Rat platelet phospholipase A_2 activity and thromboxane synthesis are concomitantly affected by dietary linoleic acid

Barbara Krumhardt* and Jacqueline Dupont

Department of Food and Nutrition, Iowa State University, Ames, Iowa, USA

Platelet thromboxane synthesis requires the release of arachidonic acid from the membrane phospholipids by phospholipase A_2. Regulation of thromboxane synthesis is by regulation of phospholipase A_2 which may be affected by the fatty acid composition of the membrane in which phospholipase resides. Male Sprague-Dawley rats were fed diets supplying 30% of energy (en%) as fat composed of corn oil and beef tallow to provide 3.0, 4.5, 6.0, 7.5, or 9.0 en% linoleate, with cholesterol added to provide equal cholesterol in all diets. Rats were fed for 30 days with eight rats/diet. Isolated platelets were assayed for fatty acid composition, net phospholipase A_2 activity, and thromboxane synthesis. Liver was analyzed for fatty acid composition. The percentage of linoleic acid in platelet and liver fatty acids rose linearly with increasing dietary linoleic acid. Phospholipase A_2 activity and thromboxane synthesis were correlated; both decreased as dietary linoleate rose. The concomitant changes in platelet fatty acid composition, phospholipase A_2 activity, and thromboxane synthesis suggest that membrane fatty acid composition is one site of control of phospholipase A_2 activity and thromboxane synthesis in rat platelets when diets provide from 4.5 to 9.0 en% linoleate. Other factors may be operative at lower linoleate concentrations.

Keywords: platelets; dietary fat; phospholipase A_2; linoleic acid; thromboxane

Introduction

Because linoleic acid is the dietary precursor of eicosanoids, much research has been done to determine the effect of increased polyunsaturated fatty acids (PUFA) in the diet on eicosanoid synthesis. A cellular mechanism has not been elucidated for the general observation that, when PUFA consumption is greater than that sufficient to alleviate the accumulation of eicosatrienoic acid indicative of essential fatty acid deficiency, eicosanoid synthesis first increases and then decreases, as the PUFA concentration of the diet rises to 10% of energy (en%) linoleate.[1]

Journal Paper No. J-13829 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project 2571, a contributing project to North Central Regional Research Project NC-167.
*Dr. Krumhardt's present address is Des Moines Area Community College, Urban Campus, 1100 7th Street, Des Moines, IA 50314.
Address reprint requests to Jacqueline Dupont, ARS, USDA, Rm. 132, Bldg. 005, BARC-W, Beltsville, MD 20705, USA.
Received October 31, 1990; accepted March 28, 1991.

In the platelet, phospholipase A_2 (PLA) releases arachidonic acid from cellular phospholipids upon cellular activation. The release of arachidonic acid is required for the synthesis of thromboxane A_2 by cyclooxygenase and thromboxane synthetase. All three enzymes required for thromboxane synthesis are located in platelet membranes.[2]

Ballou and Cheung[3,4] reported that potential PLA activity in human platelets is greater than previous studies have indicated because of the presence of an endogenous inhibitor of its activity. Studies assaying platelet microsomal PLA activity in the presence of the cell fraction containing the endogenous inhibitor or various lipids indicated that PUFA were the inhibitors of PLA activity and that this inhibition was not reversed by excess calcium, which is also required for the activity of PLA. Therefore, the role of membrane fatty acid composition effects on PLA in cellular activation and regulation of eicosanoid metabolism may be important.

