Stearic, Oleic, and Linoleic Acids Have Comparable Effects on Markers of Thrombotic Tendency in Healthy Human Subjects[1]

Myriam A.M.A. Thijssen, Gerard Hornstra, and Ronald P. Mensink[2]

Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Department of Human Biology, Maastricht University, Maastricht, The Netherlands

ABSTRACT Because human studies concerning the effects of stearic acid on thrombotic tendency are inconsistent, we compared the effects of stearic acid with those of its unsaturated derivatives, oleic acid and linoleic acid. In this randomized, crossover study, 45 subjects (27 women and 18 men) consumed, in random order, 3 experimental diets, each for 5 wk. Diets contained ~38% of energy as fat. Dietary compositions were the same except for 7% of energy from stearic, oleic, or linoleic acids. At the end of each period, ex vivo and in vitro platelet aggregation, and variables of coagulation, fibrinolysis, and hematology were evaluated. In men, ex vivo platelet aggregation time as measured by filtragometry ($P = 0.036$ for diet effects) was favorably prolonged during consumption of the linoleic acid diet compared with the stearic acid diet ($P = 0.040$), but there was no difference with consumption of the oleic acid diet ($P = 0.198$). In vitro platelet aggregation induced by collagen and ADP, and variables of coagulation (factor VII amidolytic activity and concentrations of fibrinogen and prothrombin fragment 1 and 2) and fibrinolysis [plasminogen activator inhibitor (PAI) activity and concentrations of tissue plasminogen activator (tPA)/PAI-1 complexes] did not differ among the 3 diets. The mean platelet volume of the subjects decreased during consumption of the stearic acid diet by 0.32 fL compared with the oleic acid diet ($P < 0.001$) and by 0.35 fL compared with the linoleic acid diet ($P < 0.001$). In conclusion, our results do not suggest that stearic acid is highly thrombogenic compared with oleic and linoleic acids. J. Nutr. 135: 2805–2811, 2005.

KEY WORDS: • *fatty acids* • *thrombotic tendency* • *platelet aggregation* • *coagulation* • *fibrinolysis*

The most common complications of cardiovascular disease result from the formation of an arterial thrombus (1,2), which is initiated by disturbances in the hemostatic balance. Key regulators of this delicate balance are the endothelial wall, blood platelets, and coagulation and fibrinolytic factors (3). Indeed, epidemiologic studies showed that factors related to an enhanced thrombotic tendency such as increased blood platelet aggregation (4,5), increased concentrations of coagulation and decreased concentrations of fibrinolytic factors (6–8), are positively associated with cardiovascular risk. Furthermore, it was demonstrated that the thrombotic tendency of the blood is influenced by total fat intake as well as the fatty acid composition of the diet. Although the biochemical basis of the effects of dietary fatty acids on thrombotic tendency have not been fully elucidated, dietary fatty acids can modulate the fatty acid compositions of platelets and other cell membranes, thereby changing the availability of arachidonic acid. This fatty acid is a precursor for eicosanoid synthesis, which is involved in platelet aggregation (9).

The effects of individual fatty acids on thrombotic tendency were evaluated in animal models and in human studies. In rats, arterial thrombosis tendency as measured with the aortic loop technique, was decreased by (n-6) and (n-3) PUFA, whereas SFA with 12–16 carbon atoms promoted arterial thrombus formation. The effects of oleic acid as a major monounsaturated fatty acid were neutral or even antithrombotic compared with SFA (10). In these studies, stearic acid did not seem to affect arterial thrombosis tendency, whereas other studies in rats indicated that stearic acid was highly thrombogenic in a model of venous thrombosis (11). Studies in humans also suggested that stearic acid is prothrombotic. In French farmers, for example, dietary stearic acid intake was related to increased platelet activity (12). In another study, plasma concentrations of free stearic acid correlated positively with factor VII activation (13). In contrast, later studies reported beneficial effects of stearic acid on platelet aggregation (14) and coagulation variables (15,16) compared with other SFA. Not only are the data for stearic acid inconsistent, but also the data for oleic acid and linoleic acid, two fatty acids with 18 carbon atoms (17–19). The objective of the present study was therefore to compare the effects of stearic, oleic, and linoleic acids on platelet aggregation, coagulation, fibrinolysis, and hematological variables.

SUBJECTS AND METHODS

Experimental design. The study had a randomized, multiple crossover design. The effects of stearic, oleic, and linoleic acids on lipid and lipoprotein concentrations were investigated earlier in this study, and the study protocol was described in detail (20). Briefly, each participant consumed 3 different diets in random order over three 5-wk periods. After each intervention period, there was a washout period of at least 1 wk, when participants consumed their

[1] Supported by the Dutch Dairy Association.
[2] To whom correspondence should be addressed.
E-mail: R.Mensink@hb.unimaas.nl.

