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Impact of Margarine Enriched With Plant Sterols on Blood Lipids, Platelet Function, and Fibrinogen Level in Young Men

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The effects of margarines enriched with ω-6 polyunsaturated fatty acids (PUFA), as well as those containing plant sterols or stanols, on reduction of plasma low-density lipoprotein-cholesterol (LDL-C) have been extensivly studied. However, their impact on fibrinogen (Fb) concentration and blood platelet function is much less known. Our research involved 42 healthy male students (average age, 23.7 ± 1.6) who during the research period were subjected to a controlled regime of nutrition and physical activity. After a period of diet stabilization involving 30 g butter daily in 2 servings, the subjects were randomly divided into 2 groups. In the first group, the butter was replaced by the same quantity of PUFA margarine, while the second group received margarine with added plant sterols instead of butter. The subjects consuming margarine with sterols showed a significant (11%) decrease in LDL-C ($P < .001$). Margarine rich in PUFA caused a 6% reduction in LDL-C ($P < .01$), with a simultaneous 3% reduction in high-density lipoprotein-cholesterol (HDL-C) ($P < .001$). Both types of margarine increased the concentration of Fb ($P < .001$), without exceeding the normal medium value of 2.8 g/L. After the consumption of margarine with sterols, the adhesion and aggregation time of blood platelets was significantly prolonged after collagen-epinephrine activation. Margarine with sterols, through its antiplatelet activity and the significant reduction of LDL-C, can play a vital role in the nonpharmacologic prevention of circulatory diseases.

SEVERAL DOZEN reports have been published to date on the use of margarine or yogurt with added sterol or stanol, documenting their favorable impact on the reduction of total and low-density lipoprotein-cholesterol (LDL-C) in persons with both normal and elevated cholesterolemia. The published results indicate that the addition of 2 to 3 g plant sterols to an ordinary or low-fat diet causes a 7% decrease in total cholesterol on the average and an approximately 10% reduction in LDL-C.[1-5] Some investigators suggest that the reduction in the concentration of LDL-C may reach as high as 15% or even more, but these results were obtained during the simultaneous application of a low-fat diet, supplemented by an appropriate supply of soluble fractions of dietary fiber and antioxidant vitamins.[5,6-10]

Another higher favorable phenomenon accompanying the therapeutic application of plant sterols in the treatment of patients with hypercholesterolemia is the fact that they have no effect on the level of high-density lipoprotein-cholesterol (HDL-C). This is a factor that distinguishes plant sterols in margarine from polyunsaturated fatty acids (PUFA), the application of which produces favorable hypolipemic effects through the reduction of total and LDL-C, unfortunately also causing a decrease in the level of HDL-C, averaging 2% to 4%.[11] In many cases, this is an undesirable phenomenon, especially for example, in patients with diabetes, in whom disturbances in lipid management are characterized by hypertriglyceridemia and low levels of HDL-C.[4,12-14]

Several studies have reported results suggesting that one of the consequences of the high consumption of saturated fatty acids (especially $C_{12} - C_{16}$) and trans isomers of free fatty acids, in addition to hyperlipemia, may be prothrombotic disturbances in the hemostatic system.[15,16] The causes of the prothrombotic effects of these acids should be sought in the change of the fatty acid profile in the phospholipids of the

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Table 1. Characteristics of the Subjects During the Study Time

	PUFA Group A (n = 22)		Sterol Group B (n = 20)	
	Before	After	Before	After
Age (yr)	23.2 ± 1.2		24.1 ± 1.9	
BMI (kg/m^2)	24.4 ± 2.4	24.2 ± 2.2	24.4 ± 3.2	24.0 ± 3.4
WHR	0.82 ± 0.06	0.82 ± 0.05	0.82 ± 0.07	0.81 ± 0.06
SBP (mm Hg)	121.96 ± 12,86	116.74 ± 11.34	121.36 ± 13.99	118.86 ± 12.62
DBP (mm Hg)	76.96 ± 7.35	74.13 ± 7.33	77.05 ± 7.51	73.64 ± 6.76
Glucose (mg/dL)	91.09 ± 5.07	90.04 ± 8.53	91.14 ± 7.18	89.55 ± 5.93

NOTE. Values are means ± SD (NS).
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WHR, waist-to-hip ratio.

membranes of blood platelets, which leads to increased platelet aggregation.[17-20] It is also well known that an elevated level of LDL-C has a direct impact on the aggregation of blood platelets. The capacity of LDL-C to intensify or induce blood platelet aggregation is associated with the occurrence in the platelet membrane of binding sites for LDLs; LDLs aggregate blood platelets in a receptor mechanism through apolipoprotein B (ApoB).[21]

