# The Effect of Vitamin E on Lipid Peroxidation in the Copper-Deficient Rat

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*The present study was designed to determine whether the supplementation of vitamin E in the copper-deficient diet would ameliorate the severity of copper deficiency in fructose-fed rats. Lipid peroxidation was measured in the livers and hearts of rats fed a copper-deficient diet (0.6 μg Cu/g) containing 62% fructose with adequate vitamin E (0.1 g/kg diet) or supplemented with vitamin E (1.0 g/kg diet). Hepatic lipid peroxidation was significantly reduced by vitamin E supplementation compared with the unsupplemented adequate rats. In contrast, myocardial lipid peroxidation was unaffected by the level of vitamin E. Regardless of vitamin E supplementation, all copper-deficient rats exhibited severe signs of copper deficiency, and some of the vitamin E-supplemented rats died of this deficiency. These findings suggest that although vitamin E provided protection against peroxidation in the liver, it did not protect the animals against the severity of copper deficiency induced by fructose consumption.*

**Keywords:** vitamin E; copper deficiency; fructose; lipid peroxidation.

## Introduction

Copper deficiency in fructose-fed rats causes biochemical, metabolic, and physiologic abnormalities that eventually lead to their mortality.[1-5] It is well-established that dietary copper deprivation in rats results in the reduction of the activity of the copper enzyme superoxide dismutase (SOD) in several tissues.[6-10] Superoxide dismutase plays a major role in the protection of tissues against peroxidation. Its reduced activity has been associated with the enhancement of cell damage.[8-10]

Vitamin E, the most active form of which is α-tocopherol, is a fat-soluble vitamin that functions as an intramembraneous scavenger of oxygen radicals, thereby preventing the lipid peroxidation of polyunsaturated fatty acids.[11-14]

The biologic requirements for vitamin E depend on the rate of oxidation of polyunsaturated fatty acids.[15] It has been recently shown that the degree of lipid peroxidation in copper deficiency is dependent on the type of dietary carbohydrate. It is increased in the fructose-fed, copper-deficient rats compared with starch.[9] The excessive lipid peroxidation resulting from an increase in the production of oxygen radicals can result in membrane damage. The administration of supplementary vitamin E to animals has ameliorated the cytotoxic effect of free radical-producing agents, such as carbon tetrachloride,[16] Adriamycin,[17] ozone,[18] and irradiation.[19] Therefore, it is suggested that supplemental vitamin E also may be necessary in those copper-deficient rats consuming a fructose-based diet.

The present study was designed to determine whether the supplementation of vitamin E to fructose-fed, copper-deficient rats would protect against the lethal copper-fructose interaction.

## Materials and methods

Weanling male Sprague-Dawley rats weighing approximately 50 g each were randomly assigned to one of three groups that received diets differing in the amount of copper and vitamin E: group 1, fructose, copper-deficient, 0.6 μg Cu/g diet, 0.1 g vitamin E/kg diet (n =

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15) (vitamin-adequate, copper-deficient); group 2, fructose, copper-deficient, 0.6 μg Cu/g diet, 1.0 g vitamin E/kg diet (n = 15) (vitamin-supplemented, copper-deficient); and group 3, fructose, copper-adequate, 6.0 μg Cu/g diet, 0.1 g vitamin E/kg diet (n = 10) (vitamin-adequate, copper-adequate).

All diets contained 627 g/kg carbohydrate as fructose, 200 g/kg egg white, 95 g/kg corn oil, 30 g/kg nonnutritive fiber (cellulose), 35 g/kg AIN salt mix[20] prepared in our laboratory and formulated to omit cupric carbonate from the mineral mix, 2.7 g/kg choline bitartrate, and 10 g/kg vitamin mix AIN 76A[21] supplemented with 2 mg biotin/kg diet.

The copper-supplemented diets were prepared by adding copper carbonate to the copper-deficient mineral mixture to produce a final concentration of 6.0 μg Cu/g diet.

The concentrations of copper in the liver and diets were measured by atomic absorption spectrophotometry[22] following their digestion, according to the method of Hill et al.[23] The accuracy of the analyses was verified by using the National Bureau of Standards Certified Reference Materials (Bovine Liver 1577a).

All rats were housed in individual stainless steel cages and were fed their respective diets for either 3 or 7 weeks. They were allowed free access to diets and deionized water. Body weights were recorded weekly. A total of 12 copper-deficient rats (six from group 1 and six from group 2) were killed after the third week of dietary treatment. The remaining rats were killed during the seventh week of the study.

