Long-term high copper intake: effects on copper absorption, retention, and homeostasis in men

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ABSTRACT
Background: Numerous studies have examined the effect of low and adequate intakes of copper on absorption and retention, but little information is available on the regulation of absorption and retention of copper when intake is high.

Objective: A study was conducted in men to determine the effect of long-term high copper intake on copper absorption, retention, and homeostasis.

Design: Nine men were confined to a metabolic research unit (MRU) for 18 d and were fed a 3-d rotating menu containing an average of 1.6 mg Cu/d. They continued the study under free-living conditions for 129 d, supplementing their usual diets with 7 mg Cu/d. They then returned to the MRU for 18 d and consumed the same diet as during the first period, except that copper intake was 7.8 mg/d. The stable isotope $^{63}$Cu was fed to 3 subjects and infused into the other 6 on day 7 of each MRU period, and complete urine and stool collections were made throughout the study. Total copper and $^{63}$Cu were determined by inductively coupled plasma mass spectrometry. Copper absorption, excretion, and retention were calculated on the basis of dietary, urinary, and fecal copper and $^{63}$Cu.

Results: Results were as follows when comparing the high copper intake with the usual intake: fractional copper absorption was significantly lower, but the amount absorbed was significantly higher; excretion of the infused $^{63}$Cu was significantly faster; and total retention was significantly higher.

Conclusions: Homeostatic regulation of copper absorption and retention helped to minimize the amount of copper retained with high copper intake but was not sufficient to prevent retention of $>0.6$ mg Cu/d.

KEY WORDS Copper, absorption, retention, homeostasis, stable isotope, high copper intake

INTRODUCTION
An estimated average requirement for copper of 0.7 mg/d and a recommended dietary allowance (RDA) of 0.9 mg/d for adults were introduced in 2001 in the new dietary reference intakes (1). Before, sufficient data were not available to establish an RDA for copper, but an estimated safe and adequate daily dietary intake range was suggested. The new recommendations were based on several studies conducted with low and adequate copper intakes (2–4).

A tolerable upper amount was also established, but limited data were available to set this amount. Human data, based on chronic consumption of copper gluconate, were used (1) as a basis for the upper amount. In that study 10 mg Cu/d was fed for 12 wk and liver damage was not observed (5), whereas a case report indicated that long-term intake of 60 mg Cu/d resulted in acute liver failure (6). Because no liver damage was observed at 10 mg/d, it was suggested as the amount at which no adverse effects were observed, thus, an upper amount of 10 mg/d was established. Numerous studies reported gastrointestinal effects of copper intake $<10$ mg/d, including abdominal pain, cramps, nausea, diarrhea, and vomiting, from consuming beverages or drinking water (1), and the incidence of these effects increases at copper concentrations $>3$ mg/L (7). However, those observations had limitations and the effects were considered transient. Little research has been done to examine the effect of high copper intake on copper status, metabolism, and functional effects under controlled conditions.

We have shown that, with a copper intake of 7.5 mg/d for 24 d, the efficiency of absorption was considerably less than with a lower intake, but the amount absorbed increased. Copper retention was high, averaging 0.9 mg/d, but the amount retained decreased over time (8). We hypothesized that over a longer period normal balance of close to 0 would be restored. A report on the effect of 6 mg Cu/d for 8 wk on adaptive responses found that 0.75 mg Cu/d was retained, more than when the diets contained less copper (9). The efficiency of absorption was not affected in that study, but the amount absorbed would have increased. Another study was reported in which a supplement of 6 mg Cu/d was given for 4 wk, but copper absorption and retention were not reported (10). This report describes the effect of long-term high copper intake on copper absorption, retention, and homeostasis.

SUBJECTS AND METHODS
Subjects
Eleven healthy men were selected to participate in the study. Recruitment, selection procedures, exclusion criteria, and procedures for informed consent have been described (11).

1. From the US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, Davis, California (JRT, WRK, and JMD), and the Department of Food Science and Nutrition, Soonchunhyang University, Asan City, Chungcheongnamdo, Korea (SKK).