In our previous research, we demonstrated the unfavorable effects of a fat-rich diet on the hemostatic system. We found prothrombotic effects of dietary lipemia in normolipemic patients with angiographically confirmed atherosclerosis of the coronary arteries. These effects were manifested by the increased activity of factor VII, the t-PA inhibitor (PAI-1), and the fibrinogen (Fb) concentration.[22]

It should be emphasized that the nonpharmacologic reduction of LDL-C entails the restoration of the proper equilibrium in the hemostatic system, whereby it may contribute to lowering the risk of thrombosis and coronary heart disease.[23-25] Previous research has shown that the prevention of atherosclerosis, especially primary, should include above all a change in lifestyle, including also dietary modifications. To date, there have been many reports on the effects of PUFA from the ω-6 family on lowering the level of total cholesterol and LDL-C, as well as the positive effects, associated primarily with ω-3 acids, on the hemostatic system, due to the inhibition of blood platelet aggregation.[26,27]

It seems clear, on the other hand, that margarines with a high PUFA content or with added plant sterols or stanols provide a remedy for the contemporary demands of cardiologic prophylaxis. These margarines have a stronger hypocholesterolemic effect than other margarines, thanks to the mechanism of inhibiting cholesterol absorption in the small intestine.[1,7,28] Research on the impact of sterols and stanols on hemostasis has thus far been the topic of very few reports. These have dealt with the impact of such compounds on plasma and fibrinolytic hemostasis, rather than on thrombotic. One may well expect, however, that the new generation of margarines with plant sterols, through their powerful effect in reducing LDL-C, should have some impact, even if indirect, on the hemostatic functions of blood platelets and the concentration of Fb.[24,29]

For this reason, we conducted our research on healthy young men to whose diet we added 2 margarines: one with PUFAs and one with added plant sterols, in the place of other fats used as spreads, evaluating their impact on the lipid profile and hemostatic parameters.

MATERIALS AND METHODS

Subjects and Diets

Our research involved 42 healthy male students living during the time of experiments in the same standard conditions. Their health status was evaluated on the basis of clinical examinations, routine hematologic and biochemical tests, and a patient history questionnaire (Table 1). Only healthy persons were enrolled in the study. Smokers and every day alcohol drinkers and persons taking any medications were excluded from the experiments.

After a 2-week period of dietary stabilization, the subjects were randomly divided into 2 groups, A and B. Group A included 22 men who twice daily consumed 15 g margarine (for a total of 30 g) rich in ω-6 PUFA. Group B included 20 men who twice daily consumed 15 g margarine with added vegetable oil esters (total 30 g daily). Both types of margarine used in our research were market products. The composition of the 2 types of spread is presented in Table 2. The subjects from both groups consumed the margarine for 4 weeks (double-shielded trial method). Using the computer program developed at the Institute of Food and Nutrition in Warsaw, we monitored the subjects' manner of consumption; throughout the entire experimental period they consumed the same quantities (servings) of all dietary components, the only variable being the fats that were the object of the experiment (Table 3). Throughout the observation period, the subjects did not consume any alcohol; they drank 2 cups of coffee daily, and their level of physical activity was similar, ie, consistent with the schedule of physical education classes in their academic program.

Our research project was evaluated and approved by the Ethics Commission of the Institute of Food and Nutrition in Warsaw. Each subject, upon acquainting himself with the purpose of the experiment, gave his written consent to take part in the project.

Table 2. Comparison of the Fatty Acid Contents in the 30-g Servings of Margarine Used in the Experiment

30 g/d Serving Margarine	Energy (kJ/kcal)	SFA (g)	MUFA (g)	PUFA (g)	trans-FFA (g)	Vitamin E (mg)	Sterols (g)
PUFAs	656/160	4.4	5.9	11.1	0.2	6.6	—
Sterols	405/98	2.4	2.7	5.3	0.1	19.8	2.6

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; trans-FFA, trans fatty acid.

