Oxidant Defense Systems in Testes from Zinc-Deficient Rats (44040)

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Abstract. **Previous studies have demonstrated that zinc deficiency can be associated with high rates of oxidative damage to testes lipids, proteins, and DNA in male rats. In the present work, different aspects of the oxidant defense system (enzymes and lipid-soluble antioxidant substances) were characterized in the testes of control and zinc-deficient rats. Seventeen-day-old males were given free access to either a control (25 μg Zn/g) or a zinc-deficient (0.5 μg Zn/g) diet, or the 25 μg Zn/g diet at a level of food intake similar to that of zinc-deficient rats. Animals were sacrificed 14 days after the initiation of the diet. The activities of copper-zinc superoxide dismutase (CuZn SOD) and glutathione reductase (GRed) were significantly higher (34% and 23%, respectively) in testes from the zinc-deficient animals than in those of the *ad libitum* controls. In testes, the activities of manganese superoxide dismutase (Mn SOD) and glutathione peroxidase (GPx), and the concentration of α-tocopherol and ubiquinol-9 and -10 were similar among the groups. However, the ratio of reduced/total concentration of both ubiquinols was higher in the zinc-deficient and restrict-fed animals than in the *ad libitum* controls. Testes homogenates from the zinc-deficient rats showed a low susceptibility to Fe (II)-induced oxidation, which could be explained in part by a lower peroxidation index, mainly due to the decreased testicular content of the fatty acid 20:4 observed in these animals. In summary, both undernutrition and zinc deficiency can cause an oxidative stress situation in testes, for which cells tend to compensate by increasing select components of the oxidant defense system.**

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It is well established that normal testicular maturation and function is dependent on an adequate supply of dietary zinc. In humans, one sign of developmental zinc deficiency can be hypogonadal dwarfism (1), and similar syndromes can be demonstrated in experimental animals fed low-zinc diets (1, 2). While the sensitivity of the testes to zinc deficiency has been long recognized, the mechanism underlying the effect of zinc deficiency on testicular function/maturation are poorly understood. It has been proposed that zinc deficiency impairs the maturation of spermatid cells, given that there is a low activity and concentration of angiotensin-converting enzyme in testes from zinc-deficient rats (3). Zinc deficiency–associated hypogonadism could be also due to a decreased production of testosterone (4). Low levels of serum testosterone were found in young men subjected to a short-term dietary zinc deficiency (5).

Recently, we demonstrated that zinc deficiency imposed during early postnatal development can result in high rates of oxidative damage to testes lipids, proteins, and DNA in male rats (2). Results obtained in our previous work suggested that this high level of oxidative damage in rat testes (2) could be attributed in part to low tissue concentrations of zinc, as this metal has been proposed to be part of the oxidant defense system, and in part to an increase in tissue iron con-

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centration which occurred secondary to the zinc deficiency.

Oxidative damage to cell components depends on the rate of production of reactive oxygen species (ROS), and the metabolism of these species through the oxidant defense system. Defenses against ROS are multifaceted and include the enzymes manganese (Mn) and copper-zinc (CuZn) superoxide dismutases (SOD), glutathione reductase (GRed), glutathione peroxidase (GPx) and catalase; low molecular weight compounds such as ascorbic acid, tocopherols, carotenes, glutathione, uric acid, ubiquinols, etc.; and metal binding proteins such as transferrin, lactoferrin, albumin and metallothionein (6).

In the present study, the activities of several enzymes participating in the metabolism of ROS were determined to further define the level of oxidative stress induced in testes by zinc deficiency and/or undernutrition. The concentration of lipid-soluble antioxidant substances (α-tocopherol and ubiquinols), as well as the relative concentrations of specific fatty acids (substrates of lipid peroxidation), was determined in testes from control, zinc-deficient, and diet-restricted male rats. Possible mechanisms underlying the high level of oxidative damage observed in rats that are either food-restricted or fed low-zinc diets are discussed.

Materials and Methods

Animals and Animal Care. Seventeen-day-old male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 28–32 g were individually housed in suspended stainless steel cages in a room with controlled temperature (22°–23°C) and photoperiod (12 hr/day). Animals were given free access to either a control (25 μg Zn/g) or a zinc-deficient (0.5 μg Zn/g) diet (7); or restricted access to the 25 μg Zn/g diet (restrict-fed) at a level of intake similar to that of rats fed the 0.5 μg Zn/g diet.

