Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia

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Abstract **Consumption of diets high in hydrogenated fat/*trans* fatty acids has been shown to have an adverse affect on lipoprotein profiles with respect to cardiovascular disease risk. Dietary fat and cholesterol play an important role in the regulation of immune and inflammatory responses shown to be involved in atherogenesis. We investigated the effects of diets containing hydrogenated fat on cellular immune response and production of inflammatory cytokines in human subjects with moderately elevated cholesterol levels (LDL cholesterol >130 mg/dl). In a double blind crossover study, 19 subjects consumed three diets, 30% of calories as fat, of which two thirds were provided as soybean oil, soybean oil-based stick margarine, or butter for 32 days, each in a randomized order. Production of proinflammatory mediators, prostaglandin (PG)E_2, interleukin (IL)-1β, IL-6, and tumor necrosis factor α (TNF-α); delayed type hypersensitivity (DTH) response, in vitro lymphocyte proliferation, and production of IL-2 were determined. Production of IL-6 and TNF-α was significantly higher after consumption of stick margarine diet compared with soybean oil diet. IL-1β and TNF-α production correlated positively with ratios of total cholesterol to HDL cholesterol ($r = 0.499$, $P < 0.001$ and $r = 0.291$, $P = 0.04$, respectively). There was no significant difference in DTH response, lymphocyte proliferation, or levels of IL-2 and PGE_2 produced among three groups. Our results indicate that consumption of a diet high in hydrogenated fat does not adversely affect cellular immunity but increases production of inflammatory cytokines that have been associated with the pathophysiology of atherosclerosis.**—Han, S. N., L. S. Leka, A. H. Lichtenstein, L. M. Ausman, E. J. Schaefer, and S. N. Meydani. **Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. *J. Lipid Res.* 2002.** 43: **445–452.**

Supplementary key words dietary fat • hydrogenated fat • immune response • inflammatory response • human

Evidence from epidemiological and intervention studies has strongly suggested that high intake of hydrogenated fat/*trans* fatty acids could increase the risk of developing heart disease, most likely by adversely altering the lipoprotein profile (1, 2). *Trans* isomers of fatty acids are formed during the hydrogenation process of liquid vegetable oils. Since the straighter configuration of *trans* relative to *cis* isomers resembles that of saturated fat, and *trans* isomers are derived from and replace *cis*, *cis*-linoleic acid, a precursor of prostaglandins, they could affect eicosanoid production, membrane physiology, and immune and inflammatory responses.

Dietary fat plays an essential role in modulating immune and inflammatory responses, with both quantity and quality of fats having been shown to affect these processes. A reduction in total dietary fat intake from 41% to 26% of total energy in the study by Kelley et al. (3) and from 35% to 26% of total energy in the study by Meydani et al. (4) resulted in enhanced proliferative response to T-cell mitogens, indicating that high-fat diets suppress human lymphocyte proliferation. Type and degree of unsaturation of fatty acids has also been shown to affect immune and inflammatory responses (5). The immuno-modulatory effect of dietary fatty acid is in part mediated by alteration of prostaglandin synthesis. In addition, alterations in cholesterol levels or metabolism by dietary fat can result in changes in immune and inflammatory responses. Nutritionally induced hypercholesterolemia was associated with a decreased antibody response to sheep erythrocytes and inhibition of host resistance against *Listeria monocytogenes* (6), and increased suscepti-

Abbreviations: ConA, concanavalin A; Cox, cyclooxygenase; DTH, delayed type hypersensitivity; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; PHA, phytohemagglutinin; PBMC, peripheral blood mononuclear cell; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor α.

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bility to coxsackievirus B (7). Genetically induced hypercholesterolemia significantly suppressed cellular immunity in mice infected with lymphocytic choriomeningitis virus (8).

Atherosclerosis is a chronic inflammatory disease characterized by cholesterol deposition, recruitment and activation of monocytes, macrophages, and lymphocytes, inflammation, and fibrosis (9). Interplay between lipid and cholesterol metabolism, cytokines, and cellular activity within arterial walls plays a crucial part in atherosclerosis. Interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor α (TNF-α) have been reported to affect lipid metabolism and to be involved in the development of atherosclerotic lesions (10–12). Administration of TNF-α and IL-1β to Syrian hamsters was shown to increase serum cholesterol and decrease HDL cholesterol levels (13). Increased expression of IL-1β and IL-6 in atherosclerotic lesions has been reported (10, 14).

