Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study[1–3]

David J Baer, Joseph T Judd, Beverly A Clevidence, and Russell P Tracy

ABSTRACT

Background: The effect of individual dietary fatty acids on emerging risk factors for cardiovascular disease that are associated with subclinical inflammation is unknown.

Objective: The goal was to evaluate the role of dietary fat and specific fatty acids, especially *trans* fatty acids, in altering concentrations of markers of inflammation in humans fed controlled diets.

Design: In a randomized crossover design, 50 men consumed controlled diets for 5 wk that provided 15% of energy from protein, 39% of energy from fat, and 46% of energy from carbohydrate. Eight percent of fat or fatty acids was replaced across diets with the following: cholesterol, oleic acid, *trans* fatty acids (TFAs), stearic acid (STE), TFA+STE (4% of energy each), and 12:0–16:0 saturated fatty acids (LMP).

Results: Fibrinogen concentrations were higher after consumption of the diet enriched in stearic acid than after consumption of the carbohydrate diet. C-reactive protein concentrations were higher after consumption of the TFA diet than after consumption of the carbohydrate diet, but were not significantly different after consumption of the TFA and TFA+STE diets than after consumption of the LMP diet. Interleukin 6 concentrations were lower after consumption of the oleic acid diet than after consumption of the LMP, TFA, and STE diets. E-selectin concentrations were higher after consumption of the TFA diet than after consumption of the carbohydrate diet. Consumption of the TFA but not the TFA+STE diet resulted in higher E-selectin concentrations than did the LMP diet.

Conclusions: These data provide evidence that dietary fatty acids can modulate markers of inflammation. Although stearic acid minimally affects LDL cholesterol, it does appear to increase fibrinogen concentrations. *Am J Clin Nutr* 2004;79:969–73.

KEY WORDS Inflammation, *trans* fatty acids, dietary fat, interleukins, cell adhesion molecules

INTRODUCTION

During the past decade, a body of evidence has been compiled which suggests that markers of inflammation are strong predictors of cardiovascular disease (eg, stroke, peripheral artery disease, and myocardial infarction) (1–9). These markers include proinflammatory cytokines [eg, interleukin 6 (IL-6)], adhesion molecules (eg, selectins), and acute phase proteins [eg, C-reactive protein (CRP)]. Several genetic and environmental factors that affect low-level systemic inflammation have been established (10–13); however, few data describe the effect of diet composition on subclinical inflammation (14–16).

In this report, we describe the effect of different dietary fatty acids on markers of inflammation. We previously reported the effect of these diets on lipids and lipoproteins (17). Of particular concern is the effect of dietary *trans* fatty acids on markers of inflammation compared with that of saturated fatty acids, because the hypercholesterolemic *trans* fatty acids have been implicated in increasing the risk of cardiovascular disease to an extant equal to or greater than the hypercholesterolemic saturated fatty acids (17). A second concern is the effect of stearic acid compared with that of other saturated fatty acids given its "neutral" effect on plasma LDL-cholesterol concentrations compared with that of the hypercholesterolemic saturated fatty acids. A third issue is whether lower-fat diets reduce the concentration of markers of inflammation compared with higher-fat diets containing fatty acids (eg, oleic acid) that are associated with reduced risk of cardiovascular disease. This study is among the first controlled dietary interventions to investigate the effect of dietary fats and fatty acids in modulating these emerging risk factors associated with inflammation.

SUBJECTS AND METHODS

Details of the study design were previously reported (17). Fifty adult male volunteers completed a controlled feeding study conducted at the Beltsville Human Nutrition Research Center in Beltsville, MD. Participants consumed 6 diets for 5 wk each for a total controlled feeding period of 30 wk in a randomized crossover design. During the 5th week of each period, replicate blood samples were collected on 2 d, separated by ≥24 h.

Volunteers were recruited by advertisement in the area of the US Department of Agriculture's Beltsville Agricultural Research Center. Men of all races between the ages of 25 and 60 y were recruited. From 207 respondents, 58 met the eligibility

[1] From the Diet and Human Performance Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture, Beltsville, MD (DJB, JTJ, and BAC), and the Departments of Pathology and Biochemistry, the University of Vermont, College of Medicine, Burlington, VT (RPT).