Marki and Franson[5] observed that in vitro alteration of microsomal membranes of white blood cells to in-

Table 1 Composition of experimental diets

Ingredient	Energy percentage linoleate				
	3.0	4.5	6.0	7.5	9.0
			g/100g		
Beef tallow[a]	13.70	12.20	10.80	9.20	7.80
Corn oil[b]	0.60	2.10	3.50	5.10	6.50
Casein[c]	22.35	22.35	22.35	22.35	22.35
Sucrose[b]	26.43	26.43	26.43	26.43	26.43
Corn starch[b]	26.43	26.43	26.43	26.43	26.43
Cellulose[d]	5.03	5.03	5.03	5.03	5.03
AIN mineral mixture 76[e]	3.93	3.93	3.93	3.93	3.93
AIN vitamin mixture 76[e]	1.11	1.11	1.11	1.11	1.11
L-methionine[c]	0.33	0.33	0.33	0.33	0.33
Choline chloride[f]	0.08	0.08	0.08	0.08	0.08
Ascorbic acid (antioxidant)[f]	0.01	0.01	0.01	0.01	0.01
Cholesterol (mg/100 g)[f]	0.00	1.60	3.16	4.90	6.40

[a] Purchased from Meat Laboratory, Department of Animal Science, Iowa State University, Ames, IA.
[b] Purchased from Iowa State University Food Stores: Mazola Corn Oil, Crystal Sugar, and Argo Corn Starch.
[c] Purchased from United States Biochemical Co., Cleveland, OH.
[d] Purchased from Teklad, Madison, WI.
[e] Purchased from ICN Nutritional Biochemicals, Cleveland, OH. Composition as described in *J. Nutr.* **107,** 1340–1348.
[f] Purchased from Sigma Chemical Co., St. Louis, MO.

crease PUFA proportion decreased PLA activity. Needleman et al.[6] demonstrated that incubating platelets with linoleic acid to increase the linoleic acid content of their phospholipids served to decrease thromboxane synthesis. Sato et al.[7] showed that incubation of platelets with linoleic acid, but not other 18-carbon fatty acids, interfered with platelet aggregation and the synthesis of thromboxane from exogenously added arachidonic acid. The rise in cytosolic free ionized calcium, required for activation of PLA and enzymes required for synthesis of thromboxane, was inhibited by the same concentration of linoleic acid that inhibited aggregation and thromboxane synthesis. This suggests that the action of free linoleic acid upon platelet activation is through interference with the receptor-operated calcium channel.

The conditions for stimulation of platelets to produce thromboxane determine the degree of association of thromboxane synthesis with platelet linoleate. Mild conditions (i.e., clotting blood without added factors or stimulation of isolated platelets with collagen) result in decreases of thromboxane synthesis with increases in linoleate.[8,9] The strong stimulation of platelets with thrombin, analogous to traumatic injury, however, results in maximum thromboxane synthesis and the synthesis is not correlated with either linoleate or arachidonate.[9,10]

In our study, phospholipase A_2 activity and thromboxane synthesis were measured in platelets from rats fed diets that differed primarily in fatty acid composition, with the cholesterol content of all diets held constant. Two dietary fat sources, beef tallow and corn oil, were combined in five different proportions to formulate diets that would achieve different fatty acid profiles in the rat's platelets.

Materials and methods

Animals and diets

This research was approved by the Iowa State University Vertebrate Animal Use Committee. Forty 8-week-old male Sprague-Dawley rats were purchased from Amitech (Omaha, NE). They were housed in a room maintained at 22° C and approximately 50% relative humidity. After 2 days of acclimation with feeding of Mouse/Rat Diet (6% fat, Teklad, Winfield, IA), they were assigned to experimental diets in a randomized block experimental design. The blocks represented the days that rats were sacrificed and that samples were processed.

The experimental nutritionally complete diets supplied 30 en% fat. The dietary fat was composed of different proportions of corn oil and beef tallow to provide 3.0, 4.5, 6.0, 7.5, or 9.0 en% linoleic acid, with cholesterol added as required to provide equal cholesterol in all diets (*Table 1*). The fatty acid composition of the corn oil and beef tallow used to prepare the diets was analyzed. Cholesterol was added based on previous determinations.[11] Rats were fed ad libitum with eight rats/diet. Food consumption was determined over 48 hr after 3 weeks of feeding. Tap water was available ad libitum for drinking. Rats were weighed weekly.