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study protocol was reviewed and approved by the Human Subjects Review Committee of the University of California, Davis, and by the US Department of Agriculture Human Studies Review Committee. Two subjects did not complete the study, and their data are not included. The participants ranged in age from 26 to 49 y. Mean height was 175 ± 7 cm. Body weight averaged 74 ± 13 kg at the beginning of metabolic period A (MP-A) and 76 ± 13 kg at the beginning of MP-B and did not differ significantly between MP-A and MP-B.

Experimental design

The study was part of a larger study on the effect of long-term high copper intake on indexes of copper status, immune function, and antioxidant status (11). The men lived in the Western Human Nutrition Research Center’s metabolic research unit for 18 d (MP-A) while consuming a diet containing 1.6 mg Cu/d, followed by a 129-d free-living period when they consumed their usual diets along with copper supplements containing 7 mg Cu/d. They returned to the MRU for another 18 d (MP-B), and the diets were supplemented with 6.2 mg Cu/d for a total of 7.8 mg Cu/d. They were supervised by the nursing staff at all times while living in the MRU. During the free-living period, they returned to the center once every 2 wk to receive a supply of supplements, verify compliance, monitor body weight and vital signs, and report any concerns. Body weights and vital signs were measured daily throughout the confined portions of the study.

The protocol for the study is shown in Figure 1. Complete urine and stool collections were made throughout the live-in parts of the study, and stools were collected twice during the free-living part of the study to monitor compliance in taking the copper supplements. Hair was collected on day 15 of each live-in part of the study.

Diet

A 3-d rotating menu containing at least the recommended amounts of all known essential nutrients was used in both live-in parts of the study (11). The daily energy content of the basic diet was 9.2 MJ (2200 kcal), with 87 g (15–16% of energy) from protein, 55% of energy from carbohydrate, and 30% from fat. The polyunsaturated-to-saturated fatty acid ratio was 1.10, and the diet contained 196 mg cholesterol/d. The energy intake was adjusted for each volunteer, based on body weight and diet records, so that each volunteer would maintain his initial weight throughout the study. This was done by adding energy to the basic diet with the use of an extra energy drink that contained maltodextrin, cornstarch, sugar, whipping cream, cottonseed oil, and water. If a subject gained or lost >1% of his baseline weight, his energy intake was adjusted by removing or adding the extra energy drink in 420-kJ (100-kcal) increments. Baseline weights were the average of the weights on days 2–4 of the study. Body weights remained relatively constant, averaging 74 kg in MP-A and 76 kg in MP-B.

The copper content of the diet was determined by isotope dilution inductively coupled plasma mass spectrometry (ICP-MS) as described later. Four diet composites of each of the 3 menu days were analyzed. The diet contained 1.61 ± 0.05 mg Cu on menu day 1, 1.49 ± 0.03 mg on menu day 2, and 1.77 ± 0.03 mg on menu day 3. The mean daily copper intake was 1.62 ± 0.14 mg/d. In MP-B the mean intake of copper was 7.76 ± 0.14 mg/d (7.75 ± 0.05, 7.63 ± 0.03, and 7.91 ± 0.04 mg for each of the 3 menu days, respectively). A solution containing copper sulfate was added to the extra energy drink of each meal to achieve the dietary copper intake. The solution also provided 2.7 mg Zn/d to achieve the RDA of zinc. The amount of copper was the only variable between MP-A and MP-B. During the free-living period, the subjects were instructed to consume supplements that provided 7 mg Cu/d as copper sulfate. The supplements were divided between the morning and evening meals. Subjects kept dietary records for 5 d before the study and for 5 d during the free-living period. One subject experienced nausea at the beginning of the free-living portion of the study. He did not eat breakfast and nausea followed the morning supplement. The problem was alleviated when he took the supplement with his noon meal instead. The NUTRITION DATA SYSTEM FOR RESEARCH, version 4.01 (Nutrition Coordinating Center, University of Minnesota, 1998) was used to estimate copper intake from these records. Copper intake was estimated to be an average of about 1.6 mg/d from these records.
Isotope preparation and administration

