

Effect of a therapeutic lifestyle change diet on immune functions of moderately hypercholesterolemic humans

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Abstract Hypercholesterolemia is a risk factor for coronary heart disease (CHD) and also could contribute to impaired immune response. The National Cholesterol Education Program Expert Panel recommends a therapeutic lifestyle change (TLC) diet to reduce the risk for CHD. We investigated the effects of changing from a high-fat Western diet to a low-fat diet in accordance with a TLC diet on immune functions of older adults with hypercholesterolemia to determine whether improving the lipid profile via dietary intervention would have beneficial effects on immune functions. In a double-blind study, 18 subjects consumed both a Western diet (38% fat) and a TLC diet (28% fat) for 32 days in a randomized order. Measures of cellular immune responses, including delayed-type hypersensitivity (DTH) response, in vitro lymphocyte proliferation, and interleukin (IL)-2 production, and production of proinflammatory mediators, including tumor necrosis factor- α , IL-6, IL-1 β , and prostaglandin E₂, were determined. DTH response and lymphocyte proliferative response increased significantly (29% and 27%, respectively) after consumption of a TLC diet. Our results indicate that consumption of a TLC diet enhances T cell-mediated immune functions in older adults with elevated cholesterol level. This might be a clinically important benefit, considering the decline of T cell-mediated immune functions with aging and evidence of impaired immune function associated with hypercholesterolemia.— Han, S. N., L. S. Leka, A. H. Lichtenstein, L. M. Ausman, and S. N. Meydani. **Effect of therapeutic lifestyle change diet on immune functions of moderately hypercholesterolemic humans.** *J. Lipid Res.* 2003. 44: 2304–2310.

Supplementary key words low-fat diet • immune response • dietary fat • inflammatory response

Research from animal, epidemiologic, and genetic disorder studies indicates that elevated LDL cholesterol is a major cause of coronary heart disease (CHD). These studies show that not only is hypercholesterolemia a main risk

factor for the development of atherosclerosis, it can also contribute to impaired immune response against infections. Loria, Kibrick, and Madge (1) showed that hypercholesterolemia induced by a diet high in cholesterol can alter the host defense against coxsackievirus B, as higher mortality, marked suppression of cellular infiltrates in infected tissue, and extensive pathology, including focal necrosis in liver, cholelithiasis, and cardiomyolysis were observed in hypercholesterolemic compared with normal mice. In a study by Kos et al. (2), hypercholesterolemia was associated with a 40-fold increase in susceptibility to *Listeria monocytogenes* infection and decreased antibody response to sheep erythrocytes in vivo. Netea et al. (3) showed that hyperlipoproteinemia due to the loss of the LDL receptor (LDLR) in LDLR-deficient (LDLR^{-/-}) mice had deleterious effects on the outcome of severe *Candida albicans* infection, including earlier death and higher outgrowth of *C. albicans* in the kidneys and liver, compared with wild-type littermates. In addition, the study by Ludewig et al. (4) using apolipoprotein E (apoE)-deficient mice or LDLR^{-/-} mice models showed that hypercholesterolemia had a significant suppressive effect on cellular immunity. Activation of antiviral cytotoxic T lymphocytes (CTLs), measured by ex vivo cytotoxicity and IFN- γ production, and recruitment of specific CTLs into blood and liver were impaired following lymphocytic choriomeningitis virus (LCMV) infection in hypercholesterolemic mice.

Change in lifestyle, including diet therapy, has been shown to be effective in lowering cholesterol levels, which may lead to a decrease in the risk for CHD in some populations (5, 6). Furthermore, the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) recommends therapeutic lifestyle changes (TLCs), including a TLC diet as a first line

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of therapy for clinical management of high blood cholesterol levels, except for those with CHD or a high risk of CHD who would be required to start drug therapy simultaneously with TLC (7). The major dietary components that raise LDL cholesterol are saturated fatty acids, *trans* fatty acids, and cholesterol (8, 9). Adult Treatment Panel III recommends 25–35% of total calories from fat, less than 7% of total calories as saturated fat, up to 10% of total calories from polyunsaturated fat, up to 20% of total calories from monounsaturated fat, and less than 200 mg/day of cholesterol.

In the present study, we investigated the effects of high-fat Western diet and low-fat TLC diet on immune functions of older adults with elevated serum LDL cholesterol levels to determine whether improving the lipid profile via dietary intervention would have beneficial effects on immune functions as well.

METHODS

Subjects

Eleven women and seven men over the age of 50 years with moderately elevated LDL cholesterol levels (>1.3 g/l, 3.36 mmol/l) were included in the study. All 18 subjects had no evidence of any chronic illness, including endocrine, hepatic, renal, thyroid, or cardiac dysfunction and had normal serum glucose levels after fasting. None of the subjects smoked or were taking medications known to affect serum lipid levels or nonsteroidal anti-inflammatory drugs such as aspirin. Subjects who were taking any dietary supplements known to affect immune functions were excluded from the study. All women participating in the study were postmenopausal, and none were taking hormone replacement therapy. The characteristics of the subjects at the time of screening are shown in **Table 1**. This protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University, and all subjects gave written informed consent.

