Cystine feeding enhances defects of dietary copper deficiency by a mechanism not involving oxidative stress

Norihisa Kato, Jack T. Saari, and Gwen M. Schelkoph

Department of Applied Biochemistry, Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima 724, Japan; and United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND USA

Dietary cystine supplementation exacerbates the effects of dietary copper deficiency. We examined the possibilities that this exacerbation is caused by an oxidative mechanism or by an effect on copper status. Male Sprague-Dawley rats (~120 g) were fed copper-adequate or copper-deficient diets (0.5 or 16 mg/kg diet) that were supplemented with combinations of L-cystine (20 g/kg diet), vitamin E (37 mg/kg diet), and sodium tungstate (360 mg/L in drinking water). Dietary copper deficiency depressed serum and organ copper concentrations; increased heart size; caused anemia; reduced heart and liver superoxide dismutase and cytochrome c oxidase activities; and increased serum, heart, and liver thiobarbituric acid reactive substances. Cystine feeding exacerbated the cardiac enlargement and anemia of copper deficiency and reduced liver cytochrome oxidase and superoxide dismutase activities, but had no effect on organ copper content. Cystine feeding had no effect on serum or heart thiobarbituric acid reactive substances but depressed liver thiobarbituric acid reactive substances production. Neither vitamin E nor tungsten, which was used to inhibit sulfite oxidase and its potential production of free radicals, had an effect on the copper- or cystine-dependent changes in heart size, hematocrit, or hemoglobin. Dietary vitamin E and tungsten had variable beneficial effects on copper- and cystine-dependent changes in variables related to red blood cell size, but these effects could not be consistently related to the inhibition of TBARS production caused by vitamin E and tungsten. We conclude that, while cystine feeding enhanced signs of copper deficiency, it did not do so by an oxidative mechanism or by altering copper status. (J. Nutr. Biochem. 5:99–105, 1994.)

Keywords: cystine; copper deficiency; peroxidation; vitamin E; sulfite oxidase; tungsten

Introduction

Copper deficiency causes a variety of defects having a focus on the cardiovascular system.[1] Oxygen-derived free radical damage has been proposed as a mechanism for these defects because copper-deficient animals exhibit reduced antioxidant enzyme activity,[2,3] increased susceptibility of mitochondria to in vitro oxidation,[4,5] enhanced tissue damage with oxidative stress,[6–8] and enhanced lipid peroxidation.[6,9–13]

Feeding excess sulfur amino acids to rats has been shown to exaggerate the defects of dietary copper deficiency, especially in males,[14] and to decrease indices of copper status in animals fed diets deficient, adequate, or excessive in copper.[15–18] Because copper-deficient animals are susceptible to oxidative damage, it is relevant from a mechanistic standpoint that feeding excess sulfur amino acids has been associated with reduction of antioxidant enzyme activity[17,19] and with enhanced lipid peroxidation.[15,17] The latter findings suggest that dietary sulfur amino acids may exacerbate the defects of copper deficiency by an oxidative mechanism. Furthermore, oxygen is a potential electron acceptor for the oxidation of sulfite to sulfate by sulfite oxidase, the terminal step

Address reprint requests to Dr. Jack T. Saari at USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034, USA.

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in sulfur amino acid metabolism.[20] The resulting production of oxygen-derived free radicals and reactive species, in an environment conducive to oxidative damage (copper deficiency), might thus exaggerate the defects already present.

We tested the hypothesis that cystine exacerbates defects of copper deficiency by enhancing oxidative stress. Specifically, we sought to confirm the observation that cystine enhances the defects of copper deficiency[14] to measure associated changes in indices of lipid peroxidation and to determine whether antioxidant treatment (vitamin E feeding) or sulfite oxidase inhibition (tungsten feeding) could inhibit copper deficiency-mediated defects or cystine's exacerbation of those defects.

