# Effects of Dietary Palm Oil on Arterial Thrombosis, Platelet Responses and Platelet Membrane Fluidity in Rats

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**Wistar rats were fed a control diet containing 5 energy % (en %) sunflowerseed oil or diets containing 50 en % of either palm oil, rich in saturated fatty acids, or sunflowerseed oil, high in linoleic acid, for at least eight weeks. Arterial thrombosis tendency, measured by the aorta loop technique, tended to be lowered by the palm oil diet and was lowered significantly by the sunflowerseed oil diet, compared with the control. Aggregation of platelets in whole blood activated with collagen was not altered by palm oil feeding, but was enhanced in the sunflowerseed oil group, compared with the control. The concomitant formation of thromboxane $A_2$ was decreased by palm oil feeding, although formation of prostacyclin did not change; the ratio of thromboxane/prostacyclin formed was decreased significantly in the palm oil group. Compared with the control diet, platelet membrane fluidity, measured by fluorescence polarization, was not altered in the palm oil group and was significantly increased only by sunflowerseed-oil feeding. Thus, although palm oil contains about 50% saturated fatty acids, it did not increase arterial thrombosis tendency and tended to decrease platelet aggregation, as compared with highly polyunsaturated sunflowerseed oil.**
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The type of fat in the diet has been shown to influence arterial thrombosis (1,2). Using an in vivo model for arterial thrombogenesis in rats, we have previously demonstrated that, in general, diets high in saturated fats promote arterial thrombosis, but dietary polyunsaturated fatty acids of the n-6 and n-3 families have antithrombotic effects (2,3).

Sunflowerseed oil contains large amounts of linoleic acid [18:2(n-6)], and diets enriched in this oil have a consistent antithrombotic effect, significantly increasing the obstruction time of aortic loops in rats (2). In contrast with this observation, dietary sunflowerseed oil enhances, in vitro, collagen-induced aggregation of blood platelets (4,5), cell fragments that play important roles in arterial thrombosis (6,7). This increased platelet aggregability may be due to the relatively increased production of the proaggregatory prostanoid thromboxane $A_2$ ($TXA_2$), a greater production than can be explained on the basis of the platelet phospholipid content of the thromboxane precursor, arachidonic acid (8). We have recently reported that platelets from rats fed diets high in sunflowerseed oil have an increased overall membrane fluidity (5) and, as a result, an extracellular "message," brought about by the interaction between collagen and the platelet membrane, may be transmitted more efficiently across the membrane to create a higher intracellular response (formation of $TXA_2$). Consequently, feedback amplification upon platelet stimulation may be enhanced.

This study describes investigations into the effects of another dietary fat, palm oil, that also has been shown to have antithrombotic properties (2). Palm oil is unusual in having an antithrombotic effect, as it contains a low ratio of polyunsaturated to saturated fatty acids; it consists of about 10% linoleic acid and 50% saturated fatty acids (mainly palmitic acid [16:0]). We have examined the effects of dietary palm oil on rat platelet aggregation and prostanoid production in response to stimulation with collagen in whole blood, and on fatty acid composition and membrane fluidity of the platelets, in an attempt to account for the antithrombotic effect of palm oil.

## MATERIALS AND METHODS

*Animals and diets.* Male Wistar rats (specific-pathogen-free), aged five weeks, were fed, ad libitum, one of the following three diets for at least eight weeks: a diet containing 5% of its digestible energy (en %) as sunflowerseed oil—the control diet; a diet containing 50 en % sunflowerseed oil—the sunflowerseed oil diet (which is high in polyunsaturated fatty acids); or a diet containing 50 en % palm oil—the palm oil diet (which is high in saturated and monounsaturated fatty acids). All diets contained 23 en % casein; the remaining calories were contributed by corn starch. Other dietary components (minerals, vitamins, and cellulose) were added, as previously described (2). The diets were freshly made at least once a week, and were stored at 4°C. The rats were given tap water, ad libitum.

The fatty acid compositions of the dietary fats, determined by gas liquid chromatography (GLC) after methylation (See *Lipid analyses* in this section.), are given in Table 1.

All dietary components, except the palm oil (from the Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia), were provided by Unilever Research, Vlaardingen, The Netherlands.

