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Effect of an Acute Zinc Depletion on Rat Lipoprotein Distribution and Peroxidation

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ABSTRACT

The aim of this study was to determine the extent to which zinc depletion leads to lipoprotein modifications by measuring both lipoprotein-fraction distribution and peroxidation in zinc-depleted rats.

The animals were divided into three groups and fed for 8 wk a zinc-adequate diet (100 ppm) ad libitum (AL), a zinc-deficient diet (0.2 ppm) ad libitum (ZD), or a zinc-adequate diet according to the pair feeding method (PF).

Trace-element status, tissular lipids, and lipoprotein-fraction study were performed. The MDA production by the lipoprotein fraction was measured before and after induced peroxidation.

Cholesterol and phospholipids were increased in ZD rats. An important increase of VLDL and IDL was observed and a significant enhanced production of MDA by the LDL was related to zinc deficiency. From this observation, we may conclude that LDL fractions of ZD rats are more susceptible to induced oxidative damage.

These results suggest that in zinc deficiency, the lipoprotein fragility is an aggravating factor of peroxidation and the dyslipoproteinemia may lead to an atherogenic risk.

Index Entries: Zinc-deficient rats; lipoprotein fractions; lipid peroxidation.

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INTRODUCTION

The role of trace elements in lipid metabolism as factors of the etiology of atherosclerosis (*1–3*) must be viewed as important. In this way, it has been reported that zinc interacting with dietary copper may play a role in cholesterol distribution (*4,5*). However although a significant positive relation between serum zinc and high-density-lipoprotein (HDL) cholesterol was found in rats by some researchers (*6,7*), others have been unable to find a correlation between zinc and total cholesterol in plasma (*8,9*). Thus, it may be concluded that the influence of zinc status on lipoprotein metabolism yet remains unclear. Moreover, it is known that zinc deficiency decreases free-radical protections (*10,11*) and results in enhanced lipid peroxidation (*12*). Thus, if many authors report the atherogenic role of peroxided lipoproteins (*13*), the influence of zinc status in such a modification is not established. The aim of this study was to determine the extent to which zinc depletion leads to lipoprotein modifications, by studying both lipoprotein-fraction distribution and peroxidation in ZD rats.

MATERIALS AND METHODS

Animals and Diets

Thirty male Wistar rats (initial weight 180–200 g) (IFFA CREDO F69210 L'arbesle) were housed in acid-washed stainless steel cages and allowed free access to deionized distilled water delivered via a stainless-steel watering system. We verified the absence of zinc release from these materials. The rats were divided into three groups and fed for 8 wk on a zinc-adequate diet (100 ppm) ad libitum (AL), a zinc-deficient diet (0.2 ppm) ad libitum (ZD), or a zinc-adequate diet at an intake equal to that of the deficient group (pair feeding) (PF).

The different diets were analyzed for zinc content. The AL diet contained 90 μg Zn/g dry food, and the ZD diet contained 0.27 μg Zn/g dry food (Table 1).

Food intake was recorded every day. The rats were subjected to a light cycle with a dark period from 1900 to 0700 and a light period from 0700 to 1900 throughout the experiment.

Experimental Procedure

Blood and Tissue Collection

At the end of the eighth week of dietary treatment, the animals were fasted overnight. Blood samples were taken via heart puncture, under anesthesia by intraperitoneal injection (50 mg/kg nembutal).

Two blood samples were collected: one was stored on ice for lipoprotein study, and the other in a plastic syringe also containing trace-element-free heparin for zinc and copper analysis.

Table 1
Centesimal Diet Composition[a]

Egg albumin	14.5
Corn starch	38.0
Sucrose	38.0
Corn Oil	4.5
Mineral mixture and amino acids[b]	4.0
Vitamins[c]	1.0

[a]The diets were purchased form INRA F-Clermont-Ferrand (Dr. Lamand).

[b]Salt mixture, expressed in g/10^3 g: L cystein, 2.356; L tryptophan, 0.392; Ca pantothenate, 0.392; $CaCO_3$, 9.42; KH_2PO_4, 10.68; NaCl, 7.85; NH_4, Fe citrate, 1.29; $Mn2SO_4$, 0.03; $CuSO_4$, 0.03; $CaCl_2$, 0.03; Kl, 0.00023; $MgSO_4$, 7.51.