Although several studies have indicated an important role for immune and inflammatory responses in the pathogenesis of atherosclerosis, and both *trans* and saturated fatty acids have been associated with increased risk of developing atherosclerosis, few studies have investigated the effects of *trans* fatty acids on immune and inflammatory responses. The purpose of the present study was to determine whether consumption of diets containing hydrogenated or saturated fat affects production of inflammatory cytokines and the cellular immune responses in human subjects with moderately elevated cholesterol levels.

MATERIALS AND METHODS

Subjects

Eleven women and eight men over age 50 with moderately elevated LDL cholesterol levels (>130 mg/dl or 3.36 mmol/l) were included in the study. None of the subjects had evidence of any chronic illness, including endocrine, hepatic, renal, thyroid, or cardiac dysfunction. All subjects had normal serum glucose levels, were non-smokers, and did not take nonsteroidal anti-inflammatory drugs such as aspirin, or medications known to affect serum lipid levels. The characteristics of the subjects at the time of screening are shown in **Table 1**. The study protocol was approved by the Human Investigation Review Committee (HIRC) of the New England Medical Center and Tufts University, and all subjects signed a written HIRC-approved informed consent form.

TABLE 1. Characteristics of the subjects at the time of screening

Characteristics	Women (n = 11)	Men (n = 8)	All subjects (n = 19)
Age (years)	67.5 ± 1.4	60.8 ± 1.8	64.7 ± 1.3
Body mass index (kg/m^2)	28.1 ± 0.8	29.6 ± 1.0	28.8 ± 0.6
Total serum cholesterol (mg/dl)	251 ± 10	255 + 13	253 + 8
Serum VLDL cholesterol (mg/dl)	31 ± 3	31 ± 4	31 ± 3
Serum LDL cholesterol (mg/dl)	164 ± 9	180 ± 11	171 ± 7
Serum HDL cholesterol (mg/dl)	56 ± 3	44 ± 3	51 ± 3
Serum triglyceride (mg/dl)	153 ± 17	156 ± 21	154 ± 13

Values are means ± SE.

Study design and diets

The study was composed of three 32-day diet phases with a minimum interval of 2 weeks between phases, during which the subjects consumed either their habitual diets ad libitum or an alternate experimental diet. Soybean oil, soybean oil-based margarine, or butter diet was consumed in randomized order. Previous work has indicated that under the specified study conditions, plasma lipid levels at the end of each 5-week period were independent of diet order or intervening phases (15). Additional diets were included in the randomized scheme but were not included in this analysis (2). The subjects were encouraged to maintain their habitual level of physical activity throughout the study period and body weight was kept consistent throughout the study.

Each diet was designed to provide 30% of calories as fat. The foods included in each of the three diets were identical, the only difference being that two thirds of the fat was provided in the form of soybean oil, soybean oil-based margarine, or butter. The soybean oil diet was formulated to meet National Cholesterol Education Program Step 2 criteria. These criteria were achieved by first designing a diet containing 10% of calories as fat and then adding the experimental oils to various foods to increase the fat content of the diet to 30% of calories. All foods and drinks were provided by the Metabolic Research Unit of the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University to be consumed on site or packaged for take-out. Initial caloric levels were estimated with use of the Harris-Benedict formula and adjusted, when necessary, to maintain body weight. The mean (±SE) energy intake was 2,127 ± 73 kcal for women and 2,844 ± 212 kcal for men. Analysis of the protein, carbohydrate, fat, and cholesterol content of the diets were carried out by Covance Laboratories (Madison, WI) and fatty acids by Lipton (Baltimore, MD) (**Table 2**).

Serum lipoprotein cholesterol levels

VLDL was isolated from serum by ultracentrifugation at 109,000 × *g* at 4°C (16). Serum and the infranatant were assayed for total cholesterol and triglyceride using a biochromatic analyzer (model CCX, Spectrum, Incastar, Stillwater, MN) with enzymatic reagents (17). Serum HDL cholesterol was measured in the supernatant fraction after precipitation of lipoproteins containing

TABLE 2. Composition of experimental diets

Constituent	Diet: Butter	Diet: Stick Margarine	Diet: Soybean Oil
		% *energy*	
Protein	16.9	16.7	15.7
Carbohydrate	54.0	53.5	55.8
Fat	29.1	29.7	28.5
SFA	16.7	8.5	7.3
12:0	2.5	0.6	0.63
14:0	0.1	<0.1	<0.1
16:0	7.5	4.0	3.7
18:0	3.6	2.2	1.5
MUFA	8.1	8.5	8.1
18:1	7.0	6.5	7.2
PUFA	2.4	6.3	12.5
18:2	2.1	5.6	10.7
18:3	0.3	0.7	1.7
trans	1.3	6.7	0.6
Cholesterol (mg/1,000 kcal)	121	67	66

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.

apolipoprotein B by dextran-magnesium sulfate (18). Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention (Atlanta, GA).

