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Effect of a Zinc-Deficient Diet on Lipid Peroxidation in Liver and Tumor Subcellular Membranes (42083)

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Abstract. The effect of a zinc-deficient diet on lipid peroxidation in liver and tumor mitochondrial and microsomal membrane preparations from BALB/c mice was investigated. Mitochondrial and microsomal membranes from both tissues displayed increased rates of *in vitro* peroxidation, both enzymatic and nonenzymatic. Measurement of *in vivo* peroxidation, using diene conjugation as an index of measurement revealed slight increases in tissues from zinc-deficient animals that were not statistically significant. Serum lipoperoxides analyzed from all three groups revealed no significant differences. The results point to an alteration in the peroxidation potential of mitochondrial and microsomal membranes due to zinc deficiency which may be related to an alteration in fatty acid composition.

Zinc deficiency has been shown to alter several parameters of lipid metabolism. Incorporation of [^{14}C]acetate into mono- and triglycerides as well as phospholipids increased in animals on a zinc-deficient diet (1). In addition, changes in the metabolism of polyunsaturated fatty acids have been observed. A zinc-deficient diet has been shown to decrease the levels of 22:5 fatty acids in testes microsomes (2); to increase the Δ^9-desaturation activity and to decrease the Δ^5-desaturase activity. Huang *et al.* (3) have suggested that several of the manifestations of zinc deficiency are attributable in part to a reduced conversion of 18:2w6 to 18:3w6, resulting in an accumulation of 18:2w6 in the tissues. Work from O'Dell's laboratory has shown that chicks fed a diet adequate in polyunsaturated fatty acids but low in zinc had a higher concentration of arachidonic acid in foot skin compared with that of controls (4).

Zinc deficiency has also been shown to affect lipid peroxidation. Sullivan *et al.* (5) have demonstrated increased rates of lipid peroxidation *in vitro* and *in vivo* in livers from rats fed a zinc-deficient diet. This finding was of interest for several reasons. First, we have demonstrated in our laboratory that the growth of the plasmacytoma TEPC-138 in BALB/c mice is inhibited by a zinc-deficient diet (6). Second, reports by Bartoli and Galeotti (7) have indicated that mitochondria and microsomes isolated from Morris hepatoma and Ehrlich ascites tumors exhibited lower rates of lipid peroxidation *in vitro* than control liver preparations and that the peroxidative capacity appeared to be inversely related to the growth rate of the tumor. Finally, observations in our laboratory that a zinc-deficient diet caused a decrease in state three respiration in tumor mitochondria as well as an alteration in cytochrome levels (8), coupled with the possible role of zinc in membrane stabilization (9), led us to investigate the capacity of liver and tumor mitochondrial and microsomal membrane preparations from BALB/c mice to undergo lipid peroxidation and the effect of a zinc-deficient diet on this process.

Materials and Methods. *Mice.* Female BALB/c mice (15–18 g) were obtained from Dominion Labs., McLean, Virginia.

Dietary treatment. Three groups of mice were used in this study. One group was fed a synthetic zinc-deficient diet (ZD) (TEKLAD, Madison, Wis.) containing 0.5 μg zinc/g. They were given glass distilled water from bottles fitted with polyethylene caps and stainless-steel sipper tubes. *Ad lib* (AL) animals were given a synthetic control diet (TEKLAD) in which zinc carbonate was added to give 50 μg zinc/g and tap water *ad libitum.* Pair fed (PF) animals were given the synthetic control diet in an amount equal to that of a corresponding zinc-deficient animal and tap water *ad libitum.* The composition

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of the zinc-deficient and control diets has been reported previously (10). Animals in all three groups were weighed weekly.

Experimental design. In the first experiment, animals were maintained on the appropriate diet for 3 weeks. They were then sacrificed by cervical dislocation, livers excised, and subcellular fractions prepared. In the second study, mice were maintained on the experimental diets for 3 weeks. They were then injected with 0.25 ml of a TEPC-183 cell suspension (0.5×10^7 cells/ml) subcutaneously. When the tumors reached approximately 1 cm in size the mice were sacrificed, tumors excised, and membrane fractions prepared.

Tumor transfer. TEPC-183, an IgM (k) secreting plasmacytoma, was obtained from Dr. Frances Havas, Temple University School of Medicine. It was maintained by subcutaneous transfer into BALB/c mice fed normal chow, (contains 50 ppm zinc, Ralston Purina, St. Louis, Mo.).