[c]Vitamin mixture: Vitamin A, 1980 UI; D_3, 2500 UI; B_1, 2 mg; B_2, 1.5 mg; B_3, 7 mg; B_{12}, 5 μg; C, 80 mg; E, 17 mg; PP, 10 mg.

Analytical Method

Trace-Element Analysis

Samples of diet, plasma, and femoral bone were mineralized in concentrated nitric acid. Zinc and copper were determined by flame-absorption spectroscopy (Perkin-Elmer 5010).

Nonlipid parameters of blood were determined by using an automated analyzer, Technicon RA 1000. (XF 95330 Domont)

Tissular lipids were extracted from samples of frozen, dried liver; heart; and aorta (*14*). The lipid extracts were analyzed for their content of cholesterol (*15*) and phospholipids (*16*).

Lipoprotein Analysis

The lipoprotein fractions were isolated by ultracentrifugation using the Beckman Rotor TL 100.2, according to the modified procedure described by Chung et al. (*17*). Cholesterol, triglycerides, and phospholipids concentrations in the whole serum and lipoprotein fractions were determined enzymatically, using a centrifuge analyzer (Cobas Fara from Roche Corporation). The HDL fraction was obtained by selective precipitation of the VLDL and LDL fractions from the whole serum with Mg^{2+}/phosphotungstic acid (*18*).

Malonaldehyde, generated in serum and lipoprotein fractions, was determined as a lipid-peroxidation marker. This assay was based on a modification of the method of Dousset (*19*). Fluorescence of an MDA thiobarbituric acid adduct was measured after extraction with *n*-butanol. A mixture (750 μL of thiobarbituric acid (Merck) at 8 g/L and 7% perchloric acid (Merck) (2:1, v/v) was added to 100 μL of plasma; after agitating, the mixture was placed in a 95°C water bath for 60 min, cooled in an ice bath. The fluorescent compound was extracted by mixing with

n-butanol for 2 min. After centrifuging, fluorescence in the *n*-butanol phase was determined with an Amincon Bowman fluorimeter (Maryland USA) with excitation at 532 nm and emission at 553 nm. A blank was run for each plasma sample; the calibration curve was created with a stock solution of 1,1,3,3 tetraethoxypropane (Sigma), prepared in alcohol and stored according to a method described by Wong (*20*). The production of MDA in lipoprotein fractions was also measured after lipid peroxidation induced with $FeSO^4$ (10 μM)/ascorbate (250/μM) for 30 min in a 37°C water bath, in an oxygen-free medium.

Statistical Analysis

All results are presented as the mean ± standard error (SE). When two groups were compared, Student's-test was employed. The limit of significance was set at $p < 0.05$.

RESULTS

Food Intake, Body Weight, and Zinc Status (Table 2)

During the 8-wk period of the experiment, evident external symptoms of zinc deficiency, including loss of appetite, alopecia skin lesions, and decreased statural growth (Fig. 1) were observed. The differences between the ZD group and the PF and AL control groups as well as the decline in diet consumption were observed as soon as the second week of dietary treatment.

Plasma and femoral-bone zinc levels are significantly lower in zinc-deficient rats. The level of dietary zinc does not significantly affect values for plasma copper.

Blood Nonlipid Parameters (Table 3)

In ZD as in PF rats, the protein catabolism is stimulated, resulting in an increase of urea, uric acid, and creatinemia. The calcemia is reduced. The glycemia of ZD rats is significantly lower than that found in control rats.

Serum Lipids (Table 4)

Total cholesterol and HDL cholesterol levels are not modified by zinc deprivation. However, in ZD rats, triglycerides and phospholipid levels are elevated.

Tissular Lipids (Table 5)

Zinc deficiency leads to increased cholesterol in liver and heart. The tissular phospholipids (liver, aorta, heart) are increased and we observed a raised cholesterol-to-phospholipid ratio in the livers of the rats fed a ZD diet.

Table 2
Effect of Diets on Body Weight, Food Intake, and Trace-Element Levels[a]

	ZD $n = 7$	PF $n = 7$	AL $n = 7$
Body weight, g	217 ± 3[b,c]	250 ± 5[d]	296 ± 4
Food intake, g/24 h/rat	18[c]	18[d]	30
Zn plasma, μmol/L	6.1 ± 2.1[b,c]	24.9 ± 0.5	2.56 ± 0.7
Cu plasma, μmol/L	19.6 ± 1.5[c]	16.4 ± 0.7[d]	21.5 ± 2.1
Zn femoral bone, μg/g dry	112 ± 5[b,c]	243 ± 9	252 ± 13

[a]Values are mean ± SE for n shown.
[b]$p < 0.05$ between ZD/PF.
[c]$p < 0.05$ between ZD/AL.
[d]$p < 0.05$ between PF/AL.

