

Inflammation Modifies the Effects of a Reduced-Fat Low-Cholesterol Diet on Lipids

Results From the DASH-Sodium Trial

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Background—Inflammatory mediators regulate key aspects of lipid metabolism. We hypothesized that inflammation could diminish the cholesterol-lowering effect of a reduced-fat/low-cholesterol diet.

Methods and Results—After a 2-week run-in period on a control diet (37% total fat, 16% saturated fat), 100 participants were randomized to the control or DASH diet (27% total fat, 6% saturated fat) for 12 weeks. Median C-reactive protein (CRP) at baseline was 2.37 mg/L (interquartile range, 1.20, 3.79). The DASH diet, net of control, had no effect on CRP. Overall, there were significant net reductions in total (−0.34 mmol/L), LDL (−0.29 mmol/L), and HDL (−0.12 mmol/L) cholesterol from the DASH diet (each, $P < 0.001$) and little change in triglycerides (+0.05 mmol/L, $P = 0.21$). Baseline CRP was strongly associated with lipid responsiveness to the DASH diet. Total and LDL cholesterol were reduced to a greater degree in those with a “low” (below median) compared with a “high” (above median) baseline CRP (total, −9.8% versus −3%; P for interaction = 0.006; LDL cholesterol, −11.8% versus −3%; P for interaction = 0.009). Reductions in HDL cholesterol (−8.8%) were similar in persons with low versus high CRP. Triglycerides were increased in those with a high CRP but not in those with a low CRP (19.8% versus +0%; P for interaction = 0.019).

Conclusions—In this study, the presence of increased CRP was associated with less total and LDL cholesterol reduction and a greater increase in triglycerides from a reduced-fat/low-cholesterol diet. These findings document an additional mechanism by which inflammation might increase cardiovascular disease risk. (*Circulation*. 2003;108:150-154.)

Key Words: inflammation ■ lipids ■ cholesterol ■ diet

Chronic inflammation has been hypothesized to promote the development and progression of atherosclerosis.¹ Several markers of inflammation, including high-sensitivity C-reactive protein (CRP), have been shown to predict future cardiovascular disease events.^{2–4} These studies suggest a direct adverse effect of inflammation on cardiovascular risk. However, inflammation is also associated with several traditional cardiovascular risk factors,⁵ eg, hypertension, smoking, diabetes, and elevated cholesterol and triglycerides; therefore, it is reasonable to hypothesize that inflammation might have indirect adverse effects mediated through traditional cardiovascular risk factors.

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There is a long-recognized association between inflammation and lipids.⁶ Both cholesterol and triglyceride metabolism are affected by inflammatory pathways.^{7,8} Reductions in cholesterol during acute inflammation may be a result of decreased hepatic production of lipoproteins or increased catabolism with conversion to small dense particles.^{9,10} Increased triglyceride levels as a result of increased synthesis

and secretion is a more consistent feature of inflammation-induced lipid changes.^{6,11} These observations raise the possibility that the impact of diet on plasma lipids could be modified by the degree of underlying inflammation.

In this setting, we hypothesized that inflammation could modify the lipid responsiveness to a reduced-fat/low-cholesterol diet, such that there would be a reduced cholesterol-lowering effect of the diet in the presence of inflammation. Conversely, because triglyceride levels are increased in the presence of inflammation, a diet relatively higher in carbohydrates could lead to greater increases in triglycerides in the presence of inflammation. If true, these findings could help explain the considerable interindividual variation seen in response to a lipid-lowering diet.¹²

Methods

Study Design and Participants

This study was conducted as an ancillary study to the Dietary Approaches to Stop Hypertension- Sodium (DASH-Sodium) trial, a clinical trial. This ancillary study was conducted at the Johns

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Hopkins clinical center. As an ancillary study, it was designed and analyzed only by the coauthors. Detailed descriptions of the design of the DASH-Sodium trial and of its main results have been published.¹³ The protocol was approved by a local Institutional Review Board. All participants provided written informed consent.