Assurance of Compliance with Animal Codes. All animal care procedures met the *Guide to the Use and Care of Laboratory Animals,* U.S. Public Health Service, 1985 (8), and were administered under the auspices of the Animal Resource Services of the University of California, Davis, which is accredited by the American Association for the Accreditation of Laboratory Animal Care. Experimental protocols were approved prior to implementation by the University of California, Davis, Animal Use and Care Administrative Advisory Committee and were administered through the Office of the Campus Veterinarian.

Tissue Sampling. Two weeks following the initiation of the dietary regime (at 32 days of age), animals were food deprived for 6 hr and sacrificed by overexposure to CO_2. Blood was collected by cardiac puncture into heparinized syringes (Sarstead, Princeton, NJ), centrifuged at 1700*g* for 15 min, and the plasma removed and stored at −20°C until analyzed. The testes were quickly excised, weighed, and placed in ice-cold saline. Testes were decapsulated and homogenized in 10 volumes of 50 m*M* HEPES/125 m*M* KCl (pH 7.4). 2-Thiobarbituric acid reactive substances (TBARS) in total homogenates were measured immediately, and total homogenate aliquots were stored at −80°C for further determinations of enzyme activities and antioxidant, fatty acid, and trace metal concentrations.

TBARS Determination. Testes homogenates (10 mg wet tissue) were incubated in 50 m*M* HEPES/125 m*M* KCl (pH 7.4) in a 0.5-ml reaction volume. TBARS were measured before incubation without additions or after 60 min of incubation at 37°C in the presence of 50 μ*M* $FeSO_4$. The incubation was terminated by the addition of 0.1 ml of 4% (w/v) butylated hydroxytoluene in ethanol, and lipid peroxidation products were evaluated as TBARS using the fluorometric method of Fraga *et al.* (9). TBARS values are expressed as malondialdehyde equivalents.

To test the capacity of the tissue to autoxidize, testes homogenates from six animals per dietary group were incubated at 37°C for 60 min with continuous shaking, and TBARS were determined at the end of the incubation. The autoxidation of testes homogenates was very low and no significant differences were found among the groups.

Antioxidant Determinations. Testes homogenates (20 mg) were added with 500 μl of methanol and were extracted with 4 ml of *n*-hexane. The tubes were vortexed for 1 min and then centrifuged for 5 min at 1000*g*. Three milliliters of the upper layer were evaporated under nitrogen, resuspended in 400 μl of methanol:ethanol (1:1, v/v) and filtered through a 0.22/μm-pore membrane. To determine the total concentration of ubiquinols, the extracts (250 μl) were reduced by addition of 0.5 ml of distilled water, 1 ml of methanol, and approximately 10 mg of $NaBH_4$. After vortexing, samples were incubated for 30 min at room temperature in the dark and then extracted with 4 ml of *n*-hexane following the steps described above. Ubiquinols and α-tocopherol concentrations were determined in the methanol:ethanol extracts by HPLC on a 8-C reversed-phase column with an in-line BAS LC4C electrochemical amperometric detector with a glassy-carbon working electrode at an applied oxidation potential of 0.6 V and ultraviolet detection (275/290 nm) (10). The antioxidant concentrations were quantitated using commercial standards. Values are expressed as nanomoles per gram wet tissue.

Determination of Enzyme Activities. For the determination of Mn SOD, CuZn SOD, GRed, and GPx

activities, testes homogenates were sonicated 5 sec on ice and centrifuged at 10,000*g* for 30 min at 4°C, and the assays were conducted on the supernate. The activities of Mn SOD and CuZn SOD were determined by the method of Marklund and Marklund (11). Extracellular SOD was measured as described by Olin *et al.* (12). One unit of SOD activity is defined as the amount of sample needed to obtain 50% inhibition of pyrogallol oxidation.

The activity of GPx was determined by the method of Lawrence and Burk (13). The activity of GRed was measured as described by Rogers and Augusteyn (14). One unit of GPx and GRed activity is defined as 1 nmol of NADPH oxidized/min/ml. Data are expressed as milliunits per milligram protein (units × 1000/mg protein). Protein concentrations were determined according to Bradford (15) using bovine serum albumin as the standard.