Biochemical analyses. Subcellular fractions from livers and tumors were prepared according to the procedure of Koch (11). The purity of each fraction was assessed by appropriate enzyme markers. The analysis of lipid peroxidation *in vitro* was determined by the measurement of malonaldehyde production as described by Tappel and Zalkin (12). Lipid peroxidation *in vivo* was determined by measuring conjugated dienes in $CHCl_3$ extracted membrane fractions according to the procedure of Recknagel (13). The results of diene conjugation were expressed as absorbance readings at 233 nm and adjusted to uniform base (per mg phospholipid). Phospholipid determination was conducted by measuring phosphorus levels in the extracts utilizing the procedure of Bartlett (14). Serum lipoperoxide levels were determined according to the method of Satoh (15). Zinc determinations were conducted on serum samples diluted 1/4 in 0.1 *N* HCl (Baker Analyzed) using a Varian AA-5 atomic absorption spectrophotometer with an air acetylene flame. Protein concentrations were determined according to Lowry *et al.* (16), using bovine serum albumin as a standard. Results are expressed as arithmetic means ± standard deviations. Mean values were compared by a Student's *t* test.

Results. The zinc-deficient diet markedly reduced serum zinc levels (ZD, 41.7 ± 5.1 μg/dl; PF, 123 ± 30.2 μg/dl; AL, 94.4 ± 1.3 μg/dl). Also, the mean liver to body weight ratios in the zinc-deficient group were decreased when compared to either pair fed or *ad lib* controls (data not shown).

The generation of malonaldehyde by enzymatic or nonenzymatic reactions using the thiobarbituric acid (TBA) method has been used as a measure of lipid peroxidation potential *in vitro* (17, 18). The effect of a zinc-deficient diet on this process is shown in Table I. Most evident is the five- to sixfold increase in NADPH enzymatic peroxidation in mitochondrial and microsomal liver preparations from animals on a zinc-deficient diet. Nonenzymatic peroxidation was increased as well, approximately two- to threefold. It must be pointed out that such results were not observed when livers from animals on test for 1 and 2 weeks, respectively, were utilized (data not shown).

In view of the fact that a zinc-deficient diet can inhibit the growth of plasmacytoma TEPC-183 (6), we decided to determine if the effect observed in liver subcellular fractions would also occur in tumor preparations. The results of such a study are shown in Table II. Since no differences were observed between AL and PF groups in the first study only PF and ZD groups were used. Although

TABLE I. EFFECT OF A ZINC-DEFICIENT DIET ON *IN VITRO* LIPID PEROXIDATION IN LIVER MITOCHONDRIAL AND MICROSOMAL PREPARATIONS[a]

Group	Membrane fraction	nmole malonaldehyde/mg protein: Enzymatic (Fe + ADP + NADPH)	nmole malonaldehyde/mg protein: Nonenzymatic (Fe + Ascorbate + ADP)
ZD (5)	Microsomes	14.92 ± 0.59	1.11 ± 0.15
PF (5)	Microsomes	2.92 ± 0.22[b]	0.41 ± 0.02[b]
AL (5)	Microsomes	3.42 ± 0.56b[b]	0.37 ± 0.04[b]
ZD (5)	Mitochondria	18.00 ± 0.25	1.09 ± 0.09
PF (5)	Mitochondria	3.06 ± 0.71[b]	0.64 ± 0.02[b]
AL (5)	Mitochondria	2.95 ± 0.23[b]	0.63 ± 0.06[b]

[a] Values represent the means ± standard deviations for animals after 3 weeks; number in parentheses represents number of animals.

[b] $P < 0.001$ compared with zinc-deficient group.

the rates of *in vitro* peroxidation were somewhat lower than that observed in liver, it was found that mitochondrial and microsomal membrane fractions from zinc-deficient animals displayed an increase in the rate of lipid peroxidation compared to PF controls. Enzymatic peroxidation rates from both mitochondria and microsomes were increased approximately tenfold, while the nonenzymatic rates were increased threefold in microsomes and tenfold in mitochondria.

Because dramatic differences in the rate of *in vitro* peroxidation were observed in liver and tumor preparations from zinc-deficient animals, it was of interest to determine the effect on *in vivo* peroxidation. As in the previous experiment only PF animals were compared to ZD mice. The extent of *in vivo* peroxidation was measured by determining the degree of diene conjugation. The results in Table III indicate that although microsomal values obtained from zinc-deficient animals were slightly elevated, there were no significant differences.

The high rates of *in vitro* peroxidation observed in zinc-deficient animals also led us to examine the levels of serum lipoperoxides. Serum samples were obtained from mice after 3 weeks on test and tested for malonaldehyde formation using the TBA procedure. As can be observed, no significant differences were apparent among the groups (Table IV).

Discussion. The results of this study point to an alteration in the rate of *in vitro* lipid peroxidation in livers and tumors from animals on a zinc-deficient diet, using the thiobarbituric acid method as indices of measurement. Although the specificity of the method has drawn criticism (19), the production of malonaldehyde is proportional to the content of polyunsaturated fatty acids (20) and other thiobarbituric acid reactive materials is minor compared with malonaldehyde. Liver and tumor mitochondrial and microsomal membrane preparations from zinc-deficient animals demonstrated increased peroxidative capacity compared to either pair fed or *ad lib* controls. The NADPH enzymatic lipid peroxidation was increased six- to tenfold, while the ascorbate dependent nonenzymatic lipid peroxidation was increased approximately threefold. The data presented here agrees in part with the work of Sullivan *et al.* (5), who found an enhanced rate of lipid peroxidation, both *in vitro* and *in vivo* in zinc-deficient rats, both in the presence and absence of ethanol. Our studies, while observing a slight increase in *in vivo* microsomal peroxidation, found that the difference was not statistically significant.

