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The Type and Amount of Dietary Fat Affect Plasma Factor VIIc, Fibrinogen, and PAI-1 in Healthy Individuals and Individuals at High Cardiovascular Disease Risk: 2 Randomized Controlled Trials

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ABSTRACT

Background: Factor VIIc, fibrinogen, and plasminogen activator inhibitor 1 (PAI-1) are cardiovascular disease (CVD) risk factors and are modulated, in part, by fat type and amount.

Objective: We evaluated fat type and amount on the primary outcomes: factor VIIc, fibrinogen, and PAI-1.

Methods: In the Dietary Effects on Lipoproteins and Thrombogenic Activity (DELTA) Trial, 2 controlled crossover feeding studies evaluated substituting carbohydrate or MUFAs for SFAs. Study 1: healthy participants ($n = 103$) were provided with (8 wk) an average American diet [AAD; designed to provide 37% of energy (%E) as fat, 16% SFA], a Step 1 diet (30%E fat, 9% SFA), and a diet low in SFA (Low-Sat; 26%E fat, 5% SFA). Study 2: participants ($n = 85$) at risk for CVD and metabolic syndrome (MetSyn) were provided with (7 wk) an AAD, a step 1 diet, and a high-MUFA diet (designed to provide 37%E fat, 8% SFA, 22% MUFA).

Results: Study 1: compared with AAD, the Step 1 and Low-Sat diets decreased mean factor VIIc by 1.8% and 2.6% (overall $P = 0.0001$), increased mean fibrinogen by 1.2% and 2.8% ($P = 0.0141$), and increased mean square root PAI-1 by 0.0% and 6.0% ($P = 0.0037$), respectively. Study 2: compared with AAD, the Step 1 and high-MUFA diets decreased mean factor VIIc by 4.1% and 3.2% (overall $P < 0.0001$), increased mean fibrinogen by 3.9% and 1.5% ($P = 0.0083$), and increased mean square-root PAI-1 by 2.0% and 5.8% ($P = 0.1319$), respectively.

Conclusions: Replacing SFA with carbohydrate decreased factor VIIc and increased fibrinogen in healthy and metabolically unhealthy individuals and also increased PAI-1 in healthy subjects. Replacing SFA with MUFA decreased factor VIIc and increased fibrinogen but less than carbohydrate. Our results indicate an uncertain effect of replacing SFA with carbohydrate or MUFA on cardiometabolic risk because of small changes in hemostatic factors and directionally different responses to decreasing SFA. This trial was registered at https://clinicaltrials.gov/ct2/show/NCT00000538?term=NCT00000538&rank=1 as NCT00000538. *J Nutr* 2020;150:2089–2100.

Keywords: DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity), factor VIIc, fibrinogen, plasminogen activator inhibitor, PAI-1, dietary fat

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Introduction

Cardiovascular diseases (CVDs) account for 31% of all deaths globally (1). Myocardial infarction and stroke, caused by occlusive arterial thrombosis, are the most common CVDs (1). In the United States, communities of color continue to have disproportionately higher rates of death due to coronary artery disease (CAD) and stroke (2). Several hemostatic factors have been identified that affect CVD risk (3), notably fibrinogen, factor VIIc, and plasminogen activator inhibitor 1 (PAI-1) (4). The Fibrinogen Studies Collaboration, a meta-analysis on 31 prospective studies with 154,211 participants, reported that the relative risk of coronary heart disease (CHD) and stroke increased by 1.8 (95% CI: 1.6, 2.0) units per each 1-g/L increase in plasma fibrinogen after adjustment for several CVD risk factors (5). The association between CVD and factor VIIc coagulant activity (factor VIIc) is less robust; most prospective studies have not reported an association, although in the Northwick Heart Park Study an elevated factor VIIc was associated with increased CHD mortality (6). The association between elevated PAI-1 plasma concentrations and vascular thrombosis has also been reported (7).

Epidemiologic evidence suggests that dietary total fat and SFA are positively associated with prothrombotic factors (8, 9), whereas fish, fish oil, dietary fiber, and alcohol consumption (light to moderate) are associated with lower concentrations of prothrombotic factors (10–13). Some clinical studies have suggested that reducing dietary fat and SFA and increasing dietary fiber lowers factor VIIc concentrations (14–16); however, the Women's Health Initiative reported no effect of a low-fat diet on factor VIIc activity and concentration in postmenopausal women (17). In contrast, the effects of diet composition on fibrinogen and PAI-1 concentrations have been small and inconsistent (14–16, 18), although weight loss achieved by caloric restriction has been shown to improve markers of fibrinolytic activity (19–21). Based on the research to date, Forouhi et al. (22) concluded that there is insufficient evidence on the effects of fatty acids on thrombosis. This agrees with the conclusion by Siri-Tarino et al. (23) that the effects of SFA on thrombosis are unclear.

The 2013 American Heart Association (AHA)/American College of Cardiology (ACC) Guideline on Lifestyle Management to Reduce Cardiovascular Risk (24) recommended the Dietary Approaches to Stop Hypertension (DASH) healthy, food-based dietary pattern that is low in SFA, *trans* fat, and sodium to reduce elevated LDL cholesterol and blood pressure. The recent ACC/AHA Primary Prevention Guideline (25) reinforces this healthy dietary pattern message to decrease atherosclerotic CVD risk factors. The Mediterranean-style dietary pattern, a variation of DASH (i.e., it is low in SFA and high in unsaturated fat) has remarkable cardiovascular benefits (26). There also is some evidence that a Mediterranean-style dietary pattern (26) has a protective effect on thrombosis (27, 28), which may be due to dietary unsaturated fat. We have an understanding of the effects of substituting carbohydrates and unsaturated fats for SFA on lipids and lipoproteins (29); however, little is known about the effects of macronutrient variations on hemostatic factors.

The Dietary Effects on Lipoproteins and Thrombogenic Activity (DELTA) Study was conducted to evaluate the effects of diet on the following primary outcomes: lipids, lipoproteins, and hemostatic factors in different population groups, including those at high risk of CVD (e.g., African Americans and individuals with or at risk for metabolic syndrome) (30). A major goal was to identify whether dietary carbohydrate or MUFA was the preferred caloric substitute for SFA for minimization of CVD risk factors. Study 1 evaluated the effects of a stepwise reduction in dietary total fat and SFA on plasma lipids, lipoproteins, and hemostatic factors; SFA was replaced with carbohydrate (31). Study 2 evaluated the effects on lipids, lipoproteins, and hemostatic factors of replacing SFA with either MUFA or carbohydrate in participants with an accentuated metabolic and cardiovascular risk profile (32). Including both studies enabled us to address a key nutrition question, which is whether dietary carbohydrate or unsaturated fat is the preferred macronutrient substitute for SFA?

