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Influence of a long-term zinc-deficient diet on rat platelet function and fatty acid composition

Patrice Faure, Philippe Durand*, Denis Blache*, Alain Favier & Anne Marie Roussel

**Laboratoire de Biochimie C, Hopital A. Michallon, Grenoble, France and [a]Faculté de Médecine, INSERM, Laboratoire de Biochimie des Lipoprotéines, Dijon, France*

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A reduced zinc intake is associated with numerous abnormalities and, in particular, with hemostasis dysfunction. In this report, we studied the effects of a long-term dietary zinc restriction on platelet function. Three groups of rats were analyzed: a zinc-deficient group (ZD) and two zinc-adequate fed groups, one pair-fed (PF) and one *ad libitum* fed (AL). We found that ZD diet (0.2 p.p.m.) impaired ADP-induced aggregation of washed platelet after 4 and 8 weeks of diet. Thrombin-induced aggregation was impaired in ZD rats and PF rats after 8 weeks. The thrombin-induced mobilization of radiolabeled arachidonate preincorporated into platelet phospholipids was followed as well as the subsequent formation of labeled cyclooxygenase and lipoxygenase products. Stimulated platelets of ZD rats exhibited a decreased production of cyclooxygenase and lipoxygenase products, particularly after 8 weeks of diet. Moreover, platelet thromboxane generation was decreased in the ZD group as studied using a radioimmunoassay after thrombin stimulation. In addition, we measured the total fatty acid compositions of platelet and plasma. As a whole, 20:5 ($n-3$) and 22:5 ($n-3$) fatty acids content were significantly increased in platelet lipids after 8 weeks. On the other hand, it is known that enrichment of these fatty acids through dietary studies, both in animal and human as well as *in vitro* incorporation in platelets, resulted in an inhibition of platelet function. Consequently, these changes in platelet membrane fatty acid composition may contribute to the impaired platelet aggregation observed in ZD rats.

Keywords: fatty acid, function, platelet, thromboxane, zinc deficiency

Introduction

Previous works have shown that zinc deficiency resulted in various abnormalities. Animal studies have indicated signs of retarded growth, alopecia, dermatitis, loss of appetite and reproductive failure. These studies have shown a prolonged bleeding tendency in rats fed a low zinc diet for short time periods (Gordon & Dell 1980, Emery *et al.* 1990). This hemostatic defect has also been found in humans during acute zinc deprivation and it has been attributed to an impaired platelet aggregation (Prasad 1979). By contrast, the serotonin release of rabbit and human platelets stimulated by platelet activating factor or thrombin was inhibited by *in vitro* incubation in the presence of micromolar concentrations of zinc (Huo *et al.* 1988, Nunez *et al.* 1989). However, long-term effects of zinc deficiency have not been extensively studied. Data have been reported indicating some effects on lipid metabolism and membrane fluidity (Huo *et al.* 1988). We have developed a model to study long-term zinc deficiency in rats. Using this model, we have previously reported that an increased lipoperoxidation can be found in rat plasma after an 8 week zinc deficiency period (Prasad 1979). In the present report, we used the same model to study long-term zinc deficiency on platelet reactivity and arachidonic acid metabolism. Plasma and platelet fatty acid compositions were also analyzed.

Address for correspondence: P. Faure, Laboratoire de Biochimie C, Hopital A. Michallon, BP 217X, 38043 Grenoble, France. Tel: (+33) 76 76 54 07; Fax: (+33) 76 42 66 64.

Materials and methods

Animals and diet

Male wistar rats (initial weight 180–200 g) (IFFA CREDO, L'Arbresle, France) were housed in acid-washed stainless steel cages and allowed free access to deionized distilled water delivered via a stainless steel watering system. The absence of zinc released by these materials was controlled. The rats were maintained at a constant temperature (23 °C) with a fixed (12 h) artificial light period.

For 5 days the animals were fed with a standard maintenance diet (No. 113; UAR, France). They were

then weighed and separated into three groups: Group 1 (ZD, $n = 9$) received a daily (24 h) diet of 30 g rat^{-1} zinc-deprived food described in Table 1. The food intake of the animals was recorded daily. Group 2 (PF, $n = 8$) received the same quantity of food as consumed by group 1 animals on the previous day according to the pair-feeding method. The diet was zinc adequate (100 p.p.m. of zinc in the form of $ZnSO_4 \cdot 7H_2O$). Group 3 (AL, $n = 9$) consisted of control animals which received the zinc-adequate diet (100 p.p.m.) *ad libitum*.