[2] Supported in part through a research agreement between the Agricultural Research Service, US Department of Agriculture, and the Technical Committee on Dietary Lipids, International Life Sciences Institute, NA, Washington, DC.

[3] Address reprint requests to DJ Baer, USDA, ARS, BARC-East, Beltsville Human Nutrition Research Center, Building 308, Room 218, Beltsville, MD 20705. E-mail: baer@bhnrc.arsusda.gov.

Received July 3, 2003.

Accepted for publication November 4, 2003.

TABLE 1
Baseline characteristics of the men who consumed all treatments[1]

	Value
Body weight (kg)	83.1 ± 1.7
BMI (kg/m^2)	26.2 ± 0.54
Triacylglycerols (mmol/L)	1.149 ± 0.081
Total cholesterol (mmol/L)	4.768 ± 0.092
LDL cholesterol (mmol/L)	3.082 ± 0.072
HDL cholesterol (mmol/L)	1.160 ± 0.038

[1] All values are $\bar{x}$ ± SEM; $n = 50$. The subjects' mean age was 42 y.

criteria described below and were selected to enter the study. Four of those selected did not participate in the feeding protocol. Of the 54 volunteers who started the protocol, 50 completed all 6 dietary treatments, and only data from these 50 participants are included in this report.

Eligibility criteria were based on general health, eating habits, age, body mass index, fasting plasma LDL cholesterol, HDL cholesterol, and triacylglycerol concentrations. Volunteers who reported taking lipid-lowering drugs, blood pressure medications, or dietary supplements or who had eating habits inconsistent with the study protocol were excluded. Volunteers were evaluated by a physician and were determined to be in good health with no signs or symptoms of hypertension, hyperlipidemia, diabetes, peripheral vascular disease, gout, liver or kidney disease, or endocrine disorders. Subjects selected for the study were required to have fasting plasma HDL-cholesterol concentrations >0.65 mmol/L (25 mg/dL), to have triacylglycerol concentrations <3.39 mmol/L (300 mg/dL), and to be within 85–120% of their sex-specific ideal body mass index. The baseline characteristics of the 50 participants who completed all diets are presented in **Table 1**.

Smoking and exercise habits were not selection criteria nor were they controlled during the study, but volunteers who reported participating in significant physical activities (eg, runners and weightlifters) were not selected. The subjects were encouraged to maintain their normal exercise patterns (type, duration, and frequency) throughout the study and were required to report departures from their normal pattern of exercise. Of the 50 subjects who completed the study, 3 were smokers.

Volunteers were required to read and sign a consent form detailing the study objectives and the risks and benefits before their final selection as subjects. The participants received monetary compensation commensurate with the effort required of them for the study. All procedures and payments were approved by the Johns Hopkins University Bloomberg School of Public Health's Committee on Human Research.

Data from the US Department of Agriculture (18) and analyzed values for foods were used to formulate the controlled diets. Five diets were formulated to contain 38.9% of energy from fat, 15% of energy from protein, and 46.1% of energy from digestible carbohydrates. A sixth diet was formulated to contain 30.4% of energy from fat and 54.6% of energy from carbohydrate. The range in fat concentration in the diets was selected to include the average reported fat intake, 34% of energy, for men in the United States (19). Thus, with a reported intake of 34% of energy from fat, the 5 diets varying in fatty acid content had 38.9% of energy from fat, whereas the carbohydrate diet had 30.4% of energy from fat. The 6 diets were formulated to vary by 8% of energy as follows: *1*) carbohydrate (CHO) diet, 8.5% of energy from fat replaced by digestible carbohydrate (approximately equivalent to a reduction in fatty acids of 8% of energy); *2*) oleic acid (OL) diet, 8% of energy enrichment with oleic acid; *3*) LMP diet, 8% of energy enrichment with saturated fatty acids as the sum of lauric (L), myristic (M), and palmitic (P) acids (ratio of L to M to P of 0.3:1.4:8.3); *4*) stearic acid (STE) diet, 8% of energy enrichment with stearic acid; *5*) *trans* fatty acid (TFA) diet, 8% of energy enrichment in TFAs (spectrum of *trans* 18:1 positional isomers similar to that in the US food supply); and *6*) TFA+STE diet, a combined enrichment with 4% of energy from TFAs and 4% of energy from stearic acid. Fatty acid substitutions were accomplished by providing the fatty acids as triacylglycerol (and a small amount of naturally occurring monoacyglycerols and diacylglycerols) from a variety of fat sources. Dietary cholesterol was constant at 31.1 mg $\cdot$ MJ^{-1} $\cdot$ d^{-1} across diets. Complete diet samples were collected and a composite sample was prepared that represented each day of the 7-d menu cycle. The composite samples were analyzed for protein, fat, and fatty acid composition (Corning Hazleton Inc, Madison WI). Other details about the fat sources and composition were previously described (17).

