The ability of fish oil to suppress tumor necrosis factor α production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor α production[1–3]

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ABSTRACT

Background: Tumor necrosis factor α (TNF-α) mediates inflammation. High TNF-α production has adverse effects during disease. Polymorphisms in the TNF-α and lymphotoxin α genes influence TNF-α production. Fish oil suppresses TNF-α production and has variable antiinflammatory effects on disease.

Objective: We examined the relation between TNF-α and lymphotoxin α genotypes and the ability of dietary fish oil to suppress TNF-α production by peripheral blood mononuclear cells (PBMCs) in healthy men.

Design: Polymorphisms in the TNF-α (*TNF*1* and *TNF*2*) and lymphotoxin α (*TNFB*1* and *TNFB*2*) genes were determined in 111 healthy young men. TNF-α production by endotoxin-stimulated PBMCs was measured before and 12 wk after dietary supplementation with fish oil (6 g/d).

Results: Homozygosity for *TNFB*2* was 2.5 times more frequent in the highest than in the lowest tertile of inherent TNF-α production. The percentage of subjects in whom fish oil suppressed TNF-α production was lowest (22%) in the lowest tertile and doubled with each ascending tertile. In the highest and lowest tertiles, mean TNF-α production decreased by 43% ($P < 0.05$) and increased by 160% ($P < 0.05$), respectively. In the lowest tertile of TNF-α production, only *TNFB*1/TNFB*2* heterozygous subjects were responsive to the suppressive effect of fish oil. In the middle tertile, this genotype was 6 times more frequent than the other lymphotoxin α genotypes among responsive individuals. In the highest tertile, responsiveness to fish oil appeared unrelated to lymphotoxin α genotype.

Conclusion: The ability of fish oil to decrease TNF-α production is influenced by inherent TNF-α production and by polymorphisms in the TNF-α and lymphotoxin α genes. *Am J Clin Nutr* 2002;76:454–9.

KEY WORDS TNF-α production, lymphotoxin α, genotype, fish oil, inflammation, healthy men, peripheral blood mononuclear cells, *TNF*1*, *TNF*2*, *TNFB*1*, *TNFB*2*

INTRODUCTION

Tumor necrosis factor α (TNF-α) is one of a group of proinflammatory cytokines that appears rapidly after infection and injury (1, 2). TNF-α has widespread effects. It causes loss of lean and adipose tissue, raises body temperature, reduces appetite, and stimulates production of a diverse range of immunomodulatory cytokines and oxidant molecules (3). These effects create a hostile environment for invading pathogens, provide substrate for the immune system from endogenous sources, and enhance and modify the activity of the immune system. Thus, TNF-α has a pivotal role in withstanding pathogenic invasion. However, excessive or untimely TNF-α production plays a major part in mortality and morbidity from sepsis (4), meningitis (5), and malaria (6). TNF-α also plays an important part in the pathology of inflammatory diseases such as rheumatoid arthritis (7) and inflammatory bowel disease (8), in the development of atherosclerotic plaques (9), and in the rejection of transplanted tissues (10).

TNF-α production by peripheral blood mononuclear cells (PBMCs) is remarkably constant in healthy men and postmenopausal women, each of whom has a characteristic level of production of the cytokine; however, TNF-α production varies widely between healthy persons (11). This variation results because polymorphisms in the promoter regions of the TNF-α and lymphotoxin α (also known as TNF-β) genes influence the amount of TNF-α produced after an inflammatory stimulus (12) and appear to have clinical significance. For example, persons homozygous for the TNF-α −308 (*TNF*2*) allele had 7 times the rate of malaria-related mortality and serious neurologic symptoms of persons heterozygous or homozygous for the more common *TNF*1* allele (13). Surgical patients developing sepsis who were homozygous for the lymphotoxin α +252 (*TNFB*2*) allele had 3.5

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times the mortality rates of those homozygous for the *TNFB*1* allele and 2.4 times the mortality rates of those heterozygous for the *TNFB*1* allele (14).

