUFAs are precursors of prostaglandins and leukotrienes and also affect the expression of various genes involved in inflammation (3). Tumor necrosis factor-α (TNF-α) is a major proinflammatory cytokine that has been associated with an altered lipid profile, insulin resistance, and an increased risk of cardiovascular disease (CVD) (4, 5). Different types of fatty acids, such as n−3 and n−6 PUFAs, modulate the production of TNF-α in humans (6–9). A genetic polymorphism of TNF-α has also been shown to influence the effect of dietary n−3 PUFAs on TNF-α production in humans (6). TNF-α alters lipid metabolism by inducing lipolysis in adipose tissue (10), increasing lipogenesis in the liver (11), and worsening the serum lipid profile (12, 13). TNF-α has also been shown to alter the expression of genes involved in lipid metabolism, such as lipoprotein lipase (LPL) (14); proliferator activated receptors (PPARs) (15); apolipoprotein

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[2] Supported by a grant from the Canada Institutes of Health Research (CIHR-MCT-44205) and the Advanced Foods and Materials Network. BF-B is a recipient of a Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship. AE-S holds a Canada Research Chair in Nutrigenomics.

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Received February 4, 2007.

Accepted for publication May 8, 2007.

(apo) A-I, apo A-IV, and apo E (16, 17); and lecithin:cholesterol acyltransferase (18).

Two common genetic polymorphisms in the promoter region of the TNF-α gene have been shown to alter transcriptional activity (19–21). A single nucleotide polymorphism (SNP) at position −238G→A has been shown to decrease the rate of transcription and production of the cytokine (19), whereas an SNP at position −308G→A has been shown to increase both (20, 21). TNF-α inhibits insulin action, and chronic inflammation is typically found in type 2 diabetes (22). We therefore investigated whether TNF-α genotypes modify the association between dietary PUFA intake and serum lipid concentrations among individuals with type 2 diabetes.

SUBJECTS AND METHODS

Subjects

Subjects were participants in the Canadian trial of dietary Carbohydrate in Diabetes study. Of the 164 participants recruited, blood samples for genotyping, complete dietary information, and lipid profiles were available for 109 subjects. The study participants were recruited over a 1-y period (2002–2003) in 5 cities across Canada (Edmonton, London, Montreal, Sherbrooke, and Toronto). Subjects were men ($n = 53$) and women ($n = 56$) with type 2 diabetes treated by diet alone and with a body mass index (BMI; in kg/m^2) of ≥25. The diagnosis of diabetes was made according to the Canadian Diabetes Association criteria; subjects had to have either a fasting plasma glucose concentration ≥7.0 mmol/L or a plasma glucose concentration ≥11.1 mmol/L 2 h after a 75-g oral-glucose-tolerance test within 2 mo before the start of the study. The glycated hemoglobin (Hb A_{1c}) value had to be ≤130% of the upper limit of normal of the local hospital's laboratory. Subjects taking lipid-lowering drugs [statins ($n = 46$), fibrate ($n = 2$), or both ($n = 5$) or unavailable information ($n = 8$)] were not excluded from the study to make the results more applicable to the diabetic population. Subjects were excluded if they were taking antidiabetic medications; had a major cardiovascular event or surgery within the previous 6 mo before enrollment in the study; had a serum triacylglycerol concentration >10 mmol/L; had a major debilitating disorder such as liver disease, renal failure, cancer, or gastrointestinal disorder; or used medication that was likely to alter gastrointestinal motility, nutrient absorption, or insulin sensitivity. The study protocol was approved by the ethics review committee at each participating institution, and informed consent was obtained from all subjects.

Blood measurements

Fasting serum cholesterol and triacylglycerol were measured by using the Technicon RA100 (Technicon, Miami, FL). HDL cholesterol was measured in the supernatant fluid after treatment of serum with dextran sulfate magnesium chloride. Serum free fatty acids (FFAs) were measured by enzymatic activation by long-chain fatty acid-CoA ligase (Wako Chemical Industries, Dallas, TX). Apo A-I and apo B were measured by nephelometry, and serum high-sensitivity C-reactive protein (hs-CRP) was determined by using the Behring BN100 hs-CRP reagent (Dade-Behring, Mississauga, Canada). All of the above were measured at the J Alick Little Lipid Research Laboratory of St Michael's Hospital (Toronto, Canada). Plasma insulin was measured by electrochemiluminescence immunoassay and plasma glucose by hexokinase glucose HK liquid at the University of Toronto's Banting and Best Diabetes Centre Core Laboratory. LDL cholesterol was calculated by using the Friedewald equation for samples with triacylglycerol values <4.52 mmol/L (23).