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habitual diets. The protocol of the study was approved by the Medical Ethics Committee of Maastricht University.

Diets. The prescribed nutrient composition of the 3 diets did not differ, except for the 7% of energy provided by stearic acid, oleic acid, or linoleic acid. The experimental products (margarines, bread, and sponge cakes) supplied 60% of total daily fat intake at a targeted total fat intake of 37% of energy. For the remaining 40% of the total fat intake, subjects had to consume a certain amount of "free-choice" fat-containing products. These products had to be recorded in a diary, in which alcohol consumption, medication used, any sign of illness, menstruation, and any deviations from the study protocol also were noted. At least once every week, subjects visited a dietician at the university to receive a new supply of products and to be weighed. Individual allowances were adjusted when subjects' weight differed by >1.5 kg from the initial weight during wk 1 or >2 kg during the following weeks. The mean daily energy intake and the composition of the diets were determined from FFQs, which were filled out by the subjects in wk 5 of each intervention period.

Subjects. The screening procedure and eligibility criteria of the subjects were described in detail earlier (20). Briefly, 45 healthy, nonsmoking, slightly hypercholesterolemic subjects, 18 men and 27 women, completed the study protocol. All subjects were between 28 and 66 y old (mean age was 51 ± 10 y). The men had BMIs ranging from 21.8 to 29.8 kg/m^2 (mean 26.0 ± 2.2 kg/m^2); those of the women ranged from 18.0 to 29.4 kg/m^2 (mean 24.1 ± 2.8 kg/m^2). Among the women, 16 were postmenopausal and 5 used oral contraceptives. All subjects had given their written informed consent, before they entered the study protocol.

Blood sampling. At the end of each period (in wk 4 and 5), blood samples were drawn after the subjects had fasted overnight. Blood was sampled by venipuncture with minimum stasis using a 0.9 mm-needle (PrecisionGlide, Becton-Dickinson Vacutainer Systems) in wk 4 or with a 1.0 mm-infusion needle (Microflex, Vygon) in wk 5. The first 6 mL was collected into EDTA tubes (Becton Dickinson Vacutainer Systems) and used for analysis of hematological variables. Platelet, erythrocyte, and leukocyte counts, hemoglobin concentration, and hematocrit were measured with the Coulter Microdiff 18 (Beckman Coulter). The next 9 mL was collected into sodium citrate tubes (Becton Dickinson Vacutainer Systems) and immediately placed on ice. Within 1 h of collection, plasma was separated by centrifugation at 3500 × *g* for 30 min. Plasma samples were snap-frozen in liquid nitrogen and stored at −80°C until analyses of coagulation and fibrinolytic factors. In wk 5, an extra sodium citrate tube was drawn, which was kept at 37°C, for in vitro platelet aggregation measurements. Then, 10 mL of blood was collected into a serum tube (Corvac, Becton Dickinson Vacutainer Systems) for the analysis of the fatty acid composition of serum phospholipids. At least 1 h after venipuncture, serum was obtained by centrifugation at 3500 × *g* for 30 min at 4°C and stored at −80°C. Finally, after blood sampling in wk 5, the tube of the infusion needle was connected to the filtragometer for the measurement of ex vivo platelet aggregation. A pool of citrated plasma was obtained from healthy blood donors, and prepared by methods described above.

Fatty acid composition. Fatty acid compositions of serum phospholipids in a pooled sample of wk 4 and 5 were analyzed, as described previously (20). Values were expressed as a percentage of total fatty acids (g/100 g).

Ex vivo platelet aggregation. Ex vivo platelet aggregation was measured using filtragometry. The principle of this method was described and validated by Hornstra and ten Hoor (21). Filtragometry is based on the continuous measurement of the pressure difference (ΔP)[3] across a filter with pores of 20 μm in diameter through which blood flows. Platelet aggregates, obstructing the filter, cause a change in the ΔP, which is proportional to the mass of the platelet aggregates that obstruct the filter. For measurements, blood from a forearm vein was drawn via an infusion needle by a motor-driven syringe at a flow rate of 2 mL/min. The infusion needle was connected by a tubing system with the filtragometer. Initially, the blood was anticoagulated with heparin. When ΔP reached 5 mm Hg, which corresponded to 25% of filter pore occlusion, the heparin was switched off and a citrate infusion was started, which may partially reverse the occlusion of the filter resulting in a transient decrease in ΔP. The change in ΔP was monitored continuously for 10 min after connecting the filtragometer, and registered as aggregation curves. The aggregation variables that were calculated from these aggregation curves were the aggregation time (Ta in s, the time necessary to reach a ΔP of 5 mm Hg), the aggregation slope (Ts in mm Hg/s, the slope of the tangent to the curve at ΔP = 5 mm Hg), the maximum aggregation (Amax in mmHg, the maximum height of the aggregation curve), and the disaggregation induction time (Tdi in s, time between the termination of the heparin infusion and the beginning of the disaggregation). For 3 measurements, ΔP did not reach 5 mm Hg within 10 min and the aggregation time (Ta) was then set at 600 s. For these measurements, other aggregation variables could not be calculated. Evaluation by scanning electron microscopy showed that filter occlusion is due mainly to platelet aggregates. In addition, it was demonstrated that treatment with acetylsalicylic acid prolonged ex vivo platelet aggregation (21).