Study participants were healthy adults (age ≥ 22 years) who were not receiving antihypertensive medications and who had a systolic blood pressure of 120 to 159 mm Hg and diastolic blood pressure of 80 to 95 mm Hg (average of 3 screening visits). Persons with total cholesterol >260 mg/dL were excluded from the study. In addition, persons were excluded if their LDL cholesterol (LDL-C) warranted pharmacological therapy according to the National Cholesterol Education Program Adult Treatment Program II guidelines,¹⁴ that is, LDL-C >220 mg/dL for young adults (men <35 years old and premenopausal women) without 2 or more cardiovascular disease risk factors, LDL-C >190 mg/dL for older individuals without 2 or more cardiovascular disease risk factors, and LDL-C >160 mg/dL for individuals with 2 or more cardiovascular risk factors. Persons were also excluded if they were taking cholestyramine, colestipol, or an unstable dose of a statin or any other lipid-lowering agents not already excluded. Six participants (5 control, 1 DASH) were taking a lipid-lowering agent at baseline. No participants reported starting or stopping lipid-lowering therapy during the trial. Participants were excluded if they reported drinking more than 14 alcoholic drinks per week. Of those who reported drinking (19 control, 17 DASH), the average number of drinks consumed was 3.9 per week (control) and 3.4 per week (DASH). Baseline CRP values were missing for 3 participants and they were excluded from this analysis.

The DASH-Sodium trial tested the effects of 2 dietary patterns (DASH versus control) using a parallel design and 3 dietary sodium levels (150, 100, and 50 mmol/d for a 2100-kcal diet) using a crossover design within each diet.¹³ The 2 dietary patterns in the present study corresponded to the "control" and "combination" diets in the first DASH trial.¹⁵ The combination, or DASH, diet emphasizes fruits and vegetables (total of ≈ 9 servings per day), low-fat dairy products, and other reduced-fat foods. The DASH diet provided 27% of calories from total fat: 6% from saturated fat, 13% monounsaturated fat, and 8% polyunsaturated fat. In contrast, the control diet provided 37% of calories from total fat: 16% saturated fat, 13% monounsaturated fat, and 8% polyunsaturated fat. In addition, the DASH diet provided 151 mg/d of cholesterol, compared with 300 mg/d in the control diet.

Meals were prepared in a metabolic kitchen and served in an outpatient dining facility. Throughout the 14 weeks of feeding, participants agreed to eat only the food provided to them and nothing else. Caloric intake was adjusted to maintain a stable weight.

After attending a series of 3 screening visits to determine eligibility and to collect baseline data, participants began a 2-week run-in feeding period using the control diet at the higher sodium level. Participants were then randomized to the DASH or control diet and also randomized to the sequence of sodium intake. After randomization, there were three 30-day feeding periods, 1 at each of the 3 sodium levels provided in a random order. Sampling of blood occurred at baseline (before randomization) and at the end of each 30-day period.

Measurements

Personnel involved in collection of outcome data were unaware of participants' diet assignment. Adherence to the diet was assessed by reviewing participants' food diaries and by measuring 24-hour urinary excretion of electrolytes and urea nitrogen.

Blood was drawn from the antecubital vein into a Vacutainer tube after an overnight fast and allowed to clot for 15 minutes before being centrifuged at $2000\times g$ for 15 minutes at room temperature. Plasma and serum were placed into 2-mL polyethylene storage containers and quickly frozen in a -70°C freezer until analysis.

CRP was measured from serum by high-sensitivity colorimetric competitive ELISA. In this assay, biotinylated CRP competes with CRP in the sample for coated antibody. Detection is via horseradish peroxidase conjugated in an avidin-biotin complex followed by the color reagent substrate, orthophenylene diamine. Standardization

TABLE 1. Baseline Characteristics of Participants by Diet Assignment

Characteristic	Control Diet (n=50)	DASH Diet (n=50)
Age, y	53 \pm 1.3	50 \pm 1.4
Female, n (%)	21 (42)	31 (62)
Black, n (%)	34 (68)	41 (82)
Smoking, n (%)	6 (12)	6 (12)
Cholesterol, mmol/L*		
Total	5.18 \pm 0.83	5.28 \pm 0.13
LDL	3.28 \pm 0.81	3.39 \pm 0.09
HDL	1.32 \pm 0.40	1.26 \pm 0.04
Triglycerides, mmol/L (IQR)	1.09 (0.87, 1.56)	1.01 (0.78, 1.51)
BMI, kg/m ²	30.1 \pm 0.6	29.3 \pm 0.5
CRP, ng/mL (IQR)	2.78 (1.46, 3.87)	1.74 (1.07, 3.40)

Values are mean \pm SD except for triglycerides and CRP, which are presented as medians (IQR).