Fish oil, which is rich in n−3 polyunsaturated fatty acids (PUFAs), has been shown to exert an antiinflammatory influence in many animal models of inflammation (15, 16) and produces antiinflammatory effects in rheumatoid arthritis (17), Crohn disease (18), and psoriasis (19). One of the reported antiinflammatory actions of fish oil is a reduction in the production of TNF-α by PBMCs (20). However, in studies in which fish oil has been investigated as an antiinflammatory agent, it has not been found to be effective in all individuals. For example, of 11 studies that investigated the effect of fish oil on TNF-α production by PBMCs from healthy subjects (20–30), only 6 report a suppressive effect (20, 24, 26, 27, 29, 30). All 11 studies reported large SDs in TNF-α production, which suggests a mixture of genotypes in the study population and might result in a high level of intraindividual variation in the response to fish oil. It is unknown whether polymorphisms in TNF-α and lymphotoxin α genes influence the ability of fish oil to suppress TNF-α production. An understanding of this interaction may explain inconsistencies in the literature and may permit more specific targeting of fish oil treatment for inflammatory disease. We hypothesized that persons with different genotypes that control TNF-α production would have different sensitivities to the antiinflammatory effects of fish oil. Furthermore, we hypothesized that those persons with an inherently high TNF-α production would be more sensitive to the effects of fish oil than would persons with a low inherent TNF-α production. Therefore, we measured ex vivo TNF-α production by endotoxin-stimulated PBMCs in healthy men before and after 3 mo of fish oil supplementation and related TNF-α production to polymorphisms in the TNF-α and lymphotoxin α genes of the subjects.

SUBJECTS AND METHODS

Subjects and study design

Healthy men (n = 111) with a mean (± SD) age of 28 ± 8 y (range: 20–57 y), a body weight of 77 ± 11 kg (range: 50–103 kg), and a body mass index (in kg/m^2) of 24 ± 4 (range: 18–34) were recruited from the Southampton area of the United Kingdom. Smokers and persons with inflammatory disease or taking antiinflammatory drugs were excluded from the study. Subjects maintained their usual lifestyles and diets but consumed 6 g encapsulated fish oil/d, which provided 1.8 g n−3 PUFAs/d (MaxEPA; 7 Seas Ltd, Hull, United Kingdom), for 12 wk. Before providing blood samples, subjects fasted overnight for ≥12 h. Three separate blood samples were collected sequentially at the start and at the end of fish oil supplementation. First, 20-mL blood samples were collected into evacuated tubes containing lithium heparin; these samples were used to prepare PBMCs. Next, 5-mL blood samples were collected into coagulant-free evacuated tubes; these samples were used to prepare serum for the measurement of C-reactive protein (CRP) concentrations. Serum CRP concentrations were measured to detect the presence of infection or inflammation in the subjects at the time of blood sampling. Subjects with CRP concentrations >100 mg/L in the blood sample taken before or after fish oil supplementation were excluded from the study. Finally, 5-mL blood samples were collected into evacuated tubes containing EDTA; these samples were used to prepare DNA for genotype analysis. The study was approved by the Southampton and South West Hampshire Joint Ethics Committee.

TNF-α induction and measurement

PBMCs were isolated by centrifuging heparin-treated blood on Histopaque-1077 (Sigma Chemical Co, Poole, United Kingdom) (28) and were resuspended in RMPI culture medium containing 2 mmol glutamine/L and 50 mL autologous plasma/L. PBMCs (2×10^6) were cultured in 24-well tissue culture plates at a final concentration of 15 mg *Escherichia coli* 0111:B4 endotoxin/L (Sigma Chemical Co) in a final culture volume of 2 mL. After 24 h at 37 °C in an atmosphere of 5% CO_2 and 95% air, the culture plates were centrifuged and the supernatant fluid was frozen at −80 °C until analyzed. TNF-α concentrations were measured with enzyme-linked immunosorbent assay kits (EASIA; Biosource International, Nivelles, Belgium). The inter- and intraassay CVs were <10% and the limit of detection was 3 ng/L.