Study design and diets

This study was composed of two 32 day phases, with a minimum interval of 2 weeks between diet phases, during which period of time the subjects consumed either their habitual diets ad libitum or an alternate experimental diet. All subjects were provided with a diet (termed Western diet) designed to approximate that consumed by those individuals not complying with current dietary recommendations and a diet (termed TLC diet) consistent with NCEP (Adult Treatment Panel III) recommendations.

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

Characteristics	Women ^a	Men	All Subjects
Age (years)	67.5 ± 1.4	61.2 ± 2.1	65.1 ± 1.4
Body mass index (kg/m ²)	28.1 ± 0.8	30.3 ± 0.8	29.0 ± 0.6
Serum cholesterol (mg/dl)			
Total	251 ± 10	262 ± 13	255 ± 8
VLDL	31 ± 3	33 ± 4	32 ± 3
LDL	164 ± 9	185 ± 12	172 ± 7
HDL	56 ± 3	44 ± 3	51 ± 3
Serum triglyceride (mg/dl)	153 ± 17	166 ± 21	158 ± 13

Values are means ± SE.

^a Women, n = 11; men, n = 7; all subjects, n = 18.

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 (10). Additional diets were included in the randomized scheme but were not included in this analysis (9). The subjects were encouraged to maintain their habitual level of physical activity throughout the study period.

The Western diet was designed to provide 17% of calories as protein, 45% as carbohydrate, and 38% as fat (16% saturated, 15% monounsaturated, and 7% polyunsaturated), and 164 mg cholesterol per 1,000 kcal. The TLC diet was designed to provide 16% of calories as protein, 56% as carbohydrate, and 28% as fat (7% saturated, 8% monounsaturated, and 13% polyunsaturated) and 66 mg cholesterol per 1,000 kcal. All food and drink was 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. Subjects were required to consume all that was provided to them and not to supplement with any other food or drink with the exception of water and noncaloric beverages. Initial caloric levels were estimated with the use of the Harris-Benedict formula and were adjusted, when necessary, to maintain body weight. The mean (±SE) energy intake was 2,108 ± 60 kcal for the women and 2,863 ± 258 kcal for the men. Analysis of protein, carbohydrate, fatty acid, and cholesterol contents of the diets was carried out by Covance Laboratories (Madison, WI).

Delayed-type hypersensitivity skin test

Delayed-type hypersensitivity (DTH) skin test was assessed with Multi-test CMI (Merieux Institute, Inc., Miami, FL), with a single-use disposable applicator of acrylic resin with eight heads loaded with glycerine control and the following seven recall antigens: tetanus toxoid, diphtheria toxoid, *Streptococcus* (group C), *Mycobacterium tuberculosis*, *C. albicans*, *Trichophyton metagrophytes*, and *Proteus mirabilis*. The diameter of positive reactions was measured at 24 h and 48 h after administration of the test. Maximal induration, the highest response of the two time points for each antigen, was used to calculate the cumulative score. The antigen score was calculated as the total number of positive antigens, and the cumulative score was calculated as the total diameter of induration of all positive reactions. 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 all the other positive reactions. The test was administered by the same nurse for each subject, and the diameter of induration was measured by the same person. No boosting effect was reported with repeated administration of Multi-test CMI (11).

Isolation of mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood as previously described by centrifugation over Ficoll-Hypaque (11). Cells were counted and resuspended at appropriate concentrations in RPMI 1640 supplemented with 100,000 U/1 penicillin, 100 mg/1 streptomycin, 2 mmol/1 L-glutamine, and 24 mmol/1 HEPES (Gibco Laboratories, Grand Island, NY) (complete RPMI) for cultures to measure lymphocyte proliferation and to induce cytokine and prostaglandin E₂ (PGE₂) production. Plasma isolated was heat inactivated at 56°C for 30 min to be used as autologous plasma in the cell cultures.

Complete blood count, white cell differential, and flow cytometric analysis

A complete blood count was obtained using a hematology analyzer (model Baker 9000; Sero-Baker Instrument, Inc., Allentown, PA), and the white cell differential was assessed by micro-

scopic examination of blood smears after Wright-Giemsa staining. Flow cytometric analysis was done as described (12) before using the following antibodies: anti-Leu-4 FITC (CD3), anti-Leu-3 FITC (CD4+), anti-Leu-2a PE (CD8+), and anti-Leu-12 PE (CD19+).