The two different diets were analyzed for zinc contents. The zinc-adequate diet contained 90 μg zn g^{-1} dry food and the ZD diet contained 0.20 μg zn g^{-1} dry food. The food composition and the fatty acid composition of the diet are given in Table 1.

Blood removal

The blood tests were performed twice: after 4 weeks (T1) and after 8 weeks of the ZD diet (T2). Blood was removed as in previous studies (Blache *et al.* 1987, Ciavatti *et al.* 1989) from the jugular vein of overnight fasted rats into plastic syringes with 1 volume of anticoagulant (38 mM citric acid, 75 mM sodium citrate, 136 mM glucose) for 4 volumes of blood for platelet aggregation. Another blood sample was collected in a syringe containing 1 volume of anticoagulant (EDTA 1.6 g, NaCl 0.66 g, dextrose 0.1 g per 100 ml) for 9 volumes of blood for the analysis of platelet and plasma fatty acid composition.

Zinc measurements

Blood was also withdrawn on zinc-free lithium heparinate for plasma zinc assays. Femoral bone zinc concentrations were also measured at the end of the experiment (8 weeks) after tissue mineralization in nitric acid solution. Assays of plasma and bone zinc were performed using atomic absorption spectrophotometry (Perkin Elmer 5010) as described previously (Faure *et al.* 1991).

Platelet preparation and aggregation

The platelet-rich plasma was obtained after centrifugation (150 *g*, 8 min) of the blood. The platelet pellet obtained after centrifugation (1000 *g*, 18 min) of the platelet-rich plasma was washed in Ca^{2+}-free Tyrode's buffer containing (in mM): 149 NaCl, 2.6 KCl, 9.5 $NaHCO_3$, 5.5 glucose, 0.5 $NaHPO_4$, 0.6 $MgCl_2$ and adjusted to pH 6.8 with 0.25 N HCl. Platelets were counted with a thrombocoulter (Coultronics), resuspended in Ca^{2+}-free Tyrode's buffer, (pH 7.4) and stored at 22 °C. Aggregation experiments were performed with 0.5 ml of the platelet suspension ($3–4 \times 10^8$ ml^{-1}) in disposable polystyrene cuvettes using a turbidimetric coaguloaggregometer (Renaud-Rubel). The platelet suspension was stirred at 1100 r.p.m. at 37 °C in the presence of a final Ca^{2+} concentration of 0.3 mM (resulting in maximum response) for 1 min. Platelets were then stimulated with agonists, diluted in Ca^{2+}-free Tyrode's buffer (as above) and kept on ice during the procedure. The final thrombin and ADP concentrations were, respectively, 0.08–0.1 U ml^{-1} and 0.6–1 μM.

Table 1. Diet composition

Egg albumin	14.5
Corn starch	38
Sucrose	38
Corn oil	4.5
Mineral mixture and amino acids[a]	4
Vitamins[b]	1

[a]Salt mixture (g kg^{-1}): L-cysteine 2.356; tryptophane 0.392; $CaCO_3$ 9.42; KH_2PO_4 10.68; NaCl 7.85; NH_4Fe citrate 1.29; Mn_2SO_4 0.03; $CuSO_4$ 40.03; $CaCl_2$ 0.03; KI 0.00023; $MgSO_4$ 7.51.

[b]Vitamin mixture (mg kg^{-1}): retinal palmitate 45; cholecalciferol 42; Ca pantothenate 392; thiamin HCl 23; riboflavin 15; folic acid 2; B12 22.5; ascorbic acid 150; niacin 7; biotin 45.

The fatty acid composition (%) is the following: polyunsaturated: 61.5 (18:2, 60.3; 18:3, 1.2); monounsaturated: 25.6 (16:1, 0.2; 18:1, 35.4); saturated: 12.28 (14:0, 0.08; 16:0, 10.8; 18:0, 1.4).