After 30 days of feeding, rats were fasted overnight. Food was returned to the rats the next morning to control the postprandial time. Two to three hours after food was returned to each rat, the rat was anesthetized with ether. Nine milliliters of blood was drawn from an exposed jugular vein into a syringe containing 1 ml acid citrate dextrose anticoagulant (4.5 g% sodium citrate, 2.7 g% citric acid [monohydrate], 3.6 g% dex-

trose). The rats were then killed by exsanguination by severing the aortic artery. The liver was then excised, and a 0.5-g sample in 5 ml pH 7.4 50 mM phosphate buffer was homogenized with 10 passes of a Potter-Elvejhem homogenizer. A 0.5-ml aliquot of the liver homogenate was frozen on dry ice and stored at −80° C for later fatty acid analysis.

Platelet preparation

Platelets were isolated from the anticoagulated blood by centrifugation by using a modification of the procedure of Hallam et al.[12] Anticoagulated blood was first centrifuged at 700*g* for 5 min. The supernatant platelet-rich plasma (PRP) was recentrifuged at 700*g* for 5 min to remove contaminating red and white blood cells. The PRP was then centrifuged at 400*g* for 20 min to sediment the platelets. The plasma was removed and platelets were washed twice and resuspended in an equal volume of HEPES buffered saline (HBS) (145 mM glucose, pH 7.4). This resulted in a platelet suspension containing approximately 2×10^8 cells/ml. The purity of platelet suspensions was determined by microscopic observation of several smears of PRP prepared by this procedure, which were stained with Wright's stain (Fisher Scientific, Silver Spring, MD). At least 98% of all cells observed were platelets.

Fatty acid analysis

All steps of the analysis were performed with the samples kept under a nitrogen atmosphere. Samples of beef tallow and corn oil were dissolved in chloroform: methanol (2:1), dried under a stream of nitrogen in glass culture tubes with teflon-lined caps, and saponified in 10% ethanolic KOH.

Platelet total fatty acids were determined in frozen and thawed platelets saponified in the same manner as pure fats. Neutral lipids were extracted with petroleum ether (BP 35–65° C), the residue acidified and free fatty acids were extracted with petroleum ether. The fatty acid extracts of platelet, dietary fat, and liver samples were methylated by using BF3 in methanol (Alltech, Deerfield, IL). Methylated fatty acids were analyzed by gas chromatography using a Varian 3700 gas chromatograph (Walnut Creek, CA) as previously described.[9]

Phospholipase A_2 activity

Platelet phospholipase A_2 (PLA) activity was assayed by using a modification of the procedure of Ballou and Cheung.[3] A 450 ul aliquot of the washed platelets in pH 7.5 TRIS/NaCl buffer was incubated at 37° C with 50 μl of substrate that contained approximately 0.03 μCi of 1-palmitoyl-2-[1-^{14}C]arachidonyl-sn-glycerol-3-phosphatidyl choline, specific activity 54.9 mCi/mmol (New England Nuclear, Boston, MA), in substrate buffer containing 50 mM taurocholic acid, 20 mM calcium chloride, and 0.05 g% fatty acid-free bovine serum albumin. Taurocholic acid (5 mM) was used as a biological amphipath in the assay to aid in delivery of substrate to the PLA enzyme. The concentration used was comparable to the total bile acids found in human serum.[13] The calcium chloride addition served to stimulate platelet activity.

At substrate addition and after 40 min incubation, 100 μl aliquots of the reaction mixture were added to 100 μl of 100% ethanol containing 2% glacial acetic acid and 330 μM arachidonic acid (to serve as a carrier in the thin-layer chromatography separation described below). [1-^{14}C] arachidonic acid released in each aliquot was separated from labeled substrate by using thin-layer chromatography on 250 μM silica gel G plates (Fisher Scientific) with ethyl acetate: acetic acid (99:1, vol/vol) as the solvent system. Bands of the silica gel corresponding with arachidonic acid (Nu-Chek-Prep, Inc., Elysian, MN) and phosphatidyl choline (Sigma Chemical Co., St. Louis, MO) were scraped into scintillation vials; Bioscintiverse BD (Fisher Scientific) was added, radioactivity was determined by liquid scintillation counting in a Tri-Carb Liquid Scintillation Spectrophotometer (Model C2425, Packard Instruments, Downers Grove, IL) using the external standards ratio method to correct for quenching and to calculate dpm of radioactivity.