A copper solution containing enriched $^{63}$Cu was prepared from cupric oxide powder with a $^{63}$Cu abundance of 99.89% (Oak Ridge National Laboratory, Oak Ridge, TN). The solution was used to prepare the isotope diluent, oral tracer, and infused tracer solutions as previously described (12). The oral tracer solutions contained 0.27 mg $^{63}$Cu/g solution in MP-A and 2.25 mg $^{63}$Cu/g solution in MP-B. These tracers were added to the extra energy drink of 3 of the subjects 1 h before each meal on the seventh day of each metabolic period, for total oral doses of 0.82 mg $^{63}$Cu in MP-A and 6.74 mg $^{63}$Cu in MP-B. The solution replaced the natural copper solutions (0.88 mg Cu in MP-A, 7.03 mg Cu in MP-B) added on the other days of the study. $^{63}$Cu solution (5 g), containing 0.46 mg $^{63}$Cu, was infused into arm veins of 6 subjects over a period of 1 min. The infusion took place 15 min after breakfast began on the seventh day of each metabolic period.

Dysprosium, a fecal marker not absorbed by the body (13), was administered on the day oral and intravenous isotopes were administered to confirm completeness of fecal collections. It was prepared for administration by dissolving high-purity Dy$_2$O$_3$ in hydrochloric acid. The pH was adjusted with NaOH, and the solution was diluted. The solution (1 g) containing 0.67 mg Dy was added to the extra energy drink at each meal on day 7.

Sample collection and processing

Preparations were taken to avoid contamination by trace elements during all phases of sample collection, preparation, and analysis as described previously (11). Composites of each of the 3 daily menus were collected 4 times, twice in each 18-d MRU metabolic period, for copper determination. Complete fecal collections were made throughout each 18-d metabolic period. Stools were collected in new plastic containers in 3-d pools. They were frozen after collection. Diet composites and fecal pools were thawed, homogenized, frozen, and lyophilized. When dry, they were weighed, crushed to a fine powder and mixed in large plastic bags, transferred to plastic jars, and stored in desiccators (14). Blood samples to be analyzed for copper $^{63}$Cu enrichment were drawn into trace element–free tubes that contained heparin before the isotope infusion (day 7); at 5, 15, and 30 min and 1, 2, 4, 6, 11, and 16 h after administration; and daily from day 8 to day 18. Urine was collected in 8-h or 24-h pools in plastic containers, diluted with deionized water, and acidified with 1% strength of 1% HCL for ICP-MS analysis.

Plasma samples were prepared for isotope ratio determinations with the use of a microwave digestion method modified from a procedure used for plasma molybdenum isotope ratios (15). Duplicate 0.5-g aliquots were weighed in 10-mL polytetrafluoroethylene beakers that were then placed on top of 5-mL beakers inside microwave liners. A solution containing 0.1 µg $^{60}$Cu was added to 1 aliquot of each pair. Concentrated nitric acid (6 mL) was added to the base of each microwave digestion liner, and 1.4 mL nitric acid was added to each beaker containing the plasma. Carousels of 10 samples, 1 blank and 1 plasma reference sample (from a reference pool prepared and used in our laboratory), were digested in the microwave digestion oven. After digestion they were heated to dryness in a laminar flow hood in the clean room and dissolved in 6 N HCl. The copper was separated and purified by anion exchange chromatography similar to the diet and fecal samples, except a smaller column (3 mm internal diameter) was used. The columns were rinsed with 6 N HCl (4 column volumes), then 2.5 N HCl (1 column volume) and the copper were eluted with 2.5 N HCl (2 column volumes). After separation and collection, samples were diluted to an acid strength of 1% HCl for analysis by ICP-MS.