In vitro platelet aggregation. In vitro platelet aggregation was measured in whole blood with a dual-sample aggregometer (whole blood aggro-meter model 540, Chrono-log), immediately after blood sampling. For each measurement, 1 mL of anticoagulated blood was transferred into a prewarmed plastic cuvette and electrodes were inserted into the cuvettes. The sample was kept at 37°C and stirred. After equilibration of the sample and calibration of the instrument, aggregation was induced by the addition of collagen (Chrono-PAR #385 Collagen Reagent, Chrono-log) or ADP (Chrono-PAR #384 ADP Reagent, Chrono-log). The change in impedance was monitored continuously for 15 min and registered as aggregation curves. Collagen-induced aggregation was measured at a final concentration of 2 mg/L collagen and ADP-induced aggregation was measured at final concentrations of 15 and 5 μmol/L ADP. Whole-blood platelet aggregation was quantified by measuring the aggregation time (Tai), the aggregation velocity (Va) and the maximum aggregation (Imax).

Measurements of coagulation and fibrinolysis. Before analysis of coagulation and fibrinolytic factors, equal volumes of the citrated plasma samples from wk 4 and 5 were pooled. A chromogenic assay (Coaset F.VII, Chromogenix Instrumentation Laboratory) was used to assess factor VII amidolytic (factor VIIam) activity. Activities were expressed as the percentage of a plasma pool. Prothrombin fragment 1 + 2 (PTF1 + 2) concentrations were analyzed with an enzyme immunoassay (Enzygnost F1 + 2 micro, Dade Behring). Plasma fibrinogen concentrations were measured by a clotting assay (Dade Thrombin reagent, Dade Behring) based upon the method of Clauss (22). Plasminogen activator inhibitor (PAI) activity was determined with a chromogenic assay (Spectrolyse/pL PAI, Trinity Biotech). Concentrations of tissue plasminogen activator (tPA)/PAI-1 complexes were analyzed by an ELISA (tPA/PAI-1 Complex ELISA reagent kit, Technoclone). All samples from one subject were performed in the same analytic run. The CVs within assays were 1.8% for fibrinogen, 4.2% for factor VIIam, 9.5% for PTF1 + 2, 8.3% for PAI activity, and 13.6% for the tPA/PAI-1 complexes.

Statistics. For hematological variables, the mean of the 2 plasma samples from wk 4 and 5 was calculated before statistical analyses. All data were analyzed with the general linear model (GLM) procedure of the SPSS 11 for Mac OS X package. A probability level (*P*-value) of < 0.05 was considered significant. Differences in effects were examined with diet and period as fixed factors and subject number as random factor. Because previous studies showed gender-dependent effects of dietary fatty acid intake on the variables of interest or examined effects only in men, we also analyzed data by gender. It should be noted, however, that the study was not specifically designed to look for gender-dependent effects and the statistical power may have been too limited to specifically address this question. To analyze whether effects of diet were modified by gender, the diet × gender interaction term was added to the model as a fixed factor. When the

[3] Abbreviations used: Amax, ex vivo maximum platelet aggregation; ΔP, pressure difference; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; factor VIIam, factor VII amidolytic; Imax, in vitro maximum platelet aggregation; MUFA, monounsaturated fatty acids; PAI, plasminogen activator inhibitor; PRP, platelet-rich plasma; PTF1+2, prothrombin fragment 1 and 2; Ta, ex vivo platelet aggregation time; Tai, in vitro platelet aggregation time; Tdi, ex vivo platelet disaggregation induction time; tPA, tissue plasminogen activator; Ts, ex vivo platelet aggregation slope; Va, in vitro platelet aggregation velocity.