Lymphocyte proliferation

Lymphocyte proliferation was measured by [³H]thymidine incorporation after stimulation with T and B cell mitogens. PBMCs were incubated in 96-well flat-bottom plates at 5×10^9 cells/l (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% CO₂ and 95% humidity. Final mitogen concentrations were 2.5, 5, 25, 50, and 100 mg/l for concanavalin A (ConA) (Sigma Chemical Co., St. Louis, MO), 0.5, 5, and 50 mg/l for phytohemagglutinin (PHA) (Difco Laboratories, Detroit, MI), and 0.000625%, 0.0025%, and 0.0075% for *Staphylococcus epidermidis*. Each well was pulsed with 0.5 μCi of [³H]thymidine (New England Nuclear, Boston, MA) in 20 μl for the last 4 h of the 72 h incubation. Cells were harvested onto glass microtiter filter paper using a cell harvester (Cambridge Technologies, Inc., Cambridge, MA) and radioactivity incorporation was counted in a liquid scintillation counter (Beckman Instruments, Inc., Palo Alto, CA). The results are reported as corrected cpm, which is the average cpm of mitogen-stimulated cultures minus the average cpm of cultures without mitogens.

Interleukin-2 production

PBMCs, at 1×10^9 cells/l (final concentration) in complete RPMI with 9% autologous plasma, were cultured with 10 mg/l (final concentration) of ConA or PHA for 48 h in 24-well flat-bottom plates. Cell-free supernatants were collected and stored at -70°C for analysis. Interleukin (IL)-2 activity was measured by bioassay using CTL line 2 (13). Protein levels of IL-2 were also measured using ELISA according to the manufacturer's instructions with mouse anti-human IL-2 monoclonal antibody (MAb) (PharMingen, San Diego, CA) and biotinylated anti-human IL-2 MAb.

PGE₂ production

PBMCs, at 1×10^9 cells/l (final concentration) in complete RPMI with 9% autologous plasma, were cultured with 10 mg/l or 100 mg/l (final concentration) of PHA for 48 h in 24-well flat-bottom plates. Cell-free supernatants were collected and stored at -70°C for analysis. PGE₂ was measured by radioimmunoassay as described by McCosh, Meyer, and Dupont (14). The PGE₂ antibody was provided by Dr. Dupont of Florida State University, Tallahassee and Dr. Mathias of the Agricultural Research Service in Washington, DC. The antibody has a cross-reactivity of 19% with PGE₁.

IL-1β, IL-6, and tumor necrosis factor-α production

PBMCs, at 2.5×10^9 cells/l (final concentration) in complete RPMI with 1% autologous plasma, were cultured with 1 μg/l lipopolysaccharide (LPS) (*Escherichia coli* 0111:B4; Sigma) 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 (15). Antibody to IL-1β was purchased from Cistron Biotechnology (Pine Brook, NJ). Recombinant IL-1β was purchased from Genzyme (Cambridge, MA). ¹²⁵I-labeled IL-1β was purchased from DuPont NEN. IL-6 and tumor necrosis factor-α (TNF-α) were measured by ELISA, according to the manufacturer's instructions, with rat anti-human IL-6 or mouse anti-human TNF-α MAbs (PharMingen) and biotinylated rat anti-human IL-6 or biotinylated mouse anti-human TNF-α.

Serum C-reactive protein and IL-6 levels

Serum C-reactive protein (CRP) level was measured by a Tinaquant CRP (Latex) high-sensitive immunoturbidimetric assay

(Roche Diagnostics Co., Indianapolis, IN) using a Hitachi 911 automated analyzer (Roche Diagnostics Co.). Serum IL-6 level was measured using BD OptEIA Human IL-6 ELISA kit (BD Biosciences, San Diego, CA).

Serum lipoprotein cholesterol levels

VLDL was isolated from serum by ultracentrifugation at 109,000 g at 4°C (16). Serum and the infranant (1.006 g/ml) were assayed for total cholesterol and triglyceride with 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 apoB with the use of dextran-magnesium sulfate (18). Lipid assays were standardized through the Lipid Standardization Program of the Centers for Disease Control and Prevention (Atlanta, GA).

Statistical analysis

Data were analyzed using the SYSTAT statistical package (SYSTAT 9.0, 1999; SYSTAT, Inc., Evanston, IL). A paired Student's *t*-test was carried out to test for the differences between outcome measures after Western and TLC diet consumption. Significance was set at *P* < 0.05. Appropriate transformations were done to measures that were not normally distributed.

RESULTS

Serum lipoprotein cholesterol levels

Levels of serum lipoprotein cholesterol and triglyceride after consumption of Western or TLC diets are shown in **Table 2**. Consumption of the TLC diet compared with the Western diet resulted in significant decreases in total cholesterol (8%), LDL cholesterol (10%), and HDL cholesterol (6%). Serum triglyceride level increased significantly (*P* < 0.05, 19% increase) following consumption of a TLC diet. There were no significant differences in the serum VLDL level or total cholesterol-HDL cholesterol ratio between the two diet groups.

