Human whole-body copper metabolism\textsuperscript{1,2}

Judith R Turnlund

\textbf{ABSTRACT} Whole-body copper metabolism is difficult to study in human subjects. However, the use of isotopic tracers and kinetics modeling has added a dimension beyond what can be learned in humans by direct measurement. Mechanisms regulating total body copper seem to be strong, given the relatively small and constant body pool, but they are not yet well understood. The efficiency of copper absorption varies greatly, depending on dietary intake. Changes in efficiency of absorption help to regulate the amount of copper retained by the body. In addition, endogenous excretion of copper into the gastrointestinal tract depends heavily on the amount of copper absorbed. When dietary copper is high and more is absorbed, endogenous excretion increases, protecting against excess accumulation of copper in the body. When intake is low, little endogenous copper is excreted, protecting against copper depletion. Regulation is not sufficient with very low amounts of dietary copper (0.38 mg/d) and appears to be delayed when copper intake is high. The use of isotopic tracers and kinetic modeling should aid in elucidating the regulatory mechanisms.

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\textbf{KEY WORDS} Copper, whole-body metabolism, kinetic model, compartmental model, stable isotopes, tracers, absorption

\textbf{INTRODUCTION}

\textbf{Early research on copper metabolism}

Research on copper metabolism in humans began after the discovery that copper was necessary for hemoglobin formation in rats (1). Several studies in humans of dietary copper intake, copper excretion, copper balance, and tissue copper concentrations in cadavers were reported in the 1930s and 1940s (2–5). Data were used to estimate copper requirements and make dietary intake recommendations, but the data that could be obtained at that time did not lend itself to an understanding of the metabolic fate of copper. From these studies, Tompsett (3, 4) estimated the minimum dietary copper requirement to be 0.63 mg/d, based on copper excretion after a very-low-copper diet. Chou and Adolph (2) suggested that equilibrium is reached at \( \approx 2 \) mg/d and Leverton and Binkley (5) suggested a recommended intake of 2.0–2.5 mg/d based on estimates that the average usual copper intake was 2.65 mg/d, which resulted in retention of 0.85 mg Cu/d. Concentration data at that time were generally considerably higher than present analytic values because there are now more sophisticated analytic methods available and copper contamination of samples can be well controlled.

As with all minerals, scientists face many challenges when studying whole-body copper metabolism in humans. In contrast with studies in laboratory animals, few tissues are available for analysis, limiting what can be learned directly from human studies. The metabolic fate of dietary minerals in general is illustrated in \textbf{Figure 1} (6). Of the pathways and tissues shown in the diagram, the amount of dietary minerals and the mineral content of blood and excretion products can be measured readily in human studies. Other tissues such as skin and liver can be sampled but the sampling procedures are considered invasive. Concentrations of minerals in tissues and excreta provide limited information on their metabolism. The mineral intake from one meal, food, or day cannot be separated in collected products from the endogenous mineral or from what was consumed at other times.

\textbf{Isotopic tracers for studies of copper metabolism}

The introduction of isotopic tracers has greatly facilitated research on copper metabolism, with the most definitive information being gained from laboratory animal research. Tracers permit additional information to be obtained on the metabolic fate of copper in the body. Oral and intravenous doses can be traced and separated from endogenous copper in the body. When combined with computer simulation and modeling techniques, the potential is enormous.

Radioactive isotopes were used as tracers in early animal studies of the metabolic fate of copper, the first of which were reported in the early 1940s in rats (7) and dogs (8). These studies provide information on copper absorption and retention after an oral dose (7) and appearance and disappearance from the blood after an intravenous dose (8). Research in humans with radioisotopes of copper began in 1947 (9). In the first reported human study, investigators followed the appearance and disappearance of radioactive copper in the blood after intravenous and subcutaneous injections (9).

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\footnotesize\textsuperscript{1} From the US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, San Francisco. \\
\footnotescript{2} Address reprint requests to JR Turnlund, US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, PO Box 29997, Presidio of San Francisco, CA 94129. E-mail: jturnlun@whnrc.usda.gov.
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Stable isotopes of copper have been used in a variety of human studies, primarily to measure absorption and determine bioavailability (24). Studies have been conducted in infants (25), pregnant women (26), and healthy, young and elderly adults (27). In addition, several studies have assessed interactions with dietary components, some using intrinsically labeled foods (28).

