PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 138–143 (1987)

Aortic Ascorbic Acid, Trace Elements, and Superoxide Dismutase Activity in Human Aneurysmal and Occlusive Disease (42457)

MICHAEL A. DUBICK,*‡[1] GLENN C. HUNTER,†§ SHARON M. CASEY,‖ AND CARL L. KEEN‡‖

*Departments of *Medicine and †Surgery Veterans' Administration Medical Center, Martinez, California 94553, and Departments of ‡Internal Medicine, §Surgery, and ‖Nutrition, University of California, Davis, California 95616*

Abstract. Altered trace elements and ascorbic acid metabolism have been implicated in the pathogenesis of atherosclerotic cardiovascular disease. However, their role in the disease process, or the effect of atherosclerosis on their tissue levels within plaque, is poorly understood. The present study analyzes the concentrations of Fe, Cu, Zn, and Mn, and ascorbic acid and superoxide dismutase (SOD) activity in tissue samples from 29 patients with abdominal aortic aneurysms (AAA) and 14 patients wtih atherosclerotic occlusive disease (AOD). It was observed that the Fe and Mn concentrations in AAA and AOD tissue were higher than the levels in nondiseased control aorta, whereas Cu and Zn levels in AAA and AOD tissue were similar to the levels in controls. The Zn:Cu ratio was significantly lower in the AAA tissue in comparison to both AOD and control tissue. In addition, AAA and AOD tissue had low ascorbic acid levels and low Cu,Zn-SOD activity with Cu,Zn-SOD:Mn-SOD ratios of 0.27 and 0.19, respectively, compared to a ratio of 3.20 in control aorta. These data indicate that aorta affected by aneurysms and occlusive disease have altered trace element and ascorbic acid concentrations, as well as low Cu,Zn-SOD activity. Although these observations do not directly support the hypothesis that AAA is associated with aortic Cu deficiency they do suggest a role for oxygen radicals or increased lipid peroxidation in occlusive and aneurysmal disease of the aorta.

Atherosclerosis is the most common disease of the infrarenal aorta and may result in either progressive stenosis or dilation and aneurysm formation. Although its etiology is incompletely understood, a variety of risk factors, including cigarette smoking, genetic and dietary factors, hypertension, diabetes mellitus, and free radical mechanisms, have been implicated (1–3).

In the past decade there has been considerable interest in the proposal that copper deficiency may be an important etiological factor in the development of human cardiovascular disease (4, 5). A number of studies have shown that Cu deficiency produces myocardial damage and aortic aneurysms in both experimental and domestic animals (6–10).

Despite the common association of abdominal aortic aneurysms (AAA) with atherosclerosis, the biochemical events that result in the atherosclerotic aorta becoming aneurysmal in some persons and occluded in others are poorly understood. Recently, Tilson (11) observed that patients with AAA had lower liver Cu levels than controls and proposed that tissue Cu depletion predisposes to aneurysms. Although Senapati *et al.* (12) did not observe lower Cu in AAA compared with that of controls, they did observe significantly lower zinc concentrations in aortic tissue from patients with AAA. Since dietary Cu and Zn intake can have a profound effect on lipid metabolism (7, 13), the alteration of which can be a major risk factor in atherosclerosis, we thought it appropriate to examine more fully trace element metabolism in aortic disease. In the present study we examined the concentrations of trace elements, the activities of the trace element-dependent enzymes Cu,Zn- and Mn-superoxide dismutase (SOD), as well as ascorbic acid levels in aortic tissue from patients with abdominal aortic aneurysms and atherosclerotic occlusive disease (AOD) and compared these parameters with those of control aorta to understand more fully etiological factors associated with aortic disease.

Materials and Methods. *Human aortic tissue.* Aortic specimens from the infrarenal abdominal aorta were removed at the time of

[1] To whom all correspondence should be addressed: Dr. Michael Dubick, Department of Medicine (151F) VA Medical Center, Martinez, CA 94553.

