ADVANCES IN DIET AND NUTRITION

EDITOR
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These studies may not conform to peer review standards. Therefore, the results are not conclusive.

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MECHANISM AND EFFECT OF EXCESS COPPER SUPPLEMENTATION ON BODY LIPIDS

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Introduction

Copper is an essential trace element in animals and man. Animals deficient in cop have hypercholesterolemia, hypertriglyceridemia, anatomical and functional change.. .t the heart and arteries (Allen & Klevay, 1978*a*; Klevay & Viestenz, 1979; Davis, 1976). Recent work also suggests that humoral-mediated immunity is severely impaired in copper-deficient mice (Prohaska & Lukasewuvz, 1981).

Human studies (Klevay *et al.*, 1980*b*), indicate that healthy male adults require 1.3 mg of copper/day to maintain balance, yet few self-selected diets have been found to contain more than 1 mg copper (White, 1969; Holden, Wolf & Mertz, 1979), and it has been hypothesized that copper deficiency (and its associated increase in blood cholesterol level) may contribute to the risk of heart disease. We conducted a series of preliminary investigations to address the question of whether excess copper supplementation might reduce the risk of coronary heart disease.

Results and discussion

A bioavailability study, using male Sprague-Dawley rats, indicated that a yeast (high copper yeast : Grow Company) was the best absorbed copper source in both liver and blood. We then examined the effect of copper supplementation on weight gain and body lipids in rats. A control group was fed normal laboratory rat chow (Ralston-Purina Company) sufficient in copper, and an experimental group fed the same diet to w' copper gluconate (20 ppm) had been added. Supplementation occurred for 4 mon. , after which body lipids were measured in both groups by an outside laboratory, using an enzyme technique. While there was no significant difference in the average weight gain, the excess copper gluconate appeared to have a significant effect on serum cholesterol and liver cholesterol (Table 1). The elevation in the latter appeared to be facilitated by stimulation of the HDL portion of cholesterol. Copper gluconate also lowered serum triglycerides, but not significantly so.

We investigated these findings further, using two groups of male weanling rats, and a new commercial yeast which had been found to be the most bioavailable form of copper. The experimental group received normal rat chow with a 20 ppm copper yeast supplement over 2 months, the control group a normal diet sufficient in copper. Again there was no significant difference in weight gain, but there was a significant decreas'

Table 1. *The effect of copper gluconate supplementation for 4 months on weight and body lipids of mature rats.*

Measurement	*Control group*	*Experimental group*	
Weight before supplementation, g	460 ± 75	435 ± 63	n.s.
Weight after supplementation, g	582 ± 69	550 ± 84	n.s.
Serum cholesterol after supplementation, mg/dl	277 ± 276	144 ± 131	$P < 0.05$
Serum high density cholesterol after supplementation, mg/dl	13 ± 10	18 ± 21	n.s.
Serum triglycerides after supplementation, mg/dl	176 ± 143	135 ± 12	n.s.
Liver triglycerides after supplementation, mg/dl	1045 ± 185	896 ± 370	n.s.
Liver cholesterol after supplementation, mg/g	2.8 ± 1.1	6.8 ± 4.1	$P < 0.05$

Table 2. *The effect of copper yeast supplementation for two months on weight and serum lipids of weanling rats.*

Measurement	*Control group*	*Experimental group*	
Weight before supplementation, g	49 ± 5	50 ± 8	n.s.
Weight after supplementation, g	254 ± 3	255 ± 3	n.s.
Serum cholesterol after supplementation, mg/dl	42 ± 6	34 ± 9	$P < 0.05$
Serum high density cholesterol after supplementation, mg/dl	22 ± 4	33 ± 3	$P < 0.001$
Serum triglycerides after supplementation, mg/dl	116 ± 6	89 ± 15	$P < 0.01$

serum cholesterol and triglycerides, and significnt rise in HDL cholesterol (Table 2). The ratio of cholesterol:HDL cholesterol (an indicator for risk of heart disease in mans) was 1.9 and 1.0 in the control and experimental groups respectively. The copper yeast supplement therefore had a significant effect on the cholesterol levels, and may be of potential benefit in protecting against heart diseae. In order to confirm our findings, we conducted a mechanism study to determine the effect of excess copper supplementation on cholesterol synthesis. Ten groups of five rats were used, the control groups receiving a diet sufficient in copper (*ie* supplemented to the minimum required level of 5 ppm copper), and the experimental group received a diet supplemented with copper yeast at 30 ppm. After 2 months the rats were fasted for 24 h, and injected