Genotyping

DNA was isolated from peripheral white blood cells by using the GenomicPrep Blood DNA Isolation kit (Amersham Pharmacia Biotech Inc, Piscataway, NJ). Genotyping of the −238G→A (rs361525) and −308G→A (rs1800629) polymorphisms was performed by polymerase chain reaction–restriction fragment length polymorphism analysis as previously described (24).

Dietary and physical activity assessment

Dietary intake was assessed the week before the blood sample collection with the use of a 3-d food record. Subjects recorded their food and beverage consumption during 2 weekdays and 1 weekend day. The nutrient composition of the diet was assessed by using an in-house program with a nutrient database that was based on the Canadian Nutrient File (25). Physical activity was assessed by using the Modifiable Activity Questionnaire from which an MET (metabolic equivalent) score was calculated (26).

Statistical analysis

One-factor analysis of variance was used to test for differences in general characteristics between the 3 genotypes of the TNF-α polymorphisms. The non-normally distributed variables (glucose, insulin, triacylglycerol, and C-reactive protein) were $\log_e$ transformed, and the results are presented as the antilog of the estimate. Although the distribution for apo A-I was slightly skewed, no transformation was performed to generate results that can be readily interpreted. Nevertheless, this approach gave similar results to the transformed data, but yielded a more conservative estimate. The chi-square test was used to analyze categorical variables. The 2 polymorphisms were not in linkage disequilibrium ($r^2 = 0.009$ calculated by using the HAPLOVIEW software package) (27). Because of the sample size, homozygotes for the minor allele (*AA*) were grouped with heterozygotes (*GA*) for each of the 2 polymorphisms (−238G→A and −308G→A) producing 4 possible combinations for −238/-308: 0/0, 0/1, 1/0, and 1/1, where 0 = *GG* and 1 = *AA*+*GA*. The fourth combined genotype with the 2 minor alleles was dropped from the analyses because of the small sample size ($n = 7$). Multiple linear regression was used to test whether the effect of different dietary fatty acids on serum lipid and apolipoprotein concentrations varied across the different genotypes (interaction) in the presence of various confounders. Of the variables age, energy intake, alcohol consumption, lipid-lowering drugs, Hb A_{1c}, physical activity, BMI, and sex, only the latter 2 variables were statistically significant and thus remained in the final model. No differences or interactions were found between the TNF-α genotypes and any of these potential confounders. Dietary fatty acids were adjusted for total energy intake by using both the residual and the nutrient density methods (28). Although similar results were obtained with both methods, values from the nutrient density method (% of energy of dietary fatty acids from total energy intake), which yielded more conservative estimates, are reported. Similar results were obtained when interactions were tested with PUFAs as a continuous variable and when grouped into tertiles. Men and women

TABLE 1
Clinical and metabolic characteristics and dietary intake by tumor necrosis factor-α genotype[1]