Monday through Friday, the subjects consumed breakfast and dinner at the Beltsville Human Nutrition Research Center under the supervision of a dietitian. Each morning, each subject was provided a carryout lunch for the day. Weekend meals were packaged with written instructions for home consumption and were provided to the subjects after dinner on Friday. Coffee and tea were allowed in unlimited amounts, but all additives (sugar and milk) were provided in measured quantities with the meals. Only foods provided by the study investigators were allowed to be consumed during the study.

Monday through Friday, the subjects were weighed when they arrived at the facility each morning. Energy intake was adjusted in 1.67-MJ (400-kcal) increments to maintain initial body weight. Subjects were fed the same items and the same proportions of each item relative to total dietary energy. Therefore, across subjects, the relative amounts of all nutrients were constant and directly proportional to the energy required to maintain weight. Each day, the subjects completed a questionnaire detailing beverage intake, factors related to dietary compliance, exercise, medications, and illnesses, and questions or problems with the diets. Questionnaires were routinely reviewed by a study investigator, and any problems identified were discussed with the subject during the next scheduled meal.

During the fifth week of each period, 2 blood samples were drawn after the subjects had fasted overnight. Blood was collected by venipuncture into evacuated tubes containing citrate and was promptly placed on wet ice. Within 30 min of collection, plasma was separated by centrifugation at 1400 × *g* for 20 min at 4 °C. Plasma was harvested from the tubes, divided into aliquots, and stored in cryogenic vials at −80 °C. All analyses for an analyte of samples from one subject were performed in the same analytic run.

Fibrinogen was measured in an automated clot assay by using an ST4 instrument (Diagnostica Stago, Parsippany, NJ) (20). The analytic CV for this assay was 1.7%. CRP was measured by use of a colorimetric competitive immunoassay using rabbit anti-CRP as the capture antibody (21). The CV for this assay was 5.1%. IL-6 was measured by an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis). The routine CV in the lab is 6.3%. E-selectin (endothelial leukocyte

TABLE 2
Average daily energy and nutrient intakes of the men who consumed all treatments[1]

	Diet					
Nutrients	CHO	OL	TFA	TFA + STE	STE	LMP
Energy (MJ/d)	12.92 ± 0.24	13.09 ± 0.23	13.05 ± 0.24	12.97 ± 0.24	13.07 ± 0.25	13.10 ± 0.25
Protein (g/d)	116 ± 2.2	118 ± 2.1	116 ± 2.2	115 ± 2.2	116 ± 2.2	117 ± 2.2
Digestible carbohydrate (g/d)	421 ± 7.9	364 ± 6.4	356 ± 6.7	353 ± 6.6	353 ± 6.7	355 ± 6.7
Fat (g/d)	105 ± 2.0	133 ± 2.3	137 ± 2.6	136 ± 2.6	138 ± 2.6	138 ± 2.6
Fatty acids (g/d)						
Sum L + M + P	33.6 ± 0.64	33.7 ± 0.60	35.0 ± 0.65	34.4 ± 0.65	34.7 ± 0.66	62.6 ± 1.18
Stearic acid	10.0 ± 0.19	10.1 ± 0.18	9.7 ± 0.18	23.8 ± 0.45	37.8 ± 0.72	9.4 ± 0.18
Oleic acid (*cis* 18:1)	36.0 ± 0.68	61.2 ± 1.08	36.7 ± 0.69	36.5 ± 0.68	36.4 ± 0.69	36.5 ± 0.69
trans 18:1	0.7 ± 0.01	0.3 ± 0.01	28.8 ± 0.54	14.5 ± 0.27	1.0 ± 0.02	0.7 ± 0.01
Linoleic acid	13.0 ± 0.25	13.2 ± 0.23	13.9 ± 0.26	14.8 ± 0.28	15.3 ± 0.29	14.6 ± 0.28