Genotypes for TNF-α and lymphotoxin α alleles

For each subject, an aliquot of blood that had been collected into an EDTA-containing tube was analyzed to determine single-nucleotide polymorphisms (SNPs) for the TNF-α −308 (*TNF*1* and *TNF*2*) and lymphotoxin α +252 (*TNFB*1* and *TNFB*2*) genotypes. These SNPs were selected because of their documented but variable association with TNF-α production (31, 32). Genomic DNA was extracted by a salting out procedure (33). Each SNP was detected by using a 2-reaction amplification refractory mutation system polymerase chain reaction (PCR) approach based on previously published methods (34). With this approach, 2 separate PCRs are performed per SNP. Each PCR mix also contained an additional pair of PCR primers, amplifying a sequence from the third intron of the human leukocyte antigen *DRB*1* gene to act as an internal control for successful PCRs. All PCRs were performed in 10-μL reaction volumes, and the final reagent concentrations were as follows: 1 × ABgene standard reaction buffer (Epsom, United Kingdom), 200 μmol deoxynucleotide triphosphate/L, 120 g sucrose/L, 200 μmol cresol red/L, 1 μmol/L of each specific or common primer, 0.2 μmol/L of each internal control primer, 0.25 U Thermoprime^{PLUS} DNA polymerase (ABgene), 1.75 mmol $MgCl_2$/L, and 25–100 ng DNA. PCR primer sequences and product sizes for each SNP amplicon are given in **Table 1**. PCRs were performed with the use of a Primus 96 Plus thermal cycler (MWG Biotech, Herts, United Kingdom) according to the following cycling conditions: 96 °C for 60 s followed by 10 cycles of 96 °C for 15 s, 65 °C for 50 s, and 72 °C for 40 s and then 20 cycles of 96 °C for 190 s, 60 °C for 50 s, and 72 °C for 40 s. PCR products were loaded directly onto 2% agarose gels containing 0.5 g ethidium bromide/L, electrophoresed, and visualized by photography under ultraviolet transillumination.

Plasma phospholipid fatty acid composition

Compliance with the dietary fish oil treatment was assessed by determining the fatty acid composition of plasma phospholipids. Total lipid was extracted from plasma with chloroform:methanol (2:1, vol:vol), and phospholipids were isolated by thin-layer chromatography with the use of a mixture of hexane:diethyl ether:acetic acid (90:30:1, vol:vol:vol) as the elution phase. Fatty acid methyl esters were prepared by incubation with 140 g boron trifluoride/L in methanol at 80 °C for 60 min. Fatty acid methyl esters were isolated by solvent extraction, dried, and separated by gas chromatography in a model 6890 gas chromatograph (Hewlett-Packard, Avondale, PA) fitted with a 30 m × 0.32 mm BPX70 capillary column with a film thickness of 0.25 μm. Helium at 1.0 mL/min was used as the carrier gas and the split-splitless

TABLE 1
Details of the polymerase-chain-reaction primers used[1]

SNP	Primer name	Primer sequence (5′ to 3′)	Product size (base pairs)
TNF-α −308	TNF308 common	TCT CGG TTT CTT CTC CAT CG	184
	TNF308G	ATA GGT TTT GAG GGG CAT GG	
	TNF308A	ATA GGT TTT GAG GGG CAT GA	
LT-α +252	LT252 common	AGA TCG ACA GAG AAG GGG ACA	94
	LT252G	CAT TCT CTG TTT CTG CCA TGG	
	LT252A	CAT TCT CTG TTT CTG CCA TGA	
Control primers	63	TGC CAA GTG GAG CAC CCA A	796
	64	GCA TCT TGC TCT GTG CAG AT	

[1] SNP, single-nucleotide primers; TNF, tumor necrosis factor; LT, lymphotoxin.

injector was used with a split-splitless ratio of 20:1. Injector and detector temperatures were 275 °C. The oven temperature of the column was maintained at 170 °C for 12 min after sample injection and was programmed to then increase from 170 to 210 °C at 5 °C/min before being maintained at 210 °C for 15 min. The separation was recorded with the use of HP GC CHEM STATION software (Hewlett-Packard). Fatty acid methyl esters were identified by comparison with standards run previously.

Statistical analysis

Unless otherwise indicated, values are expressed as means ± SDs. Differences in the distribution of the TNF-α and lymphotoxin α genotypes among the tertiles of TNF-α production before fish oil supplementation were examined by using the chi-square test. Differences in TNF-α production and in the proportions of various fatty acids in plasma phospholipids before and after fish oil supplementation were determined by using Student's paired *t* test. Differences in TNF-α production between subjects with different genotypes, either before or after fish oil supplementation, were determined by one-factor analysis of variance (ANOVA). The influence of genotype, of tertile of presupplementation TNF-α production, and of their interaction on the effect of fish oil on TNF-α production were determined by two-factor ANOVA. In all cases, the level of significance was set at 0.05, and Bonferonni's correction for multiple comparisons was used. All statistical comparisons were made by using SPSS version 10 (SPSS Inc, Chicago).