	−238G→A				−308G→A			
	GG (n = 82)	*GA* (n = 23)	*AA* (n = 4)	P^2	*GG* (n = 69)	*GA* (n = 35)	*AA* (n = 5)	P^2
Men/women (n)	37/45	13/10	3/1	0.35	34/35	16/19	3/2	0.82
Age (y)	60.3 ± 7.2[3]	58.5 ± 7.7	66.5 ± 8.1	0.13	59.7 ± 7.2	60.8 ± 7.8	61.8 ± 7.2	0.68
BMI (kg/m^2)	30.8 ± 4.4	30.7 ± 3.8	29.4 ± 3.0	0.81	31.3 ± 4.2	29.7 ± 4.2	30.4 ± 4.4	0.19
Hb A_{1c} (%)	6.26 ± 0.60	6.10 ± 0.62	5.92 ± 0.43	0.32	6.17 ± 0.57	6.21 ± 0.58	6.88 ± 0.88	0.04
Fasting glucose (mmol/L)	7.54 ± 1.17	7.24 ± 1.14	6.82 ± 1.14	0.31	7.39 ± 1.17	7.39 ± 1.15	8.17 ± 1.18	0.35
Fasting insulin (pmol/L)	50.4 ± 1.75	46.5 ± 1.79	41.3 ± 1.72	0.70	47.5 ± 1.70	51.4 ± 1.90	66.7 ± 1.54	0.38
Total cholesterol (mmol/L)	4.99 ± 1.02	4.98 ± 0.85	4.38 ± 0.50	0.48	4.95 ± 0.93	5.03 ± 1.07	4.70 ± 0.93	0.76
HDL cholesterol (mmol/L)	1.19 ± 0.26	1.17 ± 0.33	0.94 ± 0.09	0.20	1.15 ± 0.24	1.23 ± 0.33	1.15 ± 0.28	0.38
LDL cholesterol (mmol/L)	2.93 ± 0.86	2.94 ± 0.81	2.66 ± 0.45	0.81	2.94 ± 0.84	2.93 ± 0.85	2.73 ± 0.70	0.87
Triacylglycerol (mmol/L)	1.72 ± 1.55	1.72 ± 1.72	1.67 ± 1.38	0.99	1.77 ± 1.48	1.62 ± 1.77	1.72 ± 1.43	0.61
FFA (mEq/L)	0.61 ± 0.23	0.55 ± 0.23	0.34 ± 0.20	0.05	0.59 ± 0.23	0.61 ± 0.24	0.45 ± 0.21	0.34
Apo A–I (g/L)	1.57 ± 0.26	1.56 ± 0.29	1.36 ± 0.09	0.29	1.53 ± 0.25	1.61 ± 0.28	1.59 ± 0.31	0.35
Apo B (g/L)	1.02 ± 0.25	1.03 ± 0.24	0.99 ± 0.13	0.94	1.02 ± 0.22	1.02 ± 0.29	1.00 ± 0.20	0.98
hs-CRP (mg/L)	2.61 ± 2.94	3.42 ± 3.53	1.62 ± 3.67	0.40	2.94 ± 2.83	2.36 ± 3.60	2.29 ± 3.32	0.61
Energy intake (kcal)	1897 ± 519	1740 ± 354	1897 ± 555	0.40	1847 ± 496	1892 ± 496	1900 ± 443	0.90
Total fat (% of energy)	33.1 ± 7.3	32.4 ± 7.8	32.6 ± 7.7	0.92	33.3 ± 7.5	32.3 ± 7.4	32.3 ± 4.1	0.77
SFA (% of energy)	10.6 ± 3.4	10.0 ± 3.4	11.3 ± 2.9	0.71	10.5 ± 3.5	10.3 ± 3.3	11.5 ± 2.4	0.78
MUFA (% of energy)	13.5 ± 3.5	13.3 ± 3.7	12.7 ± 3.2	0.90	13.7 ± 3.5	13.1 ± 3.8	12.6 ± 0.8	0.64
PUFA (% of energy)	6.4 ± 2.0	6.0 ± 1.7	5.5 ± 2.1	0.53	6.3 ± 2.0	6.2 ± 1.9	5.9 ± 2.0	0.85
Cholesterol (mg/d)	288 ± 137	261 ± 127	362 ± 96	0.36	291 ± 129	275 ± 148	264 ± 117	0.79
Carbohydrates (% of energy)	45.0 ± 8.0	45.8 ± 8.9	46.3 ± 10.2	0.88	44.5 ± 8.5	46.1 ± 7.9	48.6 ± 3.8	0.39
Protein (% of energy)	20.1 ± 3.9	19.9 ± 4.4	20.1 ± 5.3	0.97	20.3 ± 4.1	19.7 ± 3.9	18.4 ± 3.8	0.49
Fiber (g/d)	23.5 ± 10.6	23.0 ± 6.8	22.8 ± 12.6	0.97	22.4 ± 9.3	25.5 ± 11.3	21.9 ± 7.2	0.29

[1] Hb A_{1c}, glycated hemoglobin; FFA, free fatty acids; apo, apolipoprotein; hsCRP, high-sensitivity C-reactive protein; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2] P values for differences between genotypes were obtained by using a one-factor ANOVA, and the chi-square test was used to test for differences between genotypes in men and women.
[3] $\bar{x}$ ± SD (all such values).