Methods

Study design

Detailed descriptions of the protocols, diet designs, and treatment effects on plasma lipids and lipoproteins have been reported previously (31, 32). The studies were conducted at the following research centers: Columbia University, Pennington Biomedical Research Center, Pennsylvania State University, and the University of Minnesota; the Coordinating Center was at the University of North Carolina. Both study 1 and study 2 used a randomized double-blind crossover design with 3 feeding periods and 6 diet sequences. After a brief run-in period to assess protocol compliance, each participant was randomly assigned to 1 of 6 diet sequences. The randomization procedure provided and controlled by the Coordinating Center was stratified by center. The duration of each feeding period was 8 wk in study 1 and 7 wk in study 2. Each period was followed by a 4- to 6-wk washout interval (or "rest interval") during which time participants resumed their habitual dietary patterns. All food was provided to participants during the feeding periods for both studies. Participants' meals were prepared individually, with all items containing fat weighed to the nearest 0.1 g; other foods were weighed to the nearest gram. For each protocol, all foods and all of the 8-d-cycle menus were identical among the 4 research centers. Diet composition was monitored by chemical analysis of duplicate meal homogenates collected daily at all research centers (33). Chemical analysis confirmed that target nutrient goals of the designed diets were met (31–33). Study participants were required to eat 2 meals on-site each weekday (either breakfast and dinner, or lunch and dinner, depending on the research center). Other meals and snacks were packed

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Abbreviations used: AAD, average American diet; ACC, American College of Cardiology; AHA, American Heart Association; BTG, B-thromboglobulin; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; DD, D-dimer; DELTA, Dietary Effects on Lipoproteins and Thrombogenic Activity; F1.2, prothrombin fragments; HOMA, homeostasis model assessment; HTE, heterogeneity of treatment effect; MetSyn, metabolic syndrome; PAI-1, plasminogen activator inhibitor 1; PAP, plasmin-antiplasmin complex; TG, plasma triglyceride.

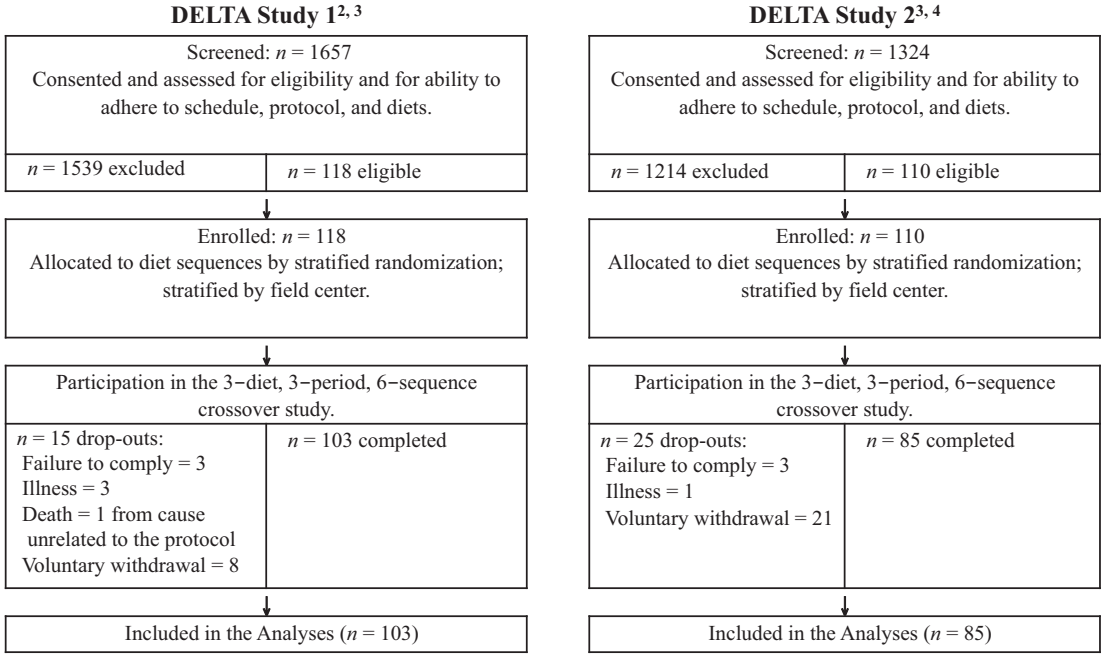

FIGURE 1 CONSORT diagrams for DELTA study 1 and study 2. [1]Methods, diets, and other information as described in Ginsberg et al. (31). [2]Each participant was randomly assigned to a sequence of 3 protocol-specific experimental diets. [3]Methods and diets as described in Berglund et al. (32); other information from unpublished data (Collaborative Studies Coordinating Center, School of Public Health, University of North Carolina, Chapel Hill, NC, 1995). CONSORT, Consolidated Standards of Reporting Trials; DELTA, Dietary Effects on Lipoproteins and Thrombogenic Activity.

and distributed to participants for consumption at a time and place of convenience. Dietary compliance was assessed by observation of 2 meals/d, daily records, and interviews. Participants were blinded to the test diets. In addition, all samples were assayed in blinded manner. The research centers began feeding on the same date and followed identical feeding schedules and study procedures for both studies. In study 1, the 3 feeding periods spanned 27 September 1993 to 4 May 1994. In study 2, the 3 feeding periods spanned 30 September 1994 to 19 May 1995.

Energy requirements were calculated using the Harris-Benedict equation with the physical activity factor derived from self-reported activity levels. If necessary, the calculated energy level was adjusted to maintain body weight. Participants were instructed not to modify exercise or other physical activity habits. These factors, the occurrence of illness, and use of medications were monitored weekly. Protocols for both studies were approved by the institutional review boards at each research center. This study is registered at ClinicalTrials.gov as NCT00000538.

Study cohorts

Participant recruitment for both studies is presented in a CONSORT (Consolidated Standards of Reporting Trials) diagram (**Figure 1**). For study 1 and study 2, we estimated that we needed targeted samples sizes that would yield ≥96 participants and 110 participants with complete data. The lengths of feeding periods were designed to allow physiological stabilization on each diet. Primary considerations in choosing the target sample sizes, the crossover experimental design, the number of longitudinal evaluations (blood draws) per period, and the lengths of the feeding periods included cost and time requirements, as well as reasonable conjectures about the anticipated levels of precision of the statistical estimators of interest, and the levels of power for the hypothesis tests of interest. The conjectures about anticipated levels of precision and power were informed by point and interval estimates of variance components obtained from our previous studies. The estimates of statistical power were obtained only for the primary hypothesis tests regarding diet effects on the lipids, lipoproteins, and hemostatic factors.

We note that all calculations of anticipated power and anticipated precision, including those used in the planning stage, have no valid role in the interpretation of study results. Once a study is completed, those historical conjectures provide no valid guidance in the analysis of the results (34–36). Rather, the CI estimates, point estimates, and hypothesis tests we have reported provide all the appropriate information and guidance for interpretation of study results.