[1] All values are $\bar{x}$ ± SEM; $n = 50$. The experimental diets were enriched by 8% of energy compared with the control carbohydrate (CHO) diet as follows: OL, enriched with oleic acid; TFA, enriched with *trans* fatty acids; TFA + STE, enriched with *trans* fatty acids and stearic acid; STE, enriched with stearic acid; LMP, enriched with 12:0–16:0 saturated fatty acids.

adhesion molecule 1, or ELAM-1 CD62E) was measured by using a commercial kit (R&D Systems). The CV for this assay was 4.0%.

The subjects were blinded to the dietary treatments. Although the study participants could recognize differences in appearance, taste, and other characteristics of the food, they were unaware of the overall nutrient and fatty acid profiles of the diets. All samples were coded to blind the analysts to the treatments.

All statistical analyses were performed by using SAS (SAS Institute Inc, Cary, NC) for WINDOWS (version 6.11) or S-PLUS (Mathsoft Engineering & Education, Cambridge, MA). The analytic plan was designed a priori and described a mixed-effects model for analysis of the data. For each variable, the average of 2 sample measurements taken during week 5 of each feeding period was analyzed by using an analysis of variance model that included terms for diet, period, subject, and carryover of a diet from one period to the next. The data were analyzed with the 3 smokers included and excluded and the results were not significantly different. The data presented include the data of the smokers. The least significant difference for the study was set at $P \leq 0.05$. Mean differences between pairs of diets were adjusted for carryover and period effects. Contrasts between pairs of diets and between the average of the 5 diets providing 39% of energy from fat compared with the CHO diet were tested by use of an F test for differences between groups, and the P values were adjusted by using Tukey's procedure for multiple mean comparisons. Data for IL-6 and CRP and were log transformed to correct for lack of heterogeneity of variance.

RESULTS

Subject compliance in consumption of the controlled diets was judged to be excellent based on observed consumption of the meals in the dining room of the Human Study Facility, review of responses to the daily questionnaires, weight maintenance at stable energy intakes over 30 wk, and frequent interviews with the subjects throughout the study. No adverse effects due to consumption of the diets were observed or reported. Average energy intake varied from 12.92 to 13.10 MJ/d (**Table 2**). The range in average energy intake across diets was small, 1.3% of the mean of all diets. Intake of nutrients was calculated from a subject's daily energy intake and the analyzed composition of the composited diets (Table 2).

E-selectin concentrations were 5.6% higher after consumption of the TFA diet than after consumption of the LMP diet (**Table 3**). However, there was no significant difference between these 2 diets with respect to IL-6, CRP, and fibrinogen concentrations. CRP and E-selectin concentrations were higher but fibrinogen and IL-6 concentrations were not significantly different after consumption of the TFA diet than after the CHO diet. There was no significant difference in CRP concentration after consumption of the TFA+STE diet compared with the CHO or LMP diet.

Consumption of the diet enriched in stearic acid resulted in higher concentrations of fibrinogen than did consumption of all other diets except the TFA+STE diet. Compared with the CHO diet, fibrinogen concentrations were 4.4% higher after consumption of the STE diet.

There were no significant differences in any of the markers of inflammation between the CHO and the OL diet, although all markers tended to be lower after consumption of the OL diet. Furthermore, there was no significant difference between the CHO diet and the average of the other 5 diets.

DISCUSSION

Markers of inflammation and hemostasis have been implicated as risk factors for several degenerative diseases. With respect to cardiovascular diseases, the data collected from cross-sectional and prospective studies provide evidence that some markers are independent risk factors, and several markers may be modifiable through lifestyle, including exercise, smoking, and diet. The present study is among the first controlled dietary intervention studies to show that specific fatty acids can modulate markers of inflammation.