0037-9727/87 $1.50

surgery from 29 patients with aneurysms and 14 patients with atherosclerotic occlusive disease. The specimens were stripped of adventitia and all surface thrombus was carefully removed. Following gentle rinsing in saline, the specimens were blotted dry, weighed, and frozen at −70°C until assayed. Aneurysmal tissue was free of clot so that only aortic wall was assayed. Aortic tissue removed from the same region of the aorta at autopsy from young adults served as controls. These aortas were kept cold until frozen, usually within 30 min of removal. Control samples were used only if they showed no evidence of vascular disease or calcified plaque.

Patients with AAA and AOD were of similar ages and had a similar smoking history. Other clinical details of the patients' history are included in Table I.

Biochemical studies. Ascorbic acid levels were determined by the method of Day *et al.* (14) as modifed for tissue homogenates (15).

Iron, Cu, Zn, and manganese were determined by flame atomic absorption spectroscopy as previously described (15). For calcium and magnesium analysis, the diluted wet ashed samples were further diluted with 0.1% lanthanum oxide to remove matrix interference (16). Standard addition of known concentrations of selected metals to the samples indicated recoveries ranging between 98 and 102% for all elements.

For determination of superoxide dismutase (SOD) activity, aortic samples were homogenized in 0.25 *M* sucrose to 10% (w/v) and then sonicated at maximum frequency three times for 5 sec each. The homogenates then were centrifuged at 4°C for 30 min at 10,000*g*. Total SOD activity was determined by the inhibition of the autoxidation of pyrogallol according to the method of Marklund and Marklund (17). Mn-SOD activity was determined under the same conditions except 1 m*M* KCN was added to the assay buffer to inhibit Cu,Zn-SOD activity. Cu,Zn-SOD activity was obtained from the difference between total SOD activity and Mn-SOD activity.

Statistics. The data were analyzed by analysis of variance or Student's *t* test, when appropriate, using a statistical package (Stat's Plus) for the Apple IIe.

Results. As expected, aortic tissue from patients with aneurysmal or occlusive disease were characterized by high Ca and Mg concentrations in comparison to control aorta (Fig. 1). Ca concentrations were also higher than control levels in diseased aorta that did not show visible evidence of calcified plaque (Fig. 1).

Aneurysmal and occlusive disease aortic samples had normal Cu and Zn concentrations, but significantly higher Mn and Fe concentrations compared to control aorta (Fig. 2). When diseased aortic tissue without evidence of plaque was analyzed, Fe and Mn remained higher and Zn concentrations were found to be significantly lower in both groups when compared to control levels (Fig. 2). In addition aneurysmal tissue had significantly higher Fe concentrations than AOD tissue, and in aneurysms without plaque, the Zn:Cu ratio was significantly lower in comparison to the other two groups. In aneurysmal tissue, Fe concentrations increased while Zn and Mn levels decreased in relation to the size of the aneurysms (Table II).

AAA and AOD tissue had significantly lower aortic ascorbic acid levels compared to

TABLE I. PATIENT DATA

	Controls ($n = 9$)	Occlusive disease ($n = 14$)	Aneurysmal disease ($n = 29$)
Age (range)	19–40	52–77	49–86
Smoking history (pack years)	Unknown	65 ± 7	59 ± 7
Samples with calcified plaque	0	11/14	11/29
Mean aneurysm size (cm)	—	—	6.8 ± 0.4
Associated diseases			
Diabetes	0	2	1
Hypertension	0	0	2
Chronic obstructive pulmonary disease	0	3	5
Myocardial infarction	0	0	5
Hyperlipidemia	0	3	0