Study 1 (amount of total and saturated fat)

In study 1, a diverse population (by age, gender, and race) with lipid/lipoprotein concentrations representative of the US population was recruited. Healthy, normolipidemic participants between 22 and 67 y of age who were taking no medications known to affect lipids or hemostatic factors were recruited. Participants were eligible if their mean fasting total cholesterol concentrations (measured at 2 separate visits) were between the 25th and 90th percentile for age, race, and gender based on NHANES III (1998–2004) (37), and their plasma triglycerides (TGs) and HDL cholesterol were between the 10th and 90th percentiles. To achieve a sample size of ≥96 participants with complete data, each research center enrolled 25–32 participants. A goal was to have ~40% males and 60% females (with similar numbers of pre- and postmenopausal women and males > and <40 y of age; i.e., older men = men ≥40 y; younger men = men <40 y of age) and 25% African Americans. Postmenopausal status was self-reported based on 12 consecutive months without a menses (38).

Study 2 (type of fat for SFA replacement)

In study 2, males and females 21–68 y of age were eligible to participate if the average of 2 screening measurements met any of the following age-, race-, and gender-adjusted criteria based on NHANES III data: HDL cholesterol ≤30th percentile, TGs ≥70th percentile, and insulin ≥70th percentile. Participants were ineligible if their *1*) average screening total cholesterol was <25th percentile or >90th percentile, *2*) LDL cholesterol was >190 mg/dL, *3*) fasting TG concentrations were <30th percentile or >500 mg/dL, or *4*) HDL cholesterol was >70th percentile. For enrolled participants, insulin resistance was defined as a baseline homeostasis model assessment (HOMA) index >3. Established criteria for MetSyn were defined after we conducted study 2 (39). In the present report, we have classified participants as having MetSyn

TABLE 1 Target nutrient composition of the experimental diets[1]

	Study 1			Study 2		
Nutrient, % of energy	AAD	Step 1	Low-Sat	AAD	Step 1	MUFA
Total fat	37	30	26	37	30	37
SFA	16	9	5	16	8	8
MUFA	14	14	14	14	15	22
PUFA	7	7	7	7	7	7
Protein	16	16	16	16	16	16
Carbohydrate	47	54	58	47	54	47
Sugar	18	21	23	19	19	18
Cholesterol (mg/d)	300	300	300	300	300	300
Fiber (g/1000 kcal)	9	10	11	8	15	8

[1] AAD, average American diet; Low-Sat, low-SFA diet.

using the Adult Treatment Panel III criteria described in a previous DELTA paper by Berglund et al. (32), as defined by Grundy et al. (39). Participants were classified as having MetSyn if any 3 of the 5 defined characteristics of MetSyn were present at baseline: elevated waist circumference, elevated TGs, reduced HDL cholesterol, elevated blood pressure, and elevated fasting glucose (37).

Diet composition

Study 1 (amount of total fat).

The study diets we designed were as follows: a high-SFA average American diet (AAD), a Step 1 diet, and a low-SFA diet (Low-Sat) (**Table 1**). The proportion of each SFA in the AAD studied was representative of the United States at the time, as well as the current US diet. *trans* Fatty acids were maintained at <1.5% of calories for each diet. Since the goal of study 1 was to evaluate the effects of reducing dietary SFA, dietary cholesterol was maintained at <300 mg/d (assayed value was 275 mg/d) for all 3 diets for all calorie levels. Dietary carbohydrate replaced SFA on the Step 1 and Low-Sat diets. The dietary carbohydrate was a mixture of whole and refined grains and included a variety of fruits and vegetables. Diets were designed to be isocaloric, and unit foods (i.e., muffins) that were compositionally similar to the experimental diet being evaluated were used to adjust calories to achieve weight maintenance.

Study 2 (type of fat).

The study diets designed were as follows: an AAD, a Step 1 diet, and a high-MUFA diet (Table 1). The high-MUFA diet was designed to match the saturated fat and polyunsaturated fat content of the Step 1 diet, and to maintain the total fat content of the AAD. For the high-MUFA diet, we replaced ~7% of calories from carbohydrate on the Step 1 diet with an equivalent proportion of calories from monounsaturated fat. All 3 diets provided ~300 mg/d of cholesterol.

Hemostasis measurement methods.

The studies were designed to provide several consecutive weekly blood samples in order to *1*) assess the time required to achieve a stable state for hemostatic factors and *2*) reduce the effects of intraindividual variation on these outcome measures. In study 1, during each of the three 8-wk feeding periods, fasting blood samples were obtained once weekly during weeks 5, 6, 7, and 8. Because plasma concentrations of hemostatic factors stabilized by week 4 in study 1, study 2 was shortened by 1 wk, and fasting blood samples were obtained once weekly during weeks 5, 6, and 7 of each feeding period. Plasma samples were collected in the morning between 06:00 and 10:00 h from the antecubital vein into citrated evacuated tubes. Tourniquet time was limited by protocol to no more than 2 min to avoid stasis. Blood was centrifuged immediately after collection at 2000 × *g* for 20 min at room temperature (factor VIIc) or 4°C (fibrinogen, PAI-1) to remove platelets and ensure that PAI-1 measurement reflected circulating plasma concentrations and not contamination from platelet PAI-1. Processing of plasma for factor VIIc was carried out at room temperature and the isolated plasma was rapidly frozen at −80°C to avoid cold activation.

Plasma aliquots were stored at −80°C until the end of each study. Immediately after each study was completed, samples were assayed at the Laboratory for Clinical Biochemistry Research, University of Vermont. Hemostatic factor assays were conducted as follows: the factor VIIc–one-stage clot-rate assay was based on the prothrombin time using human placental thromboplastin, standardized using WHO reference plasma, and performed on a semiautomated Coag-A-Mate X2 instrument (Organon-Technika Co.); PAI-1 antigen concentration was quantified using the ELISA method adapted by Cushman et al. (40), and fibrinogen was assayed using the method described by Clauss (41). Interassay CVs, based on assays of split specimens, for fibrinogen, factor VIIc, and PAI-1, were 3.1%, 5.3%, and 9.0%, respectively.

Study 1 and study 2 also included secondary outcome measures of coagulation: D-dimer (DD; ng/mL), prothrombin fragments (F1.2; nM), plasmin-antiplasmin complex (PAP; nM), C-reactive protein (CRP; μg/mL), and B-thromboglobulin (BTG; IU/mL).

In study 2, for each diet, BTG was measured only during weeks 5 and 6, while CRP, F1.2, PAP, and DD were measured only during weeks 6 and 7. In study 1, for each diet, F1.2, PAP, and DD were measured during weeks 6 and 7.

The F1.2 concentrations were measured using the Behring kit (Behringwerke) with a CV of 15% (42); PAP was measured using 2-site ELISA with murine monoclonal antibodies specific for PAP complex (43) with a CV of 3.6%; CRP immunoassay (44) used specific anticlonal antibodies and purified CRP (Calbiochem) in a competitive ELISA with a CV of 6%; and DD concentrations were measured using an ELISA 2-site immunoassay with a CV of 6% (45); BTG was measured using an immunoassay kit from Diadnotica Stago with a CV of 9%. Each of these secondary hemostasis measures was transformed to $\log_{10}$ scale for the statistical analyses.

Statistical analyses

Primary analyses.