Isotope ratio determinations

The isotope ratio of $^{65}$Cu to $^{63}$Cu ($^{63}$Cu/$^{65}$Cu) was determined in fecal, diet, urine, and plasma samples with the use of an ICP-MS equipped with AS-91 autosampler, quartz torch, nickel cones (ELAN 6000; PerkinElmer Sciei Instruments, Norwalk, CT) and ultrasonic nebulizer (U-5000AT; CETAC Technologies Inc, Omaha). The autosampler and peristaltic pump were housed inside an acrylic box with a plastic curtain covering the opening to minimize contamination during analysis. The $^{63}$Cu/$^{65}$Cu was measured with 250 sweeps and 10 replicates for an analysis time of 1.5 min, with 2-min flush and wash times. The dwell times were 20 ms for $^{65}$Cu and 10 ms for $^{63}$Cu. The radiofrequency power was 1300 W, and nebulizer gas flow was 1.07 L/min. The copper concentration in the samples was typically 30–80 ppb in 1% HCl, which yielded total copper intensities ~1 million counts/s in the
analog mode. The average reproducibility of the $^{63}\text{Cu}:{}^{65}\text{Cu}$ measurements was 0.3%.

Ultrapure 1% HCl was used as a rinse solution and for blank background determinations. Blank counts were subtracted from sample counts. The ratio of a natural copper standard (30 ppb Cu in 1% HCl) was measured to correct for instrumental mass bias.

The reference natural value for the $^{63}\text{Cu}:{}^{65}\text{Cu}$ was divided by the measured ratio to determine the ratio correction factor that was used to correct measured ratios on the day of analysis. The measured $^{63}\text{Cu}:{}^{65}\text{Cu}$ of all natural samples analyzed throughout the study was averaged for each matrix, and small matrix mass bias corrections were made.

The $^{63}\text{Cu}$ tracer and total copper content of the samples were determined by isotope dilution by the $^{63}\text{Cu}:{}^{65}\text{Cu}$ of 2 duplicate aliquots (with and without added $^{63}\text{Cu}$), natural samples of the same matrix, and the $^{63}\text{Cu}$-enriched solution, along with the weights of the sample aliquots and the added isotope diluent, the concentration of the isotope diluent, and the total pool weights. This calculation has been reported previously (12).

**Copper and dysprosium content**

Urinary copper concentrations, determined by graphite furnace atomic absorption spectrophotometry, have been reported (11). The cleaning, ashing, and graphite furnace atomic absorption spectrophotometry analysis of hair samples also have been reported (11).

Fecal samples were prepared for dysprosium analysis as described for isotope ratio measurements. After undergoing microwave digestion, the samples were diluted with deionized water to 25 mL in polyethylene volumetric flasks. Before analysis, the samples were further diluted with 1% (w/v) nitric acid to achieve final concentrations of 0–3 ppb Dy, then rhodium, an internal standard, was added at a concentration of 1 ppb. ICP-MS analysis was performed under the conditions previously described (16). Quality assurance, based on adding dysprosium to 9 samples over the range of results, showed that average recovery was 113.2% with a relative SD (RSD) of 2.6%, and analytic results were corrected for recovery. Mean dysprosium recovery in fecal collections was 101.7% (range: 89–108%) with 5.2% RSD for MP-A and 101.3% (range: 92–108%) with 4.6% RSD for MP-B.

**Absorption and excretion calculations**

Copper absorption, excretion, retention, and endogenous gastrointestinal losses were calculated on the basis of fecal monitoring as previously described (12) These calculations assume that the same fraction of dietary copper is absorbed as the oral tracer and that the same fraction of absorbed dietary copper is excreted into the gastrointestinal tract as the infused tracer. Apparent copper absorption was calculated by subtracting the amount of oral tracer recovered in the stools in a 12-d period after the feeding from the amount fed. This includes the tracer that was absorbed and then excreted during those 12 d. The fraction of infused $^{63}\text{Cu}$ excreted into the stools was measured in the 12-d periods after the infusion. True copper absorption was calculated by correcting the fraction of copper apparently absorbed by the fraction of $^{63}\text{Cu}$ excreted over the same time period. Total endogenous gastrointestinal losses were based on total fecal copper less unabsorbed dietary copper. The fast turnover pool was calculated on the basis of the losses of dietary copper consumed and absorbed in the previous 12 d. The slow turnover pool was the endogenous fecal losses not attributed to excretion of absorbed dietary copper in the first 12 d after absorption. It was calculated by subtracting excreted copper from total endogenous copper.

