Beer mitigates some effects of copper deficiency in rats[1-4]

Leslie M Klevay and Robert J Moore

ABSTRACT Because of an epidemiologic association of decreased risk of death from ischemic heart disease with moderate use of alcoholic beverages, and because numerous abnormalities found in people with ischemic heart disease are also found in animals deficient in copper, rats were fed a diet deficient in copper and were given either beer or water to drink. Rats drinking beer lived nearly six times as long and had lower plasma cholesterol, less cardiac enlargement, and higher liver copper. Apparent absorption and biological half-life of oral radiocopper were increased by beer. The effects were not attributable to alcohol, chromium, or copper in beer. Beer intakes were similar to those of some people in the United States. Results may explain seasonal cycles in plasma cholesterol and may be germane to the epidemiology of ischemic heart disease because diets in the United States seem to be low in copper. *Am J Clin Nutr* 1990;51:869-72.

KEY WORDS Beer, cardiac enlargement, cholesterol, copper absorption, copper deficiency, ischemic heart disease, seasonal cholesterol cycles, ventricular aneurysm

Introduction

The origin of ischemic heart disease, the leading cause of death in the industrialized world, remains obscure. It has been suggested that copper deficiency or abnormal metabolism of copper is of prime importance in the etiology and pathophysiology of this disease (1-4). Nearly 50 anatomical, chemical, and physiological similarities between people with ischemic heart disease and animals deficient in copper have been identified (3, 4). The most important of these similarities are glucose intolerance, hypercholesterolemia, hyperuricemia, abnormal electrocardiograms, being male, and hypertension. Dietary copper seems to be a more powerful determinant of cholesterolemia than are other agents (5). There is evidence associating hypercholesterolemia (6, 7), glucose intolerance (8), abnormal electrocardiograms (6, 9), and increased blood pressure (10) in people fed diets low in copper.

Moderate intakes of alcoholic beverages are often associated with decreased risk of death from ischemic heart disease or coronary artery occlusion (11-20). Perhaps the apparent benefits of alcoholic beverages can be explained by hidden variables (21) or associated advantageous characteristics (22). Sometimes fewer deaths from heart disease are balanced by more deaths from other causes (16). Although the types of alcoholic beverages are not usually itemized, sometimes beer seems preeminent (12, 14, 20). The objective of this study was to test the hypothesis that drinking beer could have favorable effects on rats fed a diet deficient in copper.

Materials and methods

Male weanling rats (Sprague-Dawley, Indianapolis) were fed a diet with 62% sucrose, 20% egg white, and 10% corn oil (23). Because this diet is deficient in both copper and zinc, although adequate in biotin (24), finely ground zinc acetate was added to increase dietary zinc by 13 mg/kg of diet. The amount of vitamin A is lower than that in earlier experiments (25) but it is still more than adequate. In the first four experiments two groups of 15 rats were matched by weight (51 g overall mean); in the fifth experiment the mean weight was 55 g. In the first three experiments half the rats were given demineralized water (Super Q System, Millipore Corp, Bedford, MA) to drink and half were given beer. In the fifth experiment 15 rats were given water and 19 were given beer. In the fourth experiment beer was diluted with an equal volume of demineralized water before serving. Beer (Budweiser, Anheuser Busch, St Louis) was purchased in quarts, which were opened and allowed to stand at room temperature overnight to minimize foaming. Animals were housed under standard conditions (26); experiments were done according to the guidelines of the National Research Council. Cholesterol in plasma was measured by fluorescence (27) in experiments 1-3 and by an enzymatic method (Sigma Chemical Co, St Louis) in experiment 5. Copper, zinc, and iron in organs and diet were measured by atomic absorption after oxidation of organic matter with nitric and sulfuric acids and hydrogen peroxide (28). Plasma was diluted 1 to 5 with water for measurement of zinc with flame atomic absorption spectrometry, or with a half volume of nitric acid for the measurement of copper by graphite furnace. After the first death occurred in experiment 3, organs were obtained from the remaining rats while they were under pentobarbital anesthesia.

The absorption and retention of ^{67}Cu were measured accord-

[1] From the United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND.

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[3] Presented in part at the 6th International Symposium on Trace Elements in Man and Animals, Monterey, CA, June 1987, and at the 73rd Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, March 1989.

[4] Address reprint requests to LM Klevay, USDA, ARS, Grand Forks Human Nutrition Research Center, PO Box 7166, University Station, Grand Forks, ND 58202.

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TABLE 1
Longevity*

	Median	80% dead
	d	*d*
Experiment 1		
Beer	204	428
Water	62	103
Experiment 2		
Beer	299	498
Water	42	74
Experiment 4		
Beer	303	435
Water	44	207

* After weaning and travel.

ing to the method of Lukaski et al (29) in experiment 5. On the 30th day of the experiment, after a 12-h fast, rats were given a pellet of ~1 g of diet (at 0700), which contained ~120 kBq ^{67}Cu (as chloride; Los Alamos National Laboratory, Los Alamos, NM). Then rats were fed after 2 h, and an initial whole-body count was obtained 3.5–4.5 h after administration of the radioactive copper. Additional counts were obtained daily for 13 d and were corrected for background and radioactive decay.