CRP is increasingly noted to be an independent risk factor for cardiovascular disease. In the present study, we showed that a high intake of *trans* fatty acids increases the concentration of CRP. This effect, in addition to the LDL-cholesterol–raising effect of *trans* fatty acids, may contribute to the increased risk of cardiovascular disease suggested by epidemiologic data (22). However, *trans* fatty acid consumption in the present study was high (8% of energy), and at more typical intakes, such as in the

TABLE 3
Concentrations of plasma markers of inflammation after 5 wk of consumption of each diet[1]

	Fibrinogen			C-reactive protein			Interleukin 6			E-selectin		
Diet	LS mean	Median	Interquartile range	LS mean	Median	Interquartile range	LS mean	Median	Interquartile range	LS mean	Median	Interquartile range
	g/L	*g/L*	*g/L*	ln (*mg/L*)	*mg/L*	*mg/L*	ln (*pg/mL*)	*pg/mL*	*pg/mL*	*ng/mL*	*ng/mL*	*ng/mL*
CHO	2.74[a]	2.61	2.28–3.12	0.07[a]	1.06	0.65–1.62	0.41[a,b]	1.43	0.88–2.15	40.5[a,b]	38.8	29.3–49.9
OL	2.71[a]	2.58	2.20–3.27	0.05[a]	0.97	0.64–1.60	0.31[a]	1.36	0.79–2.20	38.8[a]	37.9	28.6–48.0
TFA	2.74[a]	2.58	2.20–3.26	0.24[b]	1.04	0.66–2.07	0.46[b]	1.55	0.83–2.49	44.4[c]	42.4	33.3–53.7
TFA + STE	2.75[a,b]	2.58	2.33–3.08	0.05[a]	0.95	0.65–1.64	0.44[a,b]	1.57	0.95–2.46	41.6[b]	40.4	29.2–51.0
STE	2.86[b]	2.73	2.38–3.28	0.13[a,b]	0.98	0.64–1.69	0.48[b]	1.56	0.93–2.43	42.0[b]	38.2	30.7–49.8
LMP	2.68[a]	2.53	2.23–3.00	0.14[a,b]	1.02	0.70–1.75	0.46[b]	1.56	0.88–2.56	41.9[b]	41.1	29.0–51.5
SEE	0.08			0.09			0.10			2.0		

[1] LS, least squares. The experimental diets were enriched by 8% of energy compared with the control carbohydrate (CHO) diet as follows: OL, enriched with oleic acid; TFA, enriched with *trans* fatty acids; TFA + STE, enriched with *trans* fatty acids and stearic acid; STE, enriched with stearic acid; LMP, enriched with 12:0–16:0 saturated fatty acids. LS (adjusted) means in the same column with different superscript letters are significantly different, $P < 0.05$.

TFA+STE diet (4% of energy), they may not increase CRP relative to other fatty acids, including hypercholesterolemic saturated fatty acids.

In a meta-analysis of 22 studies, plasma fibrinogen was identified as an independent risk factor for cardiovascular disease (23). van der Bom et al (24) suggested that a 1-g/L increase in plasma fibrinogen is associated with a 45% increased risk of myocardial infarction, but that individuals with certain genetic polymorphisms that increase plasma fibrinogen may not have an increased risk of myocardial infarction. On the basis of these projections, the 0.15-g/L change in fibrinogen observed after consumption of the STE diet compared with the CHO diet could raise the risk of myocardial infarction by 7%.

The greatest effect of a fatty acid on fibrinogen in the current study was the stearic acid–induced increase in the circulating concentration of fibrinogen. After the TFA diet, fibrinogen concentrations were not significantly different than after consumption of the LMP, CHO, or OL diet. Because the fibrinogen concentration increased after consumption of the TFA+STE diet, it appears that the change elicited by the TFA+STE diet may have been a consequence of the stearic acid in this diet. These results are consistent with comparisons of diets high in stearic acid and diets high in lauric and myristic acids. In one study, fibrinogen increased after consumption of a diet rich in stearic acid compared with a diet with increased amounts of lauric and myristic acids (25). Although the amount of stearic acid in the STE diet was considerably higher than is typically consumed in the United States, these data provide evidence for increased inflammatory response at more moderate intakes (eg, the TFA+STE diet). The effect of stearic acid on LDL-cholesterol concentrations is often thought to be "neutral"; however, our study showed that stearic acid may increase the risk of cardiovascular disease through mechanisms other than cholesterol concentrations, such as an increase in fibrinogen concentrations.