Hematocrit was determined immediately after decapitation. Plasma was obtained by centrifugation and was analyzed for ceruloplasmin activity.[24] The liver, heart, thymus, and pancreas of each rat were removed and weighed. Livers and hearts were washed in ice-cold saline and homogenized with buffer containing 174 mM KCl and 25 mM Tris-HCl (pH 7.4). The preparations of the crude mitochondrial fractions from the livers and hearts were performed at 4°C as described by Paynter.[8] Non-enzymatic lipid peroxidation was assessed by the formation of thiobarbituric acid-reactive substances (malondialdehyde) in response to a challenge *in vitro* with $Fe^{2+}$.[25,26] Malondialdehyde formation was calculated from its molar extinction coefficient of $1.56 \times 10^5$.[27]

All data were subjected to one-way analysis of variance (ANOVA) with Duncan's use for means separation.[28] A value of $P < .05$ was considered statistically significant.

## Results

The supplementation of vitamin E to copper-deficient rats for 3 weeks had no effect on body weight, tissue size, and hepatic copper concentration (*Table 1*). Although hepatic lipid peroxidation in the adequate vitamin E rats was twofold higher than the corresponding values from the supplemented vitamin E rats, these differences were not significant.

Mortality occurred in rats fed excess vitamin E at the start of the sixth week of the study. By the end of this week, two of the vitamin E-supplemented rats had died.

Body weights, tissue sizes, hematocrit, hepatic copper concentration, and lipid peroxidation are presented in *Table 2*. Weight gain and related pancreatic, thymic, and myocardial sizes were greatly affected by copper deficiency compared with copper-adequate controls. Relative liver size was not affected by either vitamin E or copper. Only copper-deficient rats were anemic. The anemia was not affected by the level of vitamin E. As expected, all copper-deficient rats had nondetectable activity of ceruloplasmin. In addition, hepatic copper concentrations were significantly reduced by dietary copper deficiency compared with the copper-adequate controls.

The supplementation of vitamin E greatly reduced hepatic lipid peroxidation compared with the adequate vitamin E rats ($P < 0.01$, different by one-way ANOVA). In contrast, the lipid peroxidation of the myocardium showed no significant differences between the three groups.

## Discussion

In agreement with past studies,[1-5] all copper-deficient rats of the present study that were fed fructose were emaciated, pale, and lost weight. The pallor was evident also throughout the internal organs. In addition, all copper-deficient rats were anemic, had atrophied pancreas and thymus, and had hypertrophied hearts

**Table 1** Effect of dietary copper deficiency on body weight, liver, pancreatic, thymic and heart sizes, and hepatic copper concentrations after 3 weeks of feeding a copper-deficient diet with adequate or supplemental Vitamin E[a]

| | Adequate Vitamin E | Supplemented Vitamin E |
|---|---|---|
| Body weight | 164 ± 4.2 | 153 ± 4.40 |
| Liver size (g/100 g body wt) | 6.60 ± 0.15 | 6.50 ± 0.13 |
| Pancreatic size (g/100 g body wt) | 0.55 ± 0.02 | 0.51 ± 0.03 |
| Thymic size (g/100 g body wt) | 0.32 ± 0.02 | 0.32 ± 0.03 |
| Heart size (g/100 g body wt) | 0.46 ± 0.01 | 0.47 ± 0.02 |
| Hepatic copper (μg/g wet wt) | 1.62 ± 0.16 | 1.70 ± 0.13 |
| Hepatic malondialdehyde (nmol/g wet wt) | 16.74 ± 4.2 | 7.07 ± 2.46 |

[a] Results are expressed as mean ± SEM values of six rats per group.

**Table 2** Body weight, liver, pancreas, thymus and heart sizes, hematocrit, hepatic copper concentration, and lipid peroxidation of liver and heart of copper-deficient and -adequate rats fed fructose for 7 weeks[a]