Platelet labeling and arachidonate metabolites

Phospholipids of washed rat platelets were labeled as previously described (Blache *et al.* 1987, Blache & Ciavatti 1989), with [1-^{14}C]arachidonic acid (60 mCi mmol^{-1}; Amersham) using a modified version of the technique of Bills *et al.* (1977). After a 60 min incubation period at 37 °C in the presence of radiolabeled arachidonate, the cells were washed by centrifugation to remove the non-incorporated fatty acid. Under these conditions, 50–60% of the radiolabeled arachidonate was incorporated in platelet lipids, with 98% in the phospholipid fraction. After temperature equilibrium, the platelet suspension (2×10^9 platelets ml^{-1}) was then stimulated at 37 °C for 2 min with thrombin (final concentration 0.2 U ml^{-1}; Sigma). The reactions were terminated by addition of 0.1 volume of cold 0.5 N formic acid and 3 volumes of cold ethylacetate, the extracts were analyzed by thin layer chromatography on silica gel G plates (Merck) in $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (90/8/1/0.8, v/v). Identification, detection and quantification were performed as described (Blache *et al.* 1987, Blache & Ciavatti 1989). Platelet synthesis of thromboxane (TX) A_2 was also determinated by radioimmunoassay (RIA) of its stable hydrolysis product TXB_2 (Amersham). Washed platelets (5×10^8 in 0.5 ml) were stirred at 37 °C with thrombin (0.2 ml) for 5 min. Assays for TXB_2 were performed on diethyl ether extraction after evaporation and dilution in RIA buffer according to the manufacturer's indications. Results were expressed as pmoles TXB_2 per 10^8 platelets for 5 min stimulation.

Platelet and plasma fatty acid analysis

Washed platelets (10^9) and plasma (0.5 ml) lipids were extracted by the technique of Folch *et al.* (1957). Extracts were evaporated under nitrogen and transmethylated according to Morrisson & Smith (1964) in the presence of BF_3 in methanol and toluene for 90 min at 90 °C. The fatty acid methyl esters were separated by capillary gas liquid chromatography. Analysis were performed with a DI 200 apparatus (Delsi Nermag, France) equipped with hydrogen

flame ionization detector and a cold on-column injector. The chromatograph contained an SP-2340 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.2 μm; Supelco). Helium was used as the carrier gas at a linear velocity of 19 cm s^{-1}. The oven temperature was programmed at 5 °C min^{-1} from 90 to 135 °C, then increased to 220 °C at 2 °C min^{-1} and held at that temperature for 10 min. The temperature of the detector was 260 °C. Methyl esters were identified by comparison with standard mixtures and quantified using a computerized terminal (Chroma software, Biosystems, Dijon, France).

Statistical analysis

All results were expressed as mean ± SD. Data were analyzed statistically by analysis of variance (ANOVA) followed by Wilcoxon's *t*-test. For the data comparison between T1 and T2, paired Student's *t*-test was used. The statistical analysis was computed using PCSM computer software (Deltasoft, France).

Results

Food intake, body weight and zinc status

Throughout the 8 week period of the experiment, evident external symptoms of zinc deficiency such as loss of appetite, alopecia skin lesions and decreased statural growth were observed for the animals given the ZD diet. Clinical differences between ZD and control groups (PF and AL), as well as the decline in food consumption, were observed as early as from the second week. The final body weights of the animals were 271 ± 6, 231 ± 5 and 220 ± 4 g for the Al, PF and ZD groups, respectively.

Plasma and femoral bone zinc levels were significantly lower in ZD rats as compared to both PF and AL rats. There were no further difference in plasma zinc concentrations between the samples of 4 weeks (T1) and 8 weeks (T2) in any group (Table 2).

Platelet aggregation

As shown in Figure 1, the response of platelets to thrombin was not different at 4 weeks between the three groups of rats. On the contrary, ADP-induced aggregation was reduced after 4 weeks in the animals given the ZD diet as compared with the other groups. After 8 weeks, this marked decrease was observed for platelets stimulated with thrombin or ADP. However, after 8 weeks, the PF group showed a significant decrease of its platelet response to thrombin or ADP compared to the AL group.

