Effects of Low Copper Intake on Dimethylhydrazine-Induced Colon Cancer in Rats (43485)

ROBERT A. DISILVESTRO,*,[1] JOEL K. GREENSON,† AND ZHIMING LIAO*
Departments of Human Nutrition and Food Management and Pathology,† Ohio State University, Columbus, Ohio 43210*

Abstract. **Rats fed low copper show a high incidence of dimethylhydrazine (DMH)-induced colon tumors compared with rats fed very high Cu. The difference could be due to Cu deficiency in the low group or to Cu toxicity in the high group. In the present study, rats fed low Cu (0.2 ppm) showed greater DMH-stimulated colon tumorigenesis than rats fed adequate Cu (8 ppm). Differences were seen in the number of rats developing tumors (5 of 11 vs 1 of 10), total tumors (7 vs 2), and average tumor mass (1.02 g vs 0.29 g). Low Cu intake did not cause any general DMH toxicity as assessed by body weight gain. To prevent Cu deficiency-induced mortality, low Cu feeding was begun in postweanling rats (weight, about 80 g) housed in groups of five to six, rather than individually. This limited the effects of low Cu feeding to only a moderate Cu deficiency based on several parameters, including three Cu antioxidant enzyme activities. Group-housed rats fed marginal Cu levels (2.5 ppm) showed normal Cu status, and DMH produced only one tumor in 10 rats. In conclusion, high DMH-induced colon tumorigenesis can be found in rats with low activities of Cu antioxidant enzymes.**

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Cancer is proposed to result, at least in part, from the actions of oxygen free radicals (1). Three copper-containing enzymes are proposed to work against oxygen radical-mediated injury. Two of these, cytosolic and extracellular superoxide dismutase, are thought to eliminate superoxide radical (2, 3). Another Cu protein, ceruloplasmin, is hypothesized to inhibit iron-catalyzed radical formation (3). Because all three of these enzyme activities can be affected by deficient, and even marginal, Cu intakes (4–7), it is reasonable to suspect that Cu intake can affect cancer development.

Rats fed low Cu show a higher incidence of colon tumors after dimethylhydrazine (DMH) treatment than rats consuming very high amounts of Cu (8). However, it is not clear whether this difference results from low Cu enzyme activities in the low Cu group, or from Cu toxicity effects on potential tumor formation in the high Cu group. No Cu enzyme activities were actually measured (8), and high Cu feeding in rats is capable of suppressing chemical carcinogenesis (9). The initial study on Cu consumption and DMH-induced colon tumorigenesis did include rats fed a standard mixed feed (8). However, this group could not be compared with the low and high Cu groups because the feeds differed in many respects, including fat and vitamin contents.

The present study compared tumor development after DMH treatment in rats fed Cu levels considered either low, adequate, or marginal. The latter group was included because it resembles the Cu consumption typical of that of many humans (10). Because Cu deficiency can produce cardiovascular-related mortality (11), the effects of low Cu intake had to be moderated to allow the rats to survive the study's long feeding period. This was accomplished partially by initiating low Cu feeding in rats that had already passed through weaning, a stage at which organ development is particularly sensitive to injury. In addition, rats were housed in groups of five to six, a measure found to restrict the effects of low Cu feeding (4).

[1] To whom requests for reprints should be addressed at Department of Human Nutrition and Food Management, Ohio State University, 265 Campbell Hall, 1787 Neil Avenue, Columbus, OH 43210.

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Materials and Methods

Animal Treatment. Male Sprague-Dawley rats, weighing between 76 and 85 g, were purchased from Harlan Sprague-Dawley, Indianapolis, IN. Rats were housed in groups of five to six in stainless steel cages and given deionized water and feed *ad libitum*. The diet was purchased from ICN Biochemicals, Cleveland, OH, and was a modification of the copper-deficient diet of Mills and Murray (12), described earlier (4). The main fat source is peanut oil, which comprises 10% of the diet by weight. The basic diet contained 0.2 ppm of Cu as analyzed by wet ashing and atomic absorbance spectrometry. Adequate and marginal diets were supplemented with Cu sulfate to 8 ppm of Cu and 2.5 ppm of Cu, respectively. Rats were fed the respective diets for 5 weeks before they received the first of 12 weekly injections of freshly prepared DMH (20 mg/kg in 1 m*M* EDTA, pH 6.5, ip) or solvent alone. Rats were sacrificed 13 weeks after the last injection.