Isolation of mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by density centrifugation over Ficoll-Paque as previously described (19). Cells were counted and resuspended in RPMI 1640 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/l L-glutamine, and 24 mmol/l HEPES (Gibco Laboratories, Grand Island, NY) (complete RPMI) at appropriate concentrations for cultures to induce cytokine and prostaglandin E_2 (PGE_2) production, and lymphocyte proliferation. Plasma isolated by centrifugation was heat inactivated at 56°C for 30 min and used as autologous plasma in the cell cultures.

IL-1β, IL-6, and TNF-α production

PBMCs at 2.5×10^6 cells/ml (final concentration) in complete RPMI with 1% autologous plasma were cultured with 1 ng/ml lipopolysaccharide (LPS) (*Escherichia coli* 0111:B4, Sigma Chemical Co, St. Louis, MO) for 24 h in 24-well flat-bottom plates. Cell-free supernatants were collected and stored at −70°C for analysis. IL-1β was measured by radioimmunoassay (20). Antibody to IL-1β was purchased from Cistron Biotechnology (Pine Brook, NJ). Recombinant IL-1β was purchased from Genzyme (Cambridge, MA). [^{125}I]IL-1β was purchased from DuPont NEN (Boston, MA). IL-6 and TNF-α were measured by ELISA according to the manufacturer's instructions (PharMingen, San Diego, CA) with rat anti-human IL-6 and biotinylated rat anti-human IL-6 monoclonal antibodies (MAbs) or mouse anti-human TNF-α and biotinylated mouse anti-human TNF-α MAbs.

PGE_2 production

PBMCs at 1×10^6 cells/ml (final concentration) in complete RPMI with 9% autologous plasma were cultured with 10 or 100 μg/ml (final concentration) phytohemagglutinin (PHA) for 48 h in 24-well flat-bottom plates. Cell-free supernatants were collected and stored at −70°C for analysis. PGE_2 was measured by radioimmunoassay as described by McCosh et al. (21). The PGE_2 antibody was provided by Dr. Dupont of Florida State University, Tallahassee, FL and Dr. Mathias of the Agricultural Research Service in Washington, DC.

IL-2 production

PBMCs at 1×10^6 cells/ml (final concentration) in complete RPMI with 9% autologous plasma were cultured with 10 μg/ml (final concentration) concanavalin A (ConA) or PHA for 48 h in 24-well flat-bottom plates. Cell-free supernatants were collected and stored at −70°C for analysis. IL-2 activity was measured by bioassay as the proliferative response of the cytotoxic T lymphocytes line 2 (CTLL-2) compared with its response to recombinant human IL-2 (Genzyme, Cambridge, MA) (22).

Lymphocyte proliferation

Lymphocyte proliferation was measured by [^{3}H]thymidine incorporation after stimulation with T- and B-cell mitogens. PBMCs were incubated in 96-well flat-bottom plates at 5×10^6 cells/ml (final concentration) in complete RPMI 1640 with 5% autologous plasma and different concentrations of mitogens for 72 h at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Final mitogen concentrations were 2.5, 5, 25, 50, and 100 μg/ml for ConA (Sigma), 0.5, 5, and 50 μg/ml for PHA (Difco Laboratories, Detroit, MI), and 0.000625%, 0.0025%, and 0.0075% for *Staphylococcus epidermis*. Each well was pulsed with 0.5 μCi of [^{3}H]thymidine (New England Nuclear, Boston, MA) in 20 μl of media for the last 4 h of incubation. Cells were harvested onto glass microtiter filter paper using a cell harvester (Cambridge Technologies, Inc., Cambridge, MA), and radioactivity incorporation was counted by liquid scintillation (Beckman Instruments, Inc., Palo Alto, CA). The results are reported as corrected counts per min (ccpm) which is the average cpm of mitogen-stimulated cultures minus the average cpm of cultures without stimulation.

Delayed-type hypersensitivity skin test

Delayed-type hypersensitivity skin test was assessed with Multi Test-CMI (Merieux Institute, Inc., Miami, FL), a single-use disposable applicator of acrylic resin with eight heads each loaded with glycerine control or one of seven recall antigens: tetanus toxoid, diphtheria toxoid, *Streptococcus* (group C), *Mycobacterium tuberculosis*, *Candida albicans*, *Trichophyton metagrophytes*, or *Proteus mirabilis*. The diameter of positive reactions was measured at 24 and 48 h after administration of the test. For each time point, the antigen score was calculated as the total number of positive antigens, and the cumulative score calculated as the total diameter of induration of all positive reactions. Maximal induration was based on the largest induration at 24 or 48 h for each antigen. An induration of ⩾2 mm was considered positive. If a positive reaction to the glycerine control was observed, the diameter of its induration was subtracted from each of the other positive reactions.