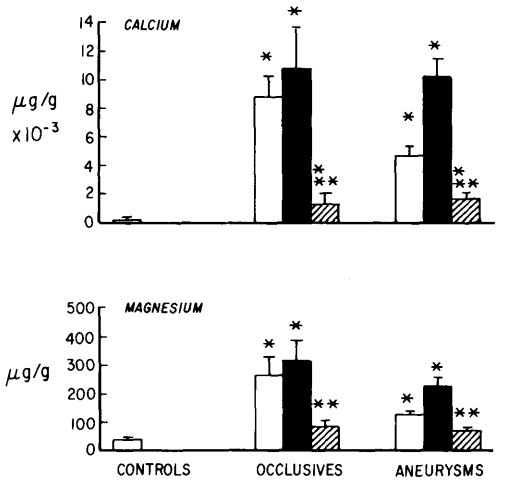

FIG. 1. Calcium and magnesium concentration in control, occlusive disease, and aneurysmal aorta. For control, $n = 9$; occlusive disease, $n = 14$ ($n = 11$ with calcified plaque); aneurysm, $n = 28$ ($n = 11$ with plaque). Combined samples are shown as open bar; samples with calcified plaque are shown as solid bar; samples without plaque are shown as hatched bar. Data represented as means ± SE. *$P < 0.05$ from controls. **$P < 0.05$ from samples with plaque.

controls (Table III), but there was no difference in ascorbic acid levels between AAA and AOD aorta. Ascorbic acid levels also were not significantly affected by the presence of calcified plaque or the size of the aneurysms.

Despite the findings of normal Cu concentrations in diseased aorta, the activity of Cu,Zn-SOD was markedly lower in AAA and AOD tissue compared to controls (Fig. 3). This observation was independent of the presence of calcified plaque. In contrast, Mn-SOD activity was significantly higher in diseased aorta in comparison to controls, but only aneurysmal Mn-SOD activity remained higher in aortic tissue without calcified plaque (Fig. 3). In aneurysmal tissue, the lowest Cu,Zn-SOD and highest Mn-SOD activity was observed in samples with a transverse diameter >9.9 cm.

Discussion. Although atherosclerosis is common to both aortic aneurysms and occlusive disease, morphologically these two diseases are strikingly different. Whereas occlusive disease is associated with increased thickness of the arterial wall, calcification and atheromatous debris, the aneurysmal aorta shows fragmentation of elastic fibers and fibrosis of the arterial wall (18).

Numerous observations have led to the concept that aortic aneurysms may be associated with, or a consequence of, copper deficiency. Copper is an important cofactor for lysyl oxidase, the enzyme essential for initiating elastin crosslinking (19, 20), and nutritional Cu deficiency can lead to decreased elastin levels and aneurysm formation in experimental and domestic animals (6, cf. 20, 21).

In addition, genetic Cu deficiency in mice (e.g., the Blotchy mouse) leads to aortic aneurysms (22). However, the results of the present study agree with the observations of Senapati *et al.* (12) that aortic aneurysmal tissue does

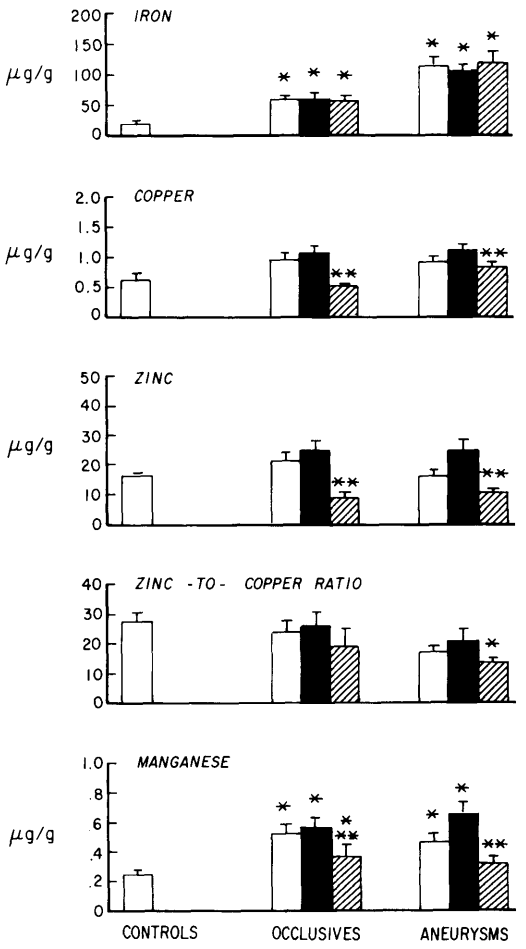

FIG. 2. Aortic iron, copper, zinc, zinc-to-copper ratio and manganese concentrations in control, occlusive disease, and aneurysmal tissue. Figure legend is the same as in Fig. 1.