TABLE 1

Daily intake of fat and fatty acids by healthy men and women during consumption of the 3 diets for 5 wk[1,2]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[3]
		% of energy		
Fat	38.2 ± 5.1	37.7 ± 5.6	38.0 ± 5.3	0.701
SFA	18.0 ± 2.3[a]	11.0 ± 2.0[b]	11.2 ± 1.9[b]	<0.001
Stearic acid [18:0][4]	7.7 ± 1.1[a]	1.2 ± 0.2[b]	1.2 ± 0.2[b]	<0.001
MUFA	12.9 ± 2.0[b]	19.1 ± 2.9[a]	12.5 ± 1.8[b]	<0.001
Oleic acid [18:1(n-9)][4]	6.8 ± 1.0[b]	13.1 ± 2.0[a]	6.6 ± 1.0[b]	<0.001
PUFA	4.7 ± 1.2[b]	5.0 ± 1.1[b]	11.8 ± 1.8[a]	<0.001
Linoleic acid [18:2(n-6)][4]	2.1 ± 0.3[b]	2.4 ± 0.3[b]	9.3 ± 1.3[a]	<0.001

[1] Values are means ± SD, $n = 45$ (18 men and 27 women) as calculated from the FFQ. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] Intakes of energy (8.5 ± 1.6 MJ), carbohydrates (46.1 ± 6.1% of energy), proteins (13.9 ± 2.0% of energy), alcohol (2.2 ± 2.3% of energy), cholesterol (17.7 ± 3.6 mg/MJ), and dietary fiber (3.1 ± 0.7 g/MJ) did not differ among the 3 diets.
[3] *P*-values for diet effects were calculated by the general linear model with subject number as random factor and diet and period as fixed factors.
[4] As provided by the experimental fats only.

analyses indicated a significant effect of diet ($P < 0.05$), the diets were compared pair wise. Between-diet comparisons were corrected for 3-group comparisons by the Bonferroni correction, and 95% CI were calculated for the differences among the diets. Values are presented as means ± SD.

RESULTS

Dietary composition. The subjects' body weights at the end of each dietary period were 72.5 ± 13.0 kg after consumption of the stearic acid diet, 72.5 ± 13.2 kg after the oleic acid diet and 72.7 ± 12.9 kg after the linoleic acid diet. Weights did not differ among those consuming the 3 diets ($P = 0.449$). Daily intakes of energy, protein, carbohydrates, fat, cholesterol, and fiber did also not differ among subjects consuming the 3 diets (**Table 1**). Fatty acid intakes were comparable, except for ~7% of energy, which was provided by stearic acid, oleic acid, or linoleic acid.

Dietary adherence of the subjects was confirmed by the fatty acid composition of serum phospholipids (**Table 2**). The 3 diets differed in their effects on the proportions of stearic acid, oleic acid, and linoleic acid ($P < 0.001$ for diet effects). The proportions of arachidonic acid ($P = 0.103$ for diet effects) and docosahexaenoic acid (DHA; $P = 0.063$ for diet effects) did not differ. The proportions of the (n-3) PUFAs, α-linolenic acid ($P < 0.001$ for diet effects) and eicosapentaenoic acid (EPA; $P < 0.001$ for diet effects) were lower during consumption of the linoleic acid diet compared with the other 2 diets.

Hematological variables. When all subjects were analyzed together, the 3 diets differed significantly in their effects on the number of erythrocytes and mean platelet volumes (**Table 3**). The number of erythrocytes tended to be lower when subjects consumed the diet high in linoleic acid rather than the stearic acid diet ($P = 0.054$, 95% CI for the difference -0.259 to -0.001×10^{12}/L). In men, the numbers of erythrocytes during consumption of the linoleic acid diet were lower than during consumption of the oleic acid diet ($P = 0.005$, 95% CI for the difference -0.365 to -0.057×10^{12}/L) and only slightly lower than during consumption of the stearic acid diet ($P = 0.106$, 95% CI for the difference -0.288 to 0.020×10^{12}/L). Hematocrit values were slightly lower in men consuming the linoleic acid diet compared with the diets high in stearic acid ($P = 0.107$, 95% CI for the difference -0.026 to 0.002 L/L) and oleic acid ($P = 0.021$, 95% CI for the

TABLE 2

Fatty acid composition of serum phospholipids in healthy men and women during consumption of the 3 diets for 5 wk[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
		g/100 g total fatty acids		
SFA	46.5 ± 1.5[a]	45.6 ± 1.5[b]	46.2 ± 1.9[a]	0.001
Stearic acid (18:0)	14.3 ± 1.2[a]	13.1 ± 1.1[c]	13.7 ± 1.3[b]	<0.001
MUFA	13.6 ± 1.1[b]	15.0 ± 1.3[a]	12.2 ± 0.9[c]	<0.001
Oleic acid [18:1(n-9)]	9.3 ± 1.1[b]	10.5 ± 1.2[a]	7.7 ± 0.8[c]	<0.001
PUFA	39.1 ± 1.6[b]	38.6 ± 1.7[b]	40.6 ± 2.1[a]	<0.001
Linoleic acid [18:2(n-6)]	20.7 ± 1.8[b]	20.5 ± 2.0[b]	23.2 ± 2.4[a]	<0.001
Arachidonic acid [20:4(n-6)]	8.9 ± 1.5	8.7 ± 1.5	8.6 ± 1.7	0.103
α-Linoleic acid [18:3(n-3)]	0.14 ± 0.04[a]	0.13 ± 0.04[a]	0.11 ± 0.04[b]	<0.001
EPA [20:5(n-3)]	0.8 ± 0.4[a]	0.7 ± 0.3[a]	0.5 ± 0.3[b]	<0.001
DHA [22:6(n-3)]	3.4 ± 0.9	3.3 ± 0.7	3.2 ± 0.7	0.063
Trans fatty acids	0.8 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	0.060