Fatty Acids Analysis. Phospholipids were extracted from testes homogenates according to Folch *et al.* (16). The washed lower phase was evaporated under nitrogen. For the esterification, 1 ml of methanol:SO_4H_2 (100:2, v/v) was added and samples were incubated at 60°C for 2 hr under nitrogen. The fatty acid methyl esters were extracted with 3 ml of chloroform and 1 ml of distilled H_2O. The lower phase was washed twice with 1 ml of H_2O, evaporated under nitrogen and then dissolved in *n*-hexane. The fatty acid methyl esters were separated by gas-liquid chromatography (model GC-8A, Shimadzu, Kyoto, Japan) on a DB-23 column (J & W Scientific, Folsom, CA) with temperature programming at 5°C/min between 140°C and 220°C. Fatty acids were identified by retention time and co-chromatography with commercial standards. The peroxidation index (PI) was calculated as PI = (monoenoic % × 0.025) + (dienoic % × 1) + (trienoic % × 2) + (tetraenoic % × 4) + (pentaenoic % × 6) + (hexaenoic % × 8) (17).

Mineral Analysis. Plasmas were wet-ashed with 16 *N* nitric acid (Baker's Instra-analyzed; J. T. Baker Co. Philipsburg, NJ), evaporated and diluted with 0.1 *N* nitric acid (Baker's Instra-analyzed) as described by Clegg *et al.* (18). Concentrations of copper and zinc were determined in the samples by flame atomic absorption spectrophotometry (model 551; Thermo Jarrel Ash, Wilmington, MA). Certified reference solutions (1000 μg metal/ml; Fisher Scientific, Santa Clara, CA) were used to generate standard curves for each element. A sample of National Bureau of Standards bovine liver (SRM 1577; U.S. Department of Commerce, National Bureau of Standards, Washington, DC) was included with the samples to ensure accuracy and reproducibility.

Statistics. Data were analyzed using one-way analysis of variance (ANOVA). Fisher least significance difference test was used to examine differences between group means. A P value ≤ 0.05 was considered statistically significant. Data are shown as mean ± SEM.

Results

Animal Outcome. After 14 days on the diets, body weights of the zinc-deficient and restrict-fed rats (42 ± 2 and 48 ± 3 g, respectively) were significantly lower than those of the control animals (99 ± 3 g). Testes weights were also significantly lower in the low-zinc and restrict-fed animals (0.41 ± 0.04 and 0.48 ± 0.03 g, respectively) than in controls (0.90 ± 0.04 g). The ratio testes to body weight was similar among the three groups.

Peroxidazibility of Testes Homogenates. Consistent with our previous findings (2) the basal level of TBARS was significantly higher in testes homogenates from the low-zinc animals compared with the *ad libitum* fed controls (Table I). The peroxidazibility of the tissue was assessed measuring TBARS production after incubating the homogenates in the presence of 50 μ*M* Fe^{2+} for 60 min at 37°C. The stimulation of TBARS production in the presence of Fe^{2+} was 45 ± 3–, 23 ± 2–, and 13 ± 2–fold above basal levels for the control, restrict-fed, and low-zinc group, respectively. Presumably, the lower peroxidazibility observed in testes homogenates from the zinc-deficient and restrict-fed animals was due to a decreased content of

Table I. TBARS Production and Peroxidation Index in Testes from Rats Fed Diets Containing 25 or 0.5 μg Zn/g Diet for 14 Days

Parameter	Dietary group		
	Control	Restrict-fed	Zn-deficient
Preincubation TBARS (nmol/g wet tissue)	26 ± 1[a]	38 ± 4[b]	36 ± 3[b]
Postincubation TBARS (nmol/g wet tissue)	1178 ± 57[a]	865 ± 67[b]	489 ± 76[c]
PI	96 ± 3[a]	92 ± 2[a]	80 ± 2[b]
TBARS/PI	0.27 ± 0.02[a]	0.39 ± 0.04[a,b]	0.48 ± 0.05[b]

Note. TBARS were measured in testes homogenates either before or after incubation for 60 min at 37°C in the presence of 50 μ*M* Fe^{2+}. Data are shown as mean ± SEM and are the average of six animals per group. PI was calculated as described in text. Data in the same row having different superscripts are significantly different ($P < 0.01$) (one-way ANOVA test).