Statistical analysis

Data were analyzed using the SYSTAT statistical package (SYSTAT 9.0, 1999; SYSTAT, Inc., Evanston, IL). An ANOVA with the main effect of diet and subject as the repeated measure was carried out for each outcome, followed by the Fisher's Least Significant Difference test for pairwise comparisons of the three diets. Significance was set at $P < 0.05$. Appropriate transformations were applied to measures that were not normally distributed.

RESULTS

Serum lipoprotein cholesterol levels

Levels of serum lipoprotein cholesterol and triglyceride are shown in **Table 3**. The source of dietary fats had a significant effect on levels of serum total, VLDL, LDL, and HDL cholesterol, and ratios of total cholesterol to HDL cholesterol. Levels of total, LDL, and HDL cholesterol were highest after subjects consumed the butter diet. VLDL cholesterol and the ratio of total cholesterol to

TABLE 3. Effects of different dietary fats on serum lipid profiles

	Diet		
Serum Lipid	Butter	Stick Margarine	Soybean Oil
Total cholesterol (mg/dl)	257 ± 10^a	245 ± 10^b	227 ± 8^c
VLDL cholesterol (mg/dl)	32 ± 4^b	36 ± 4^a	$32 \pm 3^{a,b}$
LDL cholesterol (mg/dl)	177 ± 9^a	167 ± 8^b	150 ± 7^c
HDL cholesterol (mg/dl)	48 ± 2^a	42 ± 2^c	45 ± 2^b
Triglyceride (mg/dl)	153 ± 15	166 ± 17	158 ± 16
Total cholesterol-HDL cholesterol	5.55 ± 0.28^b	6.00 ± 0.31^a	5.28 ± 0.31^b

Values are means ± SE, n = 19. Values not sharing the same letters are significantly different at $P < 0.05$ determined by Fisher's Least Significant Difference test.

HDL cholesterol were highest after consumption of stick margarine diet. HDL cholesterol level was the lowest after consumption of the stick margarine diet. Total and LDL cholesterol levels were the lowest after consumption of the soybean oil diet. Triglyceride levels were not affected by source of dietary fats.

IL-1β, IL-6, TNF-α, and PGE_2 production

Production of TNF-α and IL-6 by LPS-stimulated PBMCs were significantly affected by consumption of different diets, while production of IL-1β was not significantly affected (**Fig. 1**). TNF-α and IL-6 production was higher after the subjects consumed the stick margarine diet compared with soybean oil diet (58% and 36% higher for TNF-α and IL-6, respectively). The level of TNF-α produced after consumption of butter diet tended to be higher than that of soybean oil diet ($P < 0.1$), though it was not significantly different from that of stick margarine diet. The level of IL-6 produced after consumption of the butter diet was not significantly different from those of either diets. Significantly positive correlations were observed between ratios of total cholesterol to HDL cholesterol and IL-1β production ($r = 0.499$, $P < 0.001$), and between ratios of total cholesterol to HDL cholesterol and TNF-α production ($r = 0.291$, $P = 0.04$).

As shown in **Table 4**, there was no significant difference in PHA-stimulated PGE_2 production by PBMCs after consumption of butter, stick margarine, or soybean oil diets.

TABLE 4. Effect of different dietary fats on IL-2 and PGE_2 production

	Diet		
	Butter	Stick Margarine	Soybean Oil
IL-2 (U/ml)			
PHA (10 μg/ml)	29.4 ± 6.0	35.2 ± 10.9	34.5 ± 9.8
ConA (10 μg/ml)	3.2 ± 0.6	5.0 ± 1.3	4.6 ± 1.4
PGE_2 (pg/ml)			
PHA (10 μg/ml)	284 ± 57	224 ± 36	321 ± 95
PHA (100 μg/ml)	1,258 ± 360	1,130 ± 215	1,336 ± 340

Values are means ± SE, n = 17–19. PBMCs at 1×10^6/ml (final concentration) in complete RPMI with 9% autologous plasma were cultured with 10 μg/ml (final concentration) ConA or 10 or 100 μg/ml (final concentration) PHA for 48 h. An ANOVA with the main effect of diet and subjects as the repeated measure was carried out followed by the Fisher's Least Significant Difference test for the pairwise comparisons of the three diets. There was no significant difference among different diet groups.