[1] Values are means ± SD, $n = 45$ (18 men and 27 women) as calculated from the FFQ. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] *P*-values for diet effects were calculated by the general linear model with subject number as random factor and diet and period as fixed factors.

TABLE 3

Hematological variables during consumption of diets enriched in stearic, oleic, and linoleic acids for 5 wk by healthy men and women[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
Leukocytes, $\times 10^9$/L				
All	6.05 ± 1.28	6.23 ± 1.26	6.05 ± 1.29	0.211
Men	6.33 ± 1.20	6.41 ± 1.07	6.24 ± 1.15	0.540
Women	5.86 ± 1.32	6.12 ± 1.38	5.92 ± 1.38	0.244
Erythrocytes, $\times 10^{12}$/L				
All	4.74 ± 0.55	4.72 ± 0.49	4.61 ± 0.44	0.046
Men	4.98 ± 0.53[ab]	5.06 ± 0.47[a]	4.84 ± 0.48[b]	0.006
Women	4.59 ± 0.51	4.48 ± 0.36	4.46 ± 0.34	0.254
Hemoglobin, g/L				
All	145 ± 127	143 ± 102	142 ± 111	0.133
Men	153 ± 95	151 ± 84	151 ± 93	0.504
Women	139 ± 116	138 ± 71	136 ± 77	0.255
Hematocrit				
All	0.41 ± 0.04	0.41 ± 0.03	0.40 ± 0.03	0.108
Men	0.43 ± 0.03[ab]	0.44 ± 0.03[a]	0.42 ± 0.03[b]	0.019
Women	0.40 ± 0.04	0.39 ± 0.02	0.39 ± 0.02	0.302
Platelets, $\times 10^9$/L				
All	273 ± 64	265 ± 62	266 ± 60	0.457
Men	263 ± 57	240 ± 60	257 ± 64	0.088
Women	279 ± 69	281 ± 58	271 ± 57	0.458
Mean platelet volume, fL				
All	7.89 ± 0.76[b]	8.21 ± 0.66[a]	8.25 ± 0.72[a]	<0.001
Men	7.86 ± 0.98[b]	8.19 ± 0.82[a]	8.23 ± 0.89[a]	0.003
Women	7.92 ± 0.59[b]	8.23 ± 0.54[a]	8.26 ± 0.62[a]	0.001

[1] Values are means ± SD at the end of each intervention period (mean of wk 4 and 5), $n = 45$ (18 men and 27 women). Means in a row with superscripts without a common letter differ, $P < 0.05$.

[2] *P*-values for diet effects were calculated by the general linear model with subject number as random factor and diet and period as fixed factors.

difference −0.030 to −0.002 L/L). No other gender-dependent effects were observed. When subjects consumed the stearic acid diet, the platelet volume decreased by 0.32 fL compared with the oleic acid diet ($P < 0.001$, 95% CI for the difference −0.495 to −0.144 fL) and by 0.35 fL compared with the linoleic acid diet ($P < 0.001$, 95% CI for the difference −0.526 to −0.175 fL). These effects were evident in both men and women.

Ex vivo and in vitro platelet aggregation. In all subjects, ex vivo platelet aggregation time (Ta) as measured by filtragometry did not differ among those consuming the 3 diets ($P = 0.200$ for diet effects, **Table 4**). In men only ($P = 0.036$ for diet effects), Ta increased by 69 s during consumption of the linoleic acid diet relative to the diet high in stearic acid ($P = 0.040$, 95% CI for the difference 2 to 136 s). The aggregation time during consumption of the linoleic acid diet tended to increase compared with that when the oleic acid diet was consumed ($P = 0.198$, 95% CI for the difference −16 to 113 s). The diets high in stearic acid or oleic acid did not differ in their effects on aggregation time ($P = 1.00$, 95% CI for the difference −47 to 87 s). No diet effects were evident in women ($P = 0.713$ for diet effects).

There were no diet effects for collagen-induced platelet aggregation or ADP-induced platelet aggregation in whole blood in vitro (**Table 5**). Because effects on in vitro platelet aggregation induced by 5 μmol/L ADP were comparable to those induced by 15 μmol/L ADP, only results with a final concentration of 15 μmol/L are reported.