*Multiply cholesterol values by 38.7 and triglyceride values by 88.6 to convert to mg/dL.

was done according to the World Health Organization CRP reference standard. The analytical CV for this assay is 5.14%. Total cholesterol, HDL cholesterol (HDL-C), and triglycerides were measured by enzymatic colorimetric methods. LDL-C was calculated.

Analysis

Because the distribution of CRP was right skewed, we present medians and interquartile ranges. Baseline characteristics were compared by Student's *t* test for normally distributed continuous variables (age, cholesterol, body mass index [BMI]), Wilcoxon rank-sum test for non-normally distributed data (CRP, triglycerides), and χ^2 tests for categorical variables (sex, race, smoking status).

Lipids were measured at the end of each sodium treatment period. However, we observed no effect of sodium intake on serum lipids or CRP levels. Hence, the effect of the DASH diet on lipids was assessed independently of sodium intake. Change in lipids was calculated as the difference between baseline and the mean of the 3 end-of-period lipid levels. Changes in lipids were calculated for the entire group and according to baseline levels of CRP (above or below median at baseline). To test for the presence of inflammation-related differences in lipid responsiveness to diet, interaction terms for diet group and CRP were entered into robust multivariate regression

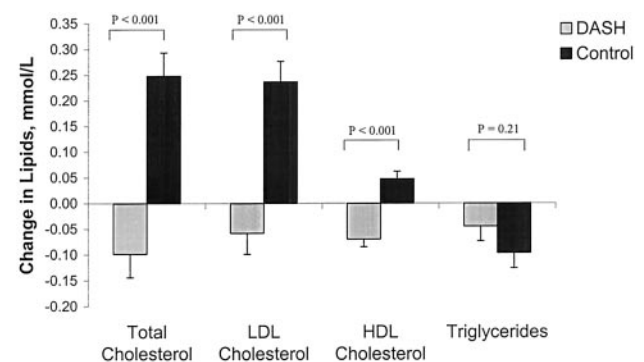


Figure 1. Change from baseline in serum lipids (mmol/L) by randomized diet assignment. Change is difference between baseline and mean of 3 end-of-period values. Gray bars (DASH diet) and black bars (control diet) reflect within-diet change. *P* values correspond to between-diet differences. Multiply cholesterol values by 38.7 and triglycerides by 88.6 to convert to mg/dL.

TABLE 2. Net Change in Serum Lipids (DASH Minus Control) Over the Entire Treatment Period by Baseline Level of CRP

	CRP <Median			CRP >Median			<i>P</i> for Interaction	Adjusted <i>P</i> for Interaction†
	Baseline*	Mean Between Diet Change (% Change)	<i>P</i>	Baseline*	Mean Between Diet Change (% Change)	<i>P</i>		
Cholesterol, mmol/L§								
Total	5.06±0.80	-0.50 (-9.8%)	<0.0001	5.37±0.85	-0.16 (-3.0%)	0.10	0.006	0.016
LDL	3.23±0.65	-0.38 (-11.8%)	<0.0001	3.46±0.80	-0.10 (-3.0%)	0.20	0.009	0.011
HDL	1.29±0.36	-0.11 (-8.8%)	<0.0001	1.29±0.36	-0.09 (-8.8%)	0.01	0.54	0.52
Triglycerides, mmol/L‡								
	1.01 (0.78, 1.53)	+0.01 (+0.001%)	0.95	1.04 (0.88, 1.42)	+0.21 (+19.8%)	<0.0001	0.019	<0.003

Change is the difference between baseline and the mean of the 3 end-of-period values.

*Baseline values are mean±SD except for triglycerides, which are medians (interquartile range).

†Adjusted for baseline lipid level, age, race, sex, smoking, and BMI.

‡Changes are medians (median % change).

§To convert to mg/dL, multiply cholesterol values by 38.7 and triglyceride values by 88.6.

analyses. Additional adjustment was made for age, sex, race (African-American versus non-African-American), smoking status (current, ever, never), and BMI. The continuous relationship between change in lipids and baseline CRP was examined by entering log-transformed CRP as a continuous interaction term with diet in multivariate models. Because of sample size considerations, we could not reliably assess higher order interactions.