TABLE II. TRACE ELEMENT CONCENTRATIONS AND SUPEROXIDE DISMUTASE ACTIVITY IN RELATION TO ANEURYSM SIZE[a]

Parameter:	Transverse diameter 4.5–6 cm ($n = 10$)	7–9 cm ($n = 11$)	>9.9 cm ($n = 5$)
Iron (μg/g)	101 ± 20	118 ± 23	156 ± 32
Copper (μg/g)	0.81 ± 0.10	1.06 ± 0.19	0.84 ± 0.12
Zinc (μg/g)	13.3 ± 2.0	12.4 ± 1.9	10.0 ± 1.3
Manganese (μg/g)	0.43 ± 0.1	0.38 ± 0.1	0.26 ± 0.02
Cu,Zn-SOD (U/g aorta)	24 ± 7 (5)	19 ± 7 (9)	7 ± 2 (5)*
Mn-SOD (U/g aorta)	97 ± 14 (5)	111 ± 13 (9)	131 ± 18 (5)*

[a] Data expressed as means ± SE. Values are calculated on a tissue wet weight basis.
* $P < 0.05$ from aneurysms <9.9 cm.

not show lower Cu concentrations than occlusive disease. In contrast, the present results show that aneurysmal Cu concentrations were somewhat higher than the levels in control aortas.

Zinc is another trace element linked to normal lipid (cf. 13) and connective tissue metabolism (23). Abnormal zinc metabolism also may be associated with atherosclerotic disease (24). In the present study the Zn concentrations in aortic tissue from patients with occlusive disease and aneurysms were not significantly different from values in control aorta. However in aneurysmal aorta without calcified plaque, Zn concentrations were significantly lower than the levels in control aorta. This resulted in a lower Zn-to-Cu ratio in aneurysmal aorta. A high Zn-to-Cu ratio has been proposed to predispose to cardiovascular disease (5). Although this ratio was reversed in the present study, its importance in other parameters of vascular disease remains to be determined.

Aortic Fe concentrations in occlusive disease and aneurysms were significantly higher in comparison with control values, and Fe concentrations in aneurysmal aorta were higher than in occlusive disease. The significance of these observations to diseased aorta remains to be established.

Despite the finding that Cu levels in diseased aorta were similar to control levels, the activity of the Cu-dependent enzyme Cu,Zn-SOD was markedly lower in these tissues compared with that of controls. Based on its antioxidant properties, it is reasonable to suggest that one consequence of low Cu,Zn-SOD activity in diseased aortic tissue would be an increased rate of tissue lipid peroxidation compared with

TABLE III. ASCORBIC ACID LEVELS IN AORTIC TISSUE[a]

	mg/100g
Controls	2.71 ± 0.37 (7)
Occlusive disease	0.86 ± 0.12 (9)*
Aneurysms	
All	1.34 ± 0.18 (22)*
<6cm	1.47 ± 0.23 (11)
>7cm	1.12 ± 0.21 (10)

[a] Data expressed as means ± SE (n).
* $P < 0.05$ from control aorta.

