Apparent copper absorption from a vegetarian diet[1–3]

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ABSTRACT

Background: Vegetarian diets often contain more copper than do nonvegetarian diets, but observations of decreased plasma copper associated with vegetarian diets suggest that these diets have lower copper bioavailability than do nonvegetarian diets.

Objective: Our objective was to determine apparent copper absorption from controlled lactoovovegetarian and nonvegetarian diets.

Design: Eighteen women aged 20–43 y consumed lactoovovegetarian and nonvegetarian weighed diets for 8 wk each in a randomized, crossover design. The lactoovovegetarian and nonvegetarian diets provided 1.45 and 0.94 mg Cu, 38 and 16 g dietary fiber, and 1584 and 518 mg phytic acid, respectively, per 9.2 MJ (2200 kcal). After the women had been consuming each diet for 4 wk, their apparent copper absorption was determined by measuring the fecal excretion of the ^{65}Cu stable isotope, extrinsically added to the entire menu as $^{65}CuCl_2$.

Results: Plasma copper and ceruloplasmin were not affected by diet. The efficiency of apparent copper absorption from the lactoovovegetarian diet was less (33%) than that from the nonvegetarian diet (42%) (pooled SD: 9%; $P < 0.05$). However, because the lactoovovegetarian diet contained ≈50% more copper, the total apparent copper absorption from the lactoovovegetarian diet (0.48 mg/d) was greater than that from the nonvegetarian diet (0.40 mg/d) (pooled SD: 0.09 mg; $P < 0.05$).

Conclusion: Although copper was less efficiently absorbed from a vegetarian diet than from a nonvegetarian diet, total apparent copper absorption was greater from the vegetarian diet because of its greater copper content.   *Am J Clin Nutr* 2001;74:803–7.

KEY WORDS   Apparent copper absorption, bioavailability, ceruloplasmin, vegetarian diets, meat, phytic acid, hormonal contraceptives, women

INTRODUCTION

In comparison with nonvegetarian diets, vegetarian diets were associated with decreased plasma copper in ≥3 longitudinal diet comparisons within the same volunteers (1–3). In cross-sectional investigations, vegetarian diets were associated (4), albeit not consistently (5, 6), with lower plasma copper.

Human copper absorption from vegetarian diets as assessed with use of isotopic tracer methods has not been reported. In one study, stable isotope measurements of apparent copper absorption were unaffected by adding α-cellulose or phytic acid to a formula diet consumed by 4 men, although serum copper was significantly decreased by each of these constituents (1). In non-pregnant women (but not in pregnant women studied at the same time), fractional apparent copper absorption from a diet of predominately plant protein was less than that from a diet of predominately animal protein, but because the plant-protein diet contained more copper, more total copper was absorbed (7). Vegetarian diets may influence copper utilization or distribution without affecting copper absorption (8).

In a previous study to measure the absorption of zinc and nonheme iron from a vegetarian diet (3), premenopausal women had a small but significant reduction in plasma copper after consuming a controlled lactoovovegetarian diet for 8 wk. The purpose of the present study was to measure apparent copper absorption from the same diet by using a stable copper isotope and to determine whether the reduction in plasma copper could be attributed to reduced apparent copper absorption.

SUBJECTS AND METHODS

General protocol

Apparent copper absorption from lactoovovegetarian and nonvegetarian diets was determined by extrinsically labeling the entire menu for each diet with a stable isotope of copper and measuring subsequent fecal excretion. In a randomized, crossover design, the women consumed each weighed diet for 8 wk. Apparent copper absorption was measured after 4 wk, and samples for blood measurements were obtained after 7 and 8 wk. Because of the extended (4 wk) equilibration period, no additional washout period was scheduled.

TABLE 1
Calculated total diet composition[1]

	Lactoovovegetarian	Nonvegetarian
Meat (3/4 beef, 1/4 chicken) (g)	0	176
Copper (mg)	2.2 (1.45)	1.5 (0.94)
Zinc (mg)	11.1 (8.8)	11.3 (10.5)
Iron (mg)	17.0	16.5
Calcium (mg)	946	944
Dietary fiber (g)	38	16
Phytic acid (mg)	1584	518
Ascorbic acid (mg)	197	162

[1] Per 9.2 MJ (2200 kcal), the average total energy intake of the participants. Phytic acid was calculated from data published by Harland and Oberleas (9) with the use of the method of the Association of Official Analytical Chemists. All other nutrients were calculated with the use of data from the US Department of Agriculture (10). Values from direct chemical analyses are in parentheses.

Subjects

Eighteen premenopausal women completed the study, but apparent copper absorption data for 3 women were eliminated as indicated below. The 18 volunteers had a mean (±SD) age of 31 ± 8 y (range: 20–43 y), a mean (±SD) body weight of 64 ± 10 kg (range: 48–83 kg), and a mean (±SD) body mass index (BMI; in kg/m^2) of 23.8 ± 3.2 (range: 18.9–29.9).