Tissue and Blood Samplings. Rats were lightly anesthetized with carbon dioxide and blood was removed from the heart into citrate-containing vacuum tubes. Rats were then sacrificed by further exposure to the carbon dioxide. The liver, heart, and large intestine were removed immediately. Hearts were weighed and then discarded. Livers were frozen at −20°C until they could be analyzed for Cu-Zn superoxide dismutase. The large intestine was inspected visually for tumors. If any were present, they were cut out, weighed, placed in formalin, and later examined by light microscopy. All tumors removed in this study were judged to be adenocarcinoma based on standard criteria (13).

Shortly after sacrifice, a small aliquot of whole blood was analyzed for hemoglobin using a kit from Sigma Chemical Co., St. Louis, MO. The remainder of the blood was centrifuged to separate out the plasma, which was stored frozen at −20°C until it could be analyzed for extracellular superoxide dismutase and ceruloplasmin.

Enzyme Assays. Ceruloplasmin was determined by oxidation of *o*-dianisidine (14). Units were arbitrarily designated as the change in absorbance at 540 nm × 100. Liver Cu-Zn and plasma extracellular superoxide dismutase activity was assayed using the modified pyrogallol method described previously (4). Mn superoxide dismutase activity in liver samples was inhibited by treatment of liver homogenates with ethanol:chloroform.

Statistical Analysis. Cu status assessor data were compared using one-way analysis of variance followed by least significance difference analysis (15). The effects of DMH on weight gain were evaluated by unpaired Student's *t* test. Tumor formation was assessed using the Mantel-Haenszel χ^2 test (15).

Results

Low, but not marginal, Cu intake produced measurable effects on Cu status (Tables I and II). Low intake caused low body weight, a slight anemia, and a small degree of cardiac hypertrophy. Plasma ceruloplasmin activities were low, but detectable. Liver Cu-Zn and plasma extracellular superoxide dismutase activities were lower in rats fed the low Cu than in rats fed adequate Cu levels. Rats fed marginal Cu did not show any significant differences from rats fed adequate Cu for any of the parameters in Tables I and II.

The number of tumors in the DMH-treated rats fed low Cu was significantly higher than that for rats consuming either of the other two Cu levels (Table III). The same was true for total numbers of tumors per dietary group. The average tumor mass in rats fed low Cu exceeded those for the other groups. Tumor mass could not be compared statistically because too few

Table I. Effects of Cu Intake on Parameters Related to Cu Deficiency[a]

Cu intake	Body wt (g)	Hemoglobin (mg/dl)	Cholesterol (mg/dl)	Heart wt/ body wt (%)
Adequate	503 ± 43	15.1 ± 0.5	78 ± 6	0.32 ± 0.01
Marginal	527 ± 13	15.3 ± 0.2	85 ± 19	0.29 ± 0.03
Low	442 ± 32[b]	12.8 ± 0.2[b]	91 ± 11[b]	0.41 ± 0.03[c]

[a] Values are the means ± SD from five solvent-treated rats.
[b] Significantly different from adequate value at $P < 0.05$ (analysis of variance + least significance difference).
[c] Significantly different from adequate value at $P < 0.01$ (analysis of variance + least significance difference).

Table II. Effects of Cu Intake on Cu Metalloenzyme Activities[a]

Cu intake	Superoxide dismutase		Plasma ceruloplasmin (units/ml)
	Plasma (units/ml)	Liver Cu-Zn (units × 10^{-3} g)	
Adequate	97 ± 17	19.4 ± 0.9	42 ± 11
Marginal	95 ± 8	19.2 ± 1.9	54 ± 8
Low	39 ± 5[b]	10.8 ± 1.4[b]	3 ± 1[b]

[a] Values are the means ± SD from five solvent-treated rats.
[b] Significantly different from adequate value at $P < 0.01$ (analysis of variance + least significance difference).

Table III. Effects of Cu Intake on DMH-Induced Tumorigenesis

Cu intake	Rats with tumors	Total tumors	Average tumor mass
Adequate	1/10	2	0.29
Marginal	1/10	1	0.49
Low	5/11[a]	7[a]	1.02 ± 0.9

[a] Significantly different from other groups at $P \leq 0.05$, Mantel-Haenszel χ^2 test.