Tests performed with platelets suspended in pH 7.0 and pH 8.0 TRIS/NaCl buffers demonstrated that PLA activity was greater at pH 8.0, but not significantly higher than activity at pH 7.5 (data not shown). PLA activity was significantly less when platelets were suspended in pH 7.0 buffer. The pH 7.5 TRIS/NaCl buffer was chosen for the assay because it reflects a more physiological pH than pH 8.0 and better mimics in vivo conditions. The 40-min incubation was selected to achieve maximum PLA activity in the least time (*Figure 1*). Fatty acids released by phospholipase

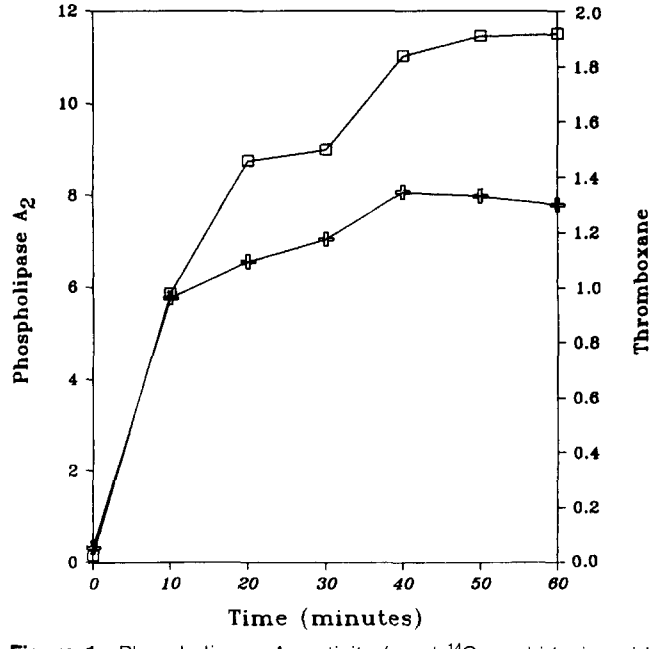

Figure 1 Phospholipase A_2 activity (pmol ^{14}C-arachidonic acid released per mg platelet protein) and thromboxane synthesis (pmol TX synthesized per mg platelet protein) during a 60 min incubation. Symbols in the figure represent: ⊟ PLA activity; ⊹ TX synthesis.

activity are reincorporated rapidly into other phospholipids or triglycerides so it is possible to quantitate only the net release of arachidonate, not the absolute amount that may have been recycled. Therefore, the PLA activity we report is actually the net release of ^{14}C arachidonic acid from the labeled phosphatidyl choline tracer used.

Thromboxane B_2 synthesis

At the same time points as for the PLA assay, 100 μl aliquots of the PLA reaction mixture were added to 20 μl of 42 mM aspirin in saline to stop TX synthesis, and the samples were frozen on dry ice and stored at −20° C. Double antibody radioimmunoassay of TX, by using ^{3}H-TX (New England Nuclear, Boston, MA) to bind antibody competitively, was performed as described previously,[14,15] with dual channel counting used to correct for the ^{14}C activity in the samples. The TX antiserum had a cross-reactivity with 6-keto prostaglandin F^1 of 20%. No correction was made for this cross-reactivity. The concentration of ^{14}C-TX in these samples was too small to determine the amount of ^{14}C-TX synthesized from the ^{14}C-labeled PLA assay substrate, so only endogenously derived TX was determined. TX synthesis over 1 hr was parallel with net PLA activity with both plateauing at 40 min.

Protein analysis

All platelet suspension remaining after use for other analyses was frozen on dry ice and stored at −20° C for later protein analysis, which was performed by using the procedure of Lowry et al.[16] Fatty acid-free bovine serum albumen fraction V (Sigma Chemical Co., St. Louis, MO) was used as the protein standard.