Table 3. Composition of the Diets in the Two Groups of Subjects

Parameter	Stabilization Diet	PUFAs Diet	Sterol Diet
Total energy kcal/kJ	2,016/8,435	1,955/8,180	1,887/7,895
Carbohydrate (g)	250.0	249.8	249.9
% energy from carbohydrate	49.6	51.1	52.9
Protein (g)	60.48	58.65	58.5
% energy from protein	12.0	12.0	12.4
Total fat (g)	85.9	80.4	78.9
% energy from total fat	38.4	36.9	34.7
SFA (g)	38.55	25.89	24.42
% energy from SFA	17.2	11.9	10.7
MUFA (g)	36.28	33.34	32.02
% energy from MUFA	16.2	15.3	14.1
PUFA (g)	11.07	21.17	16.37
% energy from PUFA	5.0	9.7	7.2
P/S	0.3	0.8	0.7
Cholesterol (mg)	408	334	334
Plant sterol (g)	—	—	2.6
Fiber (g)	21.5	21.5	21.5

Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S, polyunsaturated/saturated ratio.

The characteristics of the study groups and a comparison of the fat composition of the tested margarines are presented in Tables 1 and 2.

Hemostatic and Lipid Determinations

Blood platelet function (a simultaneous measurement of adhesion and aggregation) was determined in whole fresh citrated blood (9 parts blood + 1 part buffered 3.2% sodium citrate) using a Platelet Function Analyzer (PFA-100, manufactured by Dade Behring, Marburg, Germany), within a time not exceeding 3 hours after the blood was collected. The test was based on measuring the capillary closure time, during which appropriately activated blood from the tested sample flowed (closure time in seconds). Two types of agonists were used to activate the platelets in the blood samples: collagen with epinephrine (Coll/Epi) and collagen with adenosine diphosphate (Coll/ADP).

The Fb concentration was measured in oligothrombotic citrated plasma by the chronometric method (according to Clauss) using a Hemolab analyzer by bioMerieux (Marcy-L'Etoile, France). Determinations were performed in fresh plasma, not frozen.

The serum concentrations of total cholesterol, (TC), LDL-C and HDL-C, and triglycerides (TG) were determined. The lipid parameters were measured by the enzymatic method using ready kits by Boehringer Mannheim (Mannheim, Germany) suitable for use in a CLINILAB biochemical analyzer (bioMerieux). All determinations were performed twice, once just before the replacement of butter with margarine (after diet stabilization) and again after 4 weeks of margarine consumption.

Statistical Analysis

The impact of the 2 dietary regimes on hemostatis and lipid parameters was compared by analysis of variance (ANOVA) test for 2 factors with repeated measures (time × diet). To evaluate the effects of each of the 2 dietary regimes on hemostasis and lipid parameters, the Student *t* test for pair measures was applied. Linear correlation between changes in the hemostatic parameters and changes in the lipid parameters during the application of the diets were estimated by calculating the Pearson *r* coefficient. The STATISTICA PL5 (StatSoft, Tulsa, OK) statistical software package was used, assuming the level of statistical significance to be $P < .05$.

RESULTS

The results of the determination of lipid parameters, blood platelet aggregation and adhesion time, and Fb are presented in Tables 4 and 5. After the conclusion of the 4-week experiment, the subjects displayed no adverse health effects; the body mass index (BMI) and waist-to-hip ratio (WHR) remained unchanged, as did the blood cell count (data not show).

As shown in Table 4, a stronger hypolipemic effect from the sterol diet than the PUFA diet was observed. The percentage changes under the influence of the diet with sterols and the diet with PUFA came to 8% and 4%, respectively, in terms of total cholesterol, 11% versus 6% in LDL-C, and 6% versus 11% in the atherosclerosis index (LDL-C/HDL-C). No essential differ-

Table 4. Effects of Margarine With Omega-6 PUFA and Margarine Enriched With Plant Sterols on Lipid Parameters

Lipid Parameters	PUFA—Margarine	Plant Sterols—Margarine
Total cholesterol (mg/dL)		
Before diet	183.0 ± 32.9	185.2 ± 32.6
After diet	175.1 ± 34.6*	170.8 ± 29.7†
LDL cholesterol (mg/dL)		
Before diet	89.1 ± 27.6	89.7 ± 22.9
After diet	83.2 ± 27.3*	79.8 ± 22.9†
HDL cholesterol (mg/dL)		
Before diet	53.3 ± 10.6	54.7 ± 8.2
After diet	51.5 ± 9.5†	54.9 ± 9.3 NS
Triglycerides (mg/dL)		
Before diet	89.8 ± 35.9	87.9 ± 29.2
After diet	88.6 ± 54.9 NS	80.9 ± 28.4 NS
LDL/HDL cholesterol ratio		
Before diet	1.72 ± 0.70	1.65 ± 0.42
After diet	1.61 ± 0.68*	1.47 ± 0.40*

NOTE. Values are means ± SD.
Abbreviations: NS, not significant.
*$P < .01$, †$P < .001$ *v* before diet.