Delayed-type hypersensitivity skin response, lymphocyte proliferation, and IL-2 production

Delayed type hypersensitivity (DTH) response was affected by dietary source of fat (**Fig. 2**). DTH response increased significantly after consumption of the soybean oil diet compared with the butter diet. Total maximal induration index was significantly higher ($P < 0.05$, about 22% higher) after consumption of soybean oil diet compared with the butter diet. DTH response after consumption of

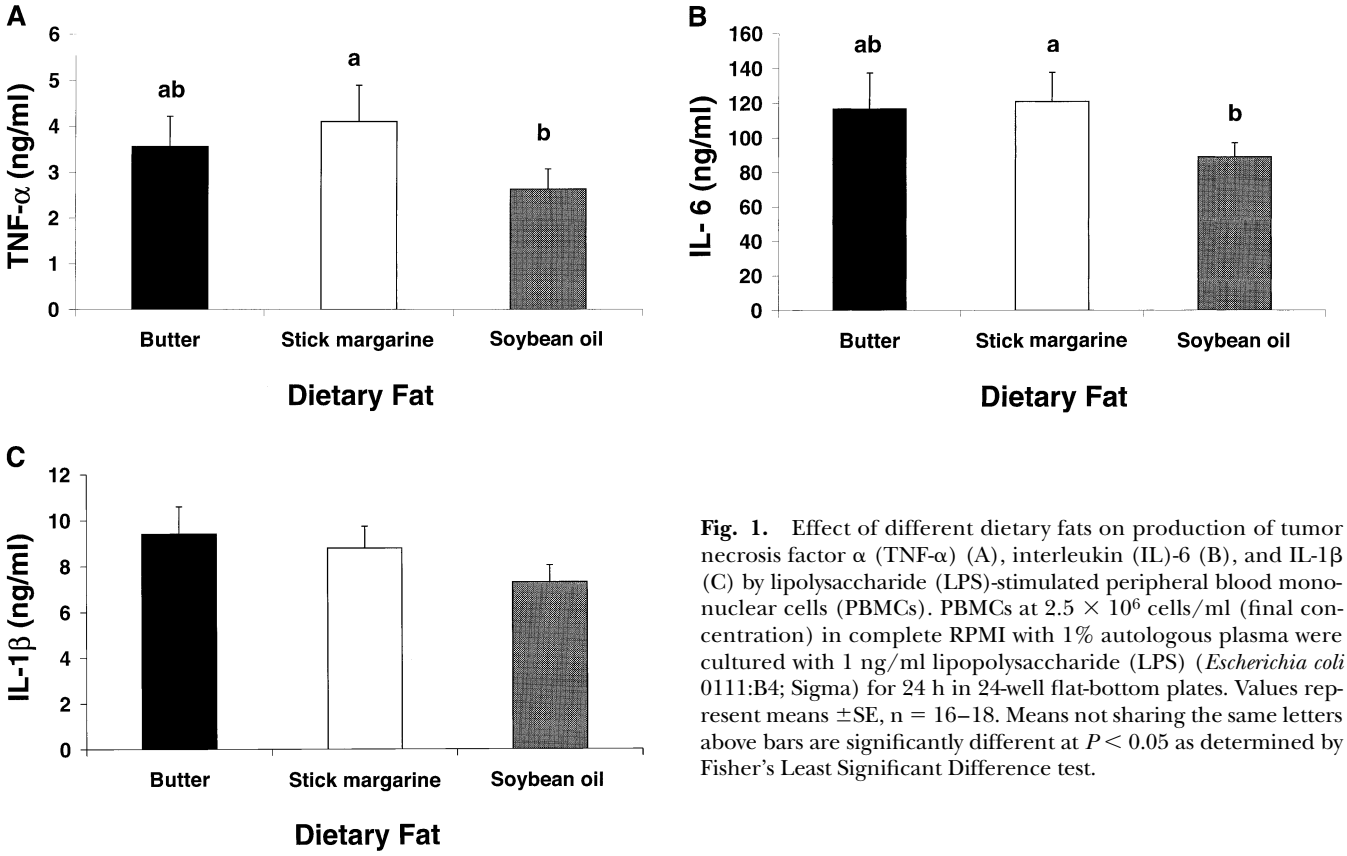

Fig. 1. Effect of different dietary fats on production of tumor necrosis factor α (TNF-α) (A), interleukin (IL)-6 (B), and IL-1β (C) by lipolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs). PBMCs at 2.5×10^6 cells/ml (final concentration) in complete RPMI with 1% autologous plasma were cultured with 1 ng/ml lipopolysaccharide (LPS) (*Escherichia coli* 0111:B4; Sigma) for 24 h in 24-well flat-bottom plates. Values represent means ±SE, n = 16–18. Means not sharing the same letters above bars are significantly different at $P < 0.05$ as determined by Fisher's Least Significant Difference test.

