Effects of age and sex on copper absorption, biological half-life, and status in humans[1-3]

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ABSTRACT Healthy, free-living men and women aged 20–83 y ($n = 127$) were studied to determine the effects of age and sex on copper absorption, biological half-life (BH), and status. Copper absorption was greater in women (71%) than in men (64%) aged 20–59 y ($P = 0.02$), but did not differ in men and women aged 60–83 y. BH of ^{67}Cu ranged from 13 to 33 d and differed between men and women aged 20–59 y ($P = 0.006$), but not between men and women aged 60–83 y. Plasma copper, enzymatic ceruloplasmin (Cp), and immunoreactive (RID) Cp were significantly higher in women than in men ($P < 0.005$), but superoxide dismutase (SOD) and in vitro ^{67}Cu uptake by red blood cells did not differ. Plasma copper, RID Cp, and cytochrome oxidase in platelets and mononuclear cells were significantly affected by age ($P < 0.005$). Oral contraceptives elevated plasma copper, enzymatic Cp, and SOD activity but not copper absorption and BH in women aged 20–39 y. Copper intake from self-selected diets was 0.9–1.2 mg/d for women and 1.2–1.3 mg/d for men, but net copper absorption (μg Cu · kg body wt^{-1} · d^{-1}) did not differ. Thus, dietary copper intake requirements may differ between men and women.   *Am J Clin Nutr* 1992;56:917–25.

KEY WORDS Copper absorption, biological half-life, age, sex, oral contraceptives, dietary intake

Introduction

The essentiality of copper for human nutrition is well accepted. However, controversy remains about what the human dietary requirement for copper is and, as a result, no recommended dietary allowance for copper has been set. Currently, the estimated safe and adequate intake of copper is set at 1.5–3 mg/d for both men and women (1).

There is considerable evidence that copper metabolism differs between male and female animals. Concentrations of liver copper (2–6) and liver superoxide dismutase (SOD) activity (5, 6) often differ between male and female rats fed similar diets; other factors such as age or strain of rats and experimental design may result in no sex difference being seen (7, 8) for these factors. Female rats may be less susceptible than males to exacerbation of copper deficiency by dietary fructose (2, 7, 8).

In humans there was no difference between males and females in liver copper concentrations of accident victims aged ≥ 2 y (8). However, Bales et al (9) found that males had more copper in salivary sediment and less copper in plasma than did females. It has long been recognized that plasma copper and ceruloplasmin are higher in women than in men (10). In separate studies

of men and women done in a metabolic unit, several indices of copper status in addition to plasma copper and ceruloplasmin were higher in women than in men (11, 12). Such differences have often been attributed to the effect of female hormones on copper metabolism (10, 13) because indices of copper status such as plasma copper and ceruloplasmin tend to increase during the use of oral contraceptives and during pregnancy (10, 13). In rats, estrogens induce de novo synthesis of ceruloplasmin (14); thus, differences in plasma copper and ceruloplasmin between men and women, as well as changes in these indices during use of oral contraceptives and during pregnancy, are likely caused by differences in the circulating concentrations of estrogens. Despite differences such as these in biochemical indices of copper metabolism, there are no data on whether men and women differ in copper absorption or in the rate of its excretion, or whether they differ in copper requirements.

Studies of the effect of aging on copper status, absorption, or requirement of humans are limited. Turnlund et al (15) found no difference in copper absorption between young and elderly men in a study with limited sample size. Salivary copper changes with aging (9), and plasma copper in men increases with age (9, 10, 16).

Because inadequate dietary copper intake has been suggested to be a factor in the etiology of ischemic heart disease (17), the incidence of which varies between men and women and with age, it would be useful to have detailed information about differences in copper metabolism between men and women at various ages. This report describes a study conducted to examine copper absorption, turnover rate, and biochemical indices of copper status in adult men and women ranging in age from 20 to 83 y. In addition, comparisons were made between women using female hormones either in the form of oral contraceptives or postmenopausal estrogen therapy and women using no hormones.

Methods

Design of study

The study was designed as a 2 × 6 factorially arranged experiment, with sex as one variable and age as the other. There were six age groups for each sex: 20–29, 30–39, 40–49, 50–59, 60–69, and ≥ 70. Additional groups of women who were using oral contraceptives (ages 20–29 and 30–39 y) or receiving estrogen therapy (age 50–59 y) were also studied to determine whether exogenous female hormones would have the same effect on copper metabolism as the innate differences between men and women. No women using either form of hormones in the age group 40–49 y volunteered for the study.