Methods and materials

Animals and diets

Seventy-two male Sprague-Dawley rats (105 to 138 g) were arranged into 12 weight-matched groups averaging 121 g each. They were fed ad libitum, in a three-way design, two concentrations of dietary copper (nominally, 0.5 or 16 mg Cu/kg diet), two concentrations of dietary L-cystine (0 or 20 g/kg diet), and three combinations of vitamin E and tungsten (0 or 37 mg/kg of DL-α-tocopherol acetate or 37 mg/kg of DL-α-tocopherol acetate plus 360 mg/L sodium tungstate in their drinking water). Composition of the basal diet was (in g/kg diet): α-corn starch, 438; sucrose, 219; casein, 200; cellulose powder, 40; soybean oil, 50; AIN-76 vitamin mixture,[21] 10; salt mixture,[22] 40; choline bitartrate, 2; L-methionine, 1. Addition of L-cystine (2.0 mg/kg diet) to this basal diet was made at the expense of α-corn starch (−1.3 g/kg diet) and sucrose (−0.7 g/kg diet). Adjustment of dietary nitrogen for the added L-cystine was not thought to be necessary because sulfur amino acids appear to act distinctly from other added amino acids, especially with respect to their effect on copper status and indicators.[14,23] DL-α-tocopherol acetate and sodium tungstate were dietary supplements. Assay (see below) of copper concentration gave a range of 0.47 to 0.56 mg Cu/kg diet for the four copper-deficient diets and a range of 15.7 to 16.3 mg Cu/kg for the four copper-adequate diets.

After 41 days on the respective diets, rats were fasted overnight in metabolic cages while their urine was collected for assay of sulfate concentration. On day 42, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg body wt, Vet Labs, Lenexa, KS USA). Blood was withdrawn from the inferior vena cava and divided into aliquots for erythrocyte counting (EDTA-treated) and serum assays (samples allowed to clot at room temperature). Hearts and livers were collected for enzyme, vitamin E, and trace element assays.

Red blood determinations

Hematocrit, hemoglobin content, mean red blood cell (RBC) volume, and red cell distribution width (defined as 100 · [SD of RBC volumes]/[mean RBC volume]) were determined on a Coulter Counter (Model S Plus 4, Hialeah, FL USA).

Mineral assays

Trace element contents of organs were determined by inductively coupled argon plasma emission spectroscopy (Jarrell-Ash, Model 1140, Waltham, MA USA) after lyophilization and digestion of organs with nitric acid and hydrogen peroxide.[24] The same procedure, without lyophilization, was followed for diet analysis. Parallel assays of National Institute of Standards and Technology (Washington DC) reference samples (#1577a, bovine liver for organs; #1572, citrus leaves for diets) yielded mineral contents within the specified ranges.

Sulfate assay of urine was done by ion chromatography using a PAX-100 column (Dionex, Sunnyvale, CA USA) and conductivity detection (Shimadzu, Wood Dale, IL USA) following deproteinization of the urine sample with trichloroacetic acid.[25]

Biochemical assays

Cytochrome c oxidase (CCO) activity was measured on whole tissue homogenates by spectrophotometrically monitoring loss of ferrocytochrome c, as described by Prohaska and Wells.[26]

For measurement of Cu,Zn superoxide dismutase (SOD) activity, tissue homogenates were treated with 0.4 volumes of a solution of ethanol and chloroform (25:15) to inactivate Mn SOD.[27] This solution was mixed well and centrifuged at 5000*g* for 15 min. Aliquots of the clear supernates were dialyzed against deionized water (4° C, 12,000 mol wt exclusion membrane) and then used to measure Cu,Zn SOD activity. Cu,Zn SOD activity was measured spectrophotometrically (Beckman, Model DU-70, Fullerton, CA USA) by using a kit (SOD-525 Method) from Bioxytech S.A. (Marne Cedex, France). The method is based on the ability of SOD to accelerate autoxidation of a proprietary reagent to a visible chromophore.

