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Bioavailability of Copper to Rats from Various Foodstuffs and in the Presence of Different Carbohydrates (42635)

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Abstract. Copper bioavailability was studied in rats using an extrinsic ^{67}Cu label. Copper absorption from sunflower seeds (46%), peanuts (41%), cooked shrimp (50%), and cooked beef (40%) was as good or better than copper sulfate (46%). Copper from plant foods (sunflower seeds, garbanzo beans, peanuts) was absorbed equally as well as copper from animal foods (beef, shrimp, chicken liver), 39 ± 7% vs 43 ± 7%, $P > 0.05$. There was no significant difference in percentage Cu absorption between intrinsically labeled chicken liver and extrinsically labeled chicken liver. In a second experiment, Cu absorption was measured in the presence of glucose, fructose, sucrose, or cornstarch. There were no significant differences in Cu absorption due to different carbohydrates in a single meal.

Copper is widely distributed in foodstuffs, and until recently, it was thought that most human diets were adequate in copper content. The estimated safe and adequate daily intake of Cu is 2–3 mg/day (1), but there is some evidence that copper intake from self-selected diets in the U.S. may fall below 1 mg/day (2). In New Zealand, half of the diets studied provided less than 2 mg Cu/day (3). The availability of dietary copper for absorption is probably a crucial determinant in the copper adequacy of the diet.

There are few data on the absorption of copper or the bioavailability of copper from foods. Lo *et al.* showed that copper is equally available to rats from soy protein or copper carbonate using a slope-ratio, depletion-repletion assay (4). About 26% of the copper in whole wheat flour was absorbed by rats (5). Copper bioavailability from raw meat is apparently poor, because rats fed raw beef developed copper deficiency (6), while rats fed cooked beef did not. Copper availability from milks and milk-based infant formulas is better than from soy-based infant formulas (7). It has been reported that the severity and rate of development of copper deficiency in rats are related to the carbohydrate component of the diet (8–12). This could be caused by a difference in bioavailability of copper in the presence of different carbohydrates, or by differences in excretion of copper when different carbohydrates are fed. Fields *et al.* (13) have reported that Cu absorption seems to be impaired in rats fed fructose compared to rats fed starch for 5 weeks. The studies reported here were designed to examine the availability of copper from various foods relatively high in copper, using an extrinsic ^{67}Cu label, to compare absorption of copper from intrinsically and extrinsically labeled liver, and to examine the effect on copper bioavailability of various carbohydrates in a single meal.

Methods: Experiment 1. Weanling female Long–Evans rats were bred at the Grand Forks Human Nutrition Research Center (GFHNRC). At 21–25 days of age they were placed in individual cages, given demineralized water, and fed a diet containing 2.5 ppm added Cu and 20 ppm Zn but otherwise based on a modified AIN-76 purified diet (14) (Table I). The analyzed Cu content of the diet was 2.6 ppm. After 1 week rats were assigned to experimental groups of seven rats, based on their weight. After 16 days they were fasted overnight (18 hr) and fed test meals containing the foods of interest labeled with ^{67}Cu. Test meals (Table II) were formulated to contain 20 μg of Cu; each was mixed with 3 μCi ^{67}Cu, except the intrinsically labeled chicken liver meals, which had an activity of 1 μCi each. After 5 hr, any test meal remaining was weighed. Within 6 hr

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TABLE I. BASAL DIET, EXPERIMENT 1

Ingredient[a]	g/kg
1. Casein	200.0
2. Cellulose	50.0
3. AIN-76 custom mineral mix[b]	35.0
4. AIN-76 vitamin mix	10.0
5. Choline chloride	1.5
6. D,L-methionine	3.0
7. Zinc mix[c]	1.0
8. Copper mix[d]	0.5
9. Sucrose	649.0
10. Corn oil	50.0

[a] All ingredients were from Teklad (Madison, WI) except sucrose and corn oil, which were purchased locally.
[b] Custom: zinc and copper free; difference made up in glucose.
[c] Zinc mix: 24.9 mg ZnO, 975.1 mg sucrose/g mix.
[d] Copper mix: 19.6 mg $CuSO_4 \cdot 5H_2O$, 980.4 sucrose/g mix. Analyzed trace mineral content of diet: 2.6 ppm Cu, 30.9 ppm Zn, 41.9 ppm Fe.

after the test meal had been presented, animals were assayed for ^{67}Cu consumption in a small animal whole body gamma counter constructed in this laboratory. They were counted daily thereafter for the duration of the experiment (13 days). Counts were corrected for ^{40}K contribution to the ^{67}Cu window, radioactive decay, coincidence loss, and electronic fluctuations. Absorption was determined as the *y* intercept of a regression on the linear portion of a plot of log percentage retention vs time.