were analyzed together because patterns were similar between them, and no interactions between sex and TNF-α genotypes on the outcome measures of interest were found. Departure of genotype distributions from Hardy-Weinberg equilibrium was assessed by using the chi-square test with 1 df and confirmed by using the HAPLOVIEW software package (27). Significant P values are 2-sided and <0.05. The Bonferroni correction for multiple comparisons was used when appropriate. All statistical analyses were performed by using SAS version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Anthropometric, biochemical, and dietary characteristics of the subjects are summarized in **Table 1**. The minor allele frequencies were 14.2% for the −238G→A polymorphism and 20.6% for the −308G→A polymorphism. The distributions of the 3 genotypes for each of the 2 SNPs (*−238GG* = 82, *GA* = 23, *AA* = 4; and *−308GG* = 69, *GA* = 35, *AA* = 5) were in Hardy-Weinberg equilibrium. No differences were observed between genotypes for the variables in Table 1, except for Hb A_{1c}, which was higher among individuals with the *−308AA* genotype than in those with the *GG* genotype ($P = 0.04$; Tukey's post hoc test). FFA was marginally significant for the −238G→A polymorphism ($P = 0.05$), but no differences between the 3 genotypes were observed when Tukey's correction for multiple comparisons was applied. All differences disappeared when individuals with the *AA* and *GA* genotypes were combined (Hb A_{1c}: $P = 0.29$; FFA: $P = 0.07$).

We assessed whether the TNF-α genotypes modified the association between dietary fatty acids (total, saturated, monounsaturated, and polyunsaturated) and circulating lipids and apolipoproteins (total, LDL, and HDL cholesterol; triacylglycerol; FFAs; apo A-I; and apo B). The results are unadjusted in the text and adjusted for sex and BMI in the tables and the figure. Significant interactions were found between dietary PUFA intake and TNF-α genotypes on plasma HDL cholesterol (PUFA × −238G→A, $P = 0.003$; PUFA × −308G→A, $P = 0.001$). PUFA intake was inversely associated with serum HDL cholesterol among individuals with the *−238GG* genotype ($P = 0.02$), but was positively associated in carriers of the *−238A* allele ($P = 0.03$). In carriers of the *−308A* allele, PUFA intake was inversely associated with serum HDL cholesterol ($P = 0.001$), but not in those with the *−308GG* genotype ($P = 0.3$) (adjusted slopes and P values for sex and BMI are shown in **Table 2**).

Because the effects of PUFA intake on HDL-cholesterol concentrations were in the opposite direction for the *−238A* and the *−308A* alleles, we grouped individuals who had the *A* allele at one position and the *GG* genotype at the other position. Forty-nine individuals had the 0/0 genotype at position −238/-308, 33 individuals had the 0/1 genotype, and 20 individuals had the 1/0 genotype, where 0 = *GG* and 1 = *GA*+*AA*. Because of the small sample size ($n = 7$) the 1/1 genotype group was dropped from the analysis. We then tested the PUFA × TNF-α interaction on HDL

TABLE 2
Association between dietary polyunsaturated fatty acid (PUFA) intake (% of energy) and HDL-cholesterol (mmol/L) or apolipoprotein (apo) A-I (g/L) concentrations for tumor necrosis factor-α (TNF-α) −238G→A and −308G→A genotypes[1]

Genotypes	HDL cholesterol[2]	P^3	P^4	R^5	Apo A-I[2]	P^3	P^4	R^5
−238 *GG* ($n = 82$)	−0.03 ± 0.01	0.03	0.004	0.45	−0.02 ± 0.01	0.19	0.05	0.45
−238 *AA+GA* ($n = 27$)	0.06 ± 0.03	0.03			0.04 ± 0.03	0.12		
−308 *GG* ($n = 69$)	0.02 ± 0.02	0.13	0.001	0.47	0.02 ± 0.01	0.13	0.002	0.51
−308 *AA+GA* ($n = 40$)	−0.07 ± 0.02	0.002			−0.06 ± 0.02	0.005		

[1] The dependent variables are either HDL cholesterol (mmol/L) or apo A-I (g/L).
[2] Values are β regression coefficients ± SE and were obtained by using a general linear model adjusted for sex and BMI.
[3] *P* test for the relation between PUFA intake and HDL-cholesterol or apo A-I concentrations for each TNF-α genotype.
[4] *P* test for the interaction between PUFA intake and the TNF-α polymorphism on HDL-cholesterol or apo A-I concentrations.
[5] Multiple correlation coefficient ($P < 0.001$ for all).