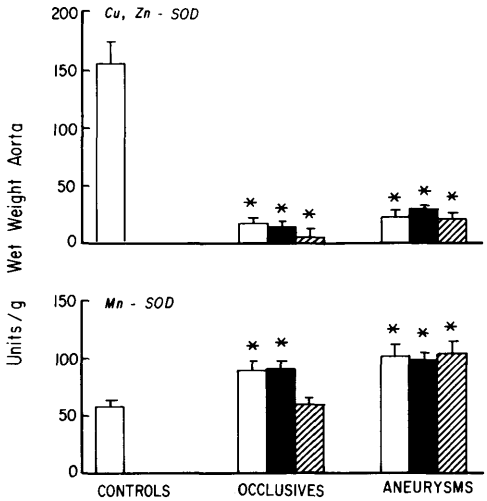

FIG. 3. Aortic Cu,Zn-, and Mn-superoxide dismutase activity in control ($n = 8$), occlusive disease ($n = 11$; $n = 8$ with plaque) and aneurysmal tissue ($n = 18$; $n = 6$ with plaque). Figure legend is the same as in Fig. 1.

that of control tissue. Consistent with this idea is the observation that Mn-SOD activity is higher in the diseased tissue than in control tissue. Compensatory changes in the SODs have been previously documented under conditions of oxidative stress (25). If this hypothesis of increased lipid peroxidation in these tissues is correct, it is tempting to speculate that the higher Fe levels in diseased aorta contribute to the peroxidative process (26). It must be mentioned, however, that direct measurements of lipid peroxidation were not made in the present study.

The observation of lower ascorbic acid levels in aneurysmal and occluded aorta compared with those of control aorta also supports this hypothesis. It is known that cigarette smoking is a major risk factor in the pathogenesis of atherosclerotic disease and particularly aortic aneurysms (1). Cigarette smokers have been shown to have lower serum ascorbic acid levels (27) and perhaps abnormal ascorbic acid metabolism (28). Abnormal ascorbic acid metabolism could affect connective tissue metabolism in aorta (20, 29, 30) and contribute to the disease process.

In conclusion, examination of ascorbic acid, trace elements, and SOD activity in aortic tissue from patients with atherosclerotic occlusive disease and abdominal aneurysms showed some marked differences in comparison with control aorta; only Fe levels differed between the two types of aortic disease. Although the control aortas obtained for the present study were from individuals slightly younger than patients in the two disease groups, no clear effect of age could be ascertained in this study. The data from the present study suggest that enhanced tissue lipid peroxidations may be one common factor underlying the pathologies of aortic aneurysms and occlusive disease. It remains to be established, however, whether the results observed are involved in the pathogenesis of vascular disease or whether they are secondary to the disease process.

This work was supported, in part, by a Grant-in-Aid from the American Heart Association, California Affiliate, and with funds contributed by the American Heart Association, Santa Clara County Chapters.

1. Auerbach O., Garfinkel L. Atherosclerosis and aneurysm of aorta in relation to smoking habits and age. Chest **78**:805–809, 1980.
2. Gordon T, Kannel WB. Predisposition to atherosclerosis in the head, heart and legs: The Framingham study. J Amer Med Assoc **221**:661–666, 1972.
3. Rosen AJ, DePalma RG, Victor Y. Risk factors in peripheral atherosclerosis. Arch Surg **107**:303–308, 1973.
4. Klevay LM, Reck SJ, Barcome DF. Evidence of dietary copper and zinc deficiencies. J Amer Med Assoc **241**:1916–1918, 1979.
5. Klevay LM. The role of copper, zinc and other chemical elements in ischemic heart disease. In: Rennert OW, Chan W-Y, Eds. Metabolism of Trace Elements in Man. Boca Raton, FL: CRC Press, Vol 1:pp129–157, 1984.
6. O'Dell BL, Hardwick BC, Reynolds G, Savage JE. Connective tissue defect in the chick resulting from copper deficiency. Proc Soc Exp Biol Med **108**:402–405, 1961.
7. Hunsaker HA, Morita M, Allen KGD. Marginal copper deficiency in rats: Aortal morphology of elastin and cholesterol values in first generation adult males. Atherosclerosis **51**:1–19, 1984.
8. Coulson WF, Carnes WWH. Cardiovascular studies on copper deficient swine. XIII. The effect of chronic copper deficiency on the cardiovascular system of miniature pigs. Lab Invest **11**:1316–1321, 1962.
9. Walsman J, Cancilla P, Coulson W. Cardiovascular studies on copper deficient swine. II. Mechanical properties of the aorta. Lab Invest **21**:548–554, 1969.
10. Prohaska JR, Hellen LF. Mechanical properties of the copper-deficient rat heart. J Nutr **112**:2142–2150, 1982.
11. Tilson MD. Decreased hepatic copper levels. A possible chemical marker for the pathogenesis of aortic aneurysms in man. Arch Surg **117**:1212–1213, 1982.
12. Senapati A, Carlsson LK, Fletcher CDM, Brouse NL, Thompson RPH. Is tissue copper deficiency associated with aortic aneurysms? Brit J Surg **72**:352–353, 1985.
13. Stemmer KL, Petering HG, Murthy L, Finelli VN, Menden EE. Copper deficiency effects on cardiovascular system and lipid metabolism in the rat: The role of dietary proteins and excessive zinc. Ann Nutr Metab **29**:332–347, 1985.
14. Day BR, Williams DR, Marsh CA. A rapid manual method for routine assay of ascorbic acid in serum and plasma. Clin Biochem **12**:22–26, 1979.
15. Dubick MA, Keen CL. Alterations in tissue trace element and ascorbic acid metabolism in phenytoin-fed rats and mice. J Nutr **115**:1481–1487, 1985.
16. Christian GV, Feldman FJ. Atomic Absorption Spectroscopy: Applications in Agriculture, Biology and Medicine. New York, Wiley, pp237–257, 1970.
17. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem **47**:469–474, 1974.
18. Zarins CK, Glagov S. Aneurysms and obstructive plaques: Differing local responses to atherosclerosis. In: Bergan JJ, Yao JST, Eds. Aneurysms Diagnosis