Estimates of lipid peroxidation in tissue and serum were obtained by fluorometric determination (Beckman, Model DU-70) of the concentration of thiobarbituric acid reactive substances (TBARS) by the methods of Ohkawa et al.[28] and Yagi.[29]

Tissue and serum α-tocopherol were determined by the method of Katayama et al.[30] using high performance liquid chromatography (HPLC). Samples were homogenized, extracted with hexane, dried under nitrogen gas at 60 to 70° C and reconstituted with the HPLC mobile phase (2% water in methanol). A reverse phase C-18 column (4.6 mm × 250 mm) was used in conjunction with fluorometric detection (Shimadzu, Wood Dale, IL USA) at 330 nm emission and 295 nm excitation wavelengths.

Liver sulfite oxidase activity was measured spectrophotometrically on whole homogenates by monitoring the reduction of ferricytochrome c, as described by Cohen et al.[31]

Statistics

Data were statistically analyzed by three-way analysis of variance (ANOVA).[32] The sources of variation were copper, cystine, and treatment (combinations of vitamin E and tungsten). Comparison of means, when necessary, was done by using Tukey's Studentized Range Test.[32]

Results

Effects of copper deficiency

Compared with rats fed copper-adequate diets, rats fed copper-deficient diets had lower heart and liver copper concentrations, lower liver zinc concentrations, and higher liver iron concentrations *(Table 1)*. Copper deficiency caused higher heart zinc concentrations innon-cystine-fed, untreated rats, but cystine feeding and treatment both altered this effect (see below). CCO and Cu,Zn SOD activities in hearts and livers were lower in copper-deficient than in copper-adequate rats *(Table 2)*. Copper deficiency caused cardiac enlargement and anemia

Table 1 Effect of dietary alteration of copper, cystine, vitamin E, and tungsten (W) on heart and liver trace elements

Diet	Heart elements (μmol/g dry weight)			Liver elements (μmol/g dry weight)		
	Cu	Zn	Fe	Cu	Zn	Fe
CuA	0.35 ± 0.13	1.04 ± 0.38	4.1 ± 1.4	0.22 ± 0.02	1.15 ± 0.08	3.9 ± 0.5
+ vit E	0.42 ± 0.02	1.25 ± 0.06	6.3 ± 1.8	0.20 ± 0.03	1.04 ± 0.11	3.9 ± 0.5
+ vit E + W	0.33 ± 0.02	0.93 ± 0.02	4.3 ± 0.9	0.22 ± 0.02	1.16 ± 0.11	3.9 ± 0.5
+ cystine	0.39 ± 0.08	1.36 ± 0.23	5.6 ± 0.9	0.19 ± 0.02	1.12 ± 0.11	4.3 ± 0.9
+ cystine + vit E	0.41 ± 0.02	1.24 ± 0.03	5.7 ± 1.4	0.20 ± 0.02	1.18 ± 0.09	4.1 ± 0.9
+ cystine + vit E + W	0.38 ± 0.03	0.95 ± 0.03	3.8 ± 0.4	0.58 ± 0.38	1.18 ± 0.09	3.8 ± 0.9
CuD	0.14 ± 0.02	1.19 ± 0.26	4.8 ± 0.7	0.08 ± 0.03	1.04 ± 0.06	5.7 ± 1.6
+ vit E	0.16 ± 0.05	1.16 ± 0.17	6.1 ± 1.6	0.06 ± 0.02	0.98 ± 0.08	7.7 ± 1.4
+ vit E + W	0.11 ± 0.02	0.84 ± 0.02	4.1 ± 0.5	0.08 ± 0.02	1.04 ± 0.09	7.3 ± 1.6
+ cystine	0.11 ± 0.02	0.93 ± 0.12	4.1 ± 0.9	0.06 ± 0.03	1.07 ± 0.12	7.2 ± 2.3
+ cystine + vit E	0.14 ± 0.03	1.13 ± 0.05	5.0 ± 1.3	0.03 ± 0.02	1.13 ± 0.12	11.5 ± 1.8
+ cystine + vit E + W	0.14 ± 0.03	0.92 ± 0.15	4.1 ± 0.4	0.06 ± 0.03	1.06 ± 0.18	10.4 ± 3.2
Source of variation	Analysis of variance, *P* values					
Copper	0.0001	0.01	NS	0.0001	0.002	0.0001
Cystine	NS	NS	NS	NS	0.04	0.0003
Copper × cystine	NS	0.02	NS	0.008	NS	0.0005
Treatment*	0.01	0.0001	0.0001	0.001	NS	0.006
Copper × treatment	NS	NS	NS	0.005	NS	0.002
Cystine × treatment	NS	NS	NS	0.005	0.05	NS
Copper × cystine × treatment	NS	0.002	NS	0.005	NS	NS