Compartmental modeling of copper kinetics

The use of isotopic tracers made it possible to conduct kinetic studies of copper metabolism (29). Early work with radioisotope tracers in humans provided kinetic information, but models were not developed (11, 13, 14, 30). Given the limitations of human research, animal studies must be relied on as a basis for human models of copper metabolism.

Pioneering work by Owen provided comprehensive information on the distribution of copper in rats (31) and on its metabolism in rats (32). Weber et al (33) developed a three-compartment model of copper metabolism in sheep, based on injections with $^{64}$Cu as a tracer, and measured radioactivity in blood, liver, urine, and bile samples. Kinetic modeling was done with the SIMULATION, ANALYSIS, AND MODELING(SAAM) program (34). Dunn et al (35) developed a model of copper metabolism in rats using $^{64}$Cu and the CONSAM modeling program, an interactive version of SAAM (36). The model described whole-body copper metabolism in rats, focusing on subcellular systems. Buckley (37) used CONSAM to develop a model of copper metabolism in dairy cows that was based on a stable isotope tracer, $^{64}$Cu. Continuous feeding of the stable isotope $^{65}$Cu was used in rats to measure organ copper turnover (38).

We used $^{65}$Cu in a study of young men to develop a model of copper metabolism, the first in humans (39). The compartmental model was developed by using CONSAM version 30.1 (36). The study was conducted in five young men who lived in a metabolic research unit for a 90-d study during which three different dietary amounts of copper were fed. $^{65}$Cu was administered orally four times and intravenously three times during the course of the study. The model contains five compartments (two plasma compartments, two liver compartments, and an “other-tissues” compartment), two delay components, and fecal and urinary excretion pathways (Figure 2). Although it is a simple model and all possible pathways could not be represented from the data.

Stable isotopes of copper have been used in a variety of other studies, including exposure to radioactivity, short half-lives, and limited availability, led investigators to explore the use of stable isotopes as tracers of copper metabolism in healthy subjects (20). Stable isotopes occur in nature in fixed natural abundances. Some minerals have only one stable isotope and they cannot be used as tracers. Other essential minerals have as many as nine stable isotopes (21). The two stable isotopes of copper, $^{64}$Cu and $^{65}$Cu, are relatively abundant. $^{64}$Cu has a natural abundance of 69.2% and $^{65}$Cu has an abundance of 30.8%. Ideally, stable isotopes to be used as tracers are of relatively low abundance naturally. Although copper has only two stable isotopes and both are relatively abundant, they have proven to be very effective in providing information related to copper absorption, bioavailability, and excretion patterns. Analytic methods of choice for isotope ratio measurements include thermal-ionization mass spectrometry (TIMS) (22) and inductively coupled-plasma mass spectrometry (ICP-MS) (23). Methods for measuring stable isotopes require specialized, costly equipment. Sample preparation is extensive, analysis is slow, and isotopes are expensive. The primary advantages of the stable isotopes of copper are that they can be used as tracers with no exposure to radioactivity and that they do not decay. They are completely safe and can be used even in pregnant women and infants. Because they do not decay, longer-term studies can be conducted than can be done with radioisotopes of copper. In addition, sample analysis can be delayed without compromising the results.

Stable isotopes of copper have been used in a variety of
collected, the model demonstrated the following: the amount of dietary copper influences the flow of copper from one liver compartment to one plasma compartment and from that plasma compartment to the other-tissues compartment; tissue uptake of oral and intravenous copper differs, with the flow from plasma to a liver compartment varying with the route of administration; and the major storage site is in the liver. Examples of the fit of the model to the $^{65}$Cu data are shown in Figure 3: A (long term) and B (short term). The model does not include a gastrointestinal tract, which will be added with additional data obtained from these subjects and from another study.