The primary hemostasis measures for study 1 and study 2 were fibrinogen, plasma factor VIIc, and PAI-1. In planning study 1, it was anticipated that each of these measures would tend to decrease as dietary intake of total fat and saturated fat decreased. Informed by the surprising results from study 1, study 2 was designed to differentiate the effect of total fat from the effect of saturated fat. In both studies, longitudinal statistical analyses using linear mixed-effects models were performed separately for each of the hemostasis outcome variables. For fibrinogen, for example, each participant contributed 9 fibrinogen values indexed by period (1, 2, or 3), diet within period, and week (fifth, sixth, or seventh) within period. This analysis strategy closely paralleled that previously described (31, 32) for the analyses of the lipid and lipoprotein outcome measures. To enable a more exact statistical analysis, the square-root scale was used for PAI-1 (ng/mL) to reduce skewness and improve distributional properties (46); similarly, the $\log_{10}$ scale had been used (31, 32) for TG concentration (mg/dL). Those transformation decisions were made a priori based on previous studies. The linear mixed-effects model represented mean response as a function of diet, gender-by-age group, race, center, amount of energy intake,

feeding period, and interactions of diet with group, race, center, and caloric intake. The 2 categories for race were "African American" and "all other." The gender-by-age groups were "premenopausal women," "postmenopausal women," "men under 40 y of age," and "men, 40 y and older." Dietary energy intake categories were 1500, 2000, 2500, 3000, and 3500 kcals/d. The "baseline" characteristics (group, race, center, energy intake) were included to potentially improve the precision of the estimators of treatment effects and period effects; however, the magnitudes of those estimators are invariant to their inclusion or exclusion. The a priori analysis plans specified criteria for dropping from the model energy intake (if no improvement in the precision of the treatment main-effect estimators) and for dropping all the interaction terms from the model (the magnitudes of the estimates of their regression coefficient being <10% of the estimate of the AAD mean, along with negligible impact on the estimates of the diet treatment main effects, i.e., the estimates changed by <10%). For both studies, total variance of the conditional distribution of assay values was assumed to be constant across all factor levels and occasions. Correlation between any 2 of an individual's assay values was assumed to be larger for same-diet pairs, smaller for different-diet pairs, but otherwise invariant. This model was represented and interpreted as a components-of-variance model with 3 components: interindividual variance of the individuals' overall mean concentrations ("subject"), interindividual variance of individuals' diet-specific mean concentrations ("diet-by-subject"), and intraindividual variance ("within subject"). In these models, missing values (if any) for the dependent variable were not imputed; the linear mixed-effects model is designed to cope with incomplete longitudinal data. Participants with some missing data values were not excluded from the computations; however, participants who dropped out of the study during the first few weeks of period #1 were excluded from the primary analyses. No baseline covariate data values were missing in these studies. Estimates of the parameters of the linear mixed-effects models were used to compute point estimates and CI estimates of diet-specific means and mean differences. In terms of mean differences, the pairwise null hypotheses ("no difference between the 2 diets in the target population") were tested using the Marcus-Peritz-Gabriel closed hierarchical multiple-comparisons procedure (47); specifically, each pairwise null hypothesis is rejected only if its *F*-test *P* value is less than $\alpha = 0.01$ and the *F*-test *P* value for the overall null hypothesis ("no differences among the 3 diets in the target population") is also less than $\alpha = 0.01$. Tests observed to be not statistically significant are inconclusive; all statistical test procedures are, by design, incapable of establishing that the null hypothesis is true. In contrast, the point and interval estimates are always informative to some degree.

The decision to choose $\alpha = 0.01$ for the primary hypothesis tests in these studies was made a priori by the research team based on investigator preferences regarding interpretation of evidence and consideration of the large number of co–primary outcome variables involved in the studies (31, 32). For consistency, 99% CIs are reported; however, the main purpose of the reported CIs is interval estimation and not service to hypothesis testing. The CIs are informative regarding the treatment effect in the target population as they identify a range of candidate values that is most compatible with the observed data. In contrast, hypothesis tests yielding a *P* value $> \alpha$ are entirely inconclusive as to whether the (null) hypothesis is true or false—that is, the result cannot be used to infer that the treatment effect is zero in the target population from which the sample of participants was drawn.

Secondary analyses of heterogeneity of treatment effects.

Subpopulation differences in diet effects (i.e., heterogeneity of treatment effects; HTEs) were investigated. For study 1 and study 2, these secondary analyses focused on interactions of diet with age-gender group and interactions of diet with race. Also, for study 2, the primary model was modified to include additional covariates representing risk categories and their interactions with diet: categories defined by the baseline eligibility criteria or risk categories defined by MetSyn (yes, no), HDL cholesterol (high, low), TGs (high, low), HOMA (high, low).

Analyses of secondary hemostasis measures.

Similar analysis strategies were applied to prothrombin fragment F1.2, PAP, DD, and BTG in studies 1 and 2, and to CRP in study 2.

Sensitivity analyses.

To guide our trust in the main results, auxiliary diagnostic procedures and variations on the analysis methods were applied to evaluate the sensitivity or robustness of the main results to reasonable perturbations of methods and assumptions used; for example, we examined *1*) temporal stabilization of hemostatic variables during the 5–8 or 5–7 wk; *2*) first-order carryover effects were investigated; *3*) variations in the number of variance-covariance parameters assumed; *4*) graphical representations of within-subject residuals, between-subject residuals for evidence of departures from modeling assumptions (via Q-Q plots, histograms, scatterplots); *5*) diagnostics for identification of extreme or influential values; and *6*) analysis of agreement between the participant's observed responses (e.g., fibrinogen values) and model-based estimates of the participant's responses—the estimates being computed by best empirical linear unbiased prediction (eBLUP) methods (48).

Computations.

All statistical computations were performed using SAS System software version 9.1 and were updated using version 9.4 (SAS Institute).

Results

The experimental diets were well accepted. Good dietary compliance, assessed by observation of 2 meals/d, daily records and interviews, was achieved as noted previously (31). In addition, participants in both studies maintained their weight (31). As verified by chemical assays, the fat and fatty acid composition of each experimental diet met protocol specifications (within 1–2% of the target value; data previously reported) (31–33). In study 1 and study 2, the estimates of diet treatment effects reported here were obtained from the reduced model, accounting only for race, gender-by-age, research center, and feeding period. We focus on the reduced model because the estimates for the interaction terms and caloric intake were negligible and had negligible impact on the estimates of the diet effects. Although not part of the criterion for removing those terms, the CIs were wide, and the hypothesis tests were inconclusive regarding the null hypotheses that those parameters are zero in the target population.

Study 1 results (amount of total fat)

Of the 118 participants who were enrolled and randomly assigned, 103 completed the study (Figure 1). Reasons for drop-out and incomplete data included scheduling issues, adherence problems with the study protocol, or short-term illness (Figure 1). The participants who dropped out did so during the first few weeks of feeding period #1 (except for the 1 death that occurred during period #2); they were excluded from the primary statistical analyses. Males and females of different ages and ethnicities were studied (**Table 2**). The demographic profiles of participants in study 1 were representative of the population at large in the United States when the study was conducted. Baseline characteristics of study participants are shown in **Table 3**. In addition, baseline percentiles of TGs and insulin are presented in **Supplemental Table 1**.

Primary analyses.