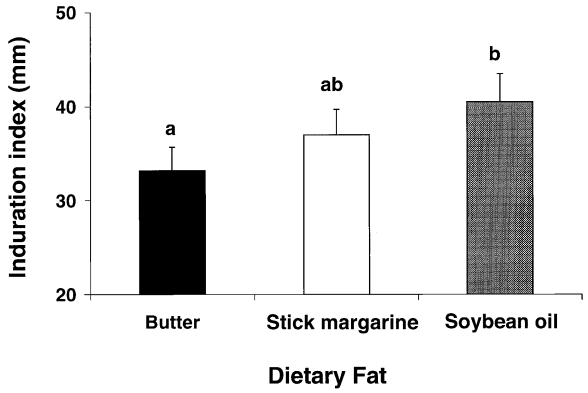

Fig. 2. Effect of different dietary fats on delayed type hypersensitivity (DTH) response. DTH response was measured by administration of seven antigens in the form of Multi-test CMI after each diet period. Diameter of induration was measured 24 and 48 h after administration. Maximal induration represents the highest response of the two time points for each antigen. Cumulative index was measured by adding the diameter of all positive responses (induration diameter of ⩾2 mm). Values represent means ± SE, n = 17–19. Means not sharing the same letters above bars are significantly different at $P < 0.05$, as determined by Fisher's Least Significant Difference test.

the stick margarine diet was intermediate between the two other diets and not significantly different from that observed after consumption of the butter diet or soybean oil diets.

Lymphocyte proliferative responses to ConA, PHA, or *Staph epi.* were not significantly different among different diet groups (**Table 5**). Thus, the consumption of a diet high in hydrogenated fat did not adversely affect lymphocyte proliferative nor DTH responses.

As shown in Table 4, there was no significant difference in ConA- or PHA-stimulated IL-2 production by PBMCs after consumption of butter, stick margarine, or soybean oil diets.

TABLE 5. Effect of different dietary fats on lymphocyte proliferative response ($\times 10^3$ ccpm)

	Diet		
Mitogen Concentration	Butter	Stick Margarine	Soybean Oil
PHA (0.5 μg/ml)	16.6 ± 4.4	18.0 ± 3.9	23.4 ± 4.4
PHA (5 μg/ml)	85.1 ± 7.6	87.1 ± 8.0	87.6 ± 7.0
PHA (50 μg/ml)	52.9 ± 6.4	52.9 ± 5.3	59.1 ± 7.3
ConA (2.5 μg/ml)	18.8 ± 3.3	20.2 ± 3.4	21.3 ± 2.6
ConA (5 μg/ml)	31.1 ± 4.2	31.1 ± 4.5	33.7 ± 3.7
ConA (25 μg/ml)	43.8 ± 5.3	42.8 ± 4.6	42.8 ± 5.4
ConA (50 μg/ml)	28.2 ± 2.9	24.1± 2.2	25.5 ± 4.3
ConA (100 μg/ml)	4.1 ± 0.6	3.4 ± 0.6	3.4 ± 0.5
Staph epi 0.000625%	1.5 ± 0.4	1.3 ± 0.2	1.8 ± 0.3
Staph epi 0.0025%	2.2 ± 0.4	2.2 ± 0.3	2.7 ± 0.4
Staph epi 0.0075%	3.3 ± 0.6	3.3 ± 0.4	3.7 ± 0.6

Values are means ± SE, n = 18–19. PBMCs at 5×10^6/ml (final concentration) in complete RPMI 1640 with 5% autologous plasma were stimulated with 2.5, 5, 25, 50, and 100 μg/ml of ConA; 0.5, 5, and 50 μg/ml of PHA; or 0.000625, 0.0025, and 0.0075% of *Staph epi.* for 72 h at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Lymphocyte proliferation was measured by incorporation of [^{3}H]thymidine for the last 4 h of incubation. Data represent corrected counts per min (ccpm) which is the average cpm of mitogen-stimulated cultures minus the average cpm of cultures without stimulation. An ANOVA with the main effect of diet and subjects as the repeated measure was carried out followed by the Fisher's Least Significant Difference test for the pairwise comparisons of the three diets. There was no significant difference among different diet groups.