The women were recruited through public advertising and were selected after an interview and blood analysis to establish that they had no apparent underlying disease and had not donated blood or used copper supplements exceeding 2 mg/d or iron or zinc supplements exceeding 20 mg/d for ≥6 mo before the study. Applicants agreed to discontinue all nutrient supplements when their application was submitted, generally 6–12 wk before the beginning of the study. Except for 4 women who routinely used hormonal contraceptives, none of the women routinely used medication. The participants gave informed consent. The University of North Dakota Institutional Review Board and the US Department of Agriculture's Human Studies Review Committee approved the study for human subjects.

Diets

Registered dietitians planned 2 experimental diets containing ordinary foods in a 2-d menu cycle. Detailed menus have been reported (3). The lactoovovegetarian and nonvegetarian diets contained 1.45 and 0.94 mg Cu/9.2 MJ, respectively, by analysis (**Table 1**). The copper content of the diet was ≈50% greater by calculation from food tables than by chemical analysis (Table 1). Grain products, fruit, and vegetables were the main sources of copper for both diets, followed by meat, poultry, and fish for the nonvegetarian diet and legumes for the lactoovovegetarian diet (**Table 2**). Dietary copper content was not standardized between the 2 diets because the differences seemed likely to reflect practical differences in copper content for lactoovovegetarian and nonvegetarian diets. The 2 diets contained 0 and 176 g (<6 oz), respectively, of meat daily (3 parts beef and 1 part chicken for the nonvegetarian diet) (Table 1). In contrast with the nonvegetarian diet, the lactoovovegetarian diet contained legumes daily and used whole-grain (rather than refined) bread and cereal products, resulting in 2.5 times more dietary fiber and 3 times more phytic acid (Table 1). Dietary phytic acid was calculated from published data based on the method of the Association of Official Analytical Chemists (9). By HPLC analyses, the lac-

toovovegetarian diet contained 4 times more total inositol phosphates than did the nonvegetarian diet (3). The lactoovovegetarian diet contained somewhat greater amounts of fruit and vegetables and ≈21% more ascorbic acid than did the nonvegetarian diet, as calculated from USDA food-composition data (10) (Table 1). The calcium contents of the 2 diets were similar (Table 1). Coffee and tea were excluded from the diets. City water, a bottled carbonated water, and chewing gum were consumed as desired, after analyses indicated minimal trace element content. Limited amounts of salt, pepper, and selected low-energy carbonated beverages were individualized to volunteers' preferences and then served consistently throughout the study.

All diet ingredients except water were weighed, prepared, and provided to the volunteers by the research center. Volunteers ate one meal at the research center on weekdays and consumed the remaining foods away from the research center after minimal reheating. Foods were weighed to 1% accuracy and consumed quantitatively. To maintain individual body weights, energy intakes were adjusted in 0.84-MJ (200-kcal) increments by proportionally changing the amounts of all foods. Mean (±SD) energy consumption was 9.2 ± 1.0 MJ (2200 ± 250 kcal).

Apparent copper absorption measurements

Apparent copper absorption was measured midway through the diet period to allow time for equilibration and subsequent measurements and to presumably represent average apparent absorption for the 8-wk period. After the women had been consuming each diet for 4 wk, the entire menu (3 meals/d for 2 d; evening snack foods were served with the third meal) was labeled with 300 mg ^{65}Cu stable isotope (Oak Ridge National Laboratory, Oak Ridge, TN), which was prepared and added to the food as ^{65}CuCl$_2$. For each meal, the tracer was pipetted onto a primary copper-containing menu item in proportion to the fraction of the total dietary copper present in each meal. Thus, the isotopic enrichment (ratio of ^{65}Cu to total copper) was constant for all meals. Although dietary energy was occasionally adjusted over time to maintain body weights, the amounts of energy served with the labeled meals were consistent between dietary treatments for each participant. All labeled meals were consumed at the research center.

Apparent absorption was determined by monitoring fecal excretion. Volunteers collected all fecal samples for 3 d before and 18 d (analyzed in 3-d aliquots) after the beginning of each

TABLE 2
Contribution of different food groups to total calculated dietary copper[1]

	Copper	
	Lactoovovegetarian	Nonvegetarian
	mg/d	
Meat, poultry, and fish	—	0.32
Milk, milk products, and eggs	0.15	0.14
Grain products	0.74	0.41
Legumes	0.40	—
Fruit and vegetables	0.69	0.46
Nuts and seeds	0.19	0.08
Fats, sweets, and beverages	0.06	0.07
Total	2.2 (1.45)	1.5 (0.94)

[1] Per 9.2 MJ (2200 kcal), the average total energy intake of the participants. Calculated with the use of data from the US Department of Agriculture (10). Values from direct chemical analyses are in parentheses.