Statistical methods

All analyses were done in duplicate for each sample. Statistical analyses were done using the General Linear Model, Least Squares Means, and Pearson Correlation Coefficients procedures of Statistical Analysis System, version 5.16, Cary, NC.

Results

Animals and diets

There were no differences in the growth rates of rats fed different diets. There was no significant difference in food consumption due to diet.

Fatty acids

The results of platelet total fatty acid analysis are shown in *Table 2*. The percentage of linoleic acid in platelets increased with increasing concentrations of dietary linoleate, resulting in a correlation between the two ($r = 0.873$, $P < 0.0001$). Concurrently, the percentage of oleic acid in platelets decreased with increasing concentrations of dietary linoleate ($r = -0.829$, $P < 0.0001$).

Phospholipase A_2 and thromboxane B_2

The effects of increasing the percentage of dietary linoleate on platelet PLA and thromboxane synthesis are shown in *Figure 2*. Platelet PLA was greatest for rats fed the 3.0 and 4.5 en% linoleate diets. There was a negative correlation between PLA and percentage of dietary linoleate ($r = -0.66$, $P < 0.0001$).

Platelet thromboxane synthesis was greatest for the rats fed the 4.5 en% linoleate diet. When the 3.0 en% linoleate diet was fed, platelet thromboxane synthesis was less than when the 4.5 en% linoleate diet was fed. A similar relationship to that of PLA and dietary linoleate is illustrated by a moderate negative correlation between thromboxane synthesis and dietary linoleate ($r = -0.459$, $P < 0.003$). The relationship between dietary linoleate and thromboxane synthesis is much stronger when the data for rats fed the 3.0 en% linoleate diet are omitted ($r = -0.740$, $P < 0.0001$). There was a trend for a quadratic relationship between dietary linoleate and thromboxane synthesis ($P < 0.08$) as might be inferred by the stronger correlation when the data for the rats fed the 3.0 en% linoleate diets are omitted.

For all diets, PLA and thromboxane synthesis were

Table 2 Platelet total fatty acids of rats fed experimental diets

	Fatty acid: Number C's:Number double bonds								
Dietary linoleic acid	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
en%					*% of total fatty acids**				
3.0	0.29	0.84	35.67	0.25	17.82	15.12[a]	4.25[a]	0.53	25.24
4.5	0.33	1.07	35.81	0.67	18.05	13.91[a]	5.17[b]	0.77	24.23
6.0	0.22	0.89	36.89	0.56	18.04	12.72[b]	5.95[c]	0.53	24.21
7.5	0.14	0.82	35.76	0.55	18.85	11.61[b]	6.65[d]	0.47	25.15
9.0	0.22	0.83	36.19	0.37	18.80	11.49[b]	6.96[d]	0.43	24.68
Pooled SEM	0.08	0.15	0.49	0.16	0.39	0.34	0.19	0.12	0.92

* Values are means of eight rats per dietary group. Means with the same superscript letter or no superscript letter are not significantly different at the 0.05 confidence level as analyzed by least squares means.

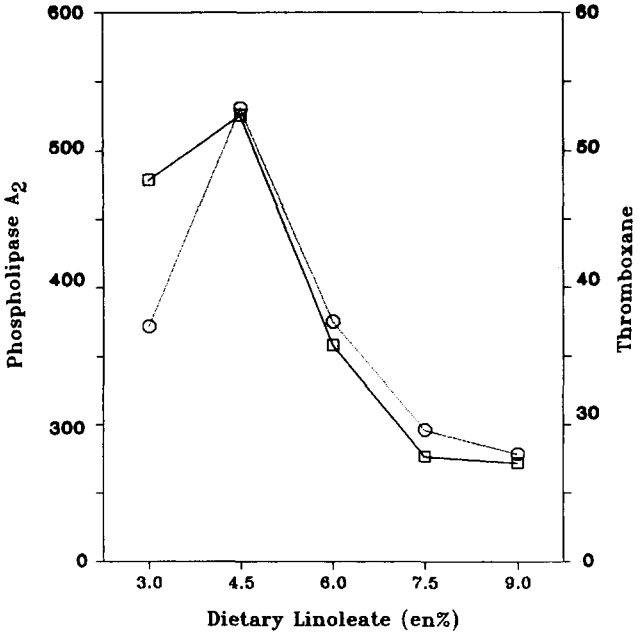

Figure 2 Effects of dietary linoleate on platelet PLA activity (pmol ^{14}C-arachidonic acid released per mg platelet protein in 40 min) and TX synthesis (pmol TX synthesized per mg platelet protein in 40 min). Points represent the mean for eight rats. Symbols in the figure represent: -⊟- PLA activity; -⊖- TX synthesis.