The effects of each diet on hemostatic responses are shown in **Table 4**. In the study 1 cohort, we observed differences in the mean concentrations of factor VIIc, fibrinogen, and PAI-1 when comparing the AAD with the Step 1 and the Low-Sat

TABLE 2 Study 1: categorization of participants by sex, age, and race

	African American	Non–African American	Total
Premenopausal women[1]	11	28	39[2]
Postmenopausal women[3]	6	12	18
Men aged <40 y	8	22	30
Men aged ≥40 y	1	15	16
Total	26	77	103

[1]Premenopausal status was self-reported based on the presence of menses, either regular or irregular periods.
[2]Because the total ($n = 103$) is very close to 100, the column percentages approximate the counts; for example, 37.9% (39/103) were premenopausal women.
[3]Postmenopausal status was self-reported based on 12 consecutive months without a menses.

diets. In terms of their estimated effects (with 99% CIs), the Step 1 and Low-Sat diets decreased the mean concentration of factor VIIc by 1.6 (0.2, 3.0) and 2.3 (0.9, 3.7) units (1.8% and 2.6%), respectively; increased mean fibrinogen by 3.3 (−3.6, 10.2) and 7.7 (0.9, 14.6) units (1.2% and 2.8%); and increased the mean of square-root PAI-1 by 0.001 (−0.15, 0.15) and 0.17 (0.01, 0.31) units (0.0% and 6.0%). For factor VIIc and for fibrinogen, but not for PAI-1, the hypothesis test procedure comparing Step 1 with Low-Sat was inconclusive as to whether the mean differences are exactly zero in the target population.

Sensitivity analyses.
In examining the robustness of the diet effect estimates, similar results were obtained with and without inclusion of energy intake and terms representing interactions of diet with gender-age group, center, race, and energy intake. For example, similar results are shown in **Supplemental Table 2** in terms of unadjusted means ± SDs for factor VIIc and fibrinogen, and as unadjusted medians and IQRs for PAI-1. Analyses of the model's residuals (total residuals, within-subject residuals, between-subject residuals) adequately supported the assumptions of the linear mixed-effects models. Identification and removal of extreme or influential observations did not occur. Model diagnostics indicated satisfactory goodness-of-fit. Stabilization of the hemostatic measures by week 4 was verified by longitudinal linear mixed-effects model analyses of the outcomes at weeks 5–8 accounting for "week" and diet-by-week interactions with use of from 6 to 12 variance-covariance parameters. For the models with 9 variance-covariance parameters, in terms of week-specific estimates of the Step 1 main-effect (relative to AAD), week 5 differed from week 8 by 0.8 units for factor VIIc, by 10 units for fibrinogen, by 0.1 units for square-root PAI-1; and similarly, for the Low-Sat main effect, these differences were 0.2, 10, and 0.2 units, respectively. Point estimates of first-order carryover effects were negligible (<5% of the estimate of the AAD mean), their CIs were wide, and the hypothesis tests were inconclusive regarding the null hypotheses that those parameters are zero in the target population. These results were obtained by inclusion of carryover terms in the models used for the main analyses. Collectively, the results of these various sensitivity analyses strengthened our trust in the main results.

Secondary analyses of HTEs.
Treatment effects were generally consistent across the subpopulations of interest and across the 4 centers, as illustrated in **Supplemental Figures 1–3**.

Analysis of secondary hemostasis measures.
For DD, F1.2, PAP, CRP, and BTG, the diet treatment effect estimates were small with wide CIs and the primary hypothesis tests were inconclusive (data not shown).

Study 2 results (type of fat)

Of the 110 participants who were enrolled and randomly assigned, 85 completed all 3 feeding periods (Figure 1). Reasons for missing data and drop-out in study 2 were similar to those reported for study 1. The participants were metabolically unhealthy as defined by their baseline characteristics (presented in Table 3) and risk factors for cardiometabolic disease (**Table 5**). The 2 most common eligibility criteria were low HDL cholesterol and low HDL cholesterol plus high TGs (Table 5). The study 2 cohort differed from the study 1 cohort in their responses to the AAD: mean factor VIIc was ∼25% higher, mean fibrinogen was 6% higher, and mean square-root PAI-1 was >100% higher.

Primary analyses.
The effects of each diet on hemostatic responses are shown in **Table 6**.

The Step 1 and high-MUFA diets, respectively, decreased the mean concentration of factor VIIc by 4.6 (2.0, 7.1) and 3.6 (1.0, 6.1) units (4.1% and 3.2%), increased mean fibrinogen by 11.9 (1.9, 21.9) and 4.6 (−5.5, 14.6) units (3.9% and 1.5%), and increased mean square-root PAI-1 by 0.09 (−0.24, 0.42) and 0.25 (−0.08, 0.58) units (2.0% and 5.8%) (Table 6). The high-MUFA diet and the Step 1 diet were similar in improving factor VIIc. The high-MUFA diet raised mean fibrinogen less than the Step 1 diet, but the Step 1 diet raised PAI-1 less than the high-MUFA diet. The hypothesis tests comparing MUFA and Step 1 were inconclusive as to whether the mean differences are exactly zero in the target population (Table 6).

Sensitivity analyses.
In Supplemental Table 2, similar results are presented as unadjusted means ± SDs for factor VIIc and fibrinogen, and as medians and IQRs for PAI-1. The study conclusions were essentially the same regardless of choice of methods and model assumptions used. As in study 1, stabilization of the hemostatic measures by week 4 was verified by longitudinal analyses for weeks 1–7, estimates of first-order carryover effects were negligible, and analyses of residuals adequately supported the assumptions of the models. Collectively, the results of these various sensitivity analyses strengthened our trust in the main results.

Secondary analyses of HTEs.
Estimation of subpopulation differences in treatment effects showed consistency in the directions and magnitudes of the diet effects across categories of gender, age, race, baseline eligibility criteria, and risk categories defined by MetSyn, low HDL cholesterol, high TGs, and high HOMA. Estimates of selected potential interactions are illustrated in **Supplemental Figures 4–6**.

For factor VIIc (Supplemental Figure 4) the interaction effect estimates ranged from 1.0 to 4.0. The observed estimates of the effect of MUFA (vs. AAD) were slightly better for categories "TG high," "HDL low," and "HOMA high," but slightly worse for "MetSyn yes." The effects for the Step 1 diet were very similar to those of MUFA.