Table IV. Effects of DMH on Body Weights[a]

Cu intake/treatment	Body wt (g)			
	At injection no.			At sacrifice
	1	6	12	
Adequate				
+ Solvent	266 ± 24	363 ± 27	430 ± 34	503 ± 43
+ DMH	277 ± 17	374 ± 31	427 ± 38	498 ± 51
Marginal				
+ Solvent	275 ± 13	377 ± 14	452 ± 14	527 ± 13
+ DMH	267 ± 16	349 ± 25[b]	402 ± 27[c]	477 ± 27[c]
Low				
+ Solvent	230 ± 27	318 ± 33	377 ± 33	442 ± 32
+ DMH	251 ± 16	327 ± 24	378 ± 30	444 ± 30

[a] Values are the means ± SD for five to six rats for the solvent treatments and 10 to 11 rats for the DMH treatment. Rats were weighed 5 days after each injection. Analysis of data from just the solvent-treated animals showed that low copper intake produced body weights that were significantly lower at each time point than those for the other two copper intakes (analysis of variance + least significance difference).
[b] Significantly different from solvent treatment, same Cu intake ($P < 0.05$, unpaired Student's t test).
[c] Significantly different from solvent treatment, same Cu intake ($P < 0.01$, unpaired Student's t test).

tumors were found in rats fed either the adequate or marginal Cu. However, it should be noted that the mass of four of the seven tumors in the low Cu group exceeded the largest tumor found for the other two dietary groups.

The effects of low Cu feeding on DMH-induced tumor formation could not be attributed to high degrees of DMH toxicity as reflected by impaired weight gain (Table IV). Inexplicably, marginal rats given DMH consistently showed lower weights than marginal rats given the solvent alone.

Discussion

Low Cu intake, in the study design used here, produced only a moderate Cu deficiency, but caused high susceptibility to DMH-induced colon tumorigenesis. The deficiency is called moderate for several reasons. First, the anemia was slight (Table I). Second, the degree of cardiac hypertrophy (Table I) was much less than that reported elsewhere (i.e., 16). Third, ceruloplasmin activity toward *o*-dianisidine, usually undetectable in Cu-deficient rats (i.e., 17), gave a small reading (Table II). Fourth, the superoxide dismutase activities (Table II) were a higher percentage of control than obtained previously by this laboratory for Cu-deficient rats (4, 6, 7).

The mechanism responsible for low Cu feeding effects on DMH-stimulated tumorigenesis was not elucidated in the present study. Low activities of Cu antioxidant metalloenzymes could be responsible because some such activities were found to be low (Table II). However, further study is needed to elucidate which, if any, Cu antioxidant enzyme activities are actually involved.

The effects of low Cu intake on tumorigenesis seen in this study occurred with a relatively mild DMH treatment. Other studies on DMH have produced a greater carcinogen stress by using more DMH injections and higher dietary fat levels (i.e., 8, 18, 19). Also, the higher than normal tumor incidence seen here for the deficient rats occurred despite slow growth rates. Conceivably, the slow growth rates could have inhibited tumor growth. This would not be a factor in marginally deficient rats with low copper enzyme activities, but normal growth. In general, the differences in tumorigenesis found here when comparing low and adequate Cu intake might be increased or diminished with more severe DMH treatments and varying severities of copper deficiency.

Unfortunately, the effects of marginal Cu status on DMH-induced colon carcinogenesis could not be evaluated in the present study. Although some rats were fed marginal Cu levels, these rats showed no evidence of marginal Cu status (Tables I and II). This was likely a result, at least to a large extent, of group housing. Marginal Cu feeding to group-housed, weanling rats produces much less of an effect on Cu status than the same Cu intake by singly housed rats (4). Possibly, in the present study, even this minimal effect on Cu status was eliminated by an adaptation in Cu retention during the long-term feeding of the marginal Cu diet. Future experiments should be carried out to determine the effects of DMH on colon carcinogenesis in singly housed rats fed marginal amounts of Cu.

Unlike selenium-deficient rats (19), rats fed low Cu did not show a toxic reaction to DMH that resulted in impaired weight gain. Thus, the effect of low Cu intake on DMH carcinogenesis did not likely result simply from a generally weakened condition. Surprisingly, rats consuming marginal Cu, despite showing no signs of poor Cu status nor high susceptibility to DMH-promoted tumorigenesis, showed some impairment of weight gain with DMH treatment (Table IV). The reason for this impairment is unclear.

In conclusion, rats showing low activities of Cu metalloenzyme activities showed high susceptibility to DMH-induced colon cancer.

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