**Statistical analysis**

Statistical analysis was performed with the personal computer version 8.2 of the STATISTICAL ANALYSIS SYSTEM (17). Descriptive statistics, including means, SDs, and plots, were tabulated and compared. PROC GLM was used to perform analysis of variance on the effect of the 2 dietary copper intakes on copper absorption and retention, fecal and urinary $^{63}\text{Cu}$ excretion, and total urinary copper. If a significant F was found, Tukey’s test was used to determine which treatment means differed. A significance level of 0.05 was used for all statistical tests.

**RESULTS**

$^{63}\text{Cu}$ excretion

The main routes of copper excretion are through the gastrointestinal tract and in the urine. Most of the infused $^{63}\text{Cu}$ that was excreted appeared in the stools, and only a small fraction was found in the urine. Mean 12-d $^{63}\text{Cu}$ fecal excretion was significantly higher ($P < 0.05$) in MP-B (46%) than in MP-A (27%; SE = 2.3), as shown in Table 1. In contrast, mean cumulative urinary $^{63}\text{Cu}$ excretion was significantly lower in MP-B (1.3%) than in MP-A (2.1%; SE = 0.14). The patterns of excretion are depicted in Figure 2. The total 12-d $^{63}\text{Cu}$ excretion by both routes was 47% of the infused dose in MP-B compared with 29% in MP-A, which was significantly lower. However, total urinary copper excretion was significantly higher in MP-B than in MP-A.

**Table 1**

Copper absorption, excretion, and retention

<table>
<thead>
<tr>
<th>Metabolic period</th>
<th>MP-A</th>
<th>MP-B</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary copper (mg/d)</td>
<td>1.6</td>
<td>7.8</td>
<td>—</td>
</tr>
<tr>
<td>Urinary copper (μg/d)</td>
<td>20</td>
<td>26</td>
<td>0.9</td>
</tr>
<tr>
<td>Fecal copper (mg/d)</td>
<td>1.6</td>
<td>7.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Urinary $^{63}\text{Cu}$ (%)</td>
<td>2.1</td>
<td>1.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Fecal $^{63}\text{Cu}$ (%)</td>
<td>27</td>
<td>40</td>
<td>2.3</td>
</tr>
<tr>
<td>Apparent absorption (%)</td>
<td>29</td>
<td>16</td>
<td>1.0</td>
</tr>
<tr>
<td>(mg/d)</td>
<td>0.48</td>
<td>1.2</td>
<td>0.05</td>
</tr>
<tr>
<td>True absorption (%)</td>
<td>40</td>
<td>29</td>
<td>—</td>
</tr>
<tr>
<td>(mg/d)</td>
<td>0.65</td>
<td>2.2</td>
<td>—</td>
</tr>
<tr>
<td>Copper retention (mg/d)</td>
<td>0.06</td>
<td>0.67</td>
<td>0.27</td>
</tr>
</tbody>
</table>

1 Means within a row with different superscript letters are significantly different, $P < 0.05$ (Tukey’s test).

2 SE of least-squares means.

3 Average daily copper excretion during the last 12 d of each metabolic period (n = 9).

4 Percentage of dose of infused $^{63}\text{Cu}$ excreted in the 12 d after infusion (n = 6).

5 Before correction for endogenous excretion (n = 3).

6 Estimated from average absorption of fed $^{63}\text{Cu}$ adjusted for average excretion of infused $^{63}\text{Cu}$.

7 Average of the last 12 d of each metabolic period (n = 9).
Plasma enrichment after infusion of $^{63}$Cu is depicted in Figure 3 (A and B). The pattern of enrichment is similar in both periods, but from 48 h after the infusion and onward enrichment differed significantly, averaging 1.1% in MP-B and 1.6% in MP-A (SE = 0.02).