Table 5. Impact of Margarine With Omega-6 PUFA and Margarine Enriched With Plant Sterols on Platelet Aggregation and Fibrinogen Level

Hemostatic Parameters	PUFAs—Margarine	Plant Sterols—Margarine
Coll/Epi (closure time, s)		
Before diet	123.3 ± 26.9	132.9 ± 14.7
After diet	126.7 ± 22.6 NS	140.9 ± 19.5*
Coll/ADP (closure time, s)		
Before diet	87.1 ± 14.7	98.0 ± 18.2
After diet	85.6 ± 11.2 NS	100.9 ± 13.1 NS
Fibrinogen (g/L)		
Before diet	2.21 ± 0.28	2.41 ± 0.34
After diet	2.65 ± 0.42†	2.74 ± 0.41†

NOTE. Values are means ± SD.
*$P = .05$, †$P < .001$ *v* before diet.

ences were observed in the concentration of TG under the influence of either diet. It is also noteworthy that the PUFA diet, in contrast to the sterol diet, significantly reduced the concentration of HDL-C (3%).

The consumption of margarine with sterols for 4 weeks was found to produce a significant prolongation of blood platelet aggregation and adhesion time after Coll/Epi activation, with no essential changes after Coll/ADP (Table 5). The margarine with PUFA did not produce any essential changes in the aggregation of blood platelets.

Each of the dietary regimes when separately applied caused a highly significant increase in the concentration of Fb (Table 5), in the PUFA diet by 19.9% and sterol diet by 13.7%. When we compared both diets by ANOVA and the Student *t* test, no statistically significant differences were found. This pertains to both lipid and hemostatic parameters.

Negative correlations were discovered in the course of the application of the sterol diet between changes in Coll/Epi platelet aggregation and changes in lipid parameters. The linear correlation coefficients were as follows: Δ Coll/Epi and Δ LDL-C: $r = -.39$, $P = .05$; Δ Coll/Epi and Δ TC: $r = -.49$, $P < .05$. Also in the case of this same sterol diet, we found a negative correlation between Δ Coll/ADP and Δ Fb ($r = -.42$, $P = .05$).

The application of the PUFA diet showed negative correlations between changes in the Fb concentration and changes in the lipid parameters. The correlation coefficients are as follows: Δ Fb and Δ TG: $r = -.46$, $P < .05$; Δ Fb and Δ TC: $r = -.43$, $P = .05$; Δ Fb and Δ LDL-C: $r = -.60$, $P < .01$. No such correlation for the sterol diet was found.

DISCUSSION

A diet rich in unsaturated fatty acids, both monounsaturated (MUFA) and PUFA has long been recommended as effectively lowering the concentration of total cholesterol and LDL-C.[30] Numerous tests have shown that a diet with added plant sterols has a significantly stronger hypolipemic effect, and this was confirmed in our study. As a result of the consumption of margarine with sterols by male subjects with normal initial lipid levels, a reduction was obtained in the classic lipid risk factors for atherosclerosis, ie, an approximately 8% reduction in total cholesterol and an 11% reduction in LDL-C, which is to say, 4% and 5% higher, respectively, than in the subjects on the PUFA diet. The results we obtained are similar to those published in recent years from experiments conducted with sterols or stenols among persons with normolipemia.[5,7,9] Thus, in our experiments, similar to other investigators, we have found no influence of sterols on HDL level, in contrast to the diet with PUFA, which lowered HDL level by an average of 3%.[1,31,32] However, this effect has been observed in a limited number of subjects, which may be related to apoA I gene polymorphism existing in this group. The most recent studies of Ordovas et al[33] have revealed substantial interactions between PUFA consumption and HDL-C level depending on the G-A ApoA I gene polymorphism.