Intrinsic labeling of chicken liver. A 6-month-old SIL-GO-LINK hen (Minnesota Hatchery, Dassel, MN)[2] was injected with 750 μCi ^{67}Cu. The isotope was diluted with saline to a volume of 1.50 ml, and 0.75 ml was injected subcutaneously near the hip joint of each leg. Five hours later the hen was killed and the liver was removed. Activity in the liver was approximately 15 μCi.

Preparation of test meals. All foods were purchased locally. Sunflower seeds were obtained from a local farm and roasted in a convection oven for 15 min at 163°C. Unsalted peanuts (K Mart) were purchased already roasted. Garbanzo beans were purchased dry and prepared by boiling. Shrimp was purchased precooked and frozen. Other foods were cooked in a microwave oven. Foods except peanuts and sunflower seeds were freeze-dried and all were ground to a powder. For sunflowers (F), garbanzo beans (G), peanuts (P), shrimp (S), beef (B), and extrinsically labeled liver (L-E), 30.5 μCi ^{67}Cu in 200 μl water was added to a small amount of the dried, ground test food, air-dried overnight, and mixed in an electric spice mill. Sufficient test food was then added to make 80 g of each test meal mixture (Table II), and mixing was continued. After addition of the sugar, the mixture was hand-mixed with a mortar and pestle for 10 min, oil was added (except group P), and mixing was continued for 10–15 min until a uniform consistency was achieved. The intrinsically labeled liver (L-I) test meals were mixed in the same way except that ^{67}Cu was already in the liver. For the control (C) group test meals, the ^{67}Cu was added to sucrose. Casein was added to the C test meals to provide protein. $^{67}Copper$ as $^{67}CuCl_2$ was obtained from Los Alamos National Laboratory. On the day of receipt it had a specific activity of 1.79 mCi/μg Cu.

Experiment 2. Weanling male Long–Evans rats were obtained from the GFH-NRC breeding colony. They were fed a diet containing equal parts of fructose, glucose, sucrose, and corn starch, and which contained 2.5 ppm added Cu (Table III). The analyzed Cu content of the diet was 3.7 ppm Cu. After 14 days rats were placed by weight in four groups of eight rats each. They were then fasted overnight (18 hr) and fed test meals (Table IV) containing one of the carbohydrates plus 25 μg of Cu as $CuSO_4 \cdot 5H_2O$ labeled with 3 μCi ^{67}Cu. At the end of 3 hr, any spilled food was weighed and rats were counted as described above. Four hours later they were given their regular diet *ad libitum* for the remainder of the study (13 days).

At the conclusion of the experiment, rats were anesthetized with sodium pentobarbital and killed via cardiac puncture. Livers were removed for Cu analysis. Ceruloplasmin activity in serum was measured by the method of Schosinsky *et al.* (15).

Preparation of test meals. Dry ingredients

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

TABLE II. COMPOSITION OF TEST MEALS, EXPERIMENT 1

Code	Food	Food Cu (ppm)[a]	Components of test meal: Test food (g)	Sucrose (g)	Corn oil (g)	Casein (g)	Analyzed Cu (ppm in meal)
F	Roasted sunflower seeds	22.1 ± 0.3	0.9	6.7	0.4		2.67 ± 0.05[a]
G	Cooked garbanzo beans	5.3 ± 0.1	3.8	3.8	0.4		2.93 ± 0.08
P	Roasted peanuts	6.7 ± 0.1	3.0	5.0	—		2.76 ± 0.03
S	Cooked shrimp	5.3 ± 0.1	4.1	3.5	0.4		2.61 ± 0.11
B	Cooked beef	3.3 ± 0.1	6.1	1.5	0.4		2.41 ± 0.04
L-E	Cooked chicken liver (extrinsic label)	13.3 ± 0.3	1.2	6.4	0.4		2.76 ± 0.03
L-I	Cooked chicken liver (intrinsic label)	[b]	1.2	6.4	0.4		1.83 ± 0.18
C	Copper sulfate control	—	0.004[c]	4.0	0.4	3.6	2.15 ± 0.95

[a] Mean of triplicate analyses.
[b] Not analyzed before test meal preparation.
[c] Cu mix: sucrose + $CuSO_4 \cdot 5H_2O$ to give 5 mg Cu/g mix.