The observation that lipid peroxidation can cause cell injury has been advanced as the cause of hepatotoxicity of a variety of agents, such as ethanol (17) and carbon tetrachloride (13). Lipoperoxides are toxic to animal tissue and hepatic microsomal lipid peroxidation *in vivo* has been proposed as a mechanism responsible for the development of a fatty liver that can progress to cell necrosis (17, 21). In view of the putative role of zinc in membrane stabilization (9), our results, which point to an alteration in the peroxidative capacity of subcellular mem-

TABLE II. *In Vitro* Lipid Peroxidation in TEPC-183 Plasmacytoma Cells from Animals on a Zinc-Deficient Diet[a]

Group	Fraction	nmole malonaldehyde/mg protein	
		Enzymatic	Nonenzymatic
ZD (5)	Microsomes	7.50 ± 2.12	0.93 ± 0.09
PF (5)	Microsomes	0.62 ± 0.21[b]	0.37 ± 0.21[b]
ZD (5)	Mitochondria	11.62 ± 4.55	5.18 ± 0.35
PF (5)	Mitochondria	1.75 ± 0.21[b]	0.55 ± 0.07[b]

[a] Values are expressed as means ± standard deviation; number in parentheses represents number of animals per group.

[b] $P < 0.001$ compared with zinc-deficient group.

TABLE III. Effect of a Zinc-Deficient Diet on Diene Conjugation in Mouse Liver Subcellular Membranes[a]

Group	Fraction	Diene conjugation[b]
ZD (3)	Microsomes	1.43 ± 0.32
PF (3)	Microsomes	1.33 ± 0.23
ZD (3)	Mitochondria	1.68 ± 0.15
PF (3)	Mitochondria	1.84 ± 0.21

[a] Values represent means ± standard deviations for animals after 4 weeks on diet; number in parentheses represents number of animals per group.

[b] Expressed as absorbance readings at 233 nm and adjusted to uniform base (mg phospholipid).

TABLE IV. EFFECT OF A ZINC-DEFICIENT DIET ON SERUM LIPOPEROXIDE LEVELS IN ANIMALS WITH AND WITHOUT TUMOR[a]

Group	Serum lipoperoxide (nmole malonaldehyde/ml)
ZD (3)	3.89 ± 0.95
PF (3)	3.65 ± 0.96
ZD with tumor (3)	3.87 ± 1.30
PF with tumor (3)	3.34 ± 1.59

[a] Values are expressed as means ± standard deviation; numbers in parentheses represent number of animals per group.

branes, contribute to this hypothesis and suggest an important role of zinc in the process.

With regard to tumor growth, studies have shown that the peroxidative capacity of Morris hepatomas is inversely related to the growth rate of the tumors (7), with the fast growing hepatomas exhibiting lower rates of peroxidation than slower growing hepatomas. The investigators felt that their studies supported the view that cell proliferation in tumors is correlated with the peroxides generated during turnover of membrane lipids. Our findings that a zinc-deficient diet inhibits the growth rate of TEPC-183 (6) as well as the fact that the rate of *in vitro* peroxidation is increased in tumor membrane fractions from zinc-deficient animals also suggests a relationship between lipid peroxidation, tumor growth, and membranes.

An interesting observation in our studies is that although there was a three- to tenfold increase in the rate of *in vitro* peroxidation, no such effect was observed in *in vivo* peroxidation or in the level of serum lipoperoxides. It seems that the increase in peroxidation potential, as measured by the generation of TBA reactive material, is not reflective of an increased rate of peroxidation *in vivo,* as measured by diene conjugation. The reason for this large increase in *in vitro* peroxidation could not be ascertained by this study. It has been reported that zinc deficiency alters the metabolism of polyunsaturated fatty acids (3, 4). In addition, Reitz *et al.* (22) have shown that tumor microsomal membranes isolated from fast growing Morris hepatoma 7777 contain about 50% less phospholipid than controls. Also, microsomes and mitochondria from this tumor have an increase in monoenoic and a decrease in polyenoic fatty acids. Feo *et al.* (23) have reported that Morris hepatomas have a different cholesterol/phospholipid ratio compared with that of controls. It is possible that a zinc-deficient diet may alter phospholipid levels, cholesterol levels, or the distribution of phospholipid subclasses as well as fatty acids, which may contribute to the results observed in these experiments. Present work in our laboratory is aimed at addressing these points.

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