One particularly interesting finding in our research was that enriching the diet with sterols caused a prolongation of the Coll/Epi time, but did not change the Coll/ADP time (no changes in the aggregation of ADP-activated platelets). Such antiaggregation activity of sterols, which can be basically compared in its biologic effect to low doses of aspirin, is difficult to explain.[34] One possible cause may be the difference in the concentration of plasma oxidized LDL (Ox-LDL) between the tested groups.In fact, it has been already proven that oxidized LDL may affect the aggregation process.[35,36]

Recently, we have shown that on the sterol diet the concentration of Ox-LDL with proaggregative activity is lowered and that the PUFA-containing diet does not produce such an effect with a comparable decrease of the LDL level.[37] The mechanism behind this new pleiotropic activity of sterols is not known, although we believe that sterols may also inhibit the absorption of certain proinflammatory oxysterols contained in food.[37]

Another fact that should be elucidated is the lack of any effect of both diets on platelet aggregation caused by Coll/ADP, despite the reduction of LDL-C in both groups studied. This is particularly important in the case of the PUFA-containing diet, which should change the fluidity of platelet cellular membranes, and in this manner, contribute to the prolongation of aggregation. This process is known to be affected by the interaction between the GP IIb/III platelet receptor, ADP, and Fb.

In our study, these interactions were manifested by the occurrence (after the application of the dietary regimes) of a negative correlation between changes in Fb and changes in the Coll/ADP time. This means that greater increases in the Fb concentration are accompanied by shorter aggregation times. Irrespective of the above, our observations should be regarded as an introduction to further studies on this subject due to a relatively weak correlation between aggregation and Fb in persons on a sterol-containing diet. However, further proof of this assertion is provided by the observation of prolonged Coll/ADP time in persons with afibrinogenemia.[38]

We found no data in the available literature regarding the inhibitory effect of sterols or stanols on blood platelet aggregation. As pertains to the fibrinolytic indicators, on the other hand, the data are controversial. In a rabbit model, some sterols (eg, sitosterol) were found to have a profibrinolytic effect, increasing the activity of t-PA.[29] In an experiment with human subjects, no changes were found in the fibrinolytic system under the impact of stanol.[24,39,40]

Clinical tests have shown that the risk of thrombosis and

circulatory diseases increases with an increased concentration of Fb. Given the fact that Fb often correlates with lipid parameters, we expected to see a reduction in Fb concentration under the influence of both diets, especially the diet enriched with plant sterols.[22,23] Contrary to our expectations, we found a significant increase in the Fb concentration under both dietary regimes. There have been previous reports suggesting that a diet containing stenols slightly increases the Fb concentration, probably due to the reduction in antioxidant vitamins, especially caroten.[24] To date, there has been considerable debate regarding the impact on the hemostatic system of various types of fatty acids (MUFA, PUFA) and antioxidants. The type of diet can directly or indirectly influence the mediation of immunologic factors and the level of Ox-LDL with varying degrees of intensity. For example, ω-3 PUFAs have a stronger anti-inflammatory effect than ω-6 PUFAs, while MUFAs inhibit the oxidative modification of LDL more strongly than PUFAs.[30,41,42] In view of these facts, the composition of the diet may be of essential importance in the regulation of the plasma concentration of Fb.

The most recent studies have shown that persons with low body mass who consume large amounts of PUFAs and antioxidants do indeed show a lower Fb concentration, while other investigators have not observed a reduction in Fb under the influence of either MUFAs or antioxidant vitamins.[23,25,43,44]

The increased Fb concentration we observed under the influence of both dietary regimes, given the simultaneous reduction in the levels of total cholesterol and LDL-C, should be regarded as a homeostatic effect. In fact, a recent pharmacoepidemiologic study showed that all types of statins tendend to increases Fb concentration by an unknown mechanism.[45] However, it is known that PUFAs may be acting as transcription factors and could intensify the biosynthesis of Fb. There is no such data in the available literature with respect to Fb. However, there are several experimental studies pointing to the impact of very–low-density lipoprotein (VLDL) realizing fatty acids as a transcription factor intensifying the expression of the promotor gene for the t-PA inhibitor (PAI-1).[46]

It should also be noted that persons consuming margarine without sterols had a significant negative correlation between lipid changes and Fb. This indicates that larger decreases in lipid values are accompanied by greater increases in the Fb concentration. The lack of such a correlation in the group consuming margarine enriched with sterols appear to serve as an argument that the properties of sterols are more advantageous than those of PUFAs in this respect.

In conclusion, 2 tested margarines increased the Fb concentration, but in reference to blood platelet function, only those enriched with plant sterols weaken the processes of adhesion and aggregation. The inhibition of blood platelet function by sterols is similar to the mechanism governing the action of aspirin and is correlated with a reduction in atherogenic lipids. Although both margarines increase the Fb concentration, it should be acknowledged that margarine enriched with sterols opens up new possibilities in the nonpharmacologic inhibition of blood platelet function and thus in the front-line prevention of circulatory diseases.

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