DISCUSSION

Our study is the first to report that consumption of diets containing hydrogenated fats high in *trans* fatty acids increases the production of the inflammatory cytokines TNF-α and IL-6 in humans with moderately elevated LDL cholesterol levels. Considering the various effects of these proinflammatory cytokines on lipid metabolism and inflammatory responses of vascular system, increased production of these cytokines after consumption of diets containing hydrogenated fats may have clinical implications and may contribute to the reported atherogenic effect of *trans* fatty acids. On the other hand, findings from the present study suggest that consumption of diets containing hydrogenated fats does not adversely affect cellular immune response in this population of subjects. Consumption of the butter diet, high in saturated fat, however, does seem to reduce cellular immune response as measured by DTH.

The quality of dietary fat has been shown to affect immune responses. Fatty acids can modulate signaling pathways at multiple levels by acting as second messengers or regulators of signal-transducing molecules (23). In recent years, several studies have demonstrated that immune cells and their products are involved in the pathogenesis of atherosclerosis (11, 24–26). Although consumption of *trans* fatty acids has been shown to be associated with increased LDL cholesterol and increased risk of atherosclerosis, very few studies have investigated the effects of *trans* fatty acids on immune functions. There are several possible mechanisms by which *trans* fatty acid could affect immune and inflammatory responses. For example, physical properties of *trans* fatty acids similar to saturated fatty acids may affect membrane physiology (27, 28), or the replacement of *cis, cis*-linoleic acid (precursor of prostaglandin synthesis) by *trans* isomers may affect production of immunosuppressive PGE_2, a product of cyclooxygenase (Cox) (29). Lee et al. (30) showed that saturated fatty acids induce nuclear factor κB (NF-κB) activation and expression of Cox-2 through Toll-like receptor 4 (Tlr4), whereas unsaturated fatty acids inhibited saturated fatty acid-induced NF-κB activation and Cox-2 expression. In addition, changes in cholesterol levels induced by *trans* fatty acids may affect immune response through its effect on lipid raft assembly and function, which are important in the signaling cascade, generating cell surface polarity and activation of immune cells. Not only the total cellular level of cholesterol, but also its distribution between membranes and within a given membrane, are important for assembly and functions of lipid rafts (31). Matthan et al. (32) reported that elevation in circulating levels of choles-

terol after consumption of hydrogenated fat containing diets might be due to impairment in the catabolic pathway of cholesterol-rich lipoproteins as cholesterol synthesis rate was lower following consumption of a hydrogenated fat diet compared with soybean oil diet.

In this study, DTH response was significantly higher following consumption of the soybean oil diet compared with the butter diet. In contrast, there was no significant difference in cell mediated immune response as determined by DTH, lymphocyte proliferation, IL-2, or PGE_2 production between soybean oil diet and stick margarine diet. As shown in Table 2, the level of linoleic acid was lower (stick margarine diet is about half of the soybean oil diet) and the level of *trans* fatty acids was higher (stick margarine diet is more than 10 times of soybean oil diet) with incorporation of hydrogenated fat. However, this amount of reduction in linoleic acid was not adequate to affect PGE_2 production. Mahfouz and Kummerow (33) also reported that consumption of hydrogenated fats high in *trans* 18:1 acids has no effect on the amount of thromboxane or prostacyclin produced by platelet or aorta in rats when the amount of linoleic acid in the diet is adequate.