Metabolism of [^{14}C]arachidonate in washed rat platelets

Preliminary time-course experiments had indicated that the loss of radiolabeled arachidonate from phospholipids leveled off after 1 min. We thus decided to carry out our investigation taking an incubation time of 2 min in the presence of thrombin.

In the three groups, we observed a decrease of platelet

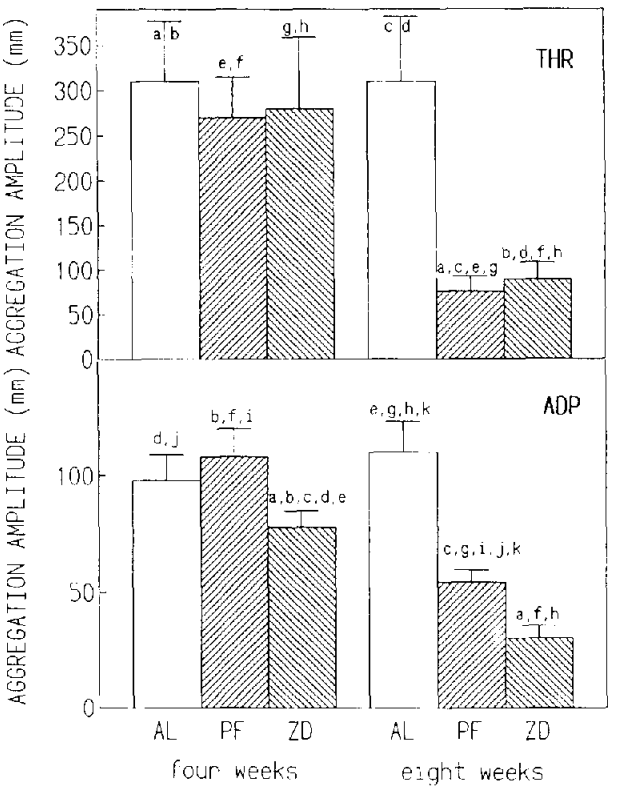

Figure 1. Aggregation of washed platelets of rats on zinc-adequate and ZD. Platelets from the AL, PF or ZDs group were prepared, at 4 and 8 weeks, and analyzed as detailed in Methods. After stimulation with low concentrations of thrombin (THR) or ADP the aggregation curves were recorded and results expressed (in mm) by means ± SD (n = 8–9). Bars with the same letter are statistically significantly different (ANOVA): $^{a,b,e,f,i,j,k}P < 0.001$; $^{c,d,g}P < 0.01$.

phospholipid-associated radioactivity and an increase of free arachidonic acid mobilized by the phospholipase A_2 activity (Table 3). More labeling was found for the two studied periods in the phospholipid fraction and less in the arachidonic acid fraction in comparison with platelets from the other groups. These results mean that phospholipase A_2 was less active in platelets from ZD rats. In addition,

Table 2. Effect of dietary zinc restriction on plasma and femoral bone zinc status

	ZD (n = 9)	PF (n = 8)	AL (n = 9)
Plasma zinc (μM)			
T1[a]	7.50 ± 0.35	23.10 ± 0.61	24.60 ± 0.71
T2[a]	7.8 ± 0.43	22.10 ± 0.78	22.50 ± 0.92
Femoral bone zinc (μg g^{-1} dry tissue)	156.20 ± 9.20	254.40 ± 10.30	261.30 ± 12.20

Values are means ± SD for the numbers of animals in each group (in parentheses).

[a]T1, 4 weeks; T2, 8 weeks. Femoral bone zinc concentrations were measured at T2. Statistical comparisons were made using ANOVA. Values from the ZD group were highly significantly different from all other groups ($P < 0.001$).

Table 3. Platelet arachidonate metabolism after 4 weeks (T1) and 8 weeks (T2) of zinc-adequate or ZD diet