cholesterol using the 3 combined genotypes and observed a stronger difference between genotypes in the association between PUFA intake and HDL-cholesterol concentration ($P = 0.0003$). Adjusted slopes and *P* values for sex and BMI are shown in **Table 3**. Among individuals with the 0/1 genotype, each 1% increase in energy from PUFA intake was associated with a 0.08 ± 0.02 mmol/L decrease in HDL-cholesterol concentration ($P = 0.0003$ unadjusted). Among individuals with the 1/0 genotype, however, the same increase in PUFA intake was associated with a 0.07 ± 0.02 mmol/L increase in HDL cholesterol ($P = 0.02$). No association between PUFA intake and HDL-cholesterol concentrations was observed among individuals with the 0/0 genotype (−0.004 ± 0.018 mmol/L of HDL per 1% PUFA; $P = 0.82$). The slope of the 0/0 group differed significantly from the slope of the 0/1 group ($P = 0.004$), and the slope of the 0/1 and 1/0 groups also differed significantly from each other ($P = 0.0001$), but a smaller difference was observed between the slopes of the 0/0 and 1/0 groups ($P = 0.06$). *P* values remained significant after a Bonferroni correction for multiple comparisons (threshold: $P < 0.017$).

Individuals were then grouped into tertiles of PUFA intake (with a median of 4%, 6%, and 8% of energy) to avoid any influential effect of outliers or assumption of linearity, and a similar interaction on HDL-cholesterol concentrations was observed ($P = 0.008$ for interaction; adjusted *P* for sex and BMI is shown **Figure 1**). Among subjects with the 0/1 genotype, the highest compared with the lowest tertile of PUFA intake was associated with a 36% lower HDL-cholesterol concentration ($P = 0.004$) and a 23% higher HDL-cholesterol concentration among those with the 1/0 genotype ($P = 0.03$). As expected, no effect of PUFA intake on HDL-cholesterol concentrations was observed among individuals with the *GG* genotype at both positions (0/0) ($P = 0.45$). Adjustment for sex and BMI did not materially alter the results. Although HDL cholesterol was negatively correlated with triacylglycerol in the entire population ($r = -0.38$, $P < 0.0001$) and within each genotype (all $P < 0.03$), triacylglycerol concentrations did not differ between genotypes (Table 1), and further adjustment of the models for triacylglycerol did not materially alter the results.

The Pearson product-moment correlation was assessed to test for colinearity among the different types of dietary fatty acids. PUFA intake was highly correlated with total fat and monounsaturated fat (both: $r > 0.6$, $P < 0.0001$), but not with saturated fat ($r = 0.11$, $P = 0.25$). However, there were no significant interactions between TNF-α genotypes and either total fat, monounsaturated fat, or saturated fat on any of the circulating lipids (data not shown).

We also tested for interactions between TNF-α genotypes and dietary PUFA intake on apo A-I concentration, which is a major constituent of the HDL particle. Consistent with the findings for HDL-cholesterol concentrations, interactions were observed between PUFA intake and each TNF-α genotype (PUFA × TNF-α −238G→A, $P = 0.02$; and PUFA × −308G→A, $P = 0.001$) as well as the combined TNF-α genotypes ($P = 0.005$) on circulating

TABLE 3
Association between dietary polyunsaturated fatty acid (PUFA) intake (% of energy) and HDL-cholesterol concentrations (mmol/L) or apolipoprotein (apo) A-I (g/L) for each combined tumor necrosis factor-α (TNF-α) genotype[1]

Combined genotypes	−238	−308	*n*	HDL cholesterol[2]	P^3	P^4	R^5	Apo A-I[2]	P^3	P^4	R^5
0/0	*GG*	*GG*	49	−0.000 ± 0.017	0.98	0.0003	0.53	0.012 ± 0.015	0.46	0.002	0.53
0/1	*GG*	*GA*	29	−0.079 ± 0.021	0.0003			−0.062 ± 0.019	0.002		
	GG	*AA*	4								
1/0	*GA*	*GG*	17	0.064 ± 0.028	0.026			0.046 ± 0.026	0.087		
	AA	*GG*	3								