The most significant effect of stick margarine diet consumption was observed in LPS- stimulated production of TNF-α and IL-6 by PBMCs. This may have important clinical implications, especially considering the fact that subjects in this study had moderately elevated LDL cholesterol levels, a risk factor for development of atherosclerosis. TNF-α plays an important role in different aspects of the atherosclerotic process. It increases permeability of endothelial cells (34), promotes monocyte adhesion (35), induces macrophage differentiation (36), promotes foam cell formation (37), and stimulates the growth of smooth muscle cells (38). It has been shown that TNF-α induces arteriosclerosis-like lesions in coronary arteries in vivo (39), and promotes in vitro vascular calcification by increasing osteoblastic differentiation of vascular cells (40). Jovinge et al. (41) showed that circulating TNF-α levels were significantly increased in patients with premature coronary heart disease compared with age-matched healthy controls. In addition, Solheim et al. (42) reported that hypercholesterolemic individuals treated with pravastatin, a cholesterol lowering agent, showed a significant reduction in the level of circulating TNF-α compared with placebo. On the other hand, in the study by Mohrschladt et al. (43), significantly higher level of ex-vivo production of TNF-α was observed in patients with endogenous hypertriglyceridemia with fibrates than in matched normolipidemic controls. Lowering of serum triglycerides in these patients resulted in a significant decrease in TNF-α production. Patients with familial hypercholesterolemia had lower production of TNF-α, and lipid lowering treatment did not affect TNF-α production in the study by Mohrschladt et al. (43). In the present study, ~58% increase in TNF-α production was observed after consumption of the stick margarine diet compared with the soybean oil diet. Levels of TNF-α produced correlated positively ($r = 0.291$, $P = 0.04$) with the ratio of total cholesterol to HDL cholesterol. However, it is not clear whether increased production of TNF-α preceded the changes in cholesterol profiles (increase in LDL and decrease in HDL) or if it was the result of changes in cholesterol profiles. Results from other studies (13, 44) show that TNF-α affects lipoprotein metabolism. Administration of TNF-α to Syrian hamsters increased serum cholesterol levels by 17% and decreased HDL cholesterol levels by 20% (13). Furthermore, exposure of rat arteries to TNF-α increased the rate of LDL accumulation (44). On the other hand, LDL can affect TNF-α expression in aorta and immune cells. Exposure of rat aortic smooth muscle cells to LDL, in vitro, stimulated TNF-α mRNA and protein expression. Accumulation of LDL in rat arteries, in vivo, after injection of LDL, induced TNF-α mRNA and immunoreactivity in the aorta and other large arteries (12). Human monocytes/macrophages cultured with oxidized LDL showed increased expression of TNF-α mRNA and increased secretion of TNF-α (38).

IL-6 is another inflammatory cytokine that has been shown to play an important role in atherogenesis. In a prospective study by Ridker et al. (45), elevated levels of plasma IL-6 were associated with increased risk of future myocardial infarction in 404 apparently healthy men during a 6-year follow-up period. IL-6 might increase atherothrombotic risk by increasing the release of adhesion molecules by the endothelium, increasing the hepatic release of fibrinogen, and having procoagulant effects on platelets (46). IL-6 also affects lipid metabolism by inhibiting lipoprotein lipase and stimulating lipolysis (47). In the present study, IL-6 production increased about 36% after consumption of the stick margarine diet compared with the soybean oil diet. In a study by Huber et al. (48), exogenously administered IL-6 significantly enhanced fatty lesion development in the atherosclerosis-prone mice, although it did not affect total cholesterol levels. However, in this study, unlike TNF-α, there was no significant correlation between IL-6 and the ratio of total cholesterol to HDL cholesterol.

IL-1β, produced mainly by blood monocytes and tissue macrophages, is expressed within the endothelium of atherosclerosis plaques, and endothelial cells are a good source of IL-1β. IL-1β promotes monocyte recruitment and infiltration into the arterial walls, increases endothelial cell adhesiveness to leukocytes by inducing adhesion molecule presentation on the cell surface of vascular endothelium, promotes vascular smooth muscle cell proliferation, and increases endothelial cell procoagulant activity (49). In the present study, levels of IL-1β produced after consumption of butter or stick margarine diets were about 28% and 20% higher, respectively, compared with that of soybean oil diet, but the difference did not reach statistical significance. A significantly positive correlation between ratios of total cholesterol to HDL cholesterol and IL-1β production ($r = 0.499$, $P < 0.001$) was observed, suggesting that factors affecting blood lipid levels may also affect IL-1β production.

In conclusion, our results suggest that consumption of hydrogenated fats may adversely affect the inflammatory process in atherosclerosis by increasing the PBMC pro-

duction of inflammatory cytokines; however, it does not seem to adversely affect cellular immune responses. The impact of saturated fat on the production of inflammatory cytokines appears to be intermediate between that of polyunsaturated fat and hydrogenated fat. When the amount of fat in the diet was similar, consumption of soybean oil as a major source of fat significantly increased DTH response compared with the consumption of butter (high in saturated fat and cholesterol) and significantly decreased inflammatory cytokine production compared with the consumption of stick margarine (high in *trans* fatty acids). Therefore, use of vegetable oil in its natural state might be preferable over a hydrogenated form for people with hypercholesterolemia.

The authors thank the staff of the Metabolic Research Unit for the expert care provided to the study subjects. We also gratefully acknowledge the cooperation of the study subjects, without whom this investigation would not have been possible. This work was supported by grant HL 54727 from the National Institutes of Health, Bethesda, MD and the US Department of Agriculture, under agreement No. 58-1950-9-001, and the USDA CRIS grant No. 51000-050. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the US Department of Agriculture.

Manuscript received 17 September 2001 and in revised form 17 December 2001.

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