TABLE 2. Effects of TLC diet on serum lipid profiles in hypercholesterolemic subjects

Serum Lipid	Diet	
	Western	TLC
Total cholesterol (mg/dl)	249 ± 10	228 ± 9 ^a
Women	240 ± 10	219 ± 10
Men	263 ± 20	242 ± 15
VLDL cholesterol (mg/dl)	28 ± 3	33 ± 3
Women	28 ± 4	28 ± 3
Men	29 ± 2	41 ± 6
LDL cholesterol (mg/dl)	168 ± 11	151 ± 8 ^a
Women	153 ± 12	143 ± 9
Men	193 ± 18	163 ± 12
HDL cholesterol (mg/dl)	47 ± 2	44 ± 2 ^a
Women	50 ± 2	48 ± 2
Men	41 ± 2	38 ± 2
Triglyceride (mg/dl)	137 ± 9	163 ± 16 ^a
Women	131 ± 11	138 ± 13
Men	148 ± 18	203 ± 33
Total HDL cholesterol	5.44 ± 0.30	5.35 ± 0.31
Women	4.83 ± 0.23	4.63 ± 0.30
Men	6.40 ± 0.41	6.48 ± 0.36

TLC, therapeutic lifestyle change. Values are means ± SE, n = 18. * *P* < 0.05 determined by paired Student's *t*-test.

White blood cell number and subpopulation

There was no significant difference in the total number of white blood cells and lymphocytes determined by differential cell counts following consumption of either diet (data not shown). As shown in **Table 3**, consumption of the TLC diet did not have significant effects on the lymphocyte subpopulation as compared with the Western diet.

DTH skin response and lymphocyte proliferation

As shown in **Fig. 1**, DTH skin response increased significantly after consumption of a TLC diet compared with the Western diet ($P = 0.002$, 31% increase). Fourteen out of the 17 subjects tested showed an increase in the maximal induration index after consumption of the TLC diet. There was no difference in the maximal number of positive antigens; however, the number of positive responses at 24 h after antigen administration was significantly higher following consumption of the TLC diet compared with the Western diet (6.1 ± 0.2 vs. 5.3 ± 0.4 , $P = 0.04$).

As shown in **Fig. 2**, lymphocyte proliferative response to T cell mitogen PHA increased significantly following consumption of the TLC diet. Consuming the TLC diet resulted in 27% ($P = 0.03$) and 13% ($P = 0.07$) higher proliferative responses to PHA, at 50 mg/l (superoptimal) and 5 mg/l (optimal) concentrations, respectively, relative to consumption of the Western diet. Proliferative response to ConA was higher (22% higher, $P = 0.07$) at a suboptimal ConA concentration (5 mg/l, data not shown). Proliferative response to B cell mitogen *S. epidermis* was not significantly different between the two diet groups (data not shown).

IL-2, PGE₂, IL-1 β , IL-6, and TNF- α production

There were no significant differences in ConA- or PHA-stimulated IL-2 or PHA-stimulated PGE₂ production by PBMCs after consumption of the TLC or Western diets (**Table 4**). PHA-stimulated IL-2 levels determined by bioassay correlated significantly ($r = 0.850$, $P < 0.001$, $n = 36$) with those determined by ELISA, indicating that biologically active IL-2 correlated tightly with protein levels.

Likewise, there were no significant differences in LPS-stimulated IL-1 β , IL-6, or TNF- α production by PBMCs after consumption of the TLC or Western diets (**Table 5**).

Serum CRP and IL-6 levels

Dietary modification had no significant effect on serum levels of CRP and IL-6 (**Table 5**).

TABLE 3. Effects of TLC diet on lymphocyte subpopulation

Lymphocyte Subpopulation	Diet	
	Western	TLC
CD3+ (T cells)	72.6 \pm 3.0 ^a	71.9 \pm 2.3
CD4+ (helper T cells)	47.0 \pm 2.2	45.9 \pm 1.7
CD8+ (cytotoxic T cells)	20.1 \pm 2.0	18.8 \pm 1.7
CD19+ (B cells)	8.2 \pm 0.7	8.2 \pm 1.0

Values are means \pm SE, $n = 18$.

^a Numbers are percentage of total lymphocyte.

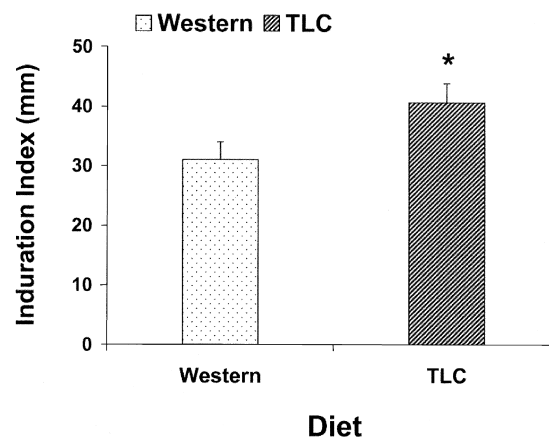


Fig. 1. Effect of the therapeutic lifestyle change (TLC) diet, relative to the Western diet, on delayed-type hypersensitivity (DTH) response. DTH response was measured by the administration of seven antigens in the form of Multi-test CMI after each diet period. Diameter of induration was measured 24 h 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 the positive responses (induration diameter of ≥ 2 mm). Values represent means \pm SE, $n = 17$. * Significantly different at $P < 0.05$ determined by Student's paired t -test.