Means were compared by *t* test (30) and data on aneurysms by the significance of difference in proportions (31). Limited necroscopy was done in experiments 1, 2, 4, and 5.

Experiments 1, 2, and 4 were studies of mortality and gross pathology. Experiment 3 was directed toward chemical analysis of plasma and organs. Retention of radiocopper was measured in experiment 5.

Results

There was no difference in growth in any experiment at the time of first death (24–35 d). Overall, median longevity was increased nearly sixfold and the time at which 80% of rats drinking beer had died was increased more than fourfold over values for rats drinking water (**Table 1**) in experiments 1, 2, and 4.

Generally, drinking beer was associated with lower plasma cholesterol (**Table 2**). Cholesterol in plasma correlated inversely with copper in liver ($r = -0.81$, $n = 28$, $p = 0.0001$) in experiment 3. Hematocrits for all groups of rats in experiments 1 and 2 were < 0.33; beer was without effect. Drinking beer was associated with a slightly higher hematocrit in experiment 5 ($p < 0.03$).

TABLE 2
Cholesterol in plasma*

Experiment	Beer	Water	*p*
	mmol/L		
1	2.64 ± 0.10	2.61 ± 0.08	NS
2	2.53 ± 0.11	3.70 ± 0.37	<0.02
3	2.20 ± 0.16	2.77 ± 0.09	<0.005
5	2.29 ± 0.17	3.47 ± 0.27	<0.01

* $\bar{x}$ ± SEM. Measurements were made after 4–8 wk. In experiments 1, 2, and 5 earlier differences were not significant. The number of measurements varied from 5 to 14 for water drinkers and 13 to 14 for beer drinkers.

TABLE 3
Organ analyses in experiment 3*

	Beer	Water	*p*
	μg/g dry wt		
Liver			
Copper	3.91 ± 0.49	1.34 ± 0.17	0.0001
Iron	910 ± 25.0	846 ± 33.7	>0.1
Zinc	73.0 ± 1.7	67.0 ± 1.6	<0.02
Heart			
Copper	4.92 ± 0.26	4.19 ± 0.20	<0.035
Iron	262 ± 6.9	228 ± 6.2	0.001
Zinc	73.9 ± 1.5	77.8 ± 0.5	<0.03

* $\bar{x}$ ± SEM. n = 14 in each group.

Ventricular aneurysms were found in 6 of 15 rats drinking beer and in 11 of 15 rats drinking water by the time that 80% of the former were dead in experiment 1 ($p = 0.066$); results for experiment 2 were 6 of 12 vs 14 of 15 ($p = 0.0108$). In experiment 3 hearts of rats drinking beer (1.12 ± 0.04 g, $\bar{x}$ ± SEM) were smaller than those of rats drinking water (1.41 ± 0.07 g), $p < 0.003$. Beer had no effect on aneurysm frequency in experiments 4 and 5. Dietary copper in these experiments ranged from 0.74 to 0.93 mg/kg. Copper in beer was 25 μg/L.

Data on organ analyses are shown in **Table 3.** Liver copper was increased approximately threefold in rats that drank beer in experiment 3 and ~50% in experiment 5. Liver zinc was increased ~9% and liver iron was unchanged in experiment 3. Copper and iron were increased and zinc was decreased in the hearts of rats drinking beer.

Rats drinking beer had 11.5 μmol zinc and 201 nmol copper, and those drinking water had 12.5 μmol zinc and 289 nmol copper/L plasma in experiment 3. The concentration of neither element was affected by treatment ($p > 0.1$).

The apparent copper absorption of rats drinking beer was greater than that of rats drinking water (**Table 4**). Biological half-life also was increased by the drinking of beer.

Discussion

Both beer and half-strength beer increased longevity of rats fed a diet deficient in copper. Tests of significance were omitted as suggested by Hill (32) because results were "grotesquely obvious." The hypothesis was tested successfully. This increase in longevity generally was associated with a number of changes in the rats, which appear to be related to copper metabolism. Copper deficiency in these experiments was verified by the presence of anemia, low copper in liver and plasma, and ven-

TABLE 4
Apparent absorption and biological half-life of ^{67}Cu for experiment 5*

	Beer	Water	*p*
Apparent absorption (%)	38 ± 4.1	27 ± 4.7	0.08
Biological half-life (d)	46 ± 4	29 ± 4	<0.02

* $\bar{x}$ ± SEM. There were 13 water drinkers and 15–17 beer drinkers.

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tricular aneurysms. Aneurysms occurred frequently in similar experiments (33–37).