Coagulation and fibrinolysis. Coagulation (factor VIIam activity, PTF1 + 2, and fibrinogen) and fibrinolytic (PAI activity and tPA/PAI-1 complexes) factors did not differ among the 3 diets (**Table 6**). These variables did also not differ between men and women.

DISCUSSION

The purpose of this well-controlled crossover study was to evaluate the effects of stearic, oleic, and linoleic acids on platelet aggregation, coagulation, and fibrinolytic factors, and hematological variables in healthy men and women. When 7% of energy of these fatty acids was exchanged among the diets, small differences on these markers of thrombotic tendency appeared. Compared with the stearic acid diet, the diet high in linoleic acid decreased ex vivo platelet aggregation in men only. Moreover, stearic acid decreased platelet volume relative to both other fatty acids in both men and women. Effects on coagulation and fibrinolytic variables did not differ after consumption of the 3 fatty acids. Therefore, the finding in previous studies (12,13) that stearic acid is highly thrombogenic is not supported by the present study.

Ex vivo platelet aggregation time in men increased with consumption of linoleic acid relative to the stearic acid diet. In 2 earlier studies with the filtragometer, linoleic acid prolonged aggregation time compared with a mixture of SFA (23) or a mixture of SFA and monounsaturated fatty acids (MUFA) (24). A prolonged aggregation time as measured by filtragometry indicates lower in vivo platelet aggregability and is negatively associated with mortality from coronary heart disease (21). In the study of Hornstra et al. (23) only men participated, whereas in the study of Fleischman et al. (24) both men and women participated. Unfortunately, in the latter study, results were not reported for men and women separately. In our study, ex vivo platelet aggregation time was significantly increased by linoleic acid consumption in men, but not in women relative to stearic acid. Compared with oleic acid, this effect tended to be significant ($P = 0.198$). These findings indicate a gender-dependent effect of dietary fatty acid intake on ex vivo platelet aggregation. In contrast to

TABLE 4

Effects on ex vivo platelet aggregation variables as measured by filtragometry during consumption of diets enriched in stearic, oleic, and linoleic acids for 5 wk in healthy men and women[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
Ta, *s*				
All	113 ± 96	109 ± 62	142 ± 125	0.200
Men	109 ± 70[b]	117 ± 61[ab]	164 ± 127[a]	0.036
Women	115 ± 110	104 ± 62	126 ± 123	0.713
Ts, *mm Hg/s*				
All	0.61 ± 0.68	0.52 ± 0.58	0.71 ± 1.06	0.243
Men	0.48 ± 0.42	0.38 ± 0.37	0.40 ± 0.60	0.789
Women	0.68 ± 0.79	0.62 ± 0.68	0.94 ± 1.26	0.259
Amax, *mm Hg*				
All	81 ± 110	97 ± 121	94 ± 123	0.795
Men	48 ± 82	74 ± 113	49 ± 95	0.657
Women	99 ± 121	113 ± 126	128 ± 133	0.786
Tdi, *s*				
All	29.5 ± 24.1	24.6 ± 11.2	24.2 ± 12.7	0.400
Men	21.7 ± 12.0	22.2 ± 5.5	23.1 ± 13.5	0.723
Women	34.1 ± 28.4	26.5 ± 14.0	25.2 ± 12.2	0.521

[1] Values are means ± SD, $n = 18$ men and 27 women, at the end of each intervention period (wk 5). Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] *P*-values for diet effects were calculated by the general linear model with subject number as random factor and diet and period as fixed factors.

the other 2 studies (23,24), we could specifically attribute the observed effects to stearic acid. Whether other saturated fatty acids would have given similar effects remains to be clarified.

Stearic acid, oleic acid, and linoleic acid did not differ in their effects on in vitro whole-blood platelet aggregation variables induced by either ADP or collagen, a technique used in many other studies. This extends the findings of Hunter et al.