Volunteers were admitted to the study after being informed of its purpose and any associated risks. The project was approved by the Institutional Review Board of the University of North Dakota (UND) and the Human Studies Committee of the United States Department of Agriculture (USDA), Agricultural Research Service, as well as by the UND Radioactive Drug Research Committee and the USDA Radiation Safety Committee. Informed consent and experimental procedures were consistent with the Declaration of Helsinki.

Subjects

All subjects were healthy, with no history of gastrointestinal disease, and all were nonsmokers. Except for women in the hormone groups, none were taking any prescription or nonprescription medication regularly. All subjects had serum cholesterol values < 6.4 mmol/L before entry into the study. All subjects were free-living and consumed self-selected diets throughout the study. All premenopausal women were screened for pregnancy before being allowed to participate in the study.

^{67}Cu with breakfast meal

Subjects reported to the Grand Forks Human Nutrition Research Center in the morning after an overnight 8-h fast. Blood was drawn for measurement of various biochemical indices of copper status. Subjects then consumed a standard breakfast that was extrinsically labeled with 92.5 kBq ^{67}Cu (Research Reactor, University of Missouri, Columbia, MO). The menu for the breakfast, which contained 0.29 mg Cu, is given in **Table 1.** Subjects were provided with a wash bottle containing demineralized water and a rubber spatula in addition to normal eating utensils; they were required to consume the meal quantitatively. Subjects were instructed not to eat or drink for 4 h after the labeled meal. They were provided with a standard sack lunch (Table 1) that contained 0.28 mg Cu. After lunch they were allowed to return to their self-selected diets.

The total dose of ^{67}Cu ingested was determined in subjects ≥ 2 h after the breakfast meal in a whole-body γ counter (18) and then three times weekly for 3 wk after the labeled meal. Subjects showered and put on clean surgical scrub suits or robes before entering the whole-body counter to reduce contamination from ^{222}Rn daughters (^{214}Bi and ^{214}Pb) carried in dust on clothes, skin, and hair.

The whole-body-counting facility includes 32 uncollimated single crystal NaI(Tl) detectors (10 × 10 × 40 cm) located in a steel room, a 512-channel multichannel pulse height analyzer per detector, and a dedicated data processor. The detectors are arrayed 16 above and 16 below the bed, which is located in a steel room. The facility has its own air-handling system that

supplies filtered air from which radon progeny have been removed.

Calculation of ^{67}Cu absorption and biological half-life

Absorption was calculated as the y intercept of a semilogarithmic plot of the percentage of ^{67}Cu retention (corrected for decay) vs time after the labeled meal (19). Biological half-life of copper was calculated as biological half-life = −ln 2/slope by using the slope of the linear portion of the semilogarithmic plot of retention vs time. The semilogarithmic plot was linear beginning at days four or five after the labeled meal.

Dietary-intake records

To estimate subjects' usual dietary intake of copper and other nutrients, they were asked to complete 3-d food diaries once during the study. This was generally done in the week preceding the labeled meal, and included two weekdays and one weekend day. Diaries were reviewed with a dietitian after completion to maximize accuracy of records. Diet diaries were analyzed by using a computerized nutrient database (20–23). In addition to published food-composition values, some locally determined data on copper content of foods (DB Milne, unpublished observations, 1980–1991) were included in the database (< 5% of values retrieved).

Biochemical indices of copper status

Blood was drawn from an antecubital vein after a 12-h fast. Plasma copper was determined by flame atomic absorption spectrophotometry (AAS) after dilution with deionized water (24). Ceruloplasmin was measured both enzymatically as p-phenylenediamine oxidase (25) and by radial immunodiffusion (RID) (Behring Diagnostics, Somerville, NJ) (26). Results of both assays were normalized to milligrams ceruloplasmin per liter by using purified human ceruloplasmin (40 U/mg protein; Sigma, St Louis) as a standard. Activity of CuZn SOD was measured in erythrocytes by the method of Winterbourne et al (27).

Blood cells, platelets, mononucleated white cells (MNCs), neutrophils, and red cells were separated with a Percoll gradient (28, 29). After treatment with Picoex S (United Technologies Packard, Downers Grove, IL), a mild detergent, and sonication, cytochrome C oxidase activity was determined in the platelets and MNCs by a method described by Prohaska and Wells (30).