	PL	TXB_2	HHT	HETE	AA
AL T1	72.12 ± 5.13[b]	2.13 ± 0.65[c]	2.25 ± 0.51	10.00 ± 0.40	12.51 ± 4.71[a]
T2	56.91 ± 4.92	6.54 ± 1.09[b]	7.83 ± 1.11[a]	12.31 ± 0.51	11.12 ± 4.11
PF T1	71.12 ± 4.41[b]	2.84 ± 1.74[c]	3.45 ± 0.21[a]	10.31 ± 0.32	10.23 ± 2.00[a]
T2	66.32 ± 1.26[b]	7.12 ± 1.74[b]	5.84 ± 0.36[a]	7.34 ± 0.64	14.72 ± 3.00
ZD T1	81.91 ± 5.56[b]	1.41 ± 0.31[c]	2.26 ± 1.00[a]	9.30 ± 0.93[b]	7.81 ± 1.00[a]
T2	59.38 ± 3.72[b]	4.14 ± 0.93[b]	3.73 ± 0.82[a]	7.14 ± 1.12	10.52 ± 4.31

Results (mean ± SD) are expressed for each compound as percentage of the total radioactivity present in platelets (AL, $n = 9$; PF, $n = 8$; ZD, $n = 9$).
PL, phospholipids; TXB_2, thromboxane B_2; HHT, 12-hydroxyheptadecanoic acid; HETE, 12-hydroxyeicosatetraenoic acid; AA, arachidonic acid.
Statistical significance (ANOVA) between ZD and zinc adequate groups (PF and AL): [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$. All the results are significantly different (paired Student's t-test) between T1 and T2.

the lipoxygenase and cyclooxygenase metabolites were also reduced to a greater extent in ZD rats than those fed with the zinc-adequate diet (Table 3). These results were confirmed by the data illustrated in Figure 2 concerning thromboxane biosynthesis measured by means of RIA after stimulation of intact platelets by thrombin for 5 min. No significant difference was obtained after 4 weeks, whereas after 8 weeks platelets from the ZD group produced less TXB_2 than both the PF and AL groups.

Platelet lipid and plasma fatty acid distribution

Platelets. As shown in Table 4, the fatty acid composition of platelet lipids differed in the animals given the ZD diet as compared to animals given the zinc-adequate diet. The percentage contents of saturated fatty acids were not changed whereas the content of 18:1 was increased, for the two studied periods, in ZD rats. After 4 weeks, the content of linoleic acid was increased and that of arachidonic acid was decreased in the ZD group. In the $n - 3$ series, the content of 18:3, 20:5 and 22:5 was decreased at 4 weeks. At 8 weeks there was a progressive increase of 20:5 and 22:5 when ZD rats were compared with PF and AL rats.

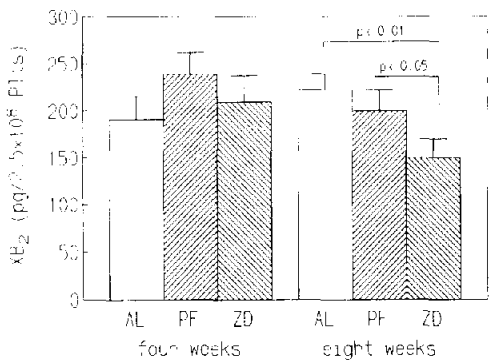

Figure 2. Thrombin-induced TXB_2 biosynthesis of platelets of rat on zinc-adequate and ZD diets. Washed platelets were prepared from the AL, PF or ZD rats after 4 or 8 weeks. After a stimulation period of 5 min at 37 °C with thrombin, TXB_2 was assayed using a RIA. Results were expressed (mean ± SD, $n = 8$–9) as pmol TXB_2 per 2.5×10^8 platelets for 5 min. The significance of the results was calculated using ANOVA.

Table 4. Main total fatty acid composition (%) of the platelets from rats fed with low or adequate zinc diet for 4 weeks (T1) and 8 weeks (T2)