TABLE 5

Effects on in vitro platelet aggregation in whole blood induced by either collagen or ADP during consumption of diets enriched in stearic, oleic, and linoleic acids for 5 wk in healthy men and women[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
		Collagen-induced platelet aggregation (2 mg/L)[3]		
Tai, *min*				
All	0.93 ± 0.23	0.99 ± 0.30	0.90 ± 0.25	0.146
Men	1.04 ± 0.22	1.17 ± 0.29	0.97 ± 0.31	0.091
Women	0.86 ± 0.22	0.87 ± 0.25	0.85 ± 0.20	0.909
Va, Ω/*min*				
All	6.11 ± 2.84	6.28 ± 3.06	5.86 ± 2.75	0.776
Men	5.21 ± 2.00	5.49 ± 1.97	5.35 ± 2.17	0.805
Women	6.73 ± 3.18	6.80 ± 3.55	6.21 ± 3.08	0.717
Imax, Ω				
All	12.4 ± 5.5	13.3 ± 4.8	12.0 ± 5.1	0.449
Men	11.3 ± 4.5	13.7 ± 4.8	12.1 ± 4.3	0.232
Women	13.2 ± 6.1	12.9 ± 4.9	11.9 ± 5.6	0.513
		ADP-induced platelet aggregation (15 μmol/L)		
Tai, *min*				
All	0.58 ± 0.23	0.57 ± 0.19	0.59 ± 0.25	0.705
Men	0.74 ± 0.20	0.65 ± 0.21	0.63 ± 0.23	0.316
Women	0.48 ± 0.18	0.52 ± 0.16	0.57 ± 0.26	0.380
Va, Ω/*min*				
All	4.65 ± 2.51	5.03 ± 2.42	4.41 ± 2.38	0.518
Men	3.53 ± 2.07	4.57 ± 1.92	4.23 ± 2.26	0.374
Women	5.42 ± 2.54	5.36 ± 2.72	4.51 ± 2.49	0.424
Imax, Ω				
All	8.8 ± 5.1	10.4 ± 4.5	8.9 ± 4.5	0.304
Men	7.7 ± 5.8	10.4 ± 4.1	8.9 ± 4.5	0.410
Women	9.6 ± 4.5	10.4 ± 4.9	8.9 ± 4.6	0.564

[1] Values are means ± SD, $n = 18$ men and 27 women at the end of each intervention period (wk 5).
[2] *P*-values for diet effects were calculated by the general linear model with subject number as random factor and diet and period as fixed factors. The 3 diets did not differ.
[3] Values are the means of 2 measurements.

TABLE 6

Coagulation and fibrinolytic variables during consumption of diets enriched in stearic, oleic, and linoleic acids for 5 wk by healthy men and women[1]

	Stearic acid diet	Oleic acid diet	Linoleic acid diet	*P* for diet effects[2]
Factor VIIam activity, *% of standard*				
All	98 ± 31	100 ± 32	97 ± 33	0.398
Men	99 ± 26	98 ± 22	99 ± 26	0.908
Women	98 ± 34	102 ± 37	96 ± 37	0.245
PTF1 + 2, *nmol/L*				
All	2.51 ± 2.98	2.51 ± 2.95	2.78 ± 3.14	0.360
Men	3.12 ± 4.01	2.67 ± 3.62	2.96 ± 3.74	0.482
Women	2.11 ± 2.05	2.40 ± 2.49	2.67 ± 2.75	0.115
Fibrinogen, *g/L*				
All	3.2 ± 0.5	3.2 ± 0.5	3.2 ± 0.7	0.940
Men	3.1 ± 0.5	3.0 ± 0.6	3.2 ± 0.9	0.599
Women	3.2 ± 0.6	3.3 ± 0.4	3.2 ± 0.5	0.389
PAI activity, *kU/L*				
All	10.47 ± 7.25	9.89 ± 7.15	9.79 ± 6.82	0.346
Men	12.76 ± 7.01	11.65 ± 6.63	10.85 ± 6.96	0.077
Women	8.94 ± 7.12	8.72 ± 7.37	9.09 ± 6.76	0.842
TPA/PAI-1 complexes, *μg/L*				
All	43.1 ± 33.6	41.2 ± 33.8	39.0 ± 23.7	0.533
Men	57.0 ± 42.1	49.9 ± 41.1	45.2 ± 22.9	0.293
Women	33.9 ± 23.1	35.4 ± 27.2	34.8 ± 23.7	0.894

[1] Values are means ± SD, n = 18 men and 27 women at the end of each intervention period (pooled wk 4 and 5).
[2] *P*-values for diet effects were calculated by the general linear model with subject number as random factor and diet and period as fixed factors. The 3 diets did not differ.

(25), who reported the effects of stearic acid, oleic acid, and linoleic acid in a crossover study with only 6 young healthy men. When experimental diets were consumed for 2 wk, ADP-induced platelet aggregation in platelet-rich plasma (PRP) was not affected (25). Several studies focused on the effects of oleic and linoleic acids, but results were inconsistent. As in our study, there was no difference in effect between oleic acid and linoleic acid in most of the studies (17,18,25–28). An exception is the study of Burri et al. (19) with 7 healthy men in which 7–8% of the energy of oleic acid and linoleic acid were exchanged. Consumption of the diet high in linoleic acid decreased ADP- and collagen-induced platelet aggregation relative to oleic acid. In another study, consumption of oleic acid as well as linoleic acid decreased collagen-induced platelet aggregation compared with a diet high in SFA (29). In the studies mentioned earlier, PRP was used to measure platelet aggregation, whereas in the last-mentioned study, platelet aggregation was analyzed in whole blood. Finally, effects depend on the way in which platelet aggregation is induced. In the study of Lahoz et al. (18), consumption of linoleic acid but not oleic acid enhanced platelet aggregation induced by ADP, relative to SFA. This proaggregatory effect was not observed when collagen or adrenalin was used as the inducer. In the other study (26), oleic acid and linoleic acid increased collagen- but not ADP-induced platelet aggregation compared with SFA. In general, in vitro platelet aggregation studies are difficult to compare because of differences in methodologies. Different anticoagulants may be used or different inducers (collagen, ADP, arachidonic acid, or thrombin) at different concentrations; in some studies, PRP was used, and in others whole blood.