correlated ($r = 0.51$, $P < 0.0008$). The correlation between PLA and thromboxane synthesis was even stronger when the data for rats fed the 3.0 en% linoleate diet were omitted ($r = 0.61$, $P < 0.0002$).

Discussion

The changes in platelet total fatty acid composition caused by diet followed the same trends demonstrated by Berlin et al.[17] These changes are significantly different due to dietary fatty acids only for the proportion of oleic and linoleic acids of the total fatty acids. Because linoleic acid is the dietary essential fatty acid precursor of the thromboxane precursor, arachidonic acid, it has sometimes been assumed that dietary linoleic acid affects thromboxane synthesis by controlling the amount of substrate available. However, platelet total fatty acid composition shows that the arachidonic acid concentration in platelets is unchanged by changes in the linoleic acid content of diet. Though this suggests that the total substrate pool of fatty acids is not the factor influencing arachidonic acid release by phospholipase A_2, we do not know that platelet total fatty acid composition reflected platelet phospholipid-bound fatty acid composition. As shown in *Figure 1*, when measured at the time of a plateau of synthesis, the absolute amount of arachidonic acid released from radiolabeled phosphatidyl choline is tenfold greater than the absolute amount of thromboxane synthesized at the same time. When considered in view of the metabolism of the whole platelet, it is obvious that even more than a tenfold difference between the amount of arachidonic acid released and thromboxane synthesized would be found because the radiolabeled phosphatidyl choline substrate represents only part of the substrate pool for the action of phospholipase A_2. Such a ratio of substrate to product might suggest that control of phospholipase A_2 activity, by any mechanism, has no bearing upon the ultimate synthesis of thromboxane in the same system.

The highly significant correlation between phospholipase activity and thromboxane synthesis, which are both significantly affected by diet, indicates otherwise. Phospholipase A_2 and the enzymes involved in synthesis of thromboxane, cyclooxygenase, and thromboxane synthetase are all enzymes residing in the cell membrane. The results of this experiment indicate it is probable that all these enzymes are, at least in part, regulated by the fatty acid composition of the phospholipid bilayer in which they reside, not by substrate availability. Ballou and Cheung[3,4] have demonstrated that micromolar concentrations of free PUFA inhibit the activity of semipurified phospholipase A_2. Additionally, increases in the PUFA content of microsomal membrane preparations of white blood cells[5] and liver,[18] produced by in vitro manipulations, also have been shown to result in a decrease in phospholipase A_2 activity. Our results indicate that inhibition of phospholipase A_2 activity and thromboxane synthesis occurs within the range of 4.5–9.0 en% dietary linoleate, but the values for these measurements with less than 4.5 en% linoleate are suggestive of other effects. When linoleate provides 3.0% of energy, factors other than diet-induced platelet fatty acid composition changes may influence platelet thromboxane synthesis, but the factors cannot be induced from this experiment. The quadratic tendencies suggested by these data indicate a complex relationship between dietary linoleate, platelet net phospholipase A_2 activity, and platelet thromboxane synthesis. Such a relationship between dietary linoleate and eicosanoid synthesis has been noted,[1] and these results suggest that phospholipase A_2 is a site for mechanism of the effect.

Acknowledgments

The authors thank Jeanne Stewart, Ellen Barber, and Susan Krueger for technical assistance.