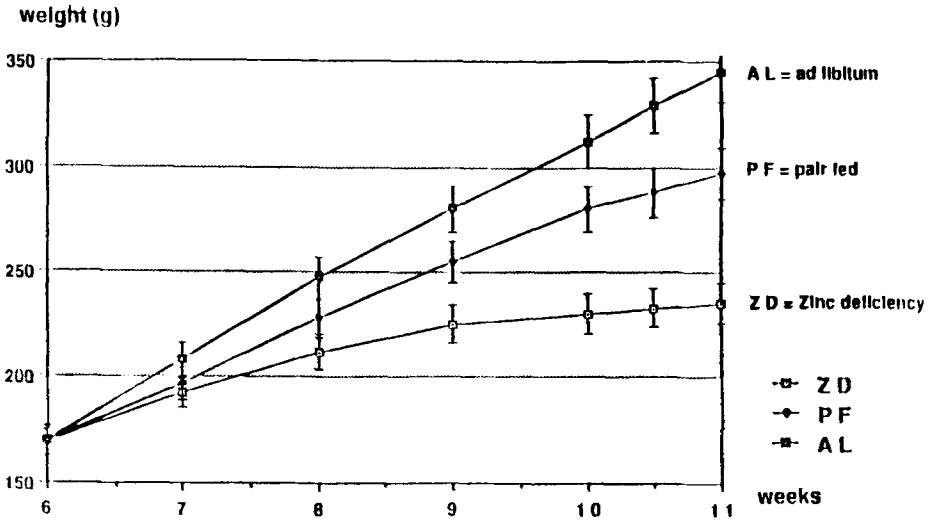

Fig. 1. Ponderal Growth of the Animals.

Lipoprotein Fractions Study

An important increase in VLDL and IDL was seen in zinc-depleted rats as well as in pair-fed rats. Lipid contents of isolated lipoprotein fractions are shown in Table 6. According to the VLDL and IDL increase, the level of triglycerides in the isolated VLDL and IDL particles is significantly higher in ZD and PF animals than in their controls. The phospholipid level in each lipoprotein fraction is higher in ZD animals than in rats fed an AL diet.

Cholesterol in lipoprotein fractions is higher in ZD rats than in PF animals, but does not differ significantly between the ZD rats and their AL-fed controls. The cholesterol HDL/cholesterol LDL ratio is the same in these two groups, but it is higher in PF rats.

Table 3
Influence of Diet on Plasma Nonlipid Parameters[a]

	ZD $n = 7$	PF $n = 7$	AL $n = 7$
Uric acid, μmol/L	89 ± 2.4[c]	86 ± 9.4[d]	61.8 ± 3.38
Ca, mmol/L	1.74 ± 0.04[c]	1.63 ± 0.82[d]	2.48 ± 0.03
P, mmol/L	3.12 ± 0.10	3.16 ± 0.09	3.14 ± 0.13
Proteins, g/L	60.75 ± 2.45[c]	57.3 ± 1.2	54.30 ± 0.61
Hematocrit	58 ± 3[b,c]	52 ± 4	52 ± 4
Cl, mmol/L	107.5 ± 0.50	106 ± 1.6	100.16 ± 1.07
Glucose, mmol/L	51 ± 0.6[b,c]	9.7 ± 0.6	7.5 ± 0.9
Creatinin, mmol/L	68.22 ± 2.1[c]	66.33 ± 3.1[d]	54.33 ± 3.8
Urea, mmol/L	10.4 ± 1.5[c]	10.7 ± 0.5[d]	8.28 ± 0.68
Na, mmol/L	144 ± 3.1	146 ± 2.24	141.3 ± 1.02
K, mmol/L	6.5 ± 0.45	7.5 ± 0.74	6 ± 0.26
CO_2, mmol/L	20 ± 2.15	19.5 ± 1.60	21 ± 1.12

[a]Values are mean ± SE for n shown.
[b]$p < 0.05$ ZD/PF.
[c]$p < 0.05$ ZD/AL.
[d]$p < 0.05$ PF/AL.