Stearic acid, oleic acid, and linoleic acid did not differ in their effects on factor VIIam activity. Hunter et al. (25) also observed comparable effects of stearic acid and these 2 *cis*-unsaturated fatty acids using 3 different methods to assess factor VII activity. In addition, when the effects of dietary oleic acid and linoleic acid were compared in a study with subjects between 45 and 65 y old, these fatty acids did not differently affect factor VII coagulant activity (30). In contrast, in 2 studies with healthy young male and female students, oleic acid consumption reduced factor VII coagulant activity compared with linoleic acid (28,31). In one of these studies, the concentration of activated factor VII (FVIIa), however, did not differ between subjects consuming the 2 diets (28). FVIIa was not measured in the other study (31). These contrasting findings (28,30,31) cannot be attributed to differences in the age of the subjects because in 2 other studies in young healthy humans, consumption of oleic acid and linoleic acid did not differently affect factor VII coagulant activity (32,33). Thus, despite the use of a wide variety of methods to measure factor VII activity, the effects of stearic, oleic, and linoleic acid consumption on this coagulation factor did not differ.

Although concentrations of fibrinogen (6,7) and PTF1 and 2 (7) are positively associated with the risk of coronary heart disease, only a few studies investigated the effects of dietary fatty acids on these coagulation variables. Although some studies reported different effects of oleic acid and linoleic acid on factor VII, concentrations of PTF1 and 2 and fibrinogen did not differ (28,31). Similar to Hunter et al. (25), we also found comparable effects of stearic, oleic, and linoleic acids on these coagulation variables. In contrast, when 8% of energy from stearic acid was replaced by oleic acid, fibrinogen concentrations increased by 0.15 g/L in a recent study by Baer et al. (34). In addition, compared with a diet rich in myristic and lauric acids, stearic acid raised fibrinogen concentrations (16). When 7–8% of energy from stearic acid was exchanged for palmitic acid, however, fibrinogen was not affected (14). The effects of lauric and palmitic acid consumption on concentrations of fibrinogen and PTF1 + 2 did not differ from those of oleic acid (35). Thus, although the effects of oleic and linoleic acid consumption on fibrinogen concentrations seem to be comparable, the effects of stearic acid relative to the other SFA or unsaturated fatty acids were inconsistent.

As markers of fibrinolytic activity, concentrations of tPA and PAI-1 antigen, tPA and PAI activities (6), and concen-

trations of tPA-PAI-1 complexes (36) are related to the risk of coronary heart disease. Various earlier studies evaluated the effects of dietary fatty acid composition on variables of the fibrinolytic system. In our study, stearic, oleic and linoleic acid consumption did not affect PAI activity and concentrations of tPA/PAI-1 complexes, which are strongly correlated with plasma levels of PAI activity or tPA antigen (36). These findings not only agree with the results of the study of Hunter et al. (25), but also with the few studies that have compared oleic acid and linoleic acid. In one study, high-fat diets rich in oleic or linoleic acids did not differ in their effects on tPA activity and PAI-1 antigen concentrations (33). Dietary oleic and linoleic acids also had comparable effects on tPA and PAI activities (28,31). Therefore, our study does not provide evidence that these 3 fatty acids have differential effects on fibrinolytic variables.

In both men and women, platelet volume was decreased after consumption of the diet high in stearic acid. Stearic acid–enriched diets also decreased platelet volume compared with diets rich in palmitic acid (14,37,38). Larger platelets are associated with increased platelet reactivity and risk of myocardial infarction (39,40). Survivors, 6 mo after a myocardial infarction, had a 0.43 fL lower platelet volume than nonsurvivors (39). In our study, platelet volumes of the subjects during consumption of the stearic acid diet were 0.32 and 0.35 fL lower than when they consumed diets high in oleic acid and linoleic acid, respectively.

To summarize, when 7% of dietary energy of stearic acid was replaced by linoleic acid, ex vivo platelet aggregation was beneficially affected in men. On the other hand, stearic acid consumption reduced platelet volume relative to the other 2 fatty acids, whereas effects on coagulation and fibrinolytic variables did not differ among the 3 fatty acids. Overall, we therefore conclude that our results do not provide evidence that stearic acid is highly thrombogenic, as suggested by some earlier studies (12,13).

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