Overview of whole-body copper metabolism and conditions influencing metabolism

Several reviews on copper metabolism since 1950 have included current knowledge of human whole-body copper metabolism. They have generally agreed on many aspects of its metabolism (30, 40–45). Dietary copper is absorbed into the body through the intestinal mucosa and transported via the portal blood to the liver. Much of that taken up by the liver is incorporated into ceruloplasmin, released into the blood, and delivered to tissues. Details on these mechanisms and cellular metabolism are covered in this supplement (46, 47). Most endogenous copper is lost via the bile, being excreted into the gastrointestinal tract. This copper combines with small amounts of copper from pancreatic and intestinal fluids and intestinal cells and is eliminated from the body. Whole-body copper retention increases with increased dietary intake. Absorption and endogenous excretion and retention are discussed in the next section. Little copper is lost in the urine and sweat. Relatively little copper is stored in the body, compared with other trace elements such as zinc and iron, and the adult body usually contains < 100 mg Cu. The highest copper concentrations are in the liver, followed by the brain, kidney, and heart.

Several conditions and diseases influence whole-body copper metabolism. The conditions include metabolic defects, such as Menkes syndrome and Wilson disease (18, 48–50), pregnancy (51), inflammation (52), and numerous diseases. Blood concentrations increase dramatically in many of these conditions. Copper metabolism changes with total parenteral nutrition, partly because of the route of administration and partly because of the underlying disease states that alter metabolism (53).

Copper absorption and endogenous excretion

A series of studies at the Western Human Nutrition Research Center showed that copper absorption is influenced markedly by the amount of dietary copper (54). The amount absorbed increases as the amount in the diet increases, but absorption is much more efficient and a higher percentage is absorbed when intake is low. Thus, a 10-fold increase in dietary copper results in only twice as much copper absorbed, as shown in Figure 4 (6). These studies were done under a variety of conditions with a variety of diets. Subjects of the studies included young men, elderly men, young women, and pregnant women. These studies suggest that the amount of dietary copper is the primary factor influencing absorption.
A recent study showed that the excretion of endogenous copper is markedly influenced by dietary copper intake (55, 56). When dietary copper is low, little endogenous copper is excreted. As the amount in the diet increases, endogenous copper excretion increases. Copper turnover is slow when dietary intake is low and high when intake is high. The regulation of absorption and endogenous excretion controls the amount of copper retained in the body and protects against copper deficiency and toxicity. The regulation of excretion appears to be more important than regulation of absorption in determining copper retention. As shown in Figure 5, fecal copper losses reflect dietary copper, with some delay when dietary intake changes, and balance can be achieved over a broad range of copper intakes. In contrast, urinary copper changes little or not at all with changes in dietary copper. Urinary losses do not contribute to regulation of copper stores and contribute an undetectable amount to copper balance.

Because of, at least in part, the regulation of body copper stores, indexes of copper status are resistant to change, except under extreme dietary conditions. We showed that when dietary intake goes from 0.8 to 7.5 mg/d, indexes of status, including plasma copper, erythrocyte superoxide dismutase, ceruloplasmin, and urinary copper excretion were not significantly different. However, when dietary copper was only 0.38 mg/d, indexes of status changed (58). For example, urinary copper, although low throughout the study, was significantly lower with the low-copper diet but did not differ between treatment periods with intakes of 0.65 and 2.48 mg/d. In another study, urinary copper did not increase when the diet contained as much as 7.53 mg Cu/d (57). It appears that the regulatory mechanisms could not compensate for the very-low dietary copper intake of 0.38 mg/d. When dietary intake is very high, regulation is challenged. At first, balance is positive but later it becomes negative as the excess copper retained early after a change in diet is eliminated (54).

In the low-copper study described above, several indexes of copper status changed with a copper intake of 0.38 mg/d, suggesting that copper intake was not adequate (58). Plasma copper, the ceruloplasmin concentration and activity, and urinary copper declined significantly. Plasma copper, the ceruloplasmin concentration, and urinary copper increased in response to repletion. The erythrocyte superoxide dismutase concentration was lower during the depletion period than during the periods before or after depletion. In addition, some functional tests suggested that a low copper intake may affect immune function (59), antioxidant status, and the protein-lysine 6-oxidase (also known as lysyl oxidase) content of the skin (60).

CONCLUSIONS

Whole-body copper metabolism is regulated by a variable efficiency of copper absorption and through excretion of endogenous copper into the gastrointestinal tract. The mechanisms of the regulation are not yet well understood. With new techniques and tools available, including stable-isotope tracers and compartmental modeling, investigators are gaining an understanding of whole-body copper metabolism in humans. These are relatively new approaches and as their use increases, the resulting research can be expected to provide a wealth of definitive information on copper metabolism in humans. Eventually the mechanisms of copper regulation will be elucidated.

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