Table 4
Influence of Diet on Plasma Lipid Parameters[a]

	ZD $n = 7$	PF $n = 7$	AL $n = 7$
Total cholesterol, mg/100 mL	54 ± 4.5	50 ± 3.4	55 ± 2.65
Triglycerides, mg/100 mL	56 ± 7.5[c]	65 ± 8.7[d]	27 ± 2.27
HDL cholesterol, mg/100 mL	45 ± 4.1	39 ± 2.6	43 ± 2.27
Phospholipids mg/100 mL	109 ± 6.1[c]	96 ± 4.5	89 ± 3.3
Chol/chol HDL	1.20	1.28	1.28

[a]Values are mean ±SEM for n shown.
[b]$p < 0.05$ ZD/PF.
[c]$p < 0.05$ ZD/AL.
[d]$p < 0.05$ PF/AL.

Lipid Peroxidation Marker (Table 7, Fig. 2)

A significant increase in the production of plasmatic MDA was found, but we did not observe the production of MDA in the lipoprotein fractions in vivo to be modified in relation to the zinc status. However, when lipoprotein peroxidation is induced by $FeSO_4$/ascorbate, we note in vitro a significantly enhanced production of MDA by the LDL in ZD rats.

Table 5
Influence of Diet on Tissular Lipids

	ZD $n = 7$	PF $n = 7$	AL $n = 7$
CHOLESTEROL	***		
Liver	0.87 ± 0.05	0.50 ± 0.16	0.60 ± 0.02
Heart, mg/g s	0.81 ± 0.04	0.60 ± 0.03	0.58 ± 0.01
Aorta	0.73 ± 0.02	0.80 ± 0.03	0.81 ± 0.03
PHOSPHOLIPID			
Liver	6.68 ± 0.20	4.5 ± 0.26	4.5 ± 0.20
Heart, mg/g s	6.07 ± 0.27	4.3 ± 0.20	4.5 ± 0.20
Aorta	1.16 ± 0.05	1.09 ± 0.08	1.06 ± 0.20
CHOL/PL			
Liver	0.16 ± 0.02	0.11 ± 0.01	0.13 ± 0.02
Heart	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.03
Aorta	0.72 ± 0.01	0.73 ± 0.02	0.76 ± 0.02

[a]Values are mean ± SE for n shown.
[b]$p < 0.05$ ZD/PF.
[c]$p < 0.05$ ZD/AL.
[d]$p < 0.05$ PF/AL.

Table 6
Influence of Diet on Lipoprotein Fractions Composition, mg/100 mL Serum

		ZD $n = 7$	PF $n = 7$	AL $n = 7$
VLDL	Chol	0.90[c] ± 0.15	0.70[d] ± 0.15	—
	TG	10.50[c] ± 2.08	16.50[c] ± 3.03	2.90 ± 0.60
	PL	1.15 ± 1.13	0.80 ± 0.37	—
IDL				
	Chol	0.75[d] ± 0.15	—	—
	TG	1.01[b] ± 0.37	0.90[d] ± 0.37	0.45 ± 0.15
	PL	0.30[d] ± 0.01	—	—
LDL				
	Chol	3.59[b] ± 0.49	1.60 ± 0.49	4.12 ± 0.75
	TG	2.26 ± 0.75	2.19 ± 0.49	3.06 ± 1.13
	PL	2.19 ± 1.26	2.60 ± 0.02	2.66 ± 0.49
HDL				
	Chol	37.05[b] ± 3.37	28.5 ± 2.25	37.50 ± 1.70
	TG	14.55 ± 3.40	13.05 ± 2.25	10.50 ± 1.70
	PL	76.35[c] ± 5.67	67.50 ± 5.10	69 ± 2.83
Chol HDL/Chol LDL		10.30	17.80	9.10

[a]Values are mean ± SEM n shown.
[b]$p < 0.05$ ZD/PF.
[c]$p < 0.05$ ZD/AL.
[d]$p < 0.05$ PF/AL.

