

# Dietary superoxide dismutase does not affect tissue levels<sup>1-3</sup>

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**ABSTRACT** The effects of dietary supplementation of superoxide dismutase on tissue superoxide dismutase levels were examined in mice. Mice were divided into two groups; the control received a complete purified diet, and the supplemented group received the same diet containing 0.004% superoxide dismutase. There were no differences in the activity of CuZn superoxide dismutase or Mn superoxide dismutase in intestine, liver, kidney, or blood. These data show that oral supplementation of superoxide dismutase does not affect tissue superoxide dismutase activity. *Am J Clin Nutr* 1983;37:5-7.

**KEY WORDS** Superoxide dismutase, CuZn superoxide dismutase, Mn superoxide dismutase, superoxide dismutase supplementation

## Introduction

Superoxide dismutase (E.C.1.15.1.1.) (SOD) is a metalloenzyme found in two forms in mammals; one has a MW of 32,000, contains copper and zinc (CuZnSOD), and is localized primarily in the cytosol while the other contains manganese (MnSOD), has a MW of 80,000, and is found primarily in the mitochondrial matrix (1). SOD catalyzes the reaction of the superoxide anion to hydrogen peroxide. Because of its catalytic function, SOD is thought to be involved in the protection of cells against damage from lipid peroxidation; when it is absent superoxide could react with hydrogen peroxide to produce the hydroxyl radical. The hydroxyl radical could subsequently initiate lipid peroxidation, potentially resulting in deleterious effects on biomembranes.

Previous investigators have shown a correlation between dietary levels of copper or manganese and SOD activity. The activity of CuZnSOD was lower in swine (3) and rats (4) fed diets deficient in copper than in controls, and depressed activity of MnSOD was observed in tissues of several species of animals fed diets deficient in manganese (5).

Articles in the popular press have implied that oral supplements of SOD have numerous beneficial effects including the prevention of aging. The July 28, 1981 issue of the *National*

*Enquirer* carried a story entitled "Look Younger—With Incredible Youth Pill." This article states: "For as little as 25¢ a day the fountain of youth can be yours—in easy-to-take pill form." At the present time, health food stores tout SOD as a miracle compound available in pill form with a recommended dosage of 2 tablets a day, equivalent to 8000 units of SOD. Passwater (an advocate of this treatment) describes SOD as a protective defense against the danger of radiation: "your radiation defense first aid kit should include kelp or potassium iodine (sic) tablets, superoxide dismutase tablets and anti-oxidants including vitamins A, C, E and the mineral selenium" (6).

However, like all enzymes, SOD is a protein, and as proteins are digested before absorption, oral consumption of SOD may not

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<sup>2</sup> Supported in part by NIH Research Grant HD-02355 from the National Institute of Child Health and Human Development.

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Received August 15, 1982.

Accepted for Publication August 18, 1982.

cause increased SOD activity in tissues. We have therefore investigated the effects of feeding SOD on the SOD activity of several tissues using the mouse as an experimental model.

### Materials and methods

Adult male Swiss-Webster mice weighing approximately 45 g were divided into two groups and fed either a purified casein-containing control diet, or the same diet containing 0.004% SOD (NF Factors, Concord, CA). The composition of the diet was protein (casein) 30%; fat 8%; cerelese 54.5%; salt mix 6.0% and vitamin mix 1.5%. Details on the salt and vitamin mix have been previously published (7). The dietary level of SOD was calculated according to the dosage recommendations of the manufacturer. The SOD was fed as a percentage of total intake 10 times the ratio recommended for humans. The activity of the SOD tablets was measured and was determined to be > 3000 units per tablet. Throughout the 7-day experimental period, daily food intake and weight gain were recorded.

At the end of the experimental period, the mice were anesthetized with ether and blood was obtained by cardiac puncture using heparinized syringes. Liver, kidney, and intestine were removed and stored at  $-70^{\circ}\text{C}$  until analysis. Tissue homogenates (10% w/v) were prepared in 0.25 M sucrose for SOD analysis. After homogenization, samples were sonicated and centrifuged at  $10,000 \times g$  for 30 min. The pellet was discarded and the supernatant used for the assay. The activity of total SOD was determined by the method of Marklund and Marklund (8). MnSOD activity was determined by the same method with the addition of 2 mM NaCN which inhibits CuZnSOD. One unit of SOD was defined as the amount required for 50% inhibition of pyrogallol oxidation.

### Results and discussion

Food intake and weight gain were similar for the two groups of mice. There were no

significant differences in the activity of CuZnSOD in any of the tissues analyzed (Table 1). In addition, the activity of MnSOD was similar between the two groups. The relative ratio of MnSOD to CuZnSOD in all tissues analyzed was also similar between the control and supplemented groups. The activity of liver CuZnSOD was about 5 times that of MnSOD in the liver, 2 times that in the intestine, and 1.5 times that in the kidney. Thus, oral supplementation of SOD did not have any effect on the SOD activity of the tissues analyzed when the animals were consuming a nutritionally adequate diet. Most probably, SOD is degraded before absorption as are dietary proteins. It must be stressed that our results do not discredit the use of the injectable form of SOD (Orgotein) as a therapeutic agent. At the present time, Orgotein is on the market for veterinary use in animals with arthritis. The Food and Drug Administration has not as yet approved it for human use.

In conclusion, oral supplementation of SOD does not affect tissue SOD activity. 

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TABLE 1  
SOD activity in tissues of control and SOD-supplemented mice  
(mean  $\pm$  SEM)

	n	Tissue			
		Liver	Kidney	Intestine	Blood
Control	5				
CuZnSOD activity*		2728 $\pm$ 225	830 $\pm$ 156	229 $\pm$ 35	413 $\pm$ 50
MnSOD activity		483 $\pm$ 57	580 $\pm$ 16	98 $\pm$ 5	
Supplemented	6				
CuZnSOD activity		2523 $\pm$ 43	950 $\pm$ 117	227 $\pm$ 5	465 $\pm$ 36
MnSOD activity		527 $\pm$ 31	537 $\pm$ 31	100 $\pm$ 12	

\* Values are expressed as SOD units per gram fresh tissue.

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