DISCUSSION

Results from the present study indicate that consumption of a TLC diet can enhance the cellular immune response in older adults with elevated LDL cholesterol levels. The levels of total and LDL cholesterol of the subjects in this study were in the "high" classification category according to the Adult Treatment Panel III classification of LDL, total, and HDL cholesterol.

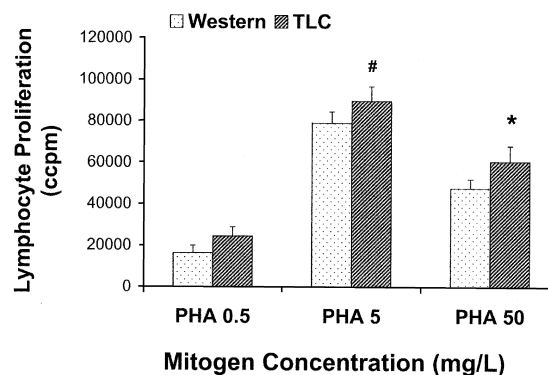


Fig. 2. Effect of the TLC diet, relative to the Western diet, on lymphocyte proliferative response. Peripheral blood mononuclear cells at $5 \times 10^9/l$ in complete RPMI with 5% autologous plasma were stimulated with phytohemagglutinin for 72 h at 37°C in an atmosphere of 5% CO₂ and 95% humidity. Lymphocyte proliferation was measured by incorporation of [³H]thymidine for the last 4 h of incubation. Data represent corrected cpm, which is the average cpm of mitogen-stimulated cultures minus the average cpm of cultures without stimulation. Values represent means \pm SE, $n = 18$. * Significantly different at $P < 0.05$ determined by Student's paired t -test. # Different at $P < 0.1$ as determined by Student's paired t -test.

TABLE 4. Effect of TLC diet on interleukin-2 and prostaglandin E₂ production in hypercholesterolemic subjects

Cytokines/PGE ₂	Diet	
	Western	TLC
IL-2 ($\times 10^3$ U/ml) ^a		
PHA at 10 mg/1	34.7 \pm 8.0	36.2 \pm 10.2
ConA at 10 mg/1	5.7 \pm 1.3	4.5 \pm 1.5
IL-2 (ng/l) ^b		
PHA at 10 mg/1	2,591 \pm 472	2,524 \pm 738
PGE ₂ (ng/l)		
PHA at 10 mg/1	265 \pm 44	306 \pm 99
PHA at 100 mg/1	1,356 \pm 285	1,106 \pm 264

ConA, concanavalin A; IL, interleukin; PBMC, peripheral blood mononuclear cell; PGE₂, prostaglandin E₂; PHA, phytohemagglutinin. Values are means \pm SE, n = 18. PBMCs at 1×10^9 cells/1 (final concentration) in complete RPMI with 9% autologous plasma were cultured with ConA or PHA at indicated concentrations for 48 h in 24-well flat-bottom plates. PGE₂ was measured by radioimmunoassay.

^a Cell-free supernatant was collected, then IL-2 activity was measured by bioassay using cytotoxic T lymphocyte line.

^b Cell-free supernatant was collected, then IL-2 activity was measured by ELISA.

Animal studies have shown that diet- or genetically induced hypercholesterolemia can increase susceptibility to Cocksackievirus B and *L. monocytogenes* and lead to a substantial impairment of antiviral cellular responses, leading to delayed viral clearance from spleen and nonlymphoid organs in LCMV (1, 2, 4). In addition to their hypercholesterolemic status, the average age of the subjects in the present study was over 60. These factors are likely to contribute adversely to the optimal immune response. It is well documented that immune functions deteriorate with aging, with the most significant changes observed in T cell function (19). Therefore, increase in T cell-mediated immune function following consumption of a TLC diet suggests that the benefits of eating diets low in saturated fat and cholesterol might go beyond their effect on improving lipids in older adults with moderate hypercholesterolemia.

TABLE 5. Effect of TLC diet on serum levels of C-reactive protein and IL-6 and production of IL-1 β , IL-6, and TNF- α by PBMC in hypercholesterolemic subjects

Inflammatory Mediators	Diet	
	Western	TLC
Serum CRP (mg/l)	2.52 \pm 0.37	2.94 \pm 0.43
Serum IL-6 (ng/l)	3.14 \pm 0.76	3.50 \pm 1.01
IL-1 β (μ g/l)		
LPS at 1 μ g/1	7.9 \pm 1.0	7.2 \pm 0.8
<i>Staphylococcus epidermis</i> at 1:20	16.7 \pm 2.7	18.3 \pm 1.9
IL-6 (μ g/l)		
LPS at 1 μ g/1	114.0 \pm 14.8	90.5 \pm 8.5
TNF- α (μ g/l)		
LPS at 1 μ g/1	2.9 \pm 0.4	2.7 \pm 0.5

CRP, C-reactive protein; LPS, lipopolysaccharide; TNF- α , tumor necrosis factor- α . Values are means \pm SE, n = 18. For determination of IL-1 β , IL-6, and TNF- α production by PBMCs, PBMCs at 2.5×10^9 cells/1 (final concentration) in complete RPMI with 1% autologous plasma were cultured with LPS or *Staphylococcus epidermis* at indicated concentrations for 24 h in 24-well flat-bottom plates. Cell-free supernatant was collected, then IL-1 β was measured using radioimmunoassay, and IL-6 and TNF- α were measured by ELISA.