TABLE 3
Plasma copper, ceruloplasmin, and apparent copper absorption in volunteers consuming lactoovovegetarian and nonvegetarian diets

	Lactoovovegetarian	Nonvegetarian	Pooled SD
Plasma copper (μmol/L)[1]	16.2	17.2	1.8
Ceruloplasmin (colorimetric, mg/L)[1]	422	442	44
Ceruloplasmin (immunochemical, mg/L)[1]	314	319	30
Apparent copper absorption (%)[2]	33.0[3]	42.1	8.6
Apparent copper absorption (mg)[2]	0.48[3]	0.40	0.09

[1] $n = 18$.
[2] $n = 15$.
[3] Significantly different from nonvegetarians, $P < 0.05$ (ANOVA).

isotope administration. Precautions to prevent trace element contamination included the use of high-purity reagents, acid-washed glassware, and deionized water. Aliquots of lyophilized, ground samples were digested by a combination of nitric acid reflux and dry ashing. Copper was extracted from the samples with freshly prepared 8-hydroxyquinoline (0.1 mol/L), and ion enrichment was determined by inductively coupled plasma mass spectrometry (PlasmaQuad 2+/Turbo; VG Elemental, Winsford, United Kingdom) as described (11). Percent apparent absorption was determined by measuring the ^{65}Cu isotope tracer that appeared in the feces, subtracting this amount from the amount of orally administered ^{65}Cu, dividing by the amount administered, and multiplying by 100. The absolute amount of apparent copper absorption was determined by multiplying the analyzed dietary copper content by the percent apparent absorption (and dividing by 100).

Apparent copper absorption results were eliminated for 3 volunteers: 1 reported an incomplete stool collection and each of the other 2 had one outstandingly high apparent absorption value (each ≈85%, and each associated with a different diet) that suggested incomplete stool collections. These latter 2 observations qualified for elimination by a statistical test for outliers (12).

Other clinical analyses

Duplicate diets were prepared for copper analyses. Aliquots of the diet composites were digested with concentrated nitric and 70% perchloric acids by method (II)A of the Analytical Methods Committee (13). The copper content of the digested aliquots was determined by inductively coupled argon plasma emission spectrophotometry. Bovine liver samples (Standard Reference Material 1577b) from the US National Institute of Standards and Technology (Gaithersburg, MD) were assayed to monitor analytic accuracy. Mean (±SD) measurements were 101 ± 4% of certified values for copper.

After an overnight fast, participants provided blood samples after 7 and 8 wk of each diet. Analyses of plasma zinc and copper, determined by atomic absorption spectrophotometry (Perkin-Elmer, Norwalk, CT), were each averaged from these 2 samples. Ceruloplasmin was determined at 8 wk by colorimetrically measuring *p*-phenylenediamine oxidase activity (14). Ceruloplasmin was also measured immunochemically by using the Behring Nephelometer 100 analyzer (Behring Diagnostics, Westwood, MA).

Statistics

Dietary treatment effects were determined by using repeated-measures analysis of variance, with individual volunteers serving as their own controls (15).

RESULTS

Percent apparent copper absorption from the vegetarian diet was significantly less than that from the nonvegetarian diet (33.0% compared with 42.1%; $n = 15$) (**Table 3**). However, because the copper content of the vegetarian diet was greater, total apparent copper absorption from the vegetarian diet was significantly higher than that from the nonvegetarian diet (0.48 compared with 0.40 mg/d). The relative difference in copper absorbed between the 2 diets was less than the relative difference in dietary copper content: although the vegetarian diet contained 50% more copper than did the nonvegetarian diet, only 20% more copper was apparently absorbed. Apparent copper absorption was unaffected by dietary sequence, confirming that the 4-wk equilibration to the diet was adequate and that a washout period between diets was not necessary.

Diet did not significantly affect plasma copper or ceruloplasmin (measured either colorimetrically or immunochemically) (Table 3). Apparent copper absorption was not correlated with plasma copper or ceruloplasmin.

Results for apparent copper absorption, plasma copper, and ceruloplasmin were not obviously different for the only participant who reported regular previous supplementation with copper (specifically, 0.5 mg Cu, 10 mg Fe, and 15 mg Zn, daily). The 4 volunteers who used hormonal contraceptives had significantly greater plasma copper and ceruloplasmin (logarithmically transformed data, $P < 0.03$ and $P < 0.05$, respectively), although only 2 of the 4 subjects accounted for this difference. Copper and ceruloplasmin concentrations in these 2 subjects were ≈2 times those of the other volunteers. However, apparent copper absorption was not related to the use of hormonal contraceptives.