TABLE 3 Baseline characteristics of study participants[1]

Baseline characteristics	Study 1 (amount of total fat)			Study 2 (type of fat)		
	Men ($n = 46$)	Women ($n = 57$)	Total ($n = 103$)	Men ($n = 52$)	Women ($n = 33$)	Total ($n = 85$)
Age, y	36.0 ± 12.0	39.4 ± 14.8	37.9 ± 13.6	33.3 ± 8.6	39.0 ± 9.7	35.5 ± 9.4
BMI, kg/m^2	24.7 ± 3.6	24.4 ± 3.0	24.5 ± 3.3	27.4 ± 4.2	27.9 ± 4.6	27.6 ± 4.4
Total cholesterol, mg/dL	198.9 ± 29.3	201.2 ± 31.8	200.1 ± 30.6	199.5 ± 34.8	199.2 ± 30.9	199.5 ± 30.9
LDL cholesterol, mg/dL	132.3 ± 29.6	128.2 ± 30.2	130.1 ± 29.9	128.8 ± 23.2	127.2 ± 27.1	128.4 ± 23.2
HDL cholesterol, mg/dL	44.5 ± 9.3	55.1 ± 9.9	50.4 ± 11.0	39.1 ± 7.7	45.6 ± 11.6	41.8 ± 7.7
Triglycerides, $\log_{10}$ mg/dL	2.01 ± 0.2	1.91 ± 0.2	1.96 ± 0.2	2.14 ± 0.3	2.08 ± 0.2	2.11 ± 0.2
Glucose, mg/dL	ND	ND	ND	93.3 ± 12.6	92.4 ± 10.8	93.0 ± 12.6
Insulin, $\log_{10}$ μU/mL	ND	ND	ND	1.06 ± 0.2	1.01 ± 0.2	1.04 ± 0.2

[1] Values are means ± 1 SD. All analytes were measured in plasma. ND, not done.

For fibrinogen (Supplemental Figure 5), the treatment effects for Step 1 and MUFA (vs. AAD) were consistent across subpopulations.

For PAI-1 (Supplemental Figure 6), the diet effect estimates for the MUFA and Step 1 diets (vs. AAD) were generally consistent across subpopulations, with 1 potential exception: the estimates suggest that individuals with MetSyn may be different from those without MetSyn. For those with MetSyn, mean square-root PAI-1 was not increased but rather was decreased by 0.15 units on the MUFA diet and was increased by 0.52 units on the Step 1 diet (Table 7).

Analysis of the secondary hemostasis measures. For DD, F1.2, PAP, and BTG, the diet treatment effect estimates were small with wide CIs, and the primary hypothesis tests were inconclusive (data not shown).

Discussion

The 2013 AHA/ACC Guideline on Lifestyle Management to Reduce Cardiovascular Risk (24), as well as the 2019 ACC/AHA Prevention Guideline (24) recommend a healthy dietary pattern

TABLE 4 Study 1: overall effects of diet on hemostatic factors in healthy and metabolically unhealthy participants[1]

	Factor VIIc ($n = 103$), %	Fibrinogen ($n = 103$), mg/dL	PAI-1 square root ($n = 102$), ng/mL
Study 1 diets			
AAD	89.1 ± 1.5	276.6 ± 4.4	2.81 ± 0.15
Step 1	87.5 ± 1.5	279.9 ± 4.4	2.81 ± 0.15
Low-Sat	86.8 ± 1.5	284.3 ± 4.4	2.97 ± 0.15
Difference: Step 1 – AAD			
Estimate ± SE	−1.6 ± 0.5	3.3 ± 2.7	0.00 ± 0.06
99% CI	(−3.0, −0.2)	(−3.6, 10.2)	(−0.15, 0.15)
Pairwise *P* value	0.0032[2]	0.2153	0.9925
Percentage change of means	−1.8 ± 0.6[3]	1.2 ± 1.0	0.0 ± 2.0
Difference: Low-Sat – AAD			
Estimate ± SE	−2.3 ± 0.5	7.7 ± 2.7	0.17 ± 0.06
99% CI	(−3.7, −0.9)	(0.9, 14.6)	(0.02, 0.31)
Pairwise *P* value	≤0.0001[2]	0.0036	0.0037
Percentage change of means	−2.6 ± 0.6	2.8 ± 1.0	6.0 ± 2.0
Difference: Low-Sat – Step 1			
Estimate ± SE	−0.7 ± 0.5	4.4 ± 2.7	0.16 ± 0.06
99% CI	(−2.1, 0.68)	(−2.4, 11.3)	(0.02, 0.31)
Pairwise *P* value	0.1853[2]	0.0938	0.0038
Percentage change of means	−0.8 ± 0.6	1.6 ± 1.0	6.0 ± 2.0
Overall *P* value	0.0001[2]	0.0141	0.0037

[1] Each entry is the estimated mean ± 1 SE obtained via a linear mixed-effects model accounting for diet, feeding period, center, race, and gender/age group. All PAI-1 values were missing for 1 participant. All analytes were measured in plasma. AAD, average American diet; Low-Sat, low-SFA diet; PAI-1, plasminogen activator inhibitor 1.

[2] In terms of mean differences, the pairwise null hypotheses ("no difference between the 2 diets in the target population") were tested using the Marcus-Peritz-Gabriel closed hierarchical multiple-comparisons procedure (47): each pairwise null hypothesis is rejected only if its *F*-test *P* value is less than $\alpha = 0.01$ and the *F*-test *P* value for the overall null hypothesis ("no differences among the 3 diets in the target population") is also less than $\alpha = 0.01$. Tests observed to be not statistically significant are inconclusive; all statistical test procedures are, by design, incapable of establishing that the null hypothesis is true. In contrast, the point and interval estimates are always informative to some degree.

[3] Percentage change in the mean from AAD ± 1 SE.

TABLE 5 Study 2: categorization of participants by eligibility criteria at baseline[1]

Criteria	*n*	Percentage
↓ HDL-C (≤ 30th percentile)[2]	26	30.6
↑ TGs (≥70th percentile) [2]	7	8.2
↑ Insulin (≥70th percentile) [2]	5	5.9
↓ HDL-C + ↑ TGs	25	29.4
↓ HDL-C + ↑ insulin	5	5.9
↑ TGs + ↑ insulin	2	2.4
↓ HDL-C + ↑ TGs + ↑ insulin	15	17.6
Total	85	100

[1]All analytes were measured in plasma. HDL-C, HDL cholesterol; TG, triglyceride.
[2]Based on the third NHANES (NHANES III) specific for age, sex, and race.

that is low in SFA and sodium to decrease CVD morbidity and mortality, which aligns with previous recommendations (25, 49–51). Higher concentrations of fibrinogen and factor VIIc have been reported with consumption of diets high in total fat and SFA and low in fiber, fruit and vegetables, and fish (52–55). In our study, type and amount of dietary fat had a very modest effect and directionally different responses on 3 hemostatic factors that affect CVD risk.

Overall findings

In both studies, decreasing saturated fat, irrespective of the total fat content of the diet, modestly lowered mean factor VIIc in both healthy participants and participants at risk for CVD. The mean fibrinogen response was adversely affected by higher-carbohydrate, lower-fat diets (i.e., the Low-Sat diet in study 1 and the Step 1 diet in study 2) but increased modestly with the high-MUFA diet in study 2. Mean square-root PAI-1 increased slightly with the Low-Sat diet in study 1 versus the AAD and increased slightly with the high-MUFA diet in study 2. As noted above, these effects were very small.