Table II. Fatty Acid Relative Content in Testes from Rats Fed Diets Containing 25 or 0.5 μg Zn/g Diet for 14 Days

Fatty acid	Dietary group		
	Control	Restrict-fed	Zn-deficient
16:0	38.1 ± 0.7	39.8 ± 1.1	39.1 ± 1.1
18:0	12.4 ± 0.7	11.2 ± 0.5	12.0 ± 0.7
18:1 ω9	16.6 ± 1.0[a]	20.2 ± 0.7[b]	21.8 ± 0.4[b]
18:2 ω6	5.0 ± 0.8	3.9 ± 0.2	3.7 ± 0.3
20:1 ω9	3.30 ± 0.05[a]	2.12 ± 0.20[b]	2.25 ± 0.34[b]
20:4 ω6	13.8 ± 0.4[a]	13.0 ± 0.2[a]	10.5 ± 0.4[b]
22:0	1.5 ± 0.2	1.1 ± 0.2	1.2 ± 0.2
22:4 ω6	8.9 ± 0.4	9.1 ± 0.3	8.4 ± 0.3
22:6 ω3	0.58 ± 0.04	0.70 ± 0.03	0.75 ± 0.08

Note. Data are shown as mean ± SEM and are the average of six animals per group. Data in the same row having different superscripts are significantly different ($P < 0.01$) (one-way ANOVA test).

Table III. Mn, CuZn Superoxide Dismutases, Glutathione Reductase, and Glutathione Peroxidase Activities in Testes from Rats Fed Diets Containing 25 or 0.5 μg Zn/g Diet for 14 Days

Enzyme	Dietary group		
	Control	Restrict-fed	Zn-deficient
CuZn SOD (U/mg protein)	4.1 ± 0.2[a]	4.9 ± 0.2[b]	5.5 ± 0.2[c]
Mn SOD (U/mg protein)	1.58 ± 0.05	1.66 ± 0.08	1.71 ± 0.04
GPx (mU/mg protein)	13.6 ± 0.4	12.0 ± 0.6	13.3 ± 0.5
GRed (mU/mg protein)	9.1 ± 0.3[a]	9.6 ± 0.2[a]	11.2 ± 0.4[b]

Note. Data are shown as mean ± SEM and are the average of five, four, and seven animals for the control, restrict-fed, and zinc-deficient groups, respectively. Data in the same row having different superscripts are significantly different ($P < 0.05$) (one-way ANOVA test).

unsaturated fatty acids (substrates of lipid peroxidation) and/or to increased levels of select components of the oxidant defense system.

The concentration of total phospholipids was similar among the three groups (6.9 ± 0.7, 6.4 ± 0.2, and 6.8 ± 0.6 mg phospholipid/g wet tissue for the control, restrict-fed, and low-zinc groups, respectively). Table II shows the fatty acid composition of testes total phospholipids from the control, restrict-fed, and low-zinc animals. The relative proportion of 14:0, 16:0, 18:2, 22:0, 22:4, and 22:6 fatty acids was similar among the groups. In contrast, the percentage of 18:1 in the testes from the restrict-fed and low zinc animals was significantly higher (20% and 22%, respectively), while the percentage of 20:1 was significantly lower (36% and 32%, respectively) than control values. The zinc-deficient animals had a 24% lower percentage of the highly peroxidizable fatty acid 20:4 compared with the two zinc-sufficient groups. As a consequence of the above, the PI was significantly lower in testes phospholipids from the zinc-deficient animals than in testes from the control and restrict-fed groups (Table I). When the basal levels of testes TBARS were expressed as a function of the PI (TBARS/PI), the ratio was significantly higher in the low-zinc animals (78%) than in controls (Table I). Fe^{2+}-induced stimulation of TBARS production, including data from the three dietary groups, was positively correlated ($P < 0.001$; $r^2 = 0.53$) with the PI. Similarly the percentage of 20:4 was positively correlated with the stimulation ($P < 0.0001$; $r^2 = 0.63$). A weak positive correlation was found between the stimulation and the percentage of 20:1 ($P < 0.02$; $r^2 = 0.28$); significant correlations were not observed between the stimulation and the percentage of either 22:4 or 22:6.

Oxidant Defense System. Table III show the activities of testes Mn SOD, CuZn SOD, GRed, and GPx. The activity of testes CuZn SOD was 20% and 34% higher in the restrict-fed and zinc-deficient animals, respectively, than in controls. Testes GRed activity was 23% higher in the zinc-deficient animals than in controls. Values for testes Mn SOD and GPx activities were similar for the three dietary groups.