The major effect of beer on trace elements in heart and liver was a threefold increase in liver copper concentration in experiment 3. Other organ changes were < 18%. Even the higher liver values associated with beer, 3.9–5.1 μg/g, were substantially lower than the 10–13 μg/g found when similar diets were supplemented with copper (34, 35, 38). Copper in plasma was ~1% of normal (38) and was unaffected by beer. Fewer analyses were done in experiment 5; liver copper was higher in rats drinking beer. In experiment 5 liver copper was increased slightly ($p = 0.06$) by beer (5.12 ± 0.43 vs 3.52 ± 0.63 μg/g dry wt, $n = 13$ and 6, respectively). Liver zinc was unchanged at 75.4 and 72.7 μg/g dry wt, respectively.

Cardiac enlargment in copper deficiency has been found many times (eg, 39, 40); however, hearts of rats drinking beer were ~20% lighter. The increases in cardiac copper and iron were similar in size and may be attributed to the smaller hearts. In contrast, cardiac zinc decreased.

The generally lower concentration of cholesterol in plasma of rats drinking beer may be related to concomitant increases in liver copper. Cholesterol in plasma often correlates inversely with liver copper (38, 41–43) or copper in liver microsomes (43, 44). This correlation was confirmed. Perhaps the greater absorption and retention of copper by rats drinking beer was associated with a greater conservation of dietary copper and/or favorable redistribution of copper that produced higher liver copper.

The mechanism by which beer produced these benefits is unknown. There is insufficient copper in beer to account for these changes. Because growth rates were similar, rats drinking beer probably consumed less copper than rats drinking water because dietary copper was ~30-fold higher than beer copper (per kilogram or per liter). The effects probably are not caused by either alcohol or chromium. In similar experiments (LM Klevay, unpublished observation, 1986) with 4% ethanol in water, a concentration similar to that in beer (45), no deaths occurred by the 26th day, two deaths occurred in each group by the 34th day, and six alcohol drinkers and seven water drinkers were dead by day 41. Chromium (5 mg/L as triacetate) also did not improve longevity and may have been harmful.

The rats that drank beer satisfied their water requirements. In experiment 4 half of the water requirement was satisfied by beer. The average human adult weighing 70 kg must drink ~1.5 L water/d when sweating does not occur (46). In 1986 178 million barrels of beer were produced in the United States (47), ~0.350 L/d per person aged > 21 y (48). On consideration of the adult water requirement and that some adults do not drink beer, it seems likely that some other adults come as close to satisfying their water requirements with beer as the rats in these experiments.

Approximately a dozen chemicals have been found to reciprocally alter the metabolism of cholesterol and copper (5, 37, 42, 49). This duality of action prompted the suggestion that a new class of chemicals, both cholesterotropic and cuprotropic, has been identified (42, 49). The origin of the suffix "tropic" is from the Greek τροπ, which indicates a turning in direction in response to a stimulus. In this sense the chemicals are the stimuli that change the directions of cholesterol and copper. Some of these chemicals, eg, aspirin and clofibrate, decrease plasma cholesterol and enhance copper metabolism. Although beer is a complex mixture, it shares some properties with these chemicals.

According to Gordon et al (50), "cyclic seasonal variation in circulating cholesterol levels has been studied . . . during the past 60 years" and "many fundamental questions about its origin and mechanisms remain unanswered." Total plasma cholesterol was 0.19 mmol/L lower at the end of June than at the end of December (50). For the 3 y ending in 1986, 27% more beer was produced in April, May, and June than in October, November, and December (47). Perhaps greater consumption of beer in warmer months contributes to the lowering of cholesterol. Perhaps this lowering of cholesterol occurs in people who habitually eat too little copper.

Daily intakes of copper by adults in the United States generally do not reach the estimated safe and adequate intakes of the National Research Council (46). The probability that < 75% of all daily diets contain 2 mg of copper and 80% of diets contain < 1.5 mg was suggested (2). A more recent estimate based on 10 surveys in which dietary copper was measured by chemical analysis reveals that only 14% of all daily diets contain > 2 mg and 35% contain < 1 mg (4). Daily intakes of < 1 mg have been found insufficient for volunteers in depletion experiments (6–10). Perhaps beer mitigates the effects of human diets low in copper.

The great variety of alcoholic beverages available complicates the epidemiology of ischemic heart disease. Some authors (13–15, 20–22) related mortality to the total amount of alcohol or alcoholic beverages consumed from all sources. Because the effect of beer in these experiments cannot be attributed to alcohol, we converted the amounts of alcohol associated with lower mortality into amounts of beer. We assume no other alcoholic beverages were drunk in this conversion.

In the United States the standard unit of beer is the bottle or can (0.354 L). Colditz et al (19) found that consuming < two-thirds of a can of beer per day was effective in reducing mortality; Gordon and Kannel (21) found the necessary dose exceeded two-thirds to one and one-third of a can per day. Shaper et al (22) noted the efficacy of one can per day and both Hennekens et al (13) and Turner et al (14) agreed that the amount should be less than four cans per day.

Beer has been brewed for at least 8000 y (51), perhaps originating in Mesopotamia (52). Katz and Voigt (52) hypothesize that the desire for beer led to the domestication of wheat and barley. This early example of food technology might have provided an evolutionary advantage with water free of pathogens and a means of preserving nutrients. Perhaps there still are advantages in drinking beer.

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