Diet-induced changes in 2 acute phase reactants, CRP and fibrinogen, may be related to diet-induced changes in IL-6. Studies have shown that increased concentrations of IL-6 are associated with increased risk of myocardial infarction in clinically healthy individuals (2). Consumption of the TFA, STE, and LMP diets in the present study increased CRP, fibrinogen, and IL-6 concentrations. The changes observed in the plasma concentrations of these clinically important inflammatory markers are consistent with the effect of *trans* fatty acids observed ex vivo (26).

Adhesion molecules play a role in the recruitment of leukocytes to endothelial sites of inflammation. Despite the limited evidence that these molecules are important prognostic indicators of coronary artery disease, several lines of evidence suggest that these molecules are important in the etiology of atherosclerotic lesions (6, 16). In the current study, E-selectin concentrations were higher after consumption of the diets enriched with *trans* and saturated fatty acids, which are associated with an increased risk of coronary artery disease, than after the oleic acid–enriched diet. The changes in E-selectin concentrations paralleled the changes in the other markers of inflammation that were analyzed. Thus, the results from our study indicate that, in addition to their hypercholesterolemic effects, *trans* and saturated fatty acids might exacerbate the risk of cardiovascular disease as a result of their effects on adhesion molecules.

In this carefully controlled dietary intervention study, diets enriched in different fatty acids modulated the concentration of proinflammatory cytokines and markers of inflammation. These data suggest that dietary fatty acids play an important role in the modulation of coronary artery disease risk above and beyond that associated with changes in LDL cholesterol. This observation is especially true for stearic acid, which is not hypercholesterolemic compared with other saturated fatty acids. Consumption of stearic acid did result in an increase in circulating IL-6 and fibrinogen. Moreover, limited pathologic data from animal studies suggest that *trans* fatty acids are hypercholesterolemic but not atherogenic (27-29). The results of the present study suggest that consumption of *trans* and saturated fatty acids might be associated with increased risk of coronary artery disease in humans as the result of effects on proinflammatory cytokines, acute phase proteins, and adhesion molecules. However, there may be a threshold intake of *trans* fatty acids needed to increase inflammation. Furthermore, with respect to the markers of inflammation measured in this study, there was no quantitative difference between the control diet and the higher-fat, oleic acid–enriched diet. This finding suggests an additional means by which dietary patterns that are high in oleic acid (eg, Mediterranean diets) may result in a lower incidence of coronary artery disease.

Notwithstanding these findings, questions remain about the role of dietary modulation of markers of inflammation. First, do changes in markers of inflammation reflect changes in inflammation itself? That is, is it possible that diet influences the metabolism of proinflammatory cytokines, such as IL-6, without a concomitant increase in the inflammatory process? Although this possibility exists, given that the diets that increased IL-6 also increased CRP or fibrinogen, it appears that a larger inflammatory process may be activated. Second, what is the biological significance of these small diet-induced changes after 5 wk? Although small changes in CRP in cross-sectional studies are predictive of future cardiovascular events, these are changes that presumably occur over a long period (albeit including those effects from long-term dietary impact). Do the short-term changes that can be measured in controlled dietary manipulations have biological significance equivalent to the changes documented in cross-sectional or prospective studies? Third, what time frame is appropriate for measuring these responses in controlled studies? Although these questions remain to be fully answered, understanding the role of dietary modulation of subclinical inflammation may be important in unraveling linkages between diet and the risk of not only cardiovascular disease but also other inflammatory diseases.

DJB, JTJ, and BAC were involved in study design, data collection, and data analysis. DJB and JTJ were involved in writing the manuscript. RPT was involved in data collection and data analysis. None of the authors had any financial or personal interest in any organization sponsoring this research project.