TABLE 1

**Fatty Acid Composition (%) of Dietary Fats**

| | Dietary fat | |
|---|---|---|
| Fatty acid | Palm oil | Sunflowerseed oil |
| 14:0 | 1 | |
| 16:0 | 44 | 6 |
| 18:0 | 5 | 4 |
| 18:1(n-9) | 39 | 22 |
| 18:2(n-6) | 10 | 66 |
| 18:3(n-3) + 20:0 | 1 | 1 |
| 22:0 | | 1 |

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Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; $TXA_2$, thromboxane $A_2$; $TXB_2$, thromboxane $B_2$; GLC, gas liquid chromatography; OT, obstruction time.

*Measurement of arterial thrombosis tendency.* After a feeding period of eight to nine weeks, the arterial thrombosis tendency of the rats was measured using the aorta loop technique, as previously described (9). The time between insertion of the loop and complete obstruction of blood flow through the loop, indicated by a change in the color of the blood from red to dark blue or black, is defined as the obstruction time (OT).

*Platelet aggregation in whole blood.* During insertion of the aorta loop, blood (1.35 ml) was collected via the loop into a prewarmed syringe containing 3.8% trisodium citrate (0.15 ml) (5). This anticoagulated blood was transferred to an aggregometer cuvette in the thermostated (37°C) cuvette holder of a whole blood aggregometer (Model 500, Chrono-log Corp., Havertown, PA). Five min after blood collection, collagen (3.3 μg/ml, final concentration; Hormon Chemie, Munich, FRG) was added, and the change in impedance, which reflects platelet aggregation around platelets adherent to the electrodes (10,11), was recorded for 15 min.

*Measurement of prostanoid formation in collagen-activated whole blood.* At completion of an aggregation measurement, the blood was immediately transferred to a small tube and centrifuged at 9,600 × *g* for 2 min. The platelet-free plasma was collected and stored at −20°C until the measurement of thromboxane $B_2$ ($TXB_2$), the stable breakdown product of $TXA_2$, using a radioimmunoassay kit (NEK-007, New England Nuclear, Dreieich, FRG) (5), and measurement of 6-keto-$PGF_{1\alpha}$, the stable metabolite of prostacyclin, also using a radioimmunoassay kit (NEK-008, NEN).

*Platelet preparation.* Suspensions of washed platelets were prepared from blood obtained by abdominal aortic puncture of rats that had been anesthetized with ether. The animals were exsanguinated at least three weeks after insertion of the aorta loop, that is, after a feeding period of at least 12 weeks. Six parts of blood were collected into one part of the anticoagulant acid-citrate-dextrose (12) and suspensions of washed platelets were prepared essentially according to the method of Ardlie and coworkers (13), as described previously (5). The final resuspension medium of the platelets was Tyrode solution containing 5 mM HEPES, pH 7.35; a portion of the final platelet suspension was used for fluidity measurements and, in some cases, the remainder was centrifuged at 1,300 × *g* for 10 min. The platelet pellet was resuspended in 0.5 ml of 139 mM NaCl, 8 mM $Na_2EDTA$, and the suspension was stored under $N_2$ at −20°C until lipid analyses were done.

*Fluidity measurements.* Platelet membrane fluidity was determined by the method of fluorescence polarization using the hydrophobic probe 1,6-diphenyl-1,3,5-hexatriene (DPH) (Molecular Probes Inc., Junction City, OR) (5,14, 15). One volume of DPH dispersion (1 μM) was added to one volume of platelet suspension ($10^8$ platelets/ml) and the mixture was incubated at 37°C for 1 hr. Steady-state fluorescence-polarization measurements were done at 37°C using an Aminco-Bowman spectrofluorimeter equipped with Glan-Thompson prism polarizers in the excitation and emission beams and $r_s$ (steady-state fluorescence anisotropy) was determined, as described previously (5). Corrections were made for scattering of polarized exciting light. To correct for slight day-to-day variations in the absolute values of $r_s$, results are expressed as comparisons with the control diet.

*Lipid analyses.* EDTA (0.5 ml of a 1% solution) was added to thawing platelet preparations (0.5 ml). A tracer amount of tritiated cholesterol ([1,2,6,7-$^3$H(N)]-cholesterol; NEN) was added to the samples to enable monitoring of recovery, and lipids were extracted according to the method of Bligh and Dyer (16). Butylated hydroxytoluene (0.005%, final concentration) was added to all organic solvents used in the lipid analyses. The phosphorus content of 5% of the total lipid extract was quantitated in duplicate, using a modification of the Fiske/Subbarow technique (17), and total platelet phospholipid content was calculated.