References

1 Dupont, J. and Dowd, M.K. (1990). Icosanoid synthesis as a functional measurement of essential fatty acid requirement. *J. Am. Coll. Nutr.* **9**, 272–276

2 Billah, M.M., Lapetina, E.G., and Cuatrecasas, P. (1980). Phospholipase A_2 and Phospholipase C Activities of Platelets. *J. Biol. Chem.* **255**, 10227–10231

3 Ballou, L.R. and Cheung, W.Y. (1983). Marked increase of human platelet phospholipase A_2 activity in vitro and demonstration of an endogenous inhibitor. *Proc. Natl. Acad. Sci. USA* **80**, 5203–5207

4 Ballou, L.R. and Cheung, W.Y. (1985). Inhibition of human platelet phospholipase A_2 activity by unsaturated fatty acids. *Proc. Natl. Acad. Sci. USA* **82**, 371–375

5 Marki, F. and Franson, R. (1986). Endogenous suppression of neutral-active and calcium-dependent phospholipase A_2 in

human polymorphonuclear leukocytes. *Biochim. Biophys. Acta* **879,** 149–156

6 Needleman, S.W., Spector, A.A., and Hoak, J.C. (1982). Enrichment of human phopholipids with linoleic acid diminishes thromboxane release. *Prostaglandins* **24,** 607–622

7 Sato, T., Nakao, K., Hashizume, T., and Fujii, T. (1987). Inhibition of platelet aggregation by unsaturated fatty acids through interference with a thromboxane-mediated process. *Biochim. Biophys. Acta* **931,** 157–164

8 Mathias, M.M. and Dupont, J. (1989). Effects of dietary fat on eicosanoid production in normal tissues. In *Carcinogenesis and Dietary Fat,* (S. Abraham, ed.), pp. 29–52, Kluwer Academic, Boston.

9 Batres-Cerezo, R., Dupont, J., Garcia, P.A., Kies, C., and Mathias, M.M. (1991). Studies of women eating diets with different fatty acid composition. III. Fatty acids and prostaglandin synthesis by platelets and cultured human endothelial cells. *J. Am. Coll. Nutr.* (in press)

10 Krumhardt, B. and Dupont, J. (1991). The relationship between thromboxane and cytosolic calcium modifiers in rat platelets. *Prostag. Leukotri. Med.* **43,** 17–23

11 Kuan, S.I. and Dupont, J. (1989). Dietary fat and cholesterol effects on cholesterol metabolism in CBA/J and C57BR/cdJ mice. *J. Nutr.* **119,** 349–355

12 Hallam, T.J., Thompson, N.T., Scrutton, M.C., and Rink, T.J. (1984). The role of cytoplasmic free calcium in the responses of quin2-loaded human platelets to vasopressin. *Biochem. J.* **221,** 897–901

13 Dupont, J., Garcia, P.A., Hennig, B., Dodd, N.K., Oh, S.Y., Sizer, C.E., Goswami, S.K., and Hyde, W.G. (1988). Bile acids in extrahepatic tissues. In *The bile acids,* Vol. 4, (K.D.R. Setchell, D. Kritchevsky, and P.P. Nair, eds.), pp. 341–372, Plenum Publishing Co., New York

14 McCosh, E.J., Meyer, D.L., and Dupont, J. (1976). Radioimmunoassay of prostaglandins E^1, E^2, and F^2 in unextracted plasma, serum and myocardium. *Prostaglandins* **12,** 471–486

15 Steinberg, L.Y., Mauldin, R.E., and Mathias, M.M. (1982). The effect of dietary lipids on clotting time and rat serum and urine prostaglandins. *Prog. Lipid Res.* **20,** 485–489

16 Lowry, O.H., Rosebrough, J., Farr, A.L., and Randall, R.L. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275

17 Berlin, E., Matusik, E.J., Jr., and Young, C., Jr. (1980). Effect of dietary fat on the fluidity of platelet membranes. *Lipids* **15,** 604–608

18 Momchilova, A., Petkova, D., Mechev, I., Dimitrov, G., and Koumanov, K. (1985). Sensitivity of 5′-nucleotidase and phospholipase A_2 towards liver plasma membranes modifications. *Int. J. Biochem.* **17,** 787–792