		Group		
		AL ($n = 9$)	PF ($n = 8$)	ZD ($n = 9$)
C18:2	T1	19.07 ± 0.73[t]	14.91 ± 1.96[at]	20.8 ± 3.90[at]
($n - 6$)	T2	8.98 ± 0.23	7.8 ± 0.43[a]	8.7 ± 0.47[a]
C18:3	T1	0.59 ± 0.04	0.52 ± 0.24	0.37 ± 0.02
($n - 3$)	T2	0.45 ± 0.12	0.5 ± 0.21	0.3 ± 0.29
C20:4	T1	25.72 ± 1.62[at]	27.55 ± 1.86[a]	21.64 ± 0.61[at]
($n - 6$)	T2	29.5 ± 0.39[a]	30.5 ± 0.32[a]	28.68 ± 0.26[a]
C20:5	T1	0.34 ± 0.06[at]	0.33 ± 0.20	0.22 ± 0.02[at]
($n - 3$)	T2	0.23 ± 0.01[a]	0.35 ± 0.80[a]	0.45 ± 0.02[a]
C22:5	T1	0.58 ± 0.11[bt]	0.44 ± 0.10[bt]	0.28 ± 0.09[bt]
($n - 3$)	T2	0.29 ± 0.12[b]	0.23 ± 0.04[b]	0.45 ± 0.16[b]
C22:6	T1	1.12 ± 0.07[t]	1.19 ± 0.24[t]	0.97 ± 0.24
($n - 3$)	T2	0.3 ± 0.02[b]	0.62 ± 0.11	0.66 ± 0.12[b]

Values are expressed as percent of mean ± SD for each fatty acid methyl ester.
Significance (ANOVA) of ZD rats versus controls (zinc adequate diet): [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$. Significance (paired Student's t-test) T1 versus T2: [t] $P < 0.05$. Platelet saturated and monounsaturated fatty acid compositions were not significantly different between the groups and are not shown.

Plasma. The major changes in the plasma fatty acid composition of ZD rats were in the contents of 18:2 ($n - 6$) and 20:4 ($n - 6$) which, respectively, increased and decreased, in particular at 8 weeks (Table 5). There was also a decrease in the content of 20:5 ($n - 3$), whereas 22:6 ($n - 3$) decreased at 4 weeks and increased at 8 weeks in plasma from ZD rats when compared to PF and AL rats.

Discussion

The present study performed in rats has shown that a long-term ZD diet progressively resulted in an impaired

Table 5. Main total fatty acid composition (%) of the plasma from rats fed low or adequate zinc diets for 4 weeks (T1) and 8 weeks (T2)

		Group		
		AL ($n = 9$)	PF ($n = 8$)	ZD ($n = 9$)
C18:2	T1	13.52 ± 2.91	11.10 ± 2.51	12.24 ± 3.08
($n - 6$)	T2	14.6 ± 3.11	7.92 ± 0.43[b]	15.19 ± 3.17[b]
C18:3	T1	0.05 ± 0.01	0.1 ± 0.09	0.05 ± 0.01
($n - 3$)	T2	0.05 ± 0.02	0.12 ± 0.01	0.05 ± 0.01
C20:4	T1	14.5 ± 6.70[a]	13.92 ± 6.51[at]	6.92 ± 1.72[at]
($n - 6$)	T2	20.5 ± 2.71[c]	30.50 ± 0.66[c]	16.36 ± 0.69[c]
C20:5	T1	0.36 ± 0.06[c]	0.3 ± 0.01	0.22 ± 0.03[c]
($n - 3$)	T2	0.23 ± 0.01	0.35 ± 0.18[a]	0.20 ± 0.03[a]
C22:5	T1	0.47 ± 0.11	0.32 ± 0.04	0.41 ± 0.21
($n - 3$)	T2	0.36 ± 0.04	0.23 ± 0.04[b]	0.36 ± 0.02[b]
C22:6	T1	1.07 ± 0.17	1.09 ± 0.69[t]	0.42 ± 0.21[t]
($n - 3$)	T2	1.28 ± 0.12	0.5 ± 0.24[b]	1.32 ± 0.21[b]

Values are expressed as percent of mean ± SD for each fatty acid methyl ester.
Significance (ANOVA) of ZD rats versus controls (zinc-adequate diet): [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$. Significance (paired Student's t-test) T1 versus T2: [t] $P < 0.05$. Plasma saturated and monounsaturated fatty acid compositions were not significantly different between the groups.