Both quantity and quality of dietary fat have been shown to modulate immune responses. Significant increases in proliferative responses to PHA, ConA, and Pokeweed mitogens were observed after consumption of diets containing 31.1% or 26.1% of energy as fat compared with the diets containing 41.1% of energy as fat in healthy women (20). However, DTH response to seven recall antigens was not significantly affected by the total amount of fat in the diet in the above study. Pedersen et al. (21) showed that natural immunity might be affected by the fat content in the diet during exercise training. Natural killer cell activity in response to endurance training decreased in the group consuming a fat-rich diet (62% energy as fat) for 7 weeks and was increased in the group consuming a carbohydrate-rich diet (65% energy as carbohydrate). On the other hand, Venkatraman et al. (22) did not observe any deleterious effects of high fat intake (41%) on the immune functions of the well-trained runners compared with the low fat intake (17%). Quality of dietary fat was shown to influence cellular membrane fatty acid composition (23) and to result in alteration of eicosanoid biosynthesis, because membrane arachidonic acid, a desaturation and elongation product of linoleic acid, serves as the substrate for the production of eicosanoids, leukotrienes, and prostaglandins (24). Eicosanoids play an important role in regulating immune and inflammatory responses. Dietary fatty acids can regulate immune and inflammatory responses by modulating signaling pathways (25). Recently, saturated fatty acids have been shown to activate nuclear transcription- κ B and to induce expression of cyclooxygenase (Cox)-2, inducible nitric oxide synthetase, and IL-1 α (26).

Following consumption of a TLC diet, the serum lipid profile improved significantly. While both total cholesterol and LDL cholesterol levels remained in the "high" classification category according to the Adult Treatment Panel III classification of LDL, total, and HDL cholesterol following the Western diet consumption, their levels registered in the "borderline high" category following the TLC diet consumption. Despite the decrease in total cholesterol levels, the total cholesterol-HDL cholesterol ratio was not significantly different between the two dietary groups, which is attributable to the decrease in HDL cholesterol levels following consumption of the TLC diet.

In this study, DTH response and lymphocyte proliferative response to the T cell mitogen PHA were significantly higher following consumption of the TLC diet compared with the Western diet; meanwhile, consumption of the TLC diet did not have a significant effect on B cells. This significant increase in T cell-mediated immunity following consumption of a TLC diet might have clinical benefits for this subject population. Wayne et al. (27) reported that anergy (response to all four antigens being less than 5 mm induration at 48 h) was associated with subsequent all-cause mortality in a group of initially healthy subjects over 60. In this study, two subjects showed anergic response (response to all seven antigens being less than 5 mm maximal induration) after consumption of the Western diet while none of the subjects showed anergic response following consumption of the TLC diet. In addition, the

average number of antigens that showed greater than 5 mm induration response was significantly higher after consumption of TLC diet compared with the Western diet (3.5 ± 0.32 vs. 2.5 ± 0.34 ; $P < 0.001$). These enhancements of T cell-mediated immune response following consumption of the TLC diet could not be explained by changes in lymphocyte population or by changes in secretory factors, immunostimulating IL-2, or immunosuppressive PGE₂, as the percentages of T cells and the T cell subpopulation and production of IL-2 and PGE₂ by PBMCs were not affected by the diet. Moreno et al. (28) reported a significant decline in T cell subset counts (CD3, CD4, and CD8) following low-fat, low-cholesterol diet therapy for 6 months in hypercholesterolemic children aged 7–16 years. In this study, neither the percentage of T cells nor that of B cells changed significantly after consumption of either diet. There was no significant change in T lymphocyte subpopulation. IL-2 plays a critical role in cellular immunity by mediating clonal expansion of activated T cells. Venkatraman et al. (22) showed that the total amount of fat in the diet could affect IL-2 production by PBMCs. IL-2 production by PBMCs was lower in runners who consumed a low-fat diet (17%) for 4 weeks compared with those who consumed a medium-fat (32%) or a high-fat (41%) diet. However, in the present study, the amount of fat in the diet did not have any significant effect on IL-2 production. The differences in results may be due to the differences in the amount of fat in low-fat diets (17% vs. 28% of calories) and the age of the subjects (35 vs. 65).