Values are means ± SD.
*Treatment with vitamin E or vitamin E + W.

Table 2 Effect of dietary alteration of copper, cystine, vitamin E, and tungsten (W) on heart and liver cytochrome c oxidase (CCO) and Cu,Zn superoxide dismutase (SOD) activities and vitamin E status

Diet	Variable				
	Heart CCO activity*	Liver CCO activity*	Heart SOD activity†	Liver SOD activity†	Liver vit E (nmol/g wet wt)
CuA	190 ± 40	100 ± 10	17 ± 2	90 ± 20	7 ± 2
+ vit E	180 ± 40	100 ± 20	17 ± 2	100 ± 10	77 ± 23
+ vit E + W	180 ± 30	110 ± 10	17 ± 1	80 ± 20	79 ± 19
+ cystine	190 ± 40	90 ± 10	18 ± 4	80 ± 10	7 ± 2
+ cystine + vit E	180 ± 40	80 ± 20	19 ± 2	70 ± 20	84 ± 14
+ cystine + vit E + W	180 ± 30	90 ± 10	15 ± 4	80 ± 20	65 ± 39
CuD	80 ± 10	50 ± 20	12 ± 2	30 ± 10	9 ± 2
+ vit E	90 ± 20	50 ± 10	13 ± 2	50 ± 10	81 ± 14
+ vit E + W	80 ± 10	60 ± 10	11 ± 2	40 ± 10	86 ± 19
+ cystine	80 ± 20	50 ± 20	10 ± 2	30 ± 10	9 ± 2
+ cystine + vit E	80 ± 20	40 ± 20	10 ± 3	30 ± 10	84 ± 9
+ cystine + vit E + W	80 ± 20	50 ± 10	12 ± 2	30 ± 10	95 ± 14
Source of variation	Analysis of variance, *P* values				
Copper	0.0001	0.0001	0.0001	0.0001	NS
Cystine	NS	0.0003	NS	0.004	NS
Copper × cystine	NS	NS	NS	NS	NS
Treatment‡	NS	NS	NS	NS	0.0001
Copper × treatment	NS	NS	NS	NS	NS
Cystine × treatment	NS	NS	NS	NS	NS
Copper × cystine × treatment	NS	NS	0.04	NS	NS

Values are means ± SD.
*Units/g wet weight of tissue; a unit of CCO activity is that amount catalyzing oxidation of 1 μmol of ferrocytochrome c to ferricytochrome c per minute.
†Units/g wet wt of tissue; a unit of SOD activity is that amount of enzyme required to double the rate of autoxidation of a proprietary reagent (Bioxytech S.A., Marne Cedex, France).
‡Treatment with vitamin E or vitamin E + W.

(Table 3). Serum, heart, and liver lipid peroxidation products, as measured by TBARS, were also increased by copper deficiency *(Table 4).*

Effects of cystine feeding

Cystine feeding did not alter heart copper concentration *(Table 1).* The significant ANOVA *P* values observed for interactions among cystine, copper, and treatment on liver copper concentration can be explained by the marked effect of tungsten feeding on liver copper in copper-adequate rats. Comparison of means showed no effect of cystine on liver copper in copper-deficient rats ($P < 0.05$). Cystine feeding depressed heart zinc in copper-deficient rats, increased liver zinc in vitamin E-treated rats, and exaggerated the increase in liver iron caused by copper deficiency *(Table 1).*