(Table IV), except for the carbohydrate and the copper mix, were mixed together and used for all four test meal formulations. Carbohydrates were ground to fine powders and mixed with the Cu mix. Each Cu–carbohydrate mix was labeled with 30 μCi ^{67}Cu/batch and allowed to dry overnight. Each was then thoroughly ground in a mortar. Weighed amounts of each labeled Cu–carbohydrate mix were added to the dry ingre-

TABLE III. BASAL MIXED-CARBOHYDRATE DIET, EXPERIMENT 2

Ingredient[a]	g/kg
1. Casein	200.0
2. Cellulose	50.0
3. Choline chloride	1.5
4. D,L-methionine	3.0
5. AIN-76 custom mineral mix[b]	35.0
6. AIN-76 vitamin mix	10.0
7. Copper mix[c]	0.5
8. Corn oil	50.0
9. Sucrose[d]	159.0
10. Glucose	163.6
11. Fructose	163.6
12. Cornstarch	163.6

[a] All ingredients were from Teklad (Madison, WI) except glucose (ICN, Cleveland, OH), fructose (Sigma, St. Louis, MO); cornstarch (Argo), corn oil (Mazola), and sucrose were purchased locally.
[b] Custom: copper free.
[c] $CuSO_4 \cdot 5H_2O$ (19.6 mg) and 980.4 mg sucrose/g mix gives 2.5 ppm Cu in diet when added at 0.05%.
[d] Mineral mix provided 4.1 g sucrose and copper mix provided 0.49 g sucrose/kg diet so that total sucrose in the diet was 163.6 g/kg.

TABLE IV. COMPOSITION OF TEST MEALS, EXPERIMENT 2

	g/100 g	g/30 g batch
Dry ingredient mix		
Casein	66.8	
Cellulose	16.7	
Choline chloride	0.5	
D,L-methionine	1.0	
AIN-76 custom mineral mix (Cu free)	11.7	
AIN-76 vitamin mix	3.3	
Test meals		
Dry ingredient mix		8.985
Cu mix[a]		0.050
Corn oil		1.500
Carbohydrate[b]		19.465

[a] Containing 250 μg Cu as $CuSO_4 \cdot 5H_2O$; remainder as sucrose. Each test meal was 3.0 g of the 30-g batch; thus each test meal contained 25 μg Cu.
[b] Glucose *or* fructose *or* sucrose *or* cornstarch.

TABLE V. COPPER ABSORPTION FROM VARIOUS FOODS

Code	Food	Cu eaten[1] in test meal (μg)	% Cu absorption	Total Cu absorbed (μg)	% Bioavailability relative to $CuSO_4$[2]
F	Roasted sunflower seeds	$10.7 \pm 2.2^{b,c,d}$	$46 \pm 10^{b,c}$	$4.8 \pm 1.2^{b,c,d}$	100
G	Cooked garbanzo beans	$18.0 \pm 3.0^{e,f}$	30 ± 4^{a}	$5.3 \pm 0.6^{c,d}$	65
P	Roasted peanuts	$13.5 \pm 1.7^{d,e}$	$41 \pm 5^{a,b,c}$	5.6 ± 1.3^{d}	89
S	Cooked shrimp	$10.9 \pm 1.7^{b,c,d}$	50 ± 5^{c}	$5.5 \pm 1.1^{c,d}$	109
B	Cooked beef	$8.0 \pm 1.0^{a,b}$	$46 \pm 8^{b,c}$	$3.7 \pm 0.9^{a,b,c}$	100
L-E	Cooked chicken liver (extrinsic label)	$9.6 \pm 2.2^{a,b,c}$	$33 \pm 7^{a,b}$	$3.2 \pm 1.1^{a,b}$	72
L-I	Cooked chicken liver (intrinsic label)	5.9 ± 1.3^{a}	31 ± 5^{a}	1.8 ± 0.5^{a}	67
C	Copper sulfate	$11.0 \pm 1.9^{b,c,d,e}$	$46 \pm 6^{b,c}$	$5.1 \pm 0.9^{c,d}$	(100)

[1] By analysis.
[2] (% Cu absorption from food/% Cu absorption from Cu sulfate) × 100%.
[3] $N = 7$ for each group; mean± SD.
[a–e] Values in a column without a common letter differ significantly, $P < 0.10$.

dients. After all dry ingredients were mixed, corn oil was added and diets were mixed to a uniform consistency.