[1] The dependent variables are either HDL cholesterol (mmol/L) or apo A-I (g/L). The fourth combined genotype with 2 minor *A* alleles (1/1) are excluded because of the small sample size (*GA+GA*, $n = 6$; *AA+AA*, $n = 1$).
[2] Values are β regression coefficients ± SE and were obtained by using a general linear model adjusted for sex and BMI.
[3] *P* test for the relation between PUFA intake and HDL-cholesterol or apo A-I concentrations among each combined genotype.
[4] *P* test for the interaction between PUFA intake and the combined genotypes on HDL-cholesterol or apo A-I concentrations.
[5] Multiple correlation coefficient ($P < 0.0001$ for HDL cholesterol and apo A-I).

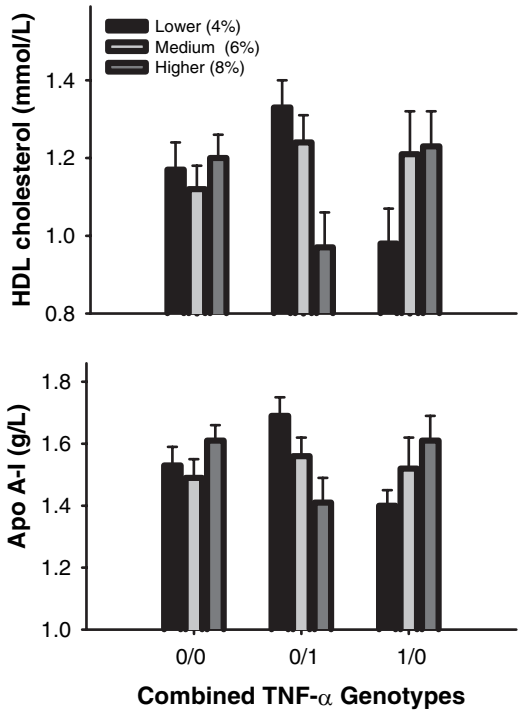

FIGURE 1. Combined genotypes of tumor necrosis factor-α (TNF-α) $-238G\rightarrow A$ and $-308G\rightarrow A$ modify the effect of polyunsaturated fatty acids (tertiles of intake − median) on circulating HDL-cholesterol concentrations ($P = 0.006$ for interaction) and apolipoprotein (apo) A-I ($P = 0.04$ for interaction). Values are means ± SEMs. Interactions were tested by using a 3×3 ANOVA adjusted for sex and BMI ($n = 109$). The fourth combined genotype with the 2 minor alleles (1/1) is not shown because of the small sample size ($n = 7$).

apo A-I concentrations. The interaction was also significant when PUFA was grouped into tertiles of intake ($P = 0.02$). Interactions remained significant after adjustment for sex and BMI (Tables 2 and 3 and Figure 1).

DISCUSSION

Our aim was to determine whether TNF-α genotypes modify the association between dietary PUFA intake and circulating lipids among individuals with type 2 diabetes. Our results showed that an increased PUFA intake is associated with higher concentrations of HDL cholesterol and apo A-I in subjects with TNF-α genotypes that are associated with lower TNF-α production. Both n−3 and n−6 PUFAs have been shown to have variable effects on HDL concentrations in humans (1, 29, 30), and our results suggest that genetic factors affecting TNF-α production may explain some of these inconsistencies. We did not observe any other significant interactions between TNF-α and other fatty acids on circulating lipids. Although the dietary analysis software used in the present study did not distinguish the n−6 from the n−3 fatty acids, we did not expect high intakes of n−3 fatty acids in our study population because these fatty acids are not abundant in the typical Canadian diet (31) and the participants were not residing in coastal cities. Thus, the effects we observed might have been due primarily to the n−6 PUFAs.

High concentrations of TNF-α in humans have been associated with low concentrations of circulating HDL cholesterol (5, 13). Several studies have shown that TNF-α can independently affect HDL by increasing endothelial lipase (32), decreasing lecithin:cholesterol acyltransferase production and activity (18), and decreasing ATP-binding cassette (ABCA1 and ABCG1) (33), scavenger receptor class B type I (34), and apo A-I and apo A-IV gene expression (16). Each of these proteins tightly regulate the reverse cholesterol transport (35). Furthermore, anti-TNF-α therapy given to patients with rheumatoid arthritis causes an increase in HDL concentrations (36).