| | Adequate Vitamin E, copper-deficient | Supplemented Vitamin E, copper-deficient | Adequate Vitamin E, copper-adequate |
|---|---|---|---|
| Body weight (g) | 256 ± 12[b] | 225 ± 14[b] | 322 ± 5[c] |
| Relative tissue sizes (g/100 g) | | | |
| Liver | 5.4 ± 0.3 | 5.5 ± 0.2 | 5.6 ± 0.3 |
| Pancreas | 0.19 ± 0.02[b] | 0.21 ± 0.03[b] | 0.60 ± 0.04[c] |
| Thymus | 0.09 ± 0.01[b] | 0.08 ± 0.01[b] | 0.21 ± 0.01[c] |
| Heart | 0.60 ± 0.06[b] | 0.63 ± 0.06[b] | 0.37 ± 0.01[c] |
| Hematocrit (%) | 18 ± 3[b] | 21 ± 4[b] | 45 ± 2[c] |
| Hepatic copper concentration (μg/g wet wt) | 0.84 ± 0.08[b] | 0.93 ± 0.09[b] | 3.83 ± 0.19[c] |
| Hepatic malondialdehyde (nmol/g wet wt) | 27 ± 2[b] | 3.9 ± 0.1[c] | 2.8 ± 0.2[d] |
| Heart malondialdehyde (nmol/g wet wt) | 1.6 ± 0.4 | 1.4 ± 0.5 | 1.2 ± 0.1 |
| Mortality | 0/9 | 2/9 | 0/10 |

[a] Results are expressed as mean ± SEM values.
Means within a row with different superscript letters are significantly different from each other at $P < 0.05$, as determined by Duncan's Range Test.

with gross abnormalities. This atrophied organ specificity has been reported to occur in copper-deficient rats consuming a fructose-based diet.[29,30]

Although mortality was confined only to those copper-deficient rats consuming the supplemental vitamin E, it is likely that all other copper-deficient rats would have died within the next few weeks.[1-5] This conclusion is based on the severe gross pathology observed in the hearts of all copper-deficient rats. Thus, the severity of copper deficiency could not be ameliorated by the additional vitamin E.

Free radicals are produced by a number of reaction mechanisms, including several enzyme systems, as part of normal cellular function.[31-33] However, these free radicals can induce the peroxidation of polyunsaturated lipids in cell membrane systems. Several enzymes play important roles in providing protection from the deleterious effects of toxic oxygen species. The copper enzyme SOD and the selenoenzyme glutathione peroxidase (GSH-Px) act intracellularly as antioxidants by decreasing free radical production and preventing the propagation of destructive radical chain reactions.[31-33] However, dietary copper deprivation has been shown to result in the depressed activities of both SOD[6-10] and GSH-Px.[9,34] The decreases in SOD and GSH-Px activities could result in increased susceptibility to free radical damage. The antioxidant status of the animal or of the tissue may be an important determinant of tissue pathology resulting from copper deficiency. Vitamin E can ameliorate this process by its antioxidant activity, thus protecting cell membranes.[15] Indeed, those rats that were supplemented with vitamin E had sixfold decreases of hepatic lipid peroxidation than the adequate unsupplemented group. Thus, vitamin E did protect the liver from peroxidative damage in the supplemented group. However, this protective effect of vitamin E was not sufficient to ameliorate the signs associated with copper deficiency.

All hearts of copper-deficient rats had extensive gross pathologic changes, and the rats that died prematurely of heart rupture were the rats that were consuming the additional dietary vitamin E. The addition of vitamin E did not prevent myocardial lipid peroxidation. Regardless of vitamin E or copper nutriture, the degree of lipid peroxidation of myocardial tissue was the same in all rats.

It is interesting to note that lipid peroxidation of heart tissue was of a lesser magnitude compared with the corresponding values from the liver. Indeed, the liver contains higher activities of SOD and GSH-Px[8] than the heart. These differences between the two organs may indicate that the heart is less susceptible to peroxidative damage than the liver and does not require greater protection. In addition, since the supplementation of vitamin E had no effect on the magnitude of myocardial lipid peroxidation, it is suggested that the heart may not be a target tissue for vitamin E. Indeed, vitamin E is stored mainly in the liver. This tissue specificity may explain why the addition of vitamin E was only beneficial in the liver.

Although only copper-deficient rats supplemented with vitamin E died of the deficiency, the mortality probably was not caused by toxicity due to excess of vitamin E. Higher levels of vitamin E supplementation than those used in the present study in rats and humans did not have any toxic effect, were beneficial in the immune system, and were proven successful in slowing the aging process.[35]

Although the reasons for the lethal fructose and copper interaction are not fully understood, it is suggested that it is the metabolic pathway of fructose[36] rather than lipid peroxidation that plays a primary role in the pathogenesis and mortality of copper deficiency. In support of this hypothesis is the finding that although copper-deficient female rats are as copper-deficient as their male counterparts, and both males and females have a similar fatty acid composition of the triglyceride and phospholipid fraction of myocardial tissue, only the females are protected against heart pathology and mortality of copper deficiency when consuming a diet containing fructose.[37]

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