In both studies, there was little or no evidence of HTEs across subpopulations; generally, the overall and subpopulation-specific treatment effects were consistently in the same direction and were of similar magnitudes. The tests of null hypotheses of the form "no HTE" were inconclusive. We noted that the point estimates and CIs in study 2 suggested that some HTE is plausible. For example, in study 2, the high-MUFA diet (vs. AAD) reduced factor VIIc slightly more for the participants with high TGs, low HDL cholesterol, and/or high HOMA, but slightly less for MetSyn participants. We found similarly weak evidence of a possible diet-by-metabolic syndrome interaction for square-root PAI-1; the MUFA diet decreased the mean concentration of square-root PAI-1, whereas the Step 1 diet increased the mean (Table 7). If so, then for individuals at risk for cardiometabolic diseases, a moderate-fat diet high in MUFA versus a lower-fat diet higher in carbohydrate may confer benefits on hemostatic factors that contribute to CVD risk. However, this needs to be studied further. Nonetheless, as we reported previously for the lipid and lipoprotein responses (30, 31), the MUFA diet provided the greatest risk reduction as a replacement for SFA because it lowered TGs and increased HDL cholesterol (vs. dietary carbohydrate).

TABLE 6 Study 2: overall effects of diet on hemostatic factors in healthy and metabolically unhealthy participants[1]

	Factor VIIc ($n = 85$), %	Fibrinogen ($n = 85$), mg/dL	PAI-1 square root ($n = 85$), ng/mL
Study 2 diets			
AAD	111.1 ± 4.3	308.0 ± 9.7	4.36 ± 0.37
Step 1	106.5 ± 4.3	319.9 ± 9.7	4.44 ± 0.37
MUFA	107.5 ± 4.3	312.5 ± 9.7	4.81 ± 0.37
Difference: Step 1 – AAD			
Estimate ± SE	−4.6 ± 1.0	11.9 ± 3.9	0.09 ± 0.13
99% CI	(−7.1, −2.0)	(1.9, 21.9)	(−0.24, 0.42)
Pairwise *P* value	≤0.0001[2]	0.0022	0.4942
Percentage change of means	−4.1 ± 0.9[3]	3.9 ± 1.3	2.0 ± 2.9
Difference: MUFA − AAD			
Estimate ± SE	−3.6 ± 1.0	4.6 ± 3.9	0.25 ± 0.13
99% CI	(−6.1, −1.0)	(−5.5, 14.6)	(−0.08, 0.58)
Pairwise *P* value	≤0.0004[2]	0.2392	0.0477
Percentage change of means	−3.2 ± 0.9	1.5 ± 1.3	5.8 ± 2.9
Difference: MUFA – Step 1			
Estimate ± SE	1.0 ± 1.0	−7.4 ± 3.9	0.17 ± 0.13
99% CI	(−1.6, 3.6)	(−17.4, 2.7)	(−0.16, 0.50)
Pairwise *P* value	≤ 0.3124[2]	0.0578	0.1929
Percentage change of means	0.9 ± 0.9	−2.4 ± 1.3	3.8 ± 2.9
Overall *P* value	<0.0001[2]	0.0083	0.1319

[1]Each entry is the estimated mean ± 1 SE obtained via a linear mixed-effects model accounting for diet, feeding period, center, race, and gender/age group. All analytes were measured in plasma. AAD, average American diet; PAI-1, plasminogen activator inhibitor 1.
[2]In terms of mean differences, the pairwise null hypotheses ("no difference between the 2 diets in the target population") were tested using the Marcus-Peritz-Gabriel closed hierarchical multiple-comparisons procedure (47): each pairwise null hypothesis is rejected only if its *F*-test *P* value is less than $\alpha = 0.01$ and the *F*-test *P* value for the overall null hypothesis ("no differences among the 3 diets in the target population") is also less than $\alpha = 0.01$. Tests observed to be not statistically significant are inconclusive; all statistical test procedures are, by design, incapable of establishing that the null hypothesis is true. In contrast, the point and interval estimates are always informative to some degree.
[3]Percentage change in the mean for AAD ± 1 SE.

TABLE 7 Study 2 heterogeneity of diet effects in PAI-1: participants with or without MetSyn[1]

	All ($n = 85$)	With MetSyn ($n = 20$)	Without MetSyn ($n = 65$)	Difference: with vs. without	Difference: *P* value
AAD	4.54 ± 0.37[2]	5.04 ± 0.48[2]	4.03 ± 0.39[2]	1.02 ± 0.47[3]	0.0317[4]
MUFA	4.65 ± 0.37	4.90 ± 0.48	4.41 ± 0.39	0.49 ± 0.47	0.2956
Step 1	4.77 ± 0.37	5.56 ± 0.48	3.99 ± 0.39	1.57 ± 0.47	0.0009
MUFA − AAD	0.12 ± 0.15[3]	−0.15 ± 0.25	0.38 ± 0.14	−0.52 ± 0.29[5]	0.0732
Step 1 − AAD	0.24 ± 0.15	0.52 ± 0.26	−0.04 ± 0.14	0.56 ± 0.29	0.0570
Step 1 − MUFA	0.12 ± 0.15	0.66 ± 0.26	−0.42 ± 0.14	1.08 ± 0.29	0.0003

[1] All analytes were measured in plasma. AAD, average American diet; MetSyn, metabolic syndrome; PAI-1, plasminogen activator inhibitor 1.
[2] Entry is the estimated mean ± 1 SE obtained via a linear mixed-effects model accounting for diet, feeding period, center, race, gender/age group, MetSyn, and terms representing diet-by-MetSyn interactions.
[3] Entry is the estimated mean difference ± 1 SE obtained from the same model.
[4] The *P* value for the test of the null hypothesis that the difference is zero in the target population from which the sample was drawn.
[5] Entry is the estimated difference of mean differences ± 1 SE obtained from the same model.

Based on our findings from both studies, decreasing SFA would be expected to lower factor VIIc. A lower-fat and -saturated-fat diet, however, tends to increase fibrinogen in healthy and at-risk individuals. However, the complexity of this finding relates to the PAI-1 responses. PAI-1 increased with the Low-Sat diet in study 1 and with the high-MUFA diet in study 2. In contrast, the Step 1 diets in both studies had little effect on PAI-1, suggesting that the lower and upper ranges of total fat recommended for heart health might not benefit PAI-1. Our secondary analyses of diet-by-subpopulation interactions suggested that the high-MUFA diet may decrease PAI-1, whereas the Step 1 diet may increase PAI-1 in individuals with MetSyn. If this hypothesis is confirmed, individuals with MetSyn might benefit from a diet low in SFA and higher in MUFA. However, further research is needed to evaluate this.

Factor VIIc

Controlled clinical studies have shown that factor VIIc activity is altered by changes in dietary fat, saturated fat, and fiber (14, 56–58). The results of a 2- to 3-wk feeding study with normolipidemic individuals demonstrated a decrease in factor VIIc of ∼5–10% with a reduction in dietary total and SFA (16). In a longer-term study (20 wk), mean factor VIIc concentrations likewise decreased (11%) when total and SFA decreased (57). The findings of study 1 are consistent with these reports; however, the magnitudes of the factor VIIc responses (reductions of 1.8% and 2.6%) were smaller than previously reported (16, 57). In study 2, the decreases were somewhat higher but still modest when reducing SFA (3.6% and 4.6%). The differences compared with other studies may be explained by differences in the diets used in the other studies (16, 58).