Although cystine feeding had no effect on heart CCO or Cu,Zn SOD activity, it depressed liver CCO and Cu,Zn SOD activities independently of copper's effect on these enzymes *(Table 2).*

Cystine feeding exaggerated the effects of copper deficiency on heart size, hematocrit, hemoglobin, mean red blood cell volume and red cell distribution width (RDW), and increased liver size in copper-deficient rats *(Table 3).*

Cystine feeding reduced lipid peroxidation (TBARS) in liver and had no effect on TBARS in serum or heart *(Ta-ble 4).*

Urine sulfate production was increased by cystine feeding *(Table 5).*

Effect of vitamin E and tungsten treatment

Vitamin E and tungsten treatment had significant effects on heart minerals, which were largely independent of copper status or amount of cystine in the diet *(Table 1).* A comparison of means ($P < 0.05$) indicates that heart copper and iron concentrations were increased by vitamin E treatment, an effect that was inhibited by tungsten treatment. Heart zinc was depressed by tungsten treatment. Regarding liver elements, tungsten greatly increased liver copper in copper-adequate, cystine-fed rats ($P < 0.05$). Vitamin E increased liver iron in copper-deficient rats ($P < 0.05$).

Neither vitamin E nor tungsten treatment had an effect on heart or liver CCO or Cu,Zn SOD activities *(Table 2).* Vitamin E treatment markedly enhanced liver vitamin E concentration *(Table 2),* as well as serum and heart vitamin E concentrations (data not shown).

Cardiovascular changes caused by copper deficiency and enhanced by cystine feeding were, for the most part, unaffected by vitamin E or tungsten treatment *(Table 3).* Tungsten did, however, improve red blood cell volume and vitamin E treatment improved RDW in cystine-fed, copper-deficient rats. An additional effect of tungsten was that it further decreased body weight in cystine-fed, copper-deficient rats.

Vitamin E and tungsten treatment each had an effect on lipid peroxidation *(Table 4).* Comparison of means indicated that serum TBARS were reduced by combined treatment with vitamin E and tungsten ($P < 0.05$), and heart TBARS were reduced by vitamin E treatment (*P*

Table 3 Effect of dietary alteration of copper, cystine, vitamin E, and tungsten (W) on variables associated with dietary copper deficiency

	Variable						
Diet	Body wt (g)	Heart wt (mg/g body wt)	Liver wt (mg/g body wt)	Hematocrit	Hemoglobin (g/L)	Mean RBC* volume (fL)	RDW† (%)
CuA	420 ± 20	2.7 ± 0.2	36 ± 1	0.43 ± 0.01	148 ± 5	55 ± 1	13 ± 1
+ vit E	450 ± 30	2.7 ± 0.1	40 ± 4	0.41 ± 0.01	141 ± 4	54 ± 2	14 ± 1
+ vit E + W	430 ± 40	2.8 ± 0.2	36 ± 2	0.43 ± 0.02	147 ± 7	55 ± 1	13 ± 1
+ cystine	400 ± 40	2.9 ± 0.1	40 ± 3	0.42 ± 0.02	146 ± 6	54 ± 2	15 ± 1
+ cystine + vit E	420 ± 40	2.8 ± 0.2	42 ± 5	0.41 ± 0.01	142 ± 5	53 ± 2	16 ± 1
+ cystine + vit E + W	420 ± 20	2.9 ± 0.2	42 ± 3	0.40 ± 0.03	137 ± 10	52 ± 2	16 ± 2
CuD	420 ± 20	3.2 ± 0.2	38 ± 4	0.35 ± 0.06	118 ± 20	46 ± 4	22 ± 6
+ vit E	410 ± 30	3.2 ± 0.4	41 ± 4	0.38 ± 0.05	127 ± 17	48 ± 1	19 ± 4
+ vit E + W	440 ± 40	3.1 ± 0.1	36 ± 4	0.37 ± 0.02	123 ± 4	49 ± 2	18 ± 2
+ cystine	390 ± 30	3.8 ± 0.5	48 ± 7	0.31 ± 0.05	95 ± 21	43 ± 3	30 ± 3
+ cystine + vit E	380 ± 20	3.6 ± 0.5	48 ± 6	0.26 ± 0.06	86 ± 24	43 ± 2	24 ± 4
+ cystine + vit E + W	320 ± 10	3.9 ± 0.7	48 ± 11	0.32 ± 0.11	102 ± 37	51 ± 7	24 ± 5
Source of variation	Analysis of variance, *P* values						
Copper	0.0002	0.0001	0.003	0.0001	0.0001	0.0001	0.0001
Cystine	0.0001	0.0001	0.0001	0.0004	0.0001	0.005	0.0001
Copper × cystine	0.004	0.003	0.02	0.01	0.003	NS	0.01
Treatment‡	NS	NS	NS	NS	NS	0.02	NS
Copper × treatment	NS	NS	NS	NS	NS	0.001	0.005
Cystine × treatment	0.03	NS	NS	NS	NS	NS	NS
Copper × cystine × treatment	0.003	NS	NS	NS	NS	0.02	NS