Table 7
Influence of Diet on MDA Production, μ*M*/L[a]

MDA	ZD $n = 7$	PF $n = 7$	AL $n = 7$
Serum[e]	3.5 ± 0.4	2.85 ± 0.2	2.7 ± 0.3
VLDL	0.90 ± 0.04	0.09 ± 0.04	0.08 ± 0.03
VLDL[f]	0.41 ± 0.18	0.44 ± 0.11	0.280 ± 0.13
IDL	0.08 ± 0.04	0.07 ± 0.04	0.07 ± 0.03
IDL[f]	0.20 ± 0.06	0.15 ± 0.01	0.15 ± 0.03
LDL	0.16[b] ± 0.05	0.110 ± 0.05	0.126 ± 0.05
LDL[f]	0.912 ± 0.24	0.480[d] ± 0.26	0.526 ± 0.26
HDL	3.72 ± 0.09	3.37 ± 0.10	3.63 ± 0.10
HDL[f]	4.52 ± 0.18	4.48 ± 0.10	4.62 ± 0.13

[a]Values are mean ± SE for *n* shown.
[b]$p < 0.05$ between ZD/PF.
[c]$p < 0.05$ between ZD/AL.
[d]$p < 0.05$ between PF/AL.
[e]Experimented on another group of rats.
[f]MDA production after induced peroxidation.

DISCUSSION

In this study, we report biological changes in animals fed a ZD diet for an 8-wk period. Under these dietary conditions, an acute zinc depletion is observed. Behavioral changes, loss of appetite, and skin lesions illustrate a significantly zinc-decreased status. Weight loss is greater in ZD rats than in PF rats, indicating that zinc deprivation, more than dietary restriction, is responsible for this effect. Also related to partial malnutrition, the protein catabolism is stimulated in both ZD and PF rats. Zincemia and femoral zinc levels in ZD rats, being good markers of zinc status, show a significant decrease in comparison to the other two groups.

Few reports have been published supporting a direct relation between zinc and triglyceride levels or metabolism. Generally, a decreased level of triglycerides in ZD rats is measured (*21*), but these observations may depend on the severity of the zinc depletion and, especially, the degree of starvation. Our results show hypertriglyceridemia both in ZD rats and in restricted-food-intake animals: the lipoprotein-fractions study reveals that this increase is represented by endogenous triglycerides transported by VLDL and IDL fractions. Our observations probably are attributable mostly to negative protein-energy intake in relation to caloric restriction, and agree with those of Petering et al. (*22*), who report a variation in triglyceridemia from 52 mg/100 mL in ZD rats to 28.8 mg/100

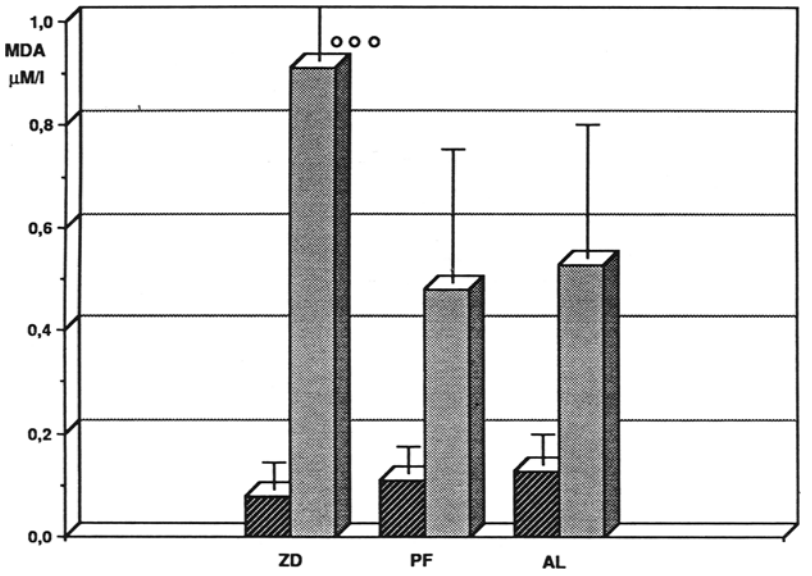

Fig. 2. Influence of diet on MDA production by LDL: dark bars, MDA production without induction; gray bars, MDA production induced by $FeSO^4$/ascorbate.