DISCUSSION

These findings that women absorbed 33.0% and 0.48 mg Cu from a vegetarian diet containing 1.45 mg Cu and 42.1% and 0.40 mg Cu from a nonvegetarian diet containing 0.94 mg Cu are consistent with other studies by Turnlund et al (1, 7) that used a similar method. For example, nonpregnant women absorbed 33.8% and 0.85 mg Cu from a plant-protein diet containing 2.5 mg Cu and 41.2% and 0.59 mg Cu from an animal-protein diet containing 1.4 mg Cu (7). Similarly, men absorbed 31–35% of the copper from a basal formula diet, but the addition of α-cellulose or phytate to this diet had no effect on apparent copper absorption (1). Johnson et al (16) reported greater apparent copper absorption measurements (50–65%). The reasons for these higher values are not totally clear, but the authors (16) partially attributed the difference to lower dietary copper content (subjects were equilibrated

to a diet containing 1.25 mg Cu/d). Consistent with such a dose-response relation, Turnlund et al (17) reported apparent copper absorption of 67%, 54%, and 44% by 4 men consuming 0.38, 0.66, and 2.49 mg Cu/d. In that report (17), true copper absorption, estimated by measuring the excretion of the injected isotope in other volunteers, was 66–77%, consistent with the greater absorption measurements of Johnson et al (18) who used a short-lived copper radioisotope (^{67}Cu) and whole-body retention curves.

The reduced fractional apparent copper absorption associated with the vegetarian diet compared with that of the nonvegetarian diet may be related to dietary inhibitors of copper absorption that reduced the copper bioavailability of the diet. As reviewed by Lonnerdal (19), the effect of phytic acid on copper absorption is inconclusive in animal studies, and, as mentioned above, phytic acid did not affect apparent copper absorption in a study in humans (1). Unlike zinc-phytate complexes, copper-phytate complexes do not precipitate at gastrointestinal pH (20). Although copper absorption is negatively affected by ascorbic acid in animal studies, probably because of reduction from the cuprous (Cu^{2+}) to the cupric (Cu^{+}) form, the effect in humans may be less pronounced (21), especially when the difference in dietary ascorbic acid is small, as in the present study (Table 1). Soluble zinc and possibly iron may competitively interact with copper in the intestinal lumen to reduce absorption (19). Such competitive inhibition does not seem to be fully consistent with the results from companion studies to the present investigation. Although 0.020 mmol less zinc (3) and 0.006 mmol less nonheme iron (22) were absorbed daily from the same vegetarian diet than from the nonvegetarian diet, the efficiency of apparent copper absorption was decreased, and the absolute apparent copper absorption from the vegetarian diet was increased by only 0.001 mmol (or 0.08 mg) daily (Table 3).

It is also possible that the difference in copper content (Table 1) could account for the different fractional apparent copper absorption from the 2 diets. Turnlund et al (23) showed in men that fractional (percent) apparent copper absorption varies inversely whereas absolute (milligrams) apparent copper absorption varies directly with the copper content of a formula-based diet.

As with other fecal recovery methods, the present absorption measurements probably underestimate true copper absorption because the fecal reexcretion of the recently absorbed isotope cannot be differentiated from the excretion of unabsorbed isotope. Thus, these are apparent absorption measurements, and the effect of the different diets on the apparent copper absorption measurements may include any dietary effects on endogenous excretion of the recently absorbed isotope.

To interpret the copper isotope apparent absorption results as indicative of apparent copper absorption from the whole diet, we assumed that the extrinsically added isotope is absorbed similarly to the copper intrinsically present in foods. There is limited data to validate extrinsic labeling for apparent copper absorption. Johnson et al (16) measured apparent copper absorption from goose liver, goose breast meat, and peanut butter in 7 women and found no difference in the apparent absorption of intrinsic and extrinsic ^{65}Cu tracers for these 3 foods.

Plasma copper and ceruloplasmin activity, which are greater in women than in men (18), were also reported to be greater in women who use hormonal contraceptives (3, 18, 24). Data from the present study demonstrate the individual variability of this relation (higher copper and ceruloplasmin in only 2 of 4 hormonal contraceptive users) and the lack of association with apparent copper absorption measurements.

Although the direction and magnitude (5–6%) of dietary effects on plasma copper and ceruloplasmin activity were nearly comparable with the results of our previous study (7–8%) (3), unlike the previous study, the differences in the present study were not statistically significant. A slightly greater decrease in plasma copper was reported when volunteers adopted a lactovegetarian diet for 3–12 mo (2) or when a formula diet was tested with added α-cellulose or phytic acid (1). Together with previous research, these results suggest that vegetarian diets may decrease plasma copper slightly, but this decrease is not caused by decreased apparent copper absorption from the diet. The results of this study do not support the proposed possibility that a vegetarian diet may decrease copper retention or help in the control of Wilson disease (25).

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