Linear regression analysis was used to assess change in serum lipid levels, except for triglycerides, for which median regression was used because of its right-skewed distribution. Change in CRP was also assessed by median regression. All models were adjusted simultaneously for baseline values of each outcome variable. All analyses were conducted according to the principle of intention to treat. All tests were 2-sided and were performed with STATA 7.0 statistical software.

Results

The mean age of participants was 52±9.9 years. Participants included 52 women and 75 African-Americans, with a mean BMI of 29.6 kg/m². There were no significant differences between diet groups at baseline (Table 1).

The DASH diet resulted in significant (*P*<0.001) reductions in total cholesterol (-0.34 mmol/L), LDL-C (-0.29 mmol/L), and HDL-C (-0.12 mmol/L) levels (Figure 1). Triglyceride levels were not changed significantly with the DASH diet (+0.05 mmol/L, *P*=0.21). These findings are consistent with results from the initial DASH trial.¹⁶ Median changes in CRP were similar in the control and DASH diets (-0.12 versus +0.02 mg/L, *P*=0.50).

Table 2 illustrates changes in lipids from the DASH diet, net of control, stratified by baseline level of CRP (below versus above median). In persons with baseline CRP levels below the median (<2.37 mg/L), the DASH diet significantly reduced total cholesterol (0.5 mmol/L [9.8%], *P*<0.0001) and LDL-C (0.38 mmol/L [11.8%], *P*<0.0001) levels. In persons with a baseline CRP above median, reductions in total cholesterol and LDL-C were modest and not significant (0.16 mmol/L [3%] and 0.10 mmol/L [3%], respectively, *P*≥0.10). Reductions in HDL-C from the DASH diet, net of control, were similar in persons with low and high baseline CRP levels (0.11 mmol/L [8.8%] and 0.09 mmol/L [8.8%], respectively, *P*≤0.01). In persons with low baseline CRP, the DASH diet had no significant effect on triglycerides (+0.01 mmol/L, *P*=0.95). However, a significant increase in

triglycerides associated with the DASH diet (0.21 mmol/L [19.8%], *P*<0.0001) was observed among persons with a high baseline CRP. Tests for interaction between diet and baseline CRP were significant for total cholesterol (*P*=0.006), LDL-C (*P*=0.009), and triglycerides (*P*=0.019) but not HDL-C (*P*=0.54). These tests for interaction remained significant after adjustment for age, race, sex, smoking, and BMI (*P*=0.016, *P*=0.011, and *P*<0.003, respectively). Evidence of a statistical interaction between diet and baseline CRP on lipid responsiveness persisted after entering log CRP as a continuous variable in fully adjusted multivariate regression models (*P* for interaction=0.001 for total cholesterol, 0.002 for LDL-C, and 0.056 for triglycerides).

In persons with low CRP, differences in lipid responses to the DASH diet by baseline CRP were evident by 4 weeks and persisted over time (Figure 2). Median triglyceride levels tended to increase; however, triglyceride measurements were less precise than corresponding cholesterol measurements.

Discussion

Our findings suggest that inflammation significantly and substantially affects the lipid response to a reduced-fat/low-cholesterol diet. In this study, the greatest degree of lipid reduction was seen in persons with low CRP. Conversely, the increase in triglycerides that was expected with greater consumption of carbohydrates occurred only in persons with elevated CRP.

Most circulating cholesterol is the result of endogenous hepatic synthesis. In animal studies, interleukin 6, a potent stimulator of CRP production, inhibits lipoprotein lipase activity in adipocytes¹⁷ and induces hepatic triglyceride secretion.¹⁸ In humans, interleukin 6 may be responsible for the lipid abnormalities found in the insulin-resistance syndrome.¹⁹

Despite substantial differences in nutrient composition, the DASH diet, net of control, had no significant effect on CRP levels. This finding is in contrast with epidemiological studies showing that diets higher in fiber or the consumption of foods with a lower glycemic index could reduce CRP levels.^{20,21} Our findings suggest that previous associations of diet and CRP could be confounded by other unmeasured factors or could be the result of residual confounding from other