PGE₂ is an immunosuppressive lipid-based mediator produced by monocytes and macrophages. Increased production of PGE₂ has been suggested to contribute to the age-associated decrease in immune function (29). Total amount, degree of saturation, and type (n-3 vs. n-6) of fat in the diet were shown to affect PGE₂ production by macrophages. A higher amount of dietary fat, especially a higher amount of n-6 fatty acids, could potentially increase the production of PGE₂ by modulating membrane fatty acid composition and increasing the availability of arachidonic acid as a substrate. However, Broughton and Wade (24) reported suppressed PGE₂ synthesis with higher total fat intake in an animal model. Saturated fatty acids were shown to induce the expression of Cox-2, an enzyme that catalyzes the conversion of arachidonic acid to PGE₂, while unsaturated fatty acids inhibited saturated fatty acid-induced Cox-2 expression in an in vitro system (26). In the present study, there was no significant difference in PGE₂ production by PBMCs between the two dietary groups. The Western and TLC diets were different in more than one factor, which could potentially affect PGE₂ production. The Western and TLC diets had differences in the total amount of fat (38% vs. 28% of energy as fat, respectively), and levels of saturated fat (16% vs. 7%, respectively) and polyunsaturated fat (7% vs. 13%, respectively). Therefore, these individual factors may have affected the PGE₂ production in opposite directions, resulting in no overall change in PGE₂ production.

Proinflammatory cytokines, including TNF- α , IL-1 β , and IL-6 contribute to atherosclerotic processes via their

effects on inflammatory responses, expression of adhesion molecules, lipid metabolism, and vascular smooth muscle cell proliferation (30, 31). Increased blood levels or increased ex vivo production of proinflammatory cytokines have often been reported in hyperlipoproteinemic subjects. Jovinge et al. (30) reported that plasma TNF- α levels correlated positively with the concentration of VLDL triglyceride and cholesterol and negatively with HDL cholesterol in hyperlipidemic patients. In vivo levels of TNF- α and IL-1 α during *C. albicans* infection and in vitro production of these cytokines by peritoneal macrophages stimulated with inactivated *C. albicans* were significantly higher in LDLR^{-/-} hyperlipoproteinemic mice (3). In the present study, consumption of the TLC diet did not have a significant effect on production of TNF- α , IL-1 β , or IL-6 by PBMCs compared with consumption of the Western diet in moderately hypercholesterolemic subjects. Shifting from a Western to a TLC diet did not have a significant effect on blood levels of the inflammatory markers CRP and IL-6.

In conclusion, consumption of a low-fat diet in accordance with a TLC diet, compared with a Western high-fat diet, significantly improved serum lipoprotein profiles and T cell-mediated immune response while it had no effect on B cell function or production of proinflammatory mediators in older adults with moderate hypercholesterolemia. Because hypercholesterolemia has been shown to suppress immune response, and aging is associated with impaired T cell-mediated immune functions, this enhancement of cellular immune response might provide an added benefit to consuming a TLC diet in this subject population. ■

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REFERENCES

1. Loria, R. M., S. Kibrick, and G. E. Madge. 1976. Infection of hypercholesteremic mice with Coxsackievirus B. *J. Infect. Dis.* **133**: 655–662.
2. Kos, W. L., R. Loria, M. J. Snodgrass, D. Cohen, T. G. Thorpe, and A. M. Kaplan. 1979. Inhibition of host resistance by nutritional hypercholesterolemia. *Infect. Immun.* **26**: 658–667.
3. Netea, M.G., P. Demacker, N. de Bont, O. C. Boerman, A. F. H. Stalenhoef, J. W. M., van der Meer, and B. J. Kullberg. 1997. Hyperlipoproteinemia enhances susceptibility to acute disseminated *Candida albicans* infection in low-density-lipoprotein-receptor-deficient mice. *Infect. Immun.* **65**: 2663–2667.
4. Ludewig, B., M. Jaggi, T. Dumrese, K. Brduscha-Riem, B. Odermatt, H. Hengartner, and R. M. Zinkernagel. 2001. Hypercholesterolemia exacerbates virus-induced immunopathologic liver disease via suppression of antiviral cytotoxic T cell responses. *J. Immunol.* **166**: 3369–3376.