Mineral analyses. Test foods, diets, and liver samples were ashed with nitric acid and hydrogen peroxide (16). Copper analyses were done by atomic absorption spectrophotometry.

Statistics. Data were analyzed using analyses of variance followed by Scheffé contrasts (17).

Results. The amount of copper absorbed from the various foods in Experiment 1 is shown in Table V. The significance level used in Table V is $P < 0.10$; when Scheffé contrasts are performed on large sets of data, this is an appropriate confidence level to use with this test (17).

Differences in palatability of the test meals fed to the rats resulted in different intakes of Cu from the various meals. With the foods tested, there was no relationship between Cu intake in the test meal and the percentage Cu absorption ($r = -0.16$, $P > 0.05$) over the range of Cu intake shown in Table V.

Copper from sunflower seeds, peanuts, cooked shrimp, and cooked beef was absorbed as well as or better than copper sulfate. There was no difference in percentage Cu absorption between plant foods (groups F, G, P), 39 ± 7%, and animal foods (groups S, B, L-E), 43 ± 7%. Copper from beef (B) was absorbed better than copper from intrinsically labeled chicken liver (L-I), and Cu from shrimp (S) was absorbed better than Cu from either liver group (L-I, L-E). There was no difference in percentage Cu absorption between extrinsically labeled chicken liver and intrinsically labeled chicken liver.

The results of Experiment 2 are shown in Table VI. There were no differences among groups in percentage Cu absorption or total μg Cu absorbed. Intake of Cu in the test meal was slightly lower for the fructose group than for the glucose or cornstarch groups. Copper status for all groups was the same. Liver copper, serum ceruloplasmin, and serum copper measured at the end of the experiment did not differ among groups (data not shown).

Discussion. Lonnerdal *et al.* (7) used 14-day-old suckling rats to assess Cu bioavail-

TABLE VI. ABSORPTION OF Cu FROM MEALS CONTAINING DIFFERENT CARBOHYDRATES

Carbohydrate	Cu eaten, test meal (μg)	Cu absorption (%)	Total Cu absorbed (μg)
Fructose ($N = 7$)	27.6 ± 1.9^{a}	38.4 ± 9.1	10.5 ± 2.0
Glucose ($N = 8$)	29.8 ± 0.3	39.7 ± 6.6	11.8 ± 2.0
Sucrose ($N = 8$)	29.1 ± 1.4	34.0 ± 5.0	9.9 ± 1.5
Cornstarch ($N = 8$)	30.1 ± 0.2	40.3 ± 7.2	12.1 ± 2.2

[a] Significantly different from glucose and cornstarch groups, $P < 0.05$.

ability from milks and infant formulas. They found that adult rats (of unspecified age) were less sensitive to differences in Cu bioavailability than suckling pups. While the suckling pup is clearly an appropriate model for investigating the bioavailability of Cu from infant foods, it is probably not appropriate to use suckling animals, with their immature intestinal tracts, to study foods not usually consumed by infants. The rats in the present study were fed test meals within 2 weeks of weaning, and were probably younger than the adult rats used by Lonnerdal *et al.* (7) or Lo *et al.* (4). Because they did discriminate among Cu sources under the conditions of the present study, weanling rats seem to be a suitable model for studying Cu bioavailability.

There was no significant difference in absorption of intrinsic or extrinsic ^{67}Cu from the cooked chicken liver, which provides support for the validity of using an extrinsic ^{67}Cu tag to measure Cu absorption from the other foods. The difference in Cu eaten by the two groups fed liver was not statistically significant, and apparently did not affect percentage Cu absorption. Any effect on percentage absorption would be expected to be such that a higher Cu intake would be associated with a lower percentage absorption. In fact the small difference seen in percentage absorption between the L-I and L-E groups was in the opposite direction. In rats (5) and humans (P. E. Johnson and G. I. Lykken, unpublished data) absorption of copper from wheat intrinsically labeled with ^{65}Cu has been found to be the same as that from wheat extrinsically labeled with stable ^{65}Cu. More foods need to be tested before firm conclusions can be drawn about the validity of extrinsic labels with copper, but the data available do not demonstrate any problems with the technique.

It is of interest that copper from liver was less well absorbed than that from beef or shrimp; although the copper content of liver is much higher (Table II) than that of beef or shrimp, significantly more Cu was absorbed from shrimp than from liver (Table V). Absorption of Cu from the limited selection of foods examined in this experiment belies the axiom that minerals are absorbed better from animal foods than from plant foods.