RESULTS

Plasma phospholipid fatty acid composition

The proportions of eicosapentaenoic and docosahexaenoic acids in plasma phospholipids increased in all subjects after fish oil supplementation, with mean increases of 370% and 94%, respectively, at the end of the supplementation period (**Table 2**).

TABLE 2
Influence of fish oil supplementation on the proportions of eicosapentaenoic, docosahexaenoic, and arachidonic acids in plasma phospholipids[1]

	Before supplementation	After supplementation
	% by wt of total fatty acids	
Eicosapentaenoic acid	0.72 ± 0.09	3.34 ± 0.29[2]
Docosahexaenoic acid	1.97 ± 0.27	3.68 ± 0.27[2]
Arachidonic acid	7.66 ± 0.69	5.23 ± 0.79[2]

[1] $\bar{x}$ ± SD; n = 111.
[2] Significantly different from presupplementation value, $P < 0.001$ (Student's paired *t* test).

An increase in fish oil–derived n−3 PUFAs was accompanied by a significant decrease in the proportion of arachidonic acid in plasma phospholipids.

Distribution of genotypes among the study population and the relation with TNF-α production

The distribution of the *TNF*1* and *TNF*2* alleles and of the *TNFB*1* and *TNFB*2* alleles among the subjects in this study is shown in **Table 3**. The percentages of subjects with the *TNF*1/TNF*1*, *TNF*1/TNF*2*, and *TNF*2/TNF*2* genotypes were ≈68%, ≈30%, and ≈2%, respectively. The percentages of subjects with the *TNFB*1/TNFB*1*, *TNFB*1/TNFB*2*, and *TNFB*2/TNFB*2* genotypes were ≈19%, ≈53%, and ≈28%, respectively. TNF-α genotype appeared to be unrelated to TNF-α production because the distribution of the *TNF*1* and *TNF*2* alleles was almost identical for the subjects in all tertiles of presupplementation TNF-α production. In contrast, the distribution of the *TNFB*1* and *TNFB*2* alleles differed among the tertiles of presupplementation TNF-α production. The distribution of lymphotoxin α genotypes among subjects in the highest tertile of presupplementation TNF-α production was significantly different from the distribution in the lowest tertile. The frequency of the *TNFB*2/TNFB*2* genotype was related positively to TNF-α production, increasing from 16% in the lowest tertile to 41% in the highest tertile, whereas the frequency of the *TNFB*1/TNFB*2* genotype decreased as inherent TNF-α production increased.

Influence of fish oil on TNF-α production

TNF-α production before and after fish oil supplementation is shown in **Table 4**. The range in TNF-α production by PBMCs

TABLE 3
Distribution of tumor necrosis factor α (TNF-α) and lymphotoxin α (LT-α) genotypes in the study population related to TNF-α production by peripheral blood mononuclear cells stimulated with endotoxin before fish oil supplementation

	TNF-α genotype			LT-α genotype		
	1/1	*1/2*	*2/2*	*B1/B1*	*B1/B2*	*B2/B2*
All subjects (*n*)	76	33	2	21	59	31
Tertile of inherent TNF-α production (*n*)						
Lowest	25	11	1	8	23	6
Middle	25	11	1	8	19	10
Highest	26	11	0	5	17	15[1]

[1] Significantly different from the lowest tertile of TNF-α production, $P < 0.001$ (chi-square test).

TABLE 4
Influence of fish oil supplementation on tumor necrosis factor α (TNF-α) production by peripheral blood mononuclear cells stimulated with endotoxin, related to presupplementation values[1]

	TNF-α production	
	Before supplementation	After supplementation
	ng/L	
All subjects (n = 111)	4821 ± 4177	4643 ± 3338
Tertile of inherent TNF-α production		
Lowest (n = 37)	1458 ± 600	3809 ± 2571[2]
Middle (n = 37)	3728 ± 936	4796 ± 3270
Highest (n = 37)	9277 ± 4338	5323 ± 3941[2]

[1] $\bar{x}$ ± SD.
[2] Significantly different from presupplementation, $P < 0.05$ (Student's paired t test).

before dietary supplementation was large. When data from all subjects were aggregated, no significant effect of fish oil supplementation was found. However, sensitivity to fish oil was influenced by presupplementation TNF-α production. Thus, TNF-α production decreased after fish oil supplementation in 22%, 43%, and 86% of the subjects in the lowest, middle, and highest presupplementation tertiles, respectively. In the highest tertile, mean production decreased significantly by 43%. TNF-α production did not decrease significantly in the middle and lowest tertiles. Indeed, paradoxically, TNF-α production increased by 160% after fish oil supplementation in subjects in the lowest tertile of presupplementation TNF-α production.