5. Schaefer, E. J., A. H. Lichtenstein, S. Lamon-Fava, J. H. Contois, Z. Li, H. Rasmussen, J. R. McNamara, and J. M. Ordovas. 1995. Efficacy of a national cholesterol education program step 2 diet in normolipidemic and hypercholesterolemic middle-aged and elderly men and women. *Arterioscler. Thromb. Vasc. Biol.* **15**: 1079–1985.
6. Watts, G. F., B. Lewis, J. N. Brunt, E. S. Lewis, D. J. Coltart, L. D. Smith, J. I. Mann, and A. V. Swan. 1992. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St Thomas' Atherosclerosis Regression Study (STARS). *Lancet.* **339**: 563–569.
7. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 2001. *J. Am. Med. Assoc.* **285**: 2486–2497.
8. Hegsted, D., L. Ausman, J. Johnson, and G. Dallal. 1993. Dietary fat and serum lipids: an evaluation of the experimental data. *Am. J. Clin. Nutr.* **57**: 875–883.
9. Lichtenstein, A. H., L. M. Ausman, S. M. Jalbert, and E. J. Schaefer. 1999. Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. *N. Engl. J. Med.* **340**: 1933–1940.
10. Lichtenstein, A. H., L. M. Ausman, W. Carrasco, J. L. Jenner, L. J. Gualtieri, B. R. Goldin, J. M. Ordovas, R. J. Nicolosi, and E. J. Schaefer. 1994. Rice bran oil consumption and plasma lipid levels in moderately hypercholesterolemic humans. *Arterioscler. Thromb.* **14**: 549–556.
11. Meydani, S. N., M. P. Barklund, S. Liu, M. Meydani, R. A. Miller, J. G. Cannon, F. D. Morrow, R. Rocklin, and J. B. Blumberg. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am. J. Clin. Nutr.* **52**: 557–563.
12. Meydani, S. N., A. H. Lichtenstein, S. Cornwall, M. Meydani, B. R. Goldin, H. Rasmussen, C. A. Dinarello, and E. J. Schaefer. 1993. Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived n-3 fatty acid enrichment. *J. Clin. Invest.* **92**: 105–113.
13. Gills, S., M. M. Ferm, W. Ou, and K. A. Smith. 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol.* **120**: 2027–2032.
14. McCosh, E., D. Meyer, and J. Dupont. 1976. Radioimmunoassay of prostaglandin E₁, E₂, F_{2α} in unextracted plasma, serum and myocardium. *Prostaglandins.* **12**: 471–486.
15. Lisi, P. J., C. W. Chu, G. A. Koch, S. Endres, G. Lonnemann, and C. A. Dinarello. 1987. Development and use of radioimmunoassay for interleukin-1β. *Lymphokine Res.* **6**: 229–244.
16. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. Distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**: 1345–1353.
17. McNamara, J. R., and E. J. Schaefer. 1987. Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin. Chim. Acta.* **166**: 1–8.
18. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28**: 1379–1388.
19. Miller, R. A. 1996. The aging immune system: primer and prospectus. *Science.* **273**: 70–74.
20. Kelley, D. S., R. M. Dougherty, L. B. Branch, P. Taylor, and J. M. Iacono. 1992. Concentration of dietary N-6 polyunsaturated fatty acids and the human immune status. *Clin. Immunol. Immunopathol.* **62**: 240–244.
21. Pedersen, B. K., J. W. Helge, E. A. Richter, T. Rohde, and B. Kiens. 2000. Training and natural immunity: effects of diets rich in fat or carbohydrate. *Eur. J. Appl. Physiol.* **82**: 98–102.
22. Venkatraman, J. T., J. A. Rowland, E. Denardin, P. J. Horvath, and D. Pendergast. 1997. Influence of the level of dietary lipid intake and maximal exercise on the immune status in runners. *Med. Sci. Sports Exerc.* **29**: 333–344.
23. Moussa, M., J. Tkaczuk, J. Ragab, J. Garcia, M. Abal, E. Ohayon, J. Ghisolfi, and J-P. Thouvenot. 2000. Relationship between the fatty acid composition of rat lymphocyte and immune function. *Br. J. Nutr.* **83**: 327–333.
24. Broughton, K. S., and J. W. Wade. 2002. Total fat and (n-3):(n-6) fat ratios influence eicosanoid production in mice. *J. Nutr.* **132**: 88–94.
25. Hwang, D., and S. H. Rhee. 1999. Receptor-mediated signaling pathways: potential targets of modulation by dietary fatty acids. *Am. J. Clin. Nutr.* **70**: 545–556.
26. Lee, J. Y., K. H. Sohn, S. H. Rhee, and D. Hwang. 2001. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptors 4. *J. Biol. Chem.* **276**: 16683–16689.
27. Wayne, S. J., R. L. Rhyne, P. J. Garry, and J. S. Goodwin. 1990. Cell-mediated immunity as a predictor of morbidity and mortality in subjects over 60. *J. Gerontol.* **45**: M45–M48.
28. Moreno, L. A., A. Sarria, A. Lazaro, M. Lasierra, L. Larrad, and M. Bueno. 1998. Lymphocyte T subset counts in children with hypercholesterolemia receiving dietary therapy. *Ann. Nutr. Metab.* **42**: 261–265.
29. Beharka, A. A., D. Wu, S. N. Han, and S. N. Meydani. 1997. Macrophage prostaglandin production contributes to the age-associated decrease in T cell function which is reversed by the dietary antioxidant vitamin E. *Mech. Ageing Dev.* **93**: 59–77.
30. Jovinge, S., A. Hamsten, P. Tornvall, P. Bavenholm, C-G. Ericsson, I. Godtsland, U. de Faire, and J. Nilsson. 1998. Evidence for a role of tumor necrosis factor-α in disturbances of triglyceride and glucose metabolism predisposing to coronary heart disease. *Metabolism.* **47**: 113–118.
31. Kaul, D. 2001. Molecular link between cholesterol, cytokines and atherosclerosis. *Mol. Cell. Biochem.* **219**: 65–71.