PUFA can incorporate into membrane phospholipids and activate the transcription nuclear transcription factor κB, which regulates the expression of the TNF-α gene (37, 38). However, studies relating the effect of PUFAs on TNF-α production have shown variable effects. Indeed, both n−3 and n−6 fatty acids have been shown to either increase or decrease TNF-α production in humans (6, 7, 9, 39, 40), animals (41–43), and cell culture (44–47). Wallace et al (48) showed that the apparent variable effects of PUFAs on TNF-α production could partly be explained by the activation state of the cells and their different capacities to produce prostaglandin E_2 (PGE_2) and leukotriene B_4, which are eicosanoids produced from PUFAs that modulate TNF-α production. They found that rats fed a diet supplemented with n−3 fatty acids, and similarly with n−6 fatty acids, had decreased TNF-α production in thioglycollate-induced inflammatory macrophages, but increased TNF-α production in noninflammatory resident macrophages (48). Therefore, the inflammatory state of the cells, which could be determined by genetic factors, appears to partly influence the effect of PUFAs on TNF-α production.

In the present study, increasing PUFA intake was associated with lower concentrations of HDL and apo A-I in individuals with a genotype associated with higher TNF-α production, but was associated with higher concentrations of HDL and apo A-I in those with a genotype associated with lower production of the cytokine. The TNF-α $-238G\rightarrow A$ polymorphism has been shown to decrease the rate of transcription and production of TNF-α (19), whereas the $-308G\rightarrow A$ polymorphism has been shown to increase both (20, 21). Grimble et al (6) showed that, among individuals with the greatest TNF-α production, those who were carriers of the TNF-α *−308A* allele had a significantly greater reduction in TNF-α production after fish-oil supplementation than did those with the *−308GG* genotype. It is possible that TNF-α polymorphisms cause changes in the production of TNF-α on dietary PUFA intake that subsequently modulate the inflammatory state of the cells and affect the reverse cholesterol transport.

The amount of PUFAs consumed also seems to be an important determinant of TNF-α production. Renz et al (49) found that PGE_2, which can be derived from PUFAs, stimulated the release of TNF-α from rat resident macrophages at lower PGE_2 concentrations but suppressed TNF-α release at higher concentrations. However, Trebble et al (39) found that supplementing the diet with n−3 fatty acids at different doses resulted in a U-shaped curve of TNF-α production in humans. Therefore, the concentration of the stimulus appears to determine whether TNF-α production is enhanced or repressed. In the present study, we showed that the HDL-cholesterol concentration appears to depend not only on the amount of PUFAs consumed, but also on the TNF-α genotype of the individual.

PUFAs are natural ligands for the transcription factors PPARs, which have been shown to increase HDL and apo A-I (50, 51). PPARs and TNF-α can reciprocally down-regulate each other, such that PPARs decrease TNF-α gene expression (52) and

TNF-α can also decrease the expression of PPARs (15, 53). Thus, high concentrations of PUFAs coupled with high concentrations of TNF-α may repress the expression of PPARs and thereby decrease HDL concentrations. However, higher concentrations of PUFAs, if coupled with lower concentrations of TNF-α, might preferentially activate PPARs and result in an increase in HDL concentrations. This may explain why an increase in PUFA intake would increase HDL concentrations among individuals with the TNF-α genotype associated with lower TNF-α production but decrease HDL among those with the genotype associated with higher TNF-α production.

In summary, we found that polymorphisms in the promoter region of the TNF-α gene alter the association between dietary PUFA and circulating HDL and apo A-I concentrations. Further studies are needed to confirm these results in other populations and to establish the mechanism of action and the type of PUFA (n−3 or n−6) responsible for the effects observed in the present study.

The authors' responsibilities were as follows—TMSW, J-LC, PWC, RGJ, LAL, PM, NWR, EAR, and AE-S: contributed to the study concept, design, and supervision; BF-B and AE-S: drafted the manuscript; BF-B and PNC: carried out the statistical analyses; PWC: provided technical and material support. All authors provided critical revision of the manuscript for important intellectual content. None of the authors had any personal or financial conflicts of interest.

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