Uptake of ^{67}Cu by red cells was measured by a modification of the procedure of Berry et al (31). Two milliliters of freshly drawn heparinized blood was incubated with approximately 37

TABLE 1
Breakfast and lunch meals fed on the day of ^{67}Cu administration

Breakfast (in g)*†		Lunch (in g)‡	
Apple juice	180	Grape juice	174
Shredded wheat	25 (1 biscuit)	White roll	40
Low-fat milk (2%)	195	Mayonnaise	12
Sugar	5	Roast turkey (white meat)	50
White bread	30	American cheese	19
Margarine	5	Iceberg lettuce	10
Grape jelly	15	Potato chips	21
Deionized water	200	Vanilla wafer cookies	30

* The meal was labeled with 92.5 kBq ^{67}Cu added to the juice.
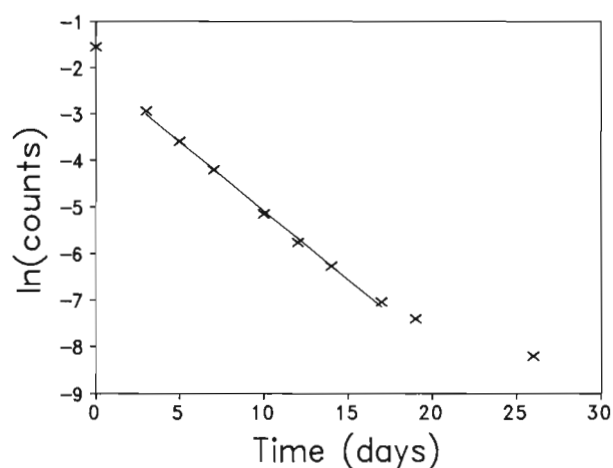† Contained 0.294 mg Cu.
‡ Contained 0.276 mg Cu.

FIG 1. A typical whole-body ^{67}Cu retention plot, showing the natural logarithm of percent retention vs time after a dose of 92.5 kBq.

kBq ^{67}Cu contained in 15–50 μL of saline (9.0 g/L) with the pH adjusted to 7.1. Before incubation, the whole-blood samples containing the isotope were counted in a Beckman (Fullerton, CA) Gamma 5500 well-type γ counter to obtain the total counts. The samples were then incubated for 2 h at 38 °C in a water bath under an atmosphere of 95% O_2 and 5% CO_2. After incubation the cells were separated from the plasma by centrifugation in a refrigerated centrifuge at 1200 × g for 15 min at 4 °C, then washed four times with isotonic saline. The isolated, washed cells were then counted as above.

Copper analysis

Duplicates of the labeled breakfast and the sack lunch were homogenized and aliquot portions were wet ashed with a mixture of trace mineral grade nitric acid and 30% hydrogen peroxide (32). Plasma copper analysis was done by AAS, and dietary copper analyses were done by using inductively coupled argon plasma spectroscopy (33). Bovine liver standard (National Institute of Standards and Technology, Gaithersburg, MD) was analyzed to monitor accuracy of the analysis. Analyzed copper content of the standard was 154.3 μg Cu/g; the certified value was 158 ± 7 μg Cu/g. Analyses of a plasma pool that were performed along with each batch of subject plasma samples from January 1990 to May 1991 gave a value of 12.7 ± 0.6 mmol Cu/L (n = 61).

Statistics

Data were analyzed by using analysis of variance (ANOVA) (34). Women using and not using hormones were compared by age group with a Student's t test. For absorption and biological half-life data, a priori contrasts between men and women ages 20–59 and 60–83 y and between the older and younger subjects were also done.

Results

Measurement of ^{67}Cu activity

Measurements of ^{67}Cu activity in our free-living subjects could be made reliably for 2 wk after the labeled meal, and occasionally longer. A typical ^{67}Cu retention curve is shown in **Figure 1**. ^{222}Rn daughter γ emissions (^{214}Bi and ^{214}Pb) originating in the body interfered with the detection of low-energy γ emissions

from absorbed ^{67}Cu, so that after 2 wk retained ^{67}Cu activity could be measured with statistical significance in some subjects but not in others. **Figure 2** shows the γ-ray spectrum of a subject after a 92.5-kBq dose of ^{67}Cu. By day 19 after the dose, ^{67}Cu was barely detectable and by day 21, only ^{214}Pb and ^{214}Bi peaks were visible in the low-energy portion of the spectrum.

Dietary intake of copper

Estimated dietary copper intake by the subjects is shown in **Table 2**. There was no significant effect of age on estimated dietary copper intake, although the youngest group of women, those in their 20s, consumed the least copper. Men consistently consumed more copper than women in every age group. On average, men are estimated to consume ≈1.3 mg Cu/d, and women ≈ 1.1 mg Cu/d. Although the men consumed almost