### Copper absorption

Copper absorption during each dietary period is shown in Table 1. The fraction absorbed was significantly higher during MP-A (29%) than during MP-B (16%; SE = 1.0). However, the amount absorbed was significantly less during MP-A (0.48 mg) than during MP-B (1.2 mg; SE = 0.05). True absorption, calculated as explained earlier, by combining the mean copper absorbed in the subjects fed $^{63}$Cu with the mean excreted over the same time period in the subjects infused, showed a similar pattern (Table 1), but the relative difference was less.

### Copper retention (balance)

Average copper retention, based on dietary, urinary, and fecal copper, for each metabolic period is also shown in Table 1. Both fecal and urinary copper were higher when dietary copper was high. Fecal copper averaged 7.1 mg/d in MP-B and 1.6 mg/d in MP-A (SE = 0.10). Urinary copper averaged 26 μg/d in MP-B and 20 μg/d in MP-A (SE = 0.9). Copper retention was markedly higher in MP-B (0.67 mg/d) than in MP-A (0.06 mg/d; SE = 0.27).

### Copper turnover

Endogenous copper losses were calculated for fast and slow turnover pools and total endogenous gastrointestinal losses for 9 subjects for the last 12 d of each metabolic period as described earlier. When dietary copper intake was 1.6 mg/d (MP-A), a total of 0.58 mg endogenous Cu/d was eliminated in the stools. Of that, 0.18 mg endogenous Cu/d was contributed by the fast turnover pool and 0.40 mg endogenous Cu/d by the slow turnover pool. When intake was 7.8 mg Cu/d (MP-B), total endogenous gastrointestinal losses were 1.56 mg Cu/d, with 1.04 mg Cu/d contributed by the fast turnover pool and 0.52 mg Cu/d by the slow turnover pool.

### DISCUSSION

We studied copper metabolism previously with high intake for 24 d, and no changes in indexes of copper status were observed (2). However, high intake resulted in marked changes in absorption and retention (8). The efficiency of absorption declined markedly, but more copper was absorbed and considerably more copper was retained. The current study was undertaken to determine whether, after long-term high copper intake, changes in copper status would occur and whether absorption and retention would adapt to long-term high intake.

In the present study, small but significant changes were observed in ceruloplasmin activity and superoxide dismutase activity, but plasma copper and ceruloplasmin concentrations did not change, as has been reported (11). We also evaluated several functions that have been associated with copper. Some of these functions suggested possible adverse effects of high copper intake. Benzylamine oxidase activity, which has been hypothesized to be associated with intestinal cell damage, increased, and...
urinary T bars, an indirect measure of oxidative damage, increased. Several indexes of immune function changed. Neutrophils decreased, lymphocytes increased, and interleukin 2 receptor decreased. Serum antibody titers after immunization were much lower in subjects who received supplements than in control subjects who did not receive supplements (11). All these changes suggest a possible adverse effect of high copper intake on immune function.

In our earlier study with high copper intake for 24 d, 0.94 mg Cu/d was retained over 24 d, but balances were highly variable and became less positive over time. We hypothesized that once equilibrium was reached, balance would return to near 0. However, when intake was high for a longer period of time in this study, an average of 0.67 mg Cu was still retained. Because total body stores of copper are only 80–100 mg, the amount of copper retained would markedly increase copper stores and could put a person at risk for cirrhosis. In conditions such as Wilson disease (18), Indian childhood cirrhosis (19), and biliary cirrhosis (20), copper amounts in liver, the storage site for copper, are high.

Copper retention is strongly regulated, but high and low copper intakes exceed the regulatory mechanisms, leading to copper depletion (12) or excess retention (8). Three sites of regulation have been identified. As copper intake increases, the efficiency of absorption decreases, but more copper is still absorbed (8, 12).

We have recently shown that some of the copper that appears to be absorbed is retained in the intestinal mucosal cells, so it does not enter into systemic circulation and is eliminated through the gastrointestinal tract when intestinal cells exfoliate (21). Finally, endogenous copper losses decline when intake is low, so less is eliminated, and increase when intake is high, so more copper is eliminated (12).