The values in Table V for bioavailability were calculated from the percentage Cu absorption from a given food relative to percentage absorption from $CuSO_4$. These values provide information about how the chemical milieu of Cu in a food affects the ability of the gut to absorb Cu from that food. Because Cu concentrations in foods differ, and serving sizes also usually differ among foods, the value of a food as a dietary Cu source will be a product of its Cu content, the amount eaten, and a bioavailability factor. Thus, liver will be a better dietary Cu source than garbanzo beans because of its higher Cu content. Similarly, though beef has the lowest Cu concentration of the foods listed in Table II, the Cu is highly available, and beef consumption in the US is quite high. Thus, beef would be a good source of highly available Cu.

In the second experiment, Cu absorption was measured from single meals containing one of four carbohydrates. Rats were adapted beforehand to a mixed diet containing equal parts of all four carbohydrates. In rats fed diets based on single carbohydrates, Cu status varies with the carbohydrate component of the diet and might affect Cu absorption. However, normal diets eaten by humans are mixtures of many carbohydrates, so the second experiment reported here was designed to simulate human diets more closely. Since all rats in this experiment were fed the same diet before the absorption experiment, their Cu status would be expected to be the same, as it was. Thus the absorption values found were not affected by differences in Cu status of the groups.

Fields *et al.* (18) used gastric intubation of starch or fructose diets labeled with ^{64}Cu. After 2 hr, they found more ^{64}Cu in the stomach and less ^{64}Cu in the intestine of rats intubated with the fructose diet than with the starch diet, regardless of to which diet the rats were adapted. However, if one totals the ^{64}Cu retention in stomach and intestine, there appears to be no effect of the type of carbohydrate in the oral dose on Cu absorption, which is consistent with the results of the present study. The emptying rate of ^{64}Cu from the stomach was greater with starch diets than for maltose, lactose, sucrose, glucose, or fructose diets (19), so that

after 2 hr, ^{64}Cu in the carcass was greater for rats dosed with starch than for rats dosed with simple sugars. In another experiment, Fields *et al.* (18) administered ^{64}Cu mixed with fructose or starch diet into ligated intestinal loops. After 1 hr, there was more ^{64}Cu in duodenal tissues of rats fed starch diets and intubated with starch than in duodenal tissue of rats fed fructose diets and intubated with fructose. However, ^{64}Cu in the carcass was not different among groups.

Fields *et al.* (13) administered intragastrically ^{67}Cu mixed with diet to Cu-supplemented or Cu-deficient rats that had been fed diets based on starch or fructose for 5 weeks. There was not a significant effect of carbohydrate on cumulative fecal excretion of ^{67}Cu in the 4 days following administration of the isotope. Likewise, carcass retention of the ^{67}Cu dose was not affected by dietary carbohydrate on Days 2–4 after the dose. Their data imply that Cu absorption was not affected by the nature of the carbohydrate when the length of the experiment was long enough to allow complete digestion of the food given in the test dose with ^{67}Cu.

None of the data of Fields *et al.* are directly comparable to the present study because they fed rats diets containing single carbohydrates for several weeks, thus altering their Cu status before the absorption experiments. In addition, several of their experiments were of short duration (1–2 hr), so that undigested food remained in stomachs and intestines of rats at the conclusion of their experiments. Nevertheless, their data, and that reported here, seem to be consistent in that there was no effect of the nature of the carbohydrate ingested on Cu bioavailability.

The data in Table VI show no differences in Cu absorption from a single meal containing either cornstarch, glucose, fructose, or sucrose. Except for the carbohydrate components of the basal diets, the absorption measurements in the second experiment were done under the same conditions as those in the first experiment, where numerous differences in Cu absorption were found that were caused by inclusion of different foods in the test meals. We reported previously that when diets made with single carbohydrates were fed (20), Cu absorption measured by isotope dilution was greater in rats fed starch than in those fed glucose, fructose, or sucrose. Subsequent work in this laboratory has failed to confirm that observation. Thus, it would appear that the nature of the carbohydrate in a meal on diet does not affect Cu bioavailability.

Summary. Copper absorption from foods was studied in rats using ^{67}Cu. Intrinsic and extrinsic ^{67}Cu were absorbed equally well from chicken liver. Other plant and animal foods were studied using an extrinsic tracer. There was no difference in Cu absorption from plant and animal foods. In rats fed a marginally Cu-adequate diet, there was no effect of glucose, fructose, sucrose, or cornstarch in a single meal on Cu absorption.

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