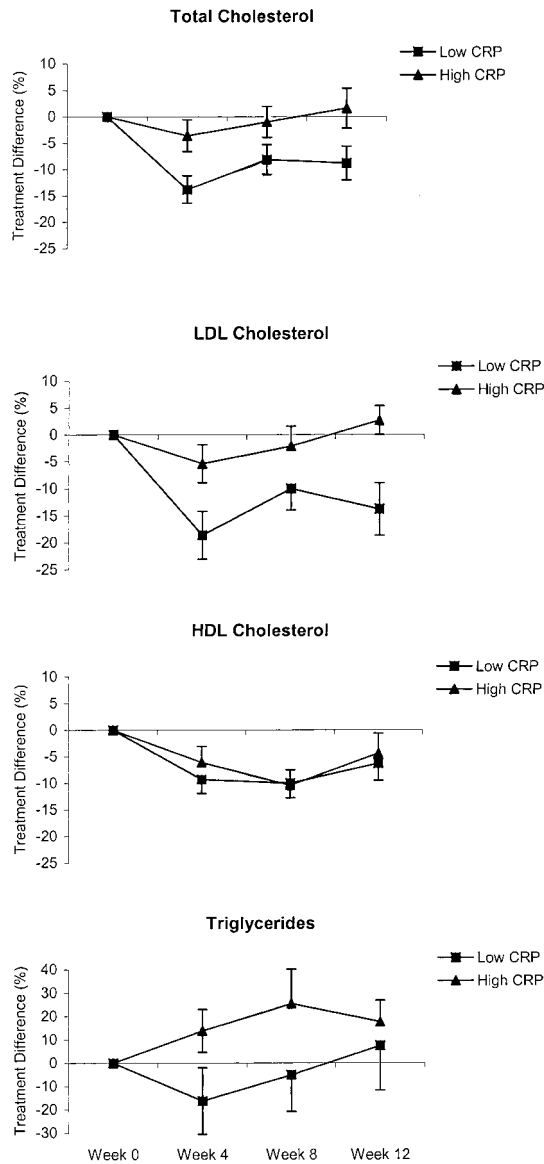


Figure 2. Change from baseline at weeks 4, 8, and 12 in blood lipids in persons with low and high baseline CRP level.

potential determinants of CRP, such as weight change.^{22,23} However, we cannot rule out the possibility that our study was underpowered to detect a small effect of diet on CRP. Overall, we had 80% power to detect a 25% change in CRP.

These findings have both clinical and scientific implications. Specifically, a reduced-fat/low-cholesterol diet that is relatively higher in carbohydrates may be extremely beneficial for persons with low levels of inflammation and could thereby mitigate the need for pharmacological therapy. In contrast, among persons with higher levels of inflammation, such diet changes might increase triglycerides and reduce HDL-C. This apparently adverse pattern of changes in triglycerides and HDL-C commonly occurs in the setting of a reduced-fat/high-carbohydrate diet. Our data suggest that inflammation is at least a marker, if not potentially a determinant, of this adverse response. Perhaps the most important implication of our findings is the use of CRP as a

means to distinguish those individuals who are likely to experience a favorable response to reduced-fat/low-cholesterol diet from those who are likely to experience an unfavorable response. In addition, these findings have important implications for the analysis and interpretation of studies examining the relationship between diet and lipids and could partially account for the considerable variability in lipid responsiveness in the literature. For example, previous studies have demonstrated less cholesterol reduction from a low-fat diet among women¹⁶ and overweight individuals.^{24–26} Additional studies are needed to determine whether inflammation could account for these observed subgroup differences.

Although we observed highly significant lipid changes in subgroups and significant interactions between subgroups, we cannot rule out the possibility of a spurious finding, ie, type I error. However, as discussed previously, there is a reasonable biological basis for postulating an interaction between diet and inflammation. Clearly, additional studies would be useful both to confirm the interaction and to better assess the point at which inflammation attenuates the beneficial effects of dietary change. Because of sample size considerations, we used median CRP as the cut point in this study.

In summary, our study suggests that inflammation modifies the effects of the DASH diet on serum lipid levels. These findings could have important implications for targeting individuals who are most likely to respond favorably to dietary changes.

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