The activity of plasma extracellular SOD and the plasma concentrations of copper and zinc are shown in Table IV. Plasma zinc concentration was markedly reduced (80%) in the zinc-deficient animals compared with both control groups. Plasma copper concentrations were similar for the three groups. The activity of plasma extracellular SOD was 15% and 28% lower in the restrict-fed and zinc-deficient animals, respectively, than in controls. The activity of plasma extracellular SOD was correlated with plasma zinc concentrations across all the groups. Significantly, a strong correlation ($r^2 = 0.6$; $P < 0.01$) between plasma extracellular SOD activity and plasma zinc concentration

Table IV. Zinc and Copper Concentrations and CuZn Superoxide Dismutase Activity in Plasma from Rats Fed Diets Containing 25 or 0.5 μg Zn/g Diet for 14 Days

Parameter	Dietary group		
	Control	Restrict-fed	Zn-deficient
Cu (μ*M*)	11.4 ± 0.9	12.3 ± 0.6	12.2 ± 0.3
Zn (μ*M*)	20.4 ± 1.2[a]	22.7 ± 0.5[a]	3.5 ± 0.3[b]
CuZn SOD (U/ml)	81 ± 3[a]	69 ± 3[b]	58 ± 4[c]

Note. Data are shown as mean ± SEM and are the average of six, six, and four animals for the control, restrict-fed and zinc-deficient groups respectively. Data in the same row having different superscripts are significantly different ($P < 0.05$) (one-way ANOVA test).

was found even when the zinc deficient group was analyzed by itself.

Table V shows the concentrations of lipid soluble antioxidants. The concentration of testes α-tocopherol and total ubiquinol-9 and ubiquinol-10 was similar for the three groups. Significantly higher levels of reduced ubiquinol-9 were found in testes from the zinc-deficient and restrict-fed animals than in controls. The ratio of reduced to total ubiquinol-9 was 32% and 84% higher for the zinc-deficient and restrict-fed animals, respectively, than in controls. Similar results were observed for ubiquinol-10 (38% and 79% higher in the zinc-deficient and restrict-fed animals, respectively).

Discussion

Zinc is involved in the protection of cells against ROS-mediated damage at multiple levels (19). First, zinc can induce metallothionein synthesis, a protein which is rich in thiol groups that can bind metals with known pro-oxidant activity (Cu^+, Cd^{2+}, Hg^{2+}); alternatively, through its thiol groups, metallothionein can scavenge hydroxyl radicals and singlet oxygen (see Ref. 20 for a review). Second, the activities of the antioxidant enzymes, CuZn SOD and extracellular SOD, strongly depend on zinc, which acts to stabilize the tertiary structure of the enzymes. Third, zinc can compete for cellular binding sites with redox active metals, such as copper and iron; in this regard, zinc has been shown to inhibit Fe^{2+}-mediated lipid peroxidation (21).

We recently demonstrated that zinc deficiency induced in rapidly growing male rats can result in an increase in oxidative damage to testes lipids, proteins, and DNA (2). We speculated that this increase in oxidative damage was due to a low tissue zinc concentration and/or to a high tissue iron concentration in the zinc-deficient animals. In our initial paper, we also observed that, while the basal levels of testes TBARS were higher in the zinc-deficient and restrict-fed animals, the susceptibility to Fe^{2+}-mediated TBARS production was markedly lower in the zinc-deficient animals than in controls; the restrict-fed group showed an intermediate response. We suggested that this differential peroxidizability was due to different levels of oxidant defenses and/or to a differential content of unsaturated fatty acids in the testes of animals from the three dietary groups.

In the present work, we observed that the PI of testes lipids was significantly lower in the zinc-deficient group than in controls. This difference in PI was primary due to a lower content of the highly oxidizable fatty acid 20:4. Consistent with the above the Fe^{2+}-mediated stimulation of TBARS production was significantly and positively correlated with the PI and with the percentage of 20:4. When basal TBARS levels (a reflection of the *in vivo* level of lipid peroxidation) were calculated based on the PI, the results indicated that lipid peroxidation rates in the zinc-deficient animals were even higher (78%), compared with control animals, than when the values were calculated on a tissue wet basis (38%). Presumably, the reduction in the PI is occurring as a consequence of a partial block in Δ5 and Δ6 desaturase activities. That the activities of these enzymes can be inhibited by either ROS or oxidation products has been suggested by numerous investigators (22, 23). The restrict-fed group showed a lower stimulation of TBARS production in the presence of Fe^{2+} compared with controls. Since the relative content of 20:4 ω6 is similar in testes from the control and restrict-fed animals, a reduction in this fatty acid is not the only factor (although potentially important) that accounts for the low susceptibility to Fe^{2+}-supported lipid peroxidation in homogenates from the zinc-deficient rats.