Influence of TNF-α and lymphotoxin α genotypes on the response to fish oil

The suppressive effect of fish oil among the high TNF-α producers occurred irrespective of TNF-α or lymphotoxin α genotype (**Table 5**). However, there was a significant interaction between TNF-α genotype and inherent TNF-α production in determining the extent of the decrease in TNF-α production that followed fish oil supplementation (P for interaction = 0.035, two-factor ANOVA). Further analysis showed that the decrease in TNF-α production among individuals in the highest tertile of presupplementation TNF-α production was significantly greater (P = 0.02) if they had the *TNF*1/TNF*2* genotype than if they had the *TNF*1/TNF*1* genotype. The interaction between lymphotoxin α genotype and inherent TNF-α production in determining the extent of the decline in TNF-α production that followed fish oil supplementation was not significant (P for interaction = 0.062, two-factor ANOVA). Fish oil suppressed the production of TNF-α by cells from some subjects in the low and middle tertiles of inherent TNF-α production. The *TNFB*1/TNFB*2* genotype appeared to be important in determining the sensitivity to fish oil among these subjects. Thus, all 8 subjects in the lowest tertile of inherent TNF-α production who responded to fish oil with a reduction in TNF-α production had the *TNFB*1/TNFB*2* genotype. In the middle tertile of inherent TNF-α production, 12 of 16 subjects who responded in this way had the *TNFB*1/TNFB*2* genotype. In the highest tertile of inherent TNF-α production, the *TNFB*1/TNFB*2* genotype only characterized one-half of the subjects (16 of 32) who responded to fish oil with a reduction in TNF-α production.

DISCUSSION

Our data suggest that the sensitivity of a person to the suppressive effects of n−3 PUFAs on TNF-α production is linked to the inherent level of production of the cytokine by cells from the person before supplementation and to genetic variation encoded by, or associated with, the TNF-α −308 and lymphotoxin α +252 SNPs. Paradoxically, fish oil appears to enhance TNF-α production in some subjects, particularly those in the lowest tertile of presupplementation production. The ability of fish oil to enhance rather than to reduce TNF-α production was not unexpected. During inflammation, phospholipase A_2 (EC 3.1.1.4) hydrolyzes membrane phospholipids, thus making arachidonic acid available for the production of the proinflammatory eicosanoids prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4). In vitro studies have shown that PGE_2 and LTB_4 have opposing effects on proinflammatory cytokine production, the former having an inhibitory and the latter a stimulatory influence (20, 35). Fish oil may alter proinflammatory cytokine production in either direction by the n−3 PUFAs

TABLE 5
Tumor necrosis factor α (TNF-α) production by peripheral blood mononuclear cells stimulated with endotoxin in relation to TNF-α and lymphotoxin α (LT-α) genotypes in the study population before fish oil (FO) supplementation and the change in production over the FO intervention[1]

Tertile of inherent TNF-α production	TNF-α genotype		LT-α genotype		
	1/1 (n = 76)	*1/2* (n = 33)	*B*1/B*1* (n = 21)	*B*1/B*2* (n = 59)	*B*2/B*2* (n = 31)
	ng/L			*ng/L*	
Lowest					
Before FO	1479 ± 602	1294 ± 713	1132 ± 556	1562 ± 592	1187 ± 757
Change	2483 ± 2543[a]	2365 ± 3040[a]	2704 ± 1345	2088 ± 2972	3442 ± 2602
Middle					
Before FO	3655 ± 962	3883 ± 953	3910 ± 1066	3544 ± 964	3884 ± 790
Change	658 ± 3066[b]	1238 ± 3365[a]	2040 ± 2558	203 ± 3398	1049 ± 2680
Highest					
Before FO	8653 ± 3126	10748 ± 6127	11570 ± 4391	7553 ± 1791	10464 ± 5507
Change	−2923 ± 4429[c]	−6388 ± 8297[b,2]	−7161 ± 7426	−3475 ± 2954	−3246 ± 7800