Values are means ± SD.
*RBC, red blood cell.
†RDW, red blood cell distribution width, defined in Methods and materials.
‡Treatment with vitamin E or vitamin E + W.

Table 4 Effect of dietary alteration of copper, cystine, vitamin E, and tungsten (W) on serum, heart, and liver content of thiobarbituric acid reactive substances (TBARS)

Diet	Variable		
	Serum TBARS (μmol/L)	Heart TBARS (nmol/g wet wt)	Liver TBARS (nmol/g wet wt)
CuA	3.3 ± 0.8	260 ± 100	90 ± 10
+ vit E	2.8 ± 1.1	160 ± 30	90 ± 20
+ vit E + W	2.6 ± 0.6	160 ± 30	80 ± 10
+ cystine	4.1 ± 1.7	230 ± 50	70 ± 20
+ cystine + vit E	2.8 ± 0.5	160 ± 30	70 ± 20
+ cystine + vit E + W	2.2 ± 0.8	150 ± 30	70 ± 20
CuD	4.2 ± 1.3	250 ± 90	100 ± 30
+ vit E	3.4 ± 0.6	180 ± 40	110 ± 20
+ vit E + W	2.8 ± 1.6	170 ± 60	100 ± 20
+ cystine	5.6 ± 3.3	260 ± 70	100 ± 30
+ cystine + vit E	4.2 ± 2.7	220 ± 50	90 ± 40
+ cystine + vit E + W	3.0 ± 1.3	200 ± 60	90 ± 30
Source of variation	Analysis of variance, *P* values		
Copper	0.02	0.04	0.0006
Cystine	NS	NS	0.01
Copper × cystine	NS	NS	NS
Treatment*	0.002	0.0001	NS
Copper × treatment	NS	NS	NS
Cystine × treatment	NS	NS	NS
Copper × cystine × treatment	NS	NS	NS

Values are means ± SD.
*Treatment with vitamin E or vitamin E + W.

< 0.05), but were reduced no further by addition of tungsten. These effects were independent of copper status or cystine feeding.

As intended by experimental design, tungsten treatment inhibited sulfite oxidase activity; tungsten treatment did not affect urinary sulfate excretion *(Table 5)*.

Discussion

Cystine enhanced the cardiac enlargement and anemia caused by copper deficiency and, in addition, caused liver enlargement in copper-deficient rats, confirming in large part the findings of Nielsen.[14] In examining the mechanism of the exaggeration of effects by cystine, however, neither an oxidative mechanism nor an effect on copper status could be implicated.

The evidence against an oxidative mechanism includes the absence of an increase in peroxidation products by cystine, and the inability of either vitamin E or tungsten to inhibit the cardiac enlargement or anemia. Although vitamin E and tungsten have effects on red cell volume and distribution width, respectively, the inconsistent nature of these findings and the absence of effects on anemia per se (hematocrit or hemoglobin) argue against an oxidative mechanism for either copper deficiency or cystine feeding and suggest diverse actions of vitamin E and tungsten.