platelet behavior and modifications of fatty acid composition. This confirms and extends earlier observations both in animals and humans carried out essentially over short-time periods (Gordon *et al.* 1980, Emery *et al.* 1990). It has been shown that because of no apparent coagulation defect, the prolonged bleeding time observed in zinc deficiency might be attributed to a defective platelet behavior. Original investigations have shown that ADP-induced aggregation was impaired using platelet-rich plasma (Gordon *et al.* 1980, Emery *et al.* 1990). This has been confirmed with washed platelets from rats given a ZD diet. Our data confirmed that, apart from ADP, other agonists such as thrombin induced a lower aggregation response. However, in contrast to the response to ADP, which was seen at 4 weeks, the thrombin reactivity was impaired between 4 and 8 weeks in our study. It is interesting to note that our results dealing with arachidonic acid metabolism fitted well with the observed time-course response to thrombin. As a matter of fact, our results obtained by means of radiolabeled arachidonic acid preincorporated into platelet membrane phospholipids demonstrated that, after thrombin stimulation, ZD rats had a slower metabolism than the other groups. Zinc deficiency induced a decreased arachidonate mobilization and metabolite synthesis from both the lipoxygenase and cyclooxygenase pathways. In particular, the production of the stable derivative of the proaggregatory TXA_2, TXB_2, also assayed by the means of RIA in thrombin-stimulated platelets was decreased after a long-term zinc deficiency (8 weeks).

Although the precise mechanism(s) leading to an impaired platelet activity is (are) still unknown (Chvapil 1973), several hypothesis can be proposed. The first one might originate from the results of plasma and platelet fatty acid compositions. We found that zinc deficiency progressively induced a reduction of the arachidonic acid content as well as an increase in long chain fatty acids from the $n - 3$ series, particularly for platelets at 8 weeks of diet feeding. These data confirm the previously reported results of Cunnane (1988) indicating a higher 18:2/20:4 ratio in plasma, liver and testes of animals given a ZD diet. Although 20:4 was still present at a relatively high concentration, this fact might explain the decreased thromboxane production and the lower reactivity of the platelets of these animals.

As far as fatty acids are concerned, it is also noteworthy that our results showed some changes in the $n - 3$ series. Platelets from ZD rats exhibited an increase of the contents of 20:5 ($n - 3$) and 22:5 ($n - 3$) after 8 weeks. These changes might explain the lower platelet reactivity of these animals. Similar changes have been obtained after fish oil supplementation, although they were usually more pronounced (Siess *et al.* 1980, Dyerberg 1981, Thorngen & Gustafson 1981, Sanders & Hochland 1983). In these studies, this enrichment in long chain fatty acids from the $n - 3$ series was accompanied by a reduction in platelet reactivity (Sanders & Hochland 1983). This has been partly attributed to a competition between 20:4 ($n - 6$) and 20:5 ($n - 3$) resulting, firstly, in a decreased biosynthesis in TXA_2 and secondly in the production of the inactive compound TXA_3 (Fisher & Weber 1983). Although in our study, we did not measure TXA_3 (or TXB_3), our data might be explained in light of the above results. Moreover, it is surprising to observe a contrast between the platelet arachidonic acid composition which increased after an 8 week ZD diet and the relative stability of this fatty acid in plasma.

In platelet activation, an increase in cytosolic free Ca^{2+} has been demonstrated (Rink *et al.* 1982). We have also shown, particularly in rat platelets, that the extracellular Ca^{2+} is a key element in this process, since the platelet response was reduced by more than 80% in the absence of external Ca^{2+} (Blache *et al.* 1987, Blache & Ciavatti 1989, Kroll & Schafer 1989). Calcium uptake has been recently measured in ZD rat platelets by O'Dell & Emery (1991). These authors have shown that these platelets took up significantly less external calcium after ADP stimulation as compared with platelets from control animals. These findings are in complete agreement with our results and might explain not only the reduction of platelet aggregation but also the decreased thromboxane production. In addition, thrombin induces the mobilization of arachidonic acid whereas threshold concentration of ADP does not (Kroll & Schafer 1989). This might explain why a decrease in the platelet reactivity was first observed with ADP by comparison with thrombin (4 versus 8 weeks).

In summary, the present study indicated that a long-term zinc deficiency induced an impaired platelet reactivity. The thrombin-induced mobilization of arachidonic

acid and the subsequent cyclooxygenase and lipoxygenase products were also decreased, essentially the active compound $TBXA_2$. These changes might result from modifications of platelet fatty acid composition, mostly in the contents of arachidonate and in the fatty acids from the $n-3$ series. These results can be explained in light of the reported data dealing with a decrease in the calcium influx. However, several unanswered questions about the mechanism warrant further studies.

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