mL in control AL-fed rats. In the same way, Clejan et al. (*23*) observe a 200% increase in triglycerides of ZD rats and put forward the hypothesis that ZD rats have a high endogenous production of lipids. If food-conversion efficiency is low in ZD rats, as reported by Cunnane (*24*), it may be that the dependence on endogenous energy sources, e.g., triglycerides, strongly occurs in acute zinc deficiency and severe restricted feeding. Concerning cholesterol and phospholipid distribution, the raised cholesterol-to-phospholipid ratio in the liver of the zinc-deficient animals is in agreement with other reports (*23,25*), suggesting that this ratio may lead to a change in the membrane composition. However, in our study, this modification is not related to a decreased phospholipid level, as described by these reports. On the contrary, we note a total elevated phospholipid level, as already observed by Lefevre (*26*), in the tissues of ZD rats. Interactions between zinc and phospholipids involve the effect of zinc status on phospholipid synthesis (*27*) and enhanced phospholipid levels in tissues. An increase in phosphatidyl choline, rich in linoleic acid, might contribute to the higher levels of this acid detected in liver phospholipids of ZD rats (*28,30*). It has been reported (*23,29*) that one possible mechanism by which zinc depletion leads to a raised phosphatidyl choline level is through a direct effect of zinc on the synthesis of phosphatidylcholine by the choline phosphotransferase pathway in liver.

In our study, zinc deficiency does not alter cholesterol concentration of HDL and LDL in ZD rats (compared with AL-fed controls), but important changes appear in comparison with FD animals. In some reports, it has been shown that zinc deficiency in rats is associated with low HDL cholesterol and that HDL cholesterol and zinc levels in serum were closely related (*31,32*). Others have been unable to find a significant correlation between zinc and total cholesterol in plasma (*8,9*). Conversely, increased zinc intake is generally associated with increased cholesterol in plasma HDL (*6,7*); these findings have recently been challenged by Lefevre et al. (*26*), who reported that zinc depletion increases cholesterol content of HDL. These conflicting results illustrate the difficult problem in evaluating a possible proatherogenic role of zinc deficiency.

The influence of the reduction in food intake must equally be viewed as important in cholesterol distribution. Schneeman et al. (*33*) observe similarities in the overall pattern of lipoprotein composition in the ZD and restricted-intake groups, and suggest that reduction in food intake may account for the differences in lipoprotein composition that were observed in zinc deficiency. Our study shows no such similarities in pattern of lipoprotein composition. In ZD rats, cholesterol LDL, as well as cholesterol HDL, is increased and the cholesterol HDL/cholesterol LDL ratio declines from 17.8 in PF rats to 10.3 in ZD rats, suggesting an enhanced atherogenic risk. Hence, it seems that, in spite of the hypocholesterolemic effect from the severe caloric restriction that is secondary to the loss of appetite in our experimental procedure, zinc deficiency *per se* is associated with an increased cholesterol content of lipoproteins. As a result of these two antagonist effects, the cholesterol distribution in ZD rats is not significantly modified in comparison to their AL-fed controls.

Another potential atherogenic effect of zinc deficiency is the lipidoperoxidation. In the second part of our work, we have studied the lipoprotein fraction peroxidation in ZD rats. The lipidoperoxidation related to zinc status must be viewed as important concerning the atherogenic effect of zinc deficiency. Peroxidation is known to result in cellular damage as well as a change in lipoprotein pathway (*34*). In our study, we established a plasmatic increase of MDA in ZD rats. However, there is no difference in MDA production by the lipoprotein fractions compared to their controls, in spite of a slightly increased LDL peroxidation in these animals. In vitro, the exposure of lipoprotein to an oxidative stress leads to a peroxidation monitored by a doubling of MDA production in ZD rats. This observation suggests that LDL lipoprotein fractions of ZD rats are more susceptible to induced oxidative damage. Modified LDL are no longer recognized by the B/E receptors, but instead bind to macrophages, scavanger receptors leading to an accumulated esterified cholesterol, and develop into foam cells (*35,36*). The enhanced production of MDA may be attributable either to a depleted antioxydant system of the lipoprotein, such as vitamin E, beta carotene, or zinc, or to an increase in polyunsaturated fatty acids.

Finally, we have to take into account that our work reports modification of lipid fractions, without studying any possible changes in apoproteins. Further studies will be necessary to clarify the effect of zinc deficiency, and we can only record the lipoprotein fragility in zinc deficiency as an aggravating factor of their peroxidation in atherogenic situations, for example, when these lipoproteins react with endothelial cells generating free radicals. The rat is not a good model of atherosclerosis; however, our results show a dyslipoproteinemia and an increased LDL peroxidation, suggesting an atherogenic risk. Evidence from human studies on the relation between zinc status and atherogenesis is no more conclusive than that from animal studies, but it is interesting to note in pathology the higher incidence of atherosclerosis as chronic renal failures, diabetes, and liver cirrhosis, which are also associated with a zinc deficiency.

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