In the present study, apparent absorption was 29% (0.48 mg Cu/d) when intake was 1.6 mg Cu/d and 16% (1.2 mg Cu/d) when intake was 7.8 mg Cu/d. When these absorption values are corrected for endogenous excretion of absorbed copper over the time of sample collection, true absorption was 40% (0.65 mg Cu/d) compared with 29% (2.2 mg Cu/d). These data show 2 of the points of regulation. The initial amount absorbed is considerably higher, but a considerable amount is immediately excreted into the gastrointestinal tract, so apparent absorption is less. The amount retained by the intestinal cells when intake is high (the other point of regulation) cannot yet be quantified, but ultimately this is eliminated in the stools along with unabsorbed dietary copper and endogenous copper.

Retention of copper in this study was 0.06 mg/d, close to 0, when dietary intake was 1.6 mg Cu/d, but increased to 0.67 mg/d when dietary intake was 7.8 mg Cu/d. Therefore, some adaptation to high dietary intake took place, as in our study with high intake for 24 d, more copper, 0.94 mg/d, was retained. However, 0.67 mg Cu/d still resulted in a significant amount of copper retained, and at this rate total body copper could double in 100–150 d.

We divided the excreted copper into 2 pools, a slow pool, the amount of endogenous copper eliminated that was not attributed to recently absorbed copper, and a fast pool, the amount eliminated rapidly after intake. The amount in the fast pool increased more as copper intake increased than did the slow pool. The fast pool was twice the size of the slow pool when intake was high; when intake was low, the slow pool was more than double the fast pool. This results in more rapid turnover of newly absorbed copper when intake is high. The rapid excretion into the gastrointestinal tract is illustrated in Figure 2. It also shows that, when intake is high, infused $^{65}$Cu is eliminated so quickly by the bile that there is less circulating isotope eliminated in the urine than is eliminated when intake is lower.

The results of this study are consistent with the results of another study in which absorption, retention, and slow and fast pools were determined (12). The results of that study show that, when intake is low, little copper is eliminated in the fast pool and this increases markedly as intake increases. The slow pool is affected to a much lesser extent. The homeostatic regulatory mechanisms work relatively well until intake exceeds 1.6 mg Cu/d, and after that retention increased markedly. Results from another study showed that even at 1.7 mg Cu/d, retention was increased to 0.17 mg Cu/d (8).

Most copper that is absorbed is lost by excretion into the gastrointestinal tract. Although gastrointestinal excretion increased markedly with high copper intake, the increase was not sufficient to remove excess copper. Copper excretion from other routes also increased in this study. Urinary copper increased significantly, from 20.3 to 25.6 μg/d (11), but this increase had no measurable effect on copper retention. Hair copper also increased significantly, from 9.2 to 21.1 μg/g hair (11), and, although we cannot estimate daily losses by this route, the effect on retention would be small.

Plasma copper enrichment also reflects the homeostatic regulation of copper metabolism. Although plasma copper concentration did not change because of dietary intake, the $^{65}$Cu enrichment was less when intake was high. Initial enrichment patterns were similar, but enrichments were consistently lower after the first 24 h, again demonstrating that the infused dose was eliminated from circulation more rapidly when intake was high.

This study shows that the homeostatic mechanisms controlling copper retention in humans are not sufficient to prevent accumulation of copper when intake is high, even over a period of several months. As indicated earlier, few changes were observed in indexes of copper status in this study, but changes in several functional markers suggest that antioxidant defense and immune function may have been adversely affected by the amount of dietary copper (11). It is possible that these effects are related to the accumulation of copper during the high copper intake. This finding suggests that the current upper amount for copper is higher than desirable, and consideration should be given to establishing a lower upper amount.

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JRT was responsible for all aspects of the study. WRK, SKK, and JMD contributed to data collection and analysis and preparation of the manuscript. None of the authors had any conflicts of interest.

REFERENCES
4. Turnlund JR, Scott KC, Peiffer GL, Jang AM, Keen CL, Sakasnki TM.
19. Pandit A, Bhave S. Present interpretation of the role of copper in Indian child cirrhosis Am J Clin Nutr 1996;63:830S–5S.