Fibrinogen

Fat reduction has been shown to decrease fibrinogen (18). In contrast, weight loss (19, 59, 60) has not had an effect on fibrinogen concentrations. In the current study, we observed small increases in mean fibrinogen concentrations when SFA was reduced in conjunction with total fat on the Low-Sat diet in study 1 and the Step 1 diet in study 2 [along with increases in TG concentrations and a decrease in HDL-cholesterol concentrations that we reported previously (31, 32)]. Collectively, the increase in mean fibrinogen along with adverse changes in TG and HDL-cholesterol concentrations in participants following a reduced-fat diet (32) may increase the CVD risk burden. Moreover, fibrinogen appears to be more responsive to the total fat versus the SFA content of the diet.

PAI-1

In our study, PAI-1 concentrations were 2-fold higher in participants at increased risk for cardiometabolic diseases (in study 2) compared with healthy participants (in study 1). We found that the lower and upper ranges of total fat recommended for heart health might not benefit PAI-1, except for individuals with MetSyn who had the best PAI-1 response with the high-MUFA diet, indicating the need for further research to better understand this observation. Moreover, the need for further research in individuals with MetSyn is reinforced by a recent study conducted by Teng et al. (61), which reported no effect of replacing SFA with MUFA or dietary carbohydrate on PAI-1. Since PAI-1 was associated with acute coronary syndrome and ischemic stroke in the European Prospective Investigation into Cancer and Nutrition–Italy Cohort (EPICOR) Study (62), a better understanding is needed about how diet, especially type and amount of dietary fat, affects PAI-1 and, in turn, CVD risk, particularly in persons with MetSyn who are at especially high CVD risk.

Strengths and limitations

Strengths of both studies were that they used a randomized, multicenter, double-blind longitudinal crossover design and very carefully prepared and monitored diets supported by central laboratories, managed by a collaborative-studies coordinating center, and funded by the NIH. The sample sizes were carefully chosen based on our previous studies. Adequacy of the repeated-measures designs and sample sizes is indicated in this report by the widths of the CIs and magnitudes of SEs. The duration of feeding periods and the numbers of weekly measurements were sufficient to ensure temporal stabilization of hemostatic factors during the feeding periods. The repeated blood sampling minimized the effects of large intraindividual variation in the hemostatic factor measures (63). Also, we have shown that the variability in hemostatic factors in premenopausal women is similar to (or better than) that for men and postmenopausal women, indicating that the menstrual cycle is not a source of variance that needed to be controlled in this study (46). In study 1, the heterogeneity of the population (45% female, 25% African American, and 17% postmenopausal females) enhanced the generalizability of the results. Study 2 addressed an important population, participants with cardiometabolic risk factors, in whom there are questions about the ideal macronutrient profile of diets that decrease the risk of thrombosis. Another strength of both studies is the carefully planned statistical analysis strategy and methods finalized prior to data collection. In the analyses of the results, we have used the current (2019) recommendations

from the American Statistical Association for our statistical inferences (64–66). Specifically, we have focused on the point and interval estimates of the population parameters of interest and interpreted P values on continuous scale. We noted that a P value that is not small (e.g., $P > \alpha$) cannot be interpreted to mean that the difference in question is zero in the target population from whom the sample of participants was drawn. When a hypothesis test yields a P value $> \alpha$, the test is entirely inconclusive with regard to whether the null hypothesis is true or false; i.e., no conclusion can be drawn and it is incorrect to say "there is no difference." In contrast, CIs are always informative to some degree. CIs identify a range of candidate values for the population parameter that is highly compatible with the observed data.

One limitation of both studies was the duration of the intervention. Although 7–8 wk of controlled feeding is a substantial period of time, this is relatively brief compared with lifestyle nutrition trials (and lifelong dietary patterns). Also, many potential dietary and lifestyle permutations were not tested. One example of this is that the type of carbohydrate that was used in the test diets was a mix of refined grains, added sugars, and some whole grains, as well as fruits and vegetables. As noted, however, our study focused on type and amount of fat and not specifically type of dietary carbohydrate. Further research is needed to understand the effect of type and amount of dietary carbohydrate on hemostatic factors. Despite a relatively higher than desired dropout rate in both studies (13% and 23%), the widths of the reported CIs indicate there were still enough participants to provide high levels of precision for the primary analyses. Furthermore, it is reasonable to assume that the reasons for dropout did not induce selection bias. It can be argued fairly that the hemostatic factor differences reported are small, but we believe that, especially in conjunction with the lipid and lipoprotein responses reported previously, long-term CVD risk might be affected; however, we hasten to add this requires further study because of the small and bidirectional changes in hemostatic factors. The changes we saw in hemostatic factor responses ranged between 0% and 6%. To put this in context, with respect to PAI-1, one study reported that cases (coronary events) had a 23% higher PAI-1 versus control participants (18.2 vs. 14.8 ng/mL) in the European Concerted Action on Thrombosis and Disabilities (ECAT) Study (67). Similarly, in the Framingham Heart Study, Jung et al. (68), reported that PAI-1 antigen concentrations were higher in those with major adverse cardiac events, with a mean difference of 6.11 ng/mL (95% CI: [3.27, 8.96]; $P < 0.001$). Also, in the Framingham cohort, Tofler et al. (69) reported a 32% increase in PAI-1 for participants with incident CVD (i.e., 29.1 vs. 22.1 ng/mL for those without incident CVD). For fibrinogen, the Northwick Park Heart Study reported a 10.3% higher concentration of fibrinogen in men and a 3.3% higher concentration in women for CHD death versus participants with no history of CHD (70). The Fibrinogen Studies Collaboration reports an HR of 2.42 for a 100-mg/dL increase in fibrinogen (5). Assuming a typical mean value for fibrinogen of ~300 mg/dL, a 100-mg/dL change corresponds to a 33% difference in risk of major CVDs. For factor VIIc, in the study by De Stavola and Meade (70), men and women who experienced CHD death had 10.9% and 16.5% higher concentrations, respectively. However, not all research shows a relation for factor VIIc and CHD (71). Finally, while the DELTA studies were conducted many years ago, the findings are still relevant because the American diet has not changed much in the past 25 years (72). Furthermore, the cohorts studied are still germane to topics of public health significance, including effects of race on diet responses and dietary management of dyslipidemia, especially given current population trends in overweight and obesity.

Conclusions

We have shown that reducing SFA modestly lowers factor VIIc in healthy and at-risk individuals. A diet low in total fat and SFA elicited a greater mean increase in fibrinogen than a moderate fat diet that was low in SFA and high in MUFA. These results indicate that, as a substitute for SFA, MUFA compared with carbohydrate results in less of an increase in fibrinogen. The results regarding the diet effects on PAI-1 were inconclusive. In secondary analyses of potential heterogeneity of diet effects, results for PAI-1 suggested that for individuals with MetSyn it is plausible that it may be beneficial to replace SFA with MUFA instead of replacing SFA with carbohydrate. Further research is needed to clarify the effect of the total fat content and the fatty acid profile of the diet on hemostatic factors in different population groups including those at high CVD risk.

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