[1] $\bar{x}$ ± SD. Data for genotype *TNF*2/TNF*2* were excluded because only 2 subjects had this genotype. Means within a column with different superscript letters are significantly different, $P < 0.001$ (one-factor ANOVA). The interaction between TNF-α genotype and change was significant, P = 0.035 (two-factor ANOVA). The interaction between LT-α genotype and change was not significant, P = 0.062 (two-factor ANOVA).
[2] Significantly different from *1/1*, P = 0.02 (one-factor ANOVA).

that it contains, replacing arachidonic acid in the cell membrane. Such an effect would decrease PGE_2 and LTB_4 production and increase the formation of PGE_3 and LTB_5. These eicosanoids have lower bioactivities than do PGE_2 and LTB_4. Thus, the overall effect on TNF-α production (inhibition or stimulation) will depend on the balance among the different stimulatory and inhibitory eicosanoids produced from arachidonic and eicosapentaenoic acids.

As outlined previously, genetic factors influence TNF-α production in an important way. The frequencies of the *TNF*1*, *TNF*2*, *TNFB*1*, and *TNFB*2* alleles in the present study agree closely with published values from studies of healthy British and other European subjects (36–38) and with values derived from independent studies in our laboratory (39). Thus, the group of subjects in the present study represent the population from which it was drawn, at least with respect to the frequencies of the TNF-α and lymphotoxin α genotypes examined. The observed positive association between *TNFB*2* homozygosity and inherent TNF-α production confirms the findings of Stuber et al (14) and Pociot et al (40). However, we did not confirm an association between TNF-α −308 genotype and TNF-α production. When the genetic characteristics of individuals in the 3 tertiles of inherent TNF-α production were examined in relation to the ability of fish oil to reduce TNF-α production, a complex interaction was apparent. The results of the present investigation suggest that *1*) most (in this case 86%) individuals with a high inherent level of TNF-α production are sensitive to the antiinflammatory effects of fish oil, *2*) medium and high inherent TNF-α production is associated with homozygosity for the *TNFB*2* allele, and *3*) individuals with medium or low levels of production are more likely to experience the antiinflammatory effects of fish oil if they are heterozygous for the *TNFB* alleles. Paradoxically, however, subjects with a *TNFB*2/TNFB*2* genotype are less likely to exhibit this phenomenon, independent of their level of inherent TNF-α production.

The present study is the largest investigation into the effects of dietary fish oil supplementation on ex vivo TNF-α production by human PBMCs currently reported in the scientific literature (41). The data from the present study, when aggregated without consideration of each subject's inherent ex vivo TNF-α production or TNF-α or lymphotoxin α genotype, agree with data from other studies that suggest that fish oil does not exert a modulatory effect on such production (21–23, 25, 28). A wide range of doses of fish oil have been used in similar studies (0.55–6.00 g n−3 PUFAs/d). Suppressive effects of fish oil on TNF-α production have generally been shown in studies that used doses of n−3 PUFAs that were greater than those used in the present study (20, 29, 30). This is not, however, universally the case because some studies that used higher doses showed no effect on TNF-α production (23, 25, 28). Of the 5 studies that used doses similar to or lower than those used in the present study (21–25), only 1 (24) showed an inhibitory effect of fish oil on TNF-α production. However, in this latter study, fish oil was given to subjects consuming a low-fat diet. In this dietary situation, competition between n−6 PUFAs from the diet and n−3 PUFAs from the supplement, for incorporation into the cell structure, would have been less than in the present study.

The results of the present study, together with the results of other studies of the effects of fish oil supplements on TNF-α production, indicate that the interaction of n−3 PUFA intake and cytokine biology is complex. Although the dose of fish oil that is given may be a determinant of whether a suppressive effect of the oil on TNF-α production can be shown at a whole-population level, our data suggest that the different sensitivities of persons to the effect of fish oil—because of the genetic variation encoded by or associated with the TNF-α −308 and lymphotoxin α +252 SNP genotypes and other individual factors that influence the inherent level of cytokine production by the subject—may limit the effectiveness of moderate doses of fish oil as an antiinflammatory agent. In the absence of a clear model to explain our results, we need to stress that the genetic influences we observed may not have been due to the SNPs we examined. It is clear that further analysis of genotype will be required if the relation between the effect of gene polymorphisms on TNF-α production and a person's sensitivity to fish oil is to be fully understood. A greater understanding of the precise nature of the genomic determinants of the ability of fish oil to act as an antiinflammatory agent will enable supplementation with this foodstuff to be used more effectively in suppressing inflammation than is presently the case.

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