REFERENCES

1. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med 2002;252:283–94.
2. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767–72.
3. Pradhan AD, Rifai N, Ridker PM. Soluble intercellular adhesion molecule-1, soluble vascular adhesion molecule-1, and the development of symptomatic peripheral arterial disease in men. Circulation 2002;106: 820–5.
4. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. Circ Res 2001;89:763–71.
5. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998;97:2007–11.
6. Malik I, Danesh J, Whincup P, et al. Soluble adhesion molecules and prediction of coronary heart disease: a prospective study and meta-analysis. Lancet 2001;358:971–6.
7. Blankenberg S, Rupprecht HJ, Bickel C, et al. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation 2001;104:1336–42.
8. Hwang SJ, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. Circulation 1997;96:4219–25.
9. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. Circulation 1998;97:425–8.
10. Barinas-Mitchell E, Cushman M, Meilahn EN, Tracy RP, Kuller LH. Serum levels of C-reactive protein are associated with obesity, weight gain, and hormone replacement therapy in healthy postmenopausal women. Am J Epidemiol 2001;153:1094–101.
11. Tracy RP, Psaty BM, Macy E, et al. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. Arterioscler Thromb Vasc Biol 1997;17:2167–76.
12. Laimer M, Ebenbichler CF, Kaser S, et al. Markers of chronic inflammation and obesity: a prospective study on the reversibility of this association in middle-aged women undergoing weight loss by surgical intervention. Int J Obes Relat Metab Disord 2002;26:659–62.
13. Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation 2002;105:564–9.
14. Liu S, Manson JE, Buring JE, Stampfer MJ, Willett WC, Ridker PM. Relation between a diet with a high glycemic load and plasma concentrations of high-sensitivity C-reactive protein in middle-aged women. Am J Clin Nutr 2002;75:492–8.
15. Jenkins DJ, Kendall CW, Connelly PW, et al. Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. Metabolism 2002;51:919–24.
16. Ridker PM. Role of inflammatory biomarkers in prediction of coronary heart disease. Lancet 2001;358:946–8.
17. Judd JT, Baer DJ, Clevidence BA, Kris-Etherton P, Muesing RA, Iwane M. Dietary *cis* and *trans* monounsaturated and saturated FA and plasma lipids and lipoproteins in men. Lipids 2002;37:123–31.
18. US Department of Agriculture, Human Nutrition Information Service. Composition of foods. Agriculture handbook no. 8, sections 1-22. Washington, DC: US Government Printing Office, 1976-1990.
19. US Department of Agriculture, Agricultural Research Service. 1997. Data tables: Results from USDA's 1996 Continuing Survey of Food Intakes by Individuals and 1996 Diet and Health Knowledge Survey. ARS Food Surveys Research Group. Internet (under "Releases"): http://www.barc.usda.gov/bhnrc/foodsurvey/home.htm (accessed April 23, 2003).
20. Geffken DF, Keating FG, Kennedy MH, Cornell ES, Bovill EG, Tracy RP. The measurement of fibrinogen in population-based research. Studies on instrumentation and methodology. Arch Pathol Lab Med 1994; 118:1106–9.
21. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997;43:52–8.
22. Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC. *Trans*-fatty acids intake and risk of myocardial infarction. Circulation 1994;89:94–101.
23. Maresca G, Di Blasio A, Marchioli R, Di Minno G. Measuring plasma fibrinogen to predict stroke and myocardial infarction: an update. Arterioscler Thromb Vasc Biol 1999;19:1368–77.
24. van der Bom JG, de Maat MP, Bots ML, et al. Elevated plasma fibrinogen: cause or consequence of cardiovascular disease? Arterioscler Thromb Vasc Biol 1998;18:621–5.
25. Bladbjerg EM, Tholstrup T, Marckmann P, Sandstrom B, Jespersen J. Dietary changes in fasting levels of factor VII coagulant activity (FVII:C) are accompanied by changes in factor VII protein and other vitamin K-dependent proteins. Thromb Haemost 1995;73:239–42.
26. Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN. Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. J Lipid Res 2002;43:445–52.
27. Kritchevsky D. *Trans* fatty acid effects in experimental atherosclerosis. Fed Proc 1982;41:2813–7.
28. Ruttenberg H, Davidson LM, Little NA, Klurfeld DM, Kritchevsky D. Influence of *trans* unsaturated fats on experimental atherosclerosis in rabbits. J Nutr 1983;113:835–44.
29. Jackson RL, Morrisett JD, Pownall HJ, et al. Influence of dietary *trans*-fatty acids on swine lipoprotein composition and structure. J Lipid Res 1977;18:182–90.