and Treatment. New York, Grune & Stratton, pp61–82, 1982.
19. Harris ED. Copper induced activation of aortic lysyl oxidase in vivo. Proc Natl Acad Sci **73**:371–374, 1976.
20. Tinker D, Rucker RB. Role of selected nutrients in the synthesis, accumulation and chemical modification of connective tissue proteins. Physiol Rev **65**: 607–657, 1985.
21. Hill KE, Davidson JM. Induction of increased collagen and elastin biosynthesis in copper-deficient pig aorta. Arteriosclerosis **6**:98–104, 1986.
22. Andrews EJ, White WJ, Bullock LP. Spontaneous aortic aneurysms in Blotchy mice. Amer J Pathol **78**: 199–210, 1975.
23. Philip B, Kurup PA. Dietary zinc and levels of collagen and elastin and carbohydrate components of glycoproteins of aorta, skin and cartilage in rats. Ind J Exp Biol **16**:370–372, 1978.
24. Klevay LM. Hypercholesterolemia in rats produced by an increase in the rate of zinc and copper ingested. Amer J Clin Nutr **26**:1060–1068, 1973.
25. Dubick MA, Keen CL. Tissue trace elements and lung superoxide dismutase activity in mice exposed to ozone. Toxicol Lett **17**:355–360, 1983.
26. Aust SD, Svingen BA. The role of iron in enzymatic lipid peroxidation. In: Pryor WA, Ed. Free Radicals in Biology. New York, Academic Press, Vol 5:pp1–28, 1982.
27. Pelletier O. Smoking and vitamin C levels in humans. Amer J Clin Nutr **21**:1259–1267, 1968.
28. Kallner AB, Hartman D, Hornig DH. On the requirements of ascorbic acid in man: Steady state turnover and body pool in smokers. Amer J Clin Nutr **34**:1347–1355, 1981.
29. Dubick MA, Last JA, Cross CE, Rucker RB. Interactions of ascorbic acid supplementation and bleomycin instillation on murine lung connective tissue metabolism. Drug-Nutr Interact **2**:105–115, 1983.
30. Critchfield JW, Dubick MA, Last J, Cross CE, Rucker RB. Changes in response to ascorbic acid administered orally to rat pups: Lung collagen, elastin and protein synthesis. J Nutr **115**:70–77, 1985.

Received July 24, 1986. P.S.E.B.M. 1987, Vol. 184.
Accepted October 13, 1986.