Table 3. *Plasma cholesterol and specific activities of lipids in rats fed copper yeast for 2 months followed by injection of C^{14}-mevalonate.*

Measurement	*Control group*	*Experimental group*	
Plasma total cholesterol, mg/dl	64 ± 9	70 ± 16	n.s.
Plasma high density cholesterol, mg/dl	41 ± 8	53 ± 12	$P < 0.1$
Specific activity of plasma total lipids, cpm/ml	984 ± 211	650 ± 181	$P < 0.05$
Specific activity of plasma free cholesterol, cpm/ml	439 ± 123	243 ± 58	$P < 0.05$
Specific activity of plasma cholesterol sters, cpm/ml	504 ± 143	349 ± 128	$P < 0.1$

subcutaneously with 4 μCi of C^{14}-mevalonate 4 h before sacrifice. Mevalonate is an obligatory intermediate in cholesterol synthesis and is formed from 3-hydroxy-3-methylglutarate and is the principal control point in cholesterol biosynthesis. Thus C^{12} incorporation provides a measure of the net influx to and efflux from the procedure of Allen & Klevay (1978*b*), and the results are shown in Tables 3 and 4. There is an interesting parallel with previous copper deficiency rat studies, with results from our copper sufficient : copper-excess groups being comparable to those from copper-deficient : copper-sufficient groups.

Table 4. *Liver cholesterol and specific activities of lipids in rats fed copper yeast for 2 months followed by injection of C^{14}-mevalonate.*

surement	*Control group*	*Experimental group*	
Liver total cholesterol, mg/dl	97 ± 54	148 ± 15	$P < 0.01$
Specific activity of liver total lipids, cpm/g	3797 ± 999	3499 ± 481	n.s.
Specific activity of free cholesterol, cpm/g	1579 ± 163	1029 ± 196	$P < 0.005$
Specific activity of liver cholesterol esters, cpm/g	219 ± 65	280 ± 53	n.s.

From this study and the previous copper gluconate study (Table 1) it appears that excess copper supplementation significantly raises liver total cholesterol. This is a result of the greater concentration of HDL cholesterol in the blood of the experimental

groups, which transports cholesterol from the plasma to the liver for storage and ultimate excretion as bile acids. The radioactivity results (Table 4) are more difficult to explain but it appears that there is greater incorporation of radioactivity in liver free cholesterol (153 per cent, $P<0.005$) but less in liver cholesterol ester (78 per cent, n.s.) for the copper sufficient group as compared to the copper excess group. The seemingly contradictory results from free versus esterified cholesterol may be the result of the time period chosen for analysis after injection of C^{14}-mevalonate, as Klevay found the same anomaly.

A preliminary study was undertaken to investigate the effect of excess copper supplementation on body lipids in humans. Eight healthy volunteers (four males and four females) were selected, aged from 20 to 61 years (average 39±14 years). Each subject took a daily supplement of 2 mg copper (four 0.5 mg copper gluconate tablets), which is the recommended daily intake of the Food & Nutrition Board of the National Academy of Sciences (National Research Council). Each subject had a fasting blood drawn before each supplementation period and the serum was analyzed for cholesterol and triglycerides by an enzymatic technique. Serum lipids were measured 1, 2, 4 and 6 months after supplementation (Table 5). There was a large increase in serum

Table 5. *The effects of copper supplementation at 2 mg/day on human serum cholesterol and triglycerides.* Significance was determined by a paired *t*-test and the data compared to the results before supplementation.

Time	*Serum cholesterol (mg/dl)*		*Serum triglycerides (mg/dl)*	
Before supplement	206 ± 29		118 ± 64	
1 month of supplement	194 ± 41	n.s.	98 ± 19	n.s.
2 months of supplement	237 ± 35	$P < 0.1$	188 ± 122	n.s.
4 months of supplement	190 ± 33	n.s.	117 ± 58	n.s.
6 months of supplement	182 ± 54	n.s.	116 ± 47	n.s.

cholesterol and triglycerides after 2 months of copper supplementation although only the former was significant ($P<0.1$). One possible hypothesis advanced is that the copper facilitated removal of atherosclerotic plaque from the arteries of volunteers, and animal studies are currently in progress to test this hypothesis. After 6 months, the serum cholesterol was lower than before supplementation, but not significantly so. A larger human study, using the increased bioavailability of copper yeast is now planned.

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