Despite the inability of vitamin E and tungsten to inhibit the defects of copper deficiency or their exacerbation by cystine feeding, both vitamin E and tungsten depressed lipid peroxidation. This depression of peroxidation was, however, statistically independent of dietary copper or cystine, thus tending to dissociate the defects of copper deficiency or their exacerbation from an oxidative mechanism. This finding is consistent with that of Silverman et al.,[33] who showed an absence of effect of vitamin E on defects of copper deficiency but a depression of lipid peroxidation.

The possibility that cystine exaggerates the effects of copper deficiency by affecting copper status was thought to be viable because homocysteine feeding has been shownto reduce indices of copper status in copper-adequate and copper-deficient animals,[17] and cysteine feeding can reduce copper status in animals suffering copper toxicity.[18] This possibility was discredited in the present study by the absence of a cystine effect on heart or liver copper concentration. Though cystine did not affect copper concentration, one might argue that it rendered copper unusable, by copper chelation, for instance. Two derivatives of cystine that are good chelators of copper are cysteine and metallothionein.[18] However, for cysteine chelation to reduce copper availability, one would have to explain why excretion of this form would not result in depression of organ copper concentrations. Enhancement of copper binding by metallothionein is most evident in models of copper loading,[34] but in the present context it would seem that enhanced copper binding would require that cystine feeding either enhance affinity of metallothionein or induce its production, which at present is uncertain.[18]

A finding that may relate to the cystine-mediated exacerbation of anemia in copper-deficient animals is

Table 5 Effect of dietary alteration of copper, cystine, vitamin E, and tungsten (W) on activity of liver sulfite oxidase and urinary sulfate excretion

Diet	Variable	
	Sulfite oxidase activity*	Urine sulfate†
CuA	41 ± 4	5.3 ± 1.5
+ vit E	33 ± 7	4.3 ± 1.6
+ vit E + W	6 ± 4	3.1 ± 0.6
+ cystine	45 ± 3	11.9 ± 1.9
+ cystine + vit E	37 ± 12	6.0 ± 1.5
+ cystine + vit E + W	2 ± 3	5.8 ± 0.3
CuD	40 ± 6	2.0 ± 0.9
+ vit E	39 ± 10	3.6 ± 0.9
+ vit E + W	11 ± 7	1.2 ± 0.6
+ cystine	37 ± 14	9.4 ± 1.6
+ cystine + vit E	38 ± 4	9.3 ± 3.4
+ cystine + vit E + W	6 ± 10	9.3 ± 2.8
Source of variation	**Analysis of variance, *P* values**	
Copper	NS	NS
Cystine	NS	0.0001
Copper × cystine	NS	0.0001
Treatment‡	0.0001	0.0001
Copper × treatment	NS	0.0001
Cystine × treatment	NS	0.007
Copper × cystine × treatment	NS	NS

Values are means ± SD.
*Units/g wet wt of liver; unit of sulfite oxidase activity is the amount of protein causing an absorbance change (at 550 nm) of 1.0/min during the reduction of ferricytochrome c.
†Unit of urine sulfate is mmol/(d · kg body wt).
‡Treatment with vitamin E or vitamin E + W.

the enhancement of liver iron by cystine. The altered metabolism of iron observed in copper deficiency has been implicated in the anemia that the deficiency causes.[35,36] Cystine may exacerbate the anemia through an additional effect on iron metabolism. A direction one might take in examining this possibility would be to look for effects of cystine on liver iron mobilization by xanthine oxidase, serum ceruloplasmin, and ascorbic acid, impairment of which was found in copper deficiency by Cohen et al.[37]

In summary, our primary hypothesis, that cystine enhances defects of dietary copper deficiency by an oxidative mechanism, appears to be negated. An unstated corollary, that components of the diet catabolized by normal metabolic pathways can act as oxidative stressors, also appears to be weakened. Finally, the absence of an apparent effect of cystine on copper status leaves open the question as to how cystine exaggerates effects of copper deficiency.

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