The higher activities of CuZn SOD and GRed found in testes from the restrict-fed and zinc-deficient rats presumably reflects a protective response to the increased oxidative damage associated with both undernutrition and zinc deficiency (2). Consistent with this concept, increased activities of antioxidant en-

Table V. α-Tocopherol and Ubiquinols Concentrations in Testes from Rats Fed Diets Containing 25 or 0.5 μg Zn/g diet for 14 Days

Parameter	Dietary group		
	Control	Restrict-fed	Zn-deficient
α-Tocopherol (nmol/g wet tissue)	16 ± 1	15 ± 1	19 ± 2
Ubiquinol-9, reduced form (nmol/g wet tissue)	15.2 ± 0.8[a]	23.5 ± 1.1[b]	22.0 ± 2[b]
Ubiquinol-9, total (nmol/g wet tissue)	42 ± 5	34 ± 2	44 ± 4
Ubiquinol-10, reduced form (nmol/g wet tissue)	2.22 ± 0.16	2.72 ± 0.09	2.90 ± 0.35
Ubiquinol-10, total (nmol/g wet tissue)	7.0 ± 1.0	4.7 ± 0.4	6.3 ± 0.8
Ubiquinol-9, reduced/total	0.38 ± 0.05[a]	0.70 ± 0.03[b]	0.50 ± 0.03[c]
Ubiquinol-10, reduced/total	0.34 ± 0.06[a]	0.61 ± 0.06[b]	0.47 ± 0.02[c]

Note. Data are shown as mean ± SEM and are the average of six animals per group. Data in the same row having different superscripts are significantly different ($P < 0.05$) (one-way ANOVA test).

zymes have been found in other conditions associated with ROS-mediated damage. For example, in the rat exposure to a single dose of x radiation can increase the activities of SOD in several organs (24). Similarly, an increase in SOD activity is observed in young *Caenorhabditis elegans* after exposure to hyperoxia and to a redox-active quinone (25). Levels of tissue oxidant defenses have also been reported to be increased after visible light exposure (26), treatment with paraquat (27), and with inhibitors of angiotensin-converting enzyme (28).

The activity of extracellular SOD, a secretory tetrameric zinc and copper-containing glycoprotein, was significantly lower in plasma from the zinc deficient animals compared with controls. In contrast, zinc-deficient animals had a higher activity of CuZnSOD in the testes. Copper ions participate in the dismutation reaction catalyzed by extracellular SOD, while zinc acts to stabilize the enzyme. The significant lower plasma zinc concentration in the zinc-deficient animals presumably limited the amount of zinc available for the enzyme. Consistent with this, plasma zinc concentration was positively correlated with extracellular SOD in the zinc-deficient group. In contrast, the 30% lower concentration of zinc found in testes from the zinc-deficient animals did not affect the activity of testes CuZnSOD. This different effect of zinc deficiency on testes CuZnSOD activity and plasma extracellular SOD could represent a differential regulation of these two enzymes (although similar in function, the two enzymes are distinct), or, alternatively, it might simply reflect the more significant reduction which occurred in plasma zinc concentration, relative to the reduction which occurred in testes zinc concentration.

Lipid soluble antioxidants protect membranes from oxidation by inhibiting the propagation of lipid peroxidation, thus preventing the release of products of lipid degradation that can modify other cellular components. α-Tocopherol is the main antioxidant in hydrophobic environments. Ubiquinols, besides their participation in mitochondrial electron transport, have been proposed to play an important role as antioxidants in lipid domains (29).

In the present investigation, α-tocopherol and total ubiquinols concentrations in testes were not affected by either undernutrition or zinc deficiency. However, the ratios of reduced to total ubiquinol-9 and -10 were markedly higher in the restrict-fed and zinc-deficient animals than in controls. The functional significance of this change in the ratio needs to be ascertained; however, it is indicating a better reducing state of the cell.

In summary, the higher activities of testes CuZn SOD and GRed, together with a higher proportion of reduced ubiquinols indicates that both, undernutrition and zinc deficiency cause an oxidative stress situation which cells tend to compensate for by increasing select components of the oxidant defense system. The lower content of the fatty acid 20:4 found in testes phospholipids from zinc-deficient animals suggests that the extent of lipid peroxidation previously reported (2) was underestimated. A downregulation of Δ5 and Δ6 desaturase activities could be one mechanism of protection against tissue oxidative damage in zinc deficiency.

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