Cholesterol and total phospholipids were separated from other lipids by thin layer chromatography and free cholesterol was determined as described previously, correcting for recovery of tritiated cholesterol (5).

Total platelet phospholipids were eluted and transmethylated with boron trifluoride. Methyl esters of the fatty acids from the phospholipids were extracted with pentane and were analyzed by GLC with a Hewlett-Packard 5840A gas chromatograph using columns packed with 5% DEGS on Chromosorb WHP, 100/120 mesh (Chrompack, Middelburg, The Netherlands) and 2% Apiezon L on Chromosorb WHP, 100/120 mesh, as previously described (5).

Using computer-assisted analysis, chromatograms were corrected for blank runs originating from extraction of platelet-suspending medium and were adjusted with respect to the internal standard, the methyl ester of 15:0. Results are expressed as area percentage of total fatty acids. Identifications were made by comparison of retention times with those of standard mixtures of fatty acid methyl esters (from Unilever Research and Chrompack).

*Analysis of data.* Because OTs of aorta loops show a log-normal pattern of distribution (2), logarithmic transformation of these values was necessary for statistical analysis. The same was found to be true for $TXB_2$ and 6-keto-$PGF_{1\alpha}$ formed in whole blood in response to collagen. Analysis of variance, Bonferroni inequality test, Student's t-test or paired t-test were used as indicated in the Results and Discussion section to determine significance of differences. Two-tailed tests were performed.

## RESULTS AND DISCUSSION

At the time of insertion of the aorta loops, that is, after a feeding period of eight to nine weeks, there was no difference in mean body weights (approximately 370 g) of the rats fed the three different diets.

Increasing the amount of sunflowerseed oil in the diet from 5 en % to 50 en % was associated with a significant prolongation of the mean log OT of the aorta loops ($P < 0.05$) (Fig. 1), indicating a decreased arterial thrombosis tendency. Rats fed the palm oil diet tended to have longer mean log OTs also, but this difference did not reach statistical significance ($P = 0.10$). Thus, palm oil feeding again tended to lower arterial thrombosis tendency. That a significant lowering of arterial thrombosis tendency was not observed in the present study may have been due to factors other than diet. For example, the variability in OTs of aorta loops is quite high and depends on the skill of the surgeon who inserts the loops. In an earlier study (3), feeding of 50 en % palm oil significantly lowered arterial thrombosis tendency compared with feeding

high-fat (50 en %) diets containing two different types of saturated triglycerides, whale oil or hydrogenated coconut oil. Further confirmation of the antithrombotic effect of palm oil was obtained in a later study (18) in which palm oils again prolonged the OT, compared with a low-fat control group (5 en % sunflowerseed oil), but no significant difference was observed in comparison with the OT of animals on a 50 en % sunflowerseed-oil diet. When results from more recent, as yet unpublished, experiments are included, arterial thrombosis tendency of rats fed a high-fat palm oil diet has been compared with that of rats fed a high-fat sunflowerseed oil diet in 10 studies, and that of rats fed control diets of low-fat (5 en %) sunflowerseed oil or 5 en % sunflowerseed oil + 45 en % hydrogenated coconut oil (This contains a high proportion of saturated fatty acids.) in five studies each. The mean OT (± SEM) of the palm oil groups was 92 ± 3.8% of that of the high sunflowerseed-oil groups ($P > 0.10$), but those of the low-fat and high-fat control groups were 65 ± 3.8% and 63 ± 2.5%, respectively ($P < 0.001$). This clearly demonstrates that dietary palm oil has a distinctive antithrombotic effect when compared with a low-fat or a high-saturated-fat diet.

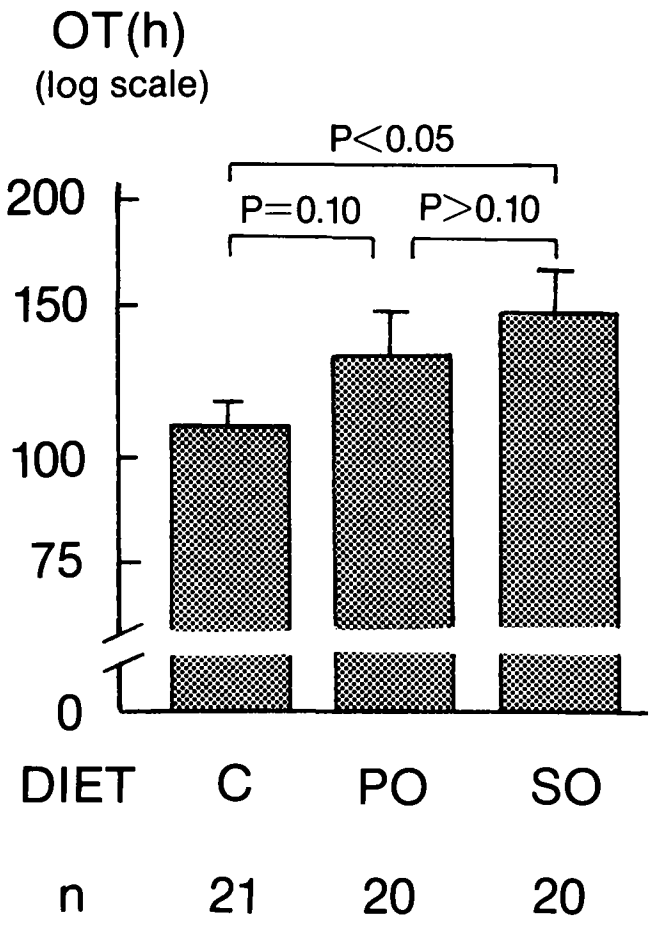


**FIG. 1. Arterial thrombosis tendency, as determined by obstruction time (OT) of aorta loops of rats fed the control diet (C), the palm oil diet (PO) or the sunflowerseed oil diet (SO). See Materials and Methods for further details of the diets and aorta loop technique. Values are geometric means ± SEM. Analysis of variance and the Bonferroni inequality test (2-tailed) were used to determine significances of differences.**

As measured by the maximum change in impedance, in ohms, there was a significantly greater extent of platelet aggregation in whole blood in the sunflowerseed-oil group (6.8 ± 0.81, n = 19), compared with the control group (4.25 ± 0.55, n = 19) ($P < 0.01$). There was no difference in the extent of platelet aggregation in the palm oil group (4.59 ± 0.65, n = 20), compared with the control group. The values tended to be lower than those observed for the sunflowerseed-oil group ($0.1 > P > 0.05$, means ± SEM, analysis of variance and Bonferroni inequality test).

The present study confirms earlier results (2,4,5) demonstrating that dietary sunflowerseed oil has a distinct antithrombotic effect that is associated, unexpectedly, with enhanced platelet aggregability in vitro in response to collagen. Palm oil feeding was associated with a tendency towards an antithrombotic effect, but no change in platelet aggregability, as compared with the control group. Consequently, no relationship was observed between arterial thrombosis tendency in vivo and platelet aggregability to collagen in vitro.

While sunflowerseed-oil feeding had no effect on the production of immunoreactive thromboxane or prostacyclin in collagen-activated whole blood, dietary palm oil significantly reduced thromboxane formation, leaving production of prostacyclin unaffected (Table 2). This

**TABLE 2**

**Effect of Dietary Fat on Prostanoid Formation in Citrated Whole Blood Activated with Collagen (3.3 μg/ml)[a]**

| Parameter | Diet | | |
|---|---|---|---|
| | Control | Palm oil | Sunflowerseed oil |
| $TXB_2$ (log ng/ml) | 1.70 ± 0.066(20) | 1.55 ± 0.041(20)[b] | 1.64 ± 0.082(19) |
| (ng/ml) | 50.1 | 35.5 | 43.7 |
| 6-keto-$PGF_{1\alpha}$ (log pg/ml) | 2.95 ± 0.027(20) | 2.99 ± 0.039(16) | 3.05 ± 0.030(14) |
| (pg/ml) | 891 | 977 | 1122 |
| TX/PG ratio[c] | 64.3 ± 7.32(20) | 40.2 ± 4.34(13)[b] | 61.8 ± 11.40(14) |

[a] Values are means ± SEM. Number of determinations is given in parentheses.

[b] Significantly different than control ($P < 0.01$, Bonferroni inequality test).

[c] Calculated on the basis of nontransformed data.

resulted in a significantly lower thromboxane/prostacyclin ratio (Table 2), which has been suggested to determine platelet reactivity and thrombosis tendency (19). It is unlikely that the reduced thromboxane formation by collagen-stimulated platelets from rats fed the palm oil diet was due to a lower platelet count in these animals, because similar numbers of platelets were recovered from the rats fed the different diets (data not shown).

The formation of 6-keto-$PGF_{1\alpha}$ in collagen-activated whole blood most likely reflects prostacyclin production by monocytes (20). Whether these cells are activated themselves and use endogenous arachidonic acid for prostanoid formation or whether they "steal" arachidonic acid or endoperoxides from activated platelets (21) remains to be determined. Activated platelets were most likely the main source of thromboxane produced by collagen-activated whole blood.

Although thromboxane production in vitro has been demonstrated to be positively related to the arachidonic acid content of platelet phospholipids (8), the lower thromboxane production in activated whole blood of palm oil-fed animals cannot be explained on the basis of this relationship, because the arachidonic acid content of platelet phospholipids was not detectably affected by dietary palm oil (Table 3).

Lipid fluidity has been defined as the reciprocal of the lipid structural order parameter, and lower values of $r_s$ (steady-state fluorescence anisotropy), measured using fluorescence polarization, indicate decreased structural order parameters and increased membrane fluidity (22). As observed earlier (5), the administration of a diet rich in sunflowerseed oil significantly enhanced the overall fluidity of platelet membranes, measured with the hydrophobic probe DPH (Table 4). It has been suggested that this greater fluidity promotes stimulus-response coupling, and, as a result, platelet aggregation is increased and $TXA_2$ production in relation to arachidonic acid availability is enhanced (5). If this is true for palm oil also, then the lower $TXA_2$ formation by collagen-activated platelets observed after palm oil feeding should be associated with a reduced fluidity of platelet membranes. Compared with the control, however, palm oil feeding did not modify the overall fluidity of platelet membranes (Table 4).

Parameters that are known to modulate membrane fluidity, the cholesterol/phospholipid molar ratio (C/P ratio) and the fatty acid composition of the membrane phospholipids (23,24), were determined in platelets from rats fed the control, palm-oil and sunflowerseed-oil diets. There were no significant differences among the C/P ratios of platelets from rats fed the control diet (0.60 ± 0.01, n = 3), the sunflowerseed-oil diet (0.54 ± 0.02, n = 4) or the palm-oil diet (0.56 ± 0.01, n = 5) (means ± SEM, analysis of variance). We have previously shown that the C/P ratio of platelets from rats fed the control diet and sunflowerseed-oil diet are essentially identical (5).

Feeding of the highly polyunsaturated sunflowerseed-oil diet, compared with the control diet, was associated with differences in the fatty acid composition of the total platelet phospholipids (Table 3) that reflected the fatty acid composition of the sunflowerseed oil (Table 1). For example, as shown previously (5), significantly higher levels of 18:2(n-6) (linoleic acid) and of its elongation product 24:2(n-6) were found in the sunflowerseed-oil group

**TABLE 3**

**Fatty Acid Composition (%) of Phospholipids of Platelets from Rats Fed the Control, Palm Oil and Sunflowerseed Oil Diets[a]**

| Fatty acid[b] | Diet | | |
|---|---|---|---|
| | Control (n = 3) | Palm oil (n = 4) | Sunflowerseed oil (n = 5) |
| 14:0 | 0.6 ± 0.04 | 0.4 ± 0.04 | 0.5 ± 0.05 |
| 16:0 | 27.3 ± 0.15 | 27.5 ± 0.63 | 24.6 ± 0.37[e] |
| 16:1(n-7) | 1.3 ± 0.02 | 0.7 ± 0.08 | 0.9 ± 0.31 |
| 18:0 | 13.2 ± 1.56 | 12.6 ± 0.98 | 16.3 ± 1.12 |
| 18:1(n-9) | 7.9 ± 0.65 | 6.7 ± 0.15 | 4.3 ± 0.19[e] |
| 18:2(n-6) | 4.5 ± 0.18 | 4.0 ± 0.36 | 9.9 ± 0.32[e] |
| 20:0 | NS | 0.9 ± 0.13 | 0.7 ± 0.04 |
| 20:1(n-9) | 0.5 ± 0.08 | 0.8 ± 0.04 | 0.5 ± 0.03 |
| 20:3(n-9) | 0.3 ± 0.04 | NS[c] | 0.1 ± 0.04[e] |
| 20:3(n-6) | 0.6 ± 0.01 | 0.5 ± 0.07 | 0.7 ± 0.03[f] |
| 20:4(n-6) | 25.3 ± 0.89 | 25.5 ± 0.57 | 22.7 ± 1.34 |
| 20:5(n-3) | NS | — | 0.3 ± 0.05[e] |
| 22:0 | 0.6 ± 0.02 | 0.7 ± 0.02[e] | 0.7 ± 0.03[d] |
| 22:1(n-9) | 1.0 ± 0.03 | 1.1 ± 0.01[f] | 0.8 ± 0.04[e] |
| 22:4(n-6) | 5.3 ± 0.24 | 6.0 ± 1.05 | 5.4 ± 0.50 |
| 22:6(n-3) | 0.2 ± 0.02 | NS | —[d] |
| 24:0 | 0.8 ± 0.03 | 0.7 ± 0.09 | 0.9 ± 0.04 |
| 24:1(n-9) | 1.8 ± 0.15 | 2.3 ± 0.20 | 1.2 ± 0.03[e] |
| 24:2(n-6) | 1.0 ± 0.09 | 0.6 ± 0.12[d] | 2.4 ± 0.16[f] |
| UI[g] | 150.2 ± 3.5 | 148.3 ± 3.8 | 148.6 ± 4.3 |

[a] Values are means ± SEM. A line (—) indicates that the fatty acid was not detected, and NS indicates that the mean value was not significantly different than zero.

[b] 18:3(n-3) was not detected in the samples.

Significance of difference compared with control (Bonferroni inequality test):

[c] $P < 0.05$;

[d] $P < 0.025$;

[e] $P < 0.005$;

[f] $P < 0.001$.

[g] UI, the unsaturation index, is calculated by summing the percentage of each fatty acid in a sample multipled by the number of double bonds contained in that fatty acid (24).

(Table 3). However, feeding of the highly saturated/monounsaturated palm-oil diet was not associated with any major differences in fatty acid composition of total platelet phospholipids, compared with the control diet (Table 3).

There were no significant differences among the unsaturation indices calculated from the fatty acid compositions of total phospholipids of platelets from rats fed the three different diets (Table 3). Membrane unsaturation was also calculated in several other ways, by determining the total proportion of unsaturated fatty acids, the ratio of the unsaturation index to the proportion of unsaturated fatty acids, and the ratio of the proportion of unsaturated fatty acids to saturated fatty acids (data not shown) (24–26). There were no significant differences among the unsaturation of the membranes of the platelets from rats fed the different diets calculated in these ways. We agree with the conclusion of Lands (27), that it is difficult to correlate membrane fluidity with unsaturation of the phospholipids of membranes, calculated by means of simple formulae.

**TABLE 4**

**Effect of Dietary Fat on Steady-State Fluorescence Anisotropy, $r_s$, of DPH-labeled Rat Platelets[a]**

| | Diet | | |
|---|---|---|---|
| | Control | Palm oil | Sunflowerseed oil |
| Mean $r_s$ | 0.218 ± 0.0035 | 0.218 ± 0.0039 | 0.212 ± 0.0033 |
| Paired difference than control | — | 0.001 ± 0.0018 | 0.007 ± 0.0018[b] |
| Paired difference than palm oil | 0.001 ± 0.0018 | — | 0.006 ± 0.0015[b] |

[a]Means ± SEM are of 9-10 values. Paired differences are mean paired differences ± SEM of 9-10 values.
[b]Significantly different than zero ($P < 0.01$; paired t-test).

Thus, on the basis of the C/P ratios and the fatty acid data, it is not surprising that differences were not observed in membrane fluidity between the control and palm oil diets. It must be noted, however, that the membrane fluidity measured in these studies is an average membrane fluidity, as DPH partitions equally well into solid and fluid lipid domains of membranes (28), and that the membrane lipid composition that was determined is an overall composition. No information can be obtained from our studies about fluidity or lipid composition of domains of lipids that exist in the membranes (29). It remains possible that compositional and fluidity shifts occur in specific lipid domains and that such shifts were not detected with the methodology used in these studies.

This study demonstrates that palm oil, although it contains a high content of saturated fatty acids, does not promote arterial thrombus formation but, rather, tends to inhibit it. This beneficial antithrombotic effect is associated with a reduced formation of $TXA_2$ by activated blood platelets, although prostacyclin formation in whole blood is not affected. Collagen-induced platelet aggregation in citrated whole blood and platelet membrane fluidity are not altered in palm oil-fed animals. Upon sunflowerseed-oil feeding, however, collagen-induced platelet aggregation is enhanced and this is associated with an increased fluidity of platelet membranes. The unexpected antithrombotic effect of dietary palm oil, as well as its effect on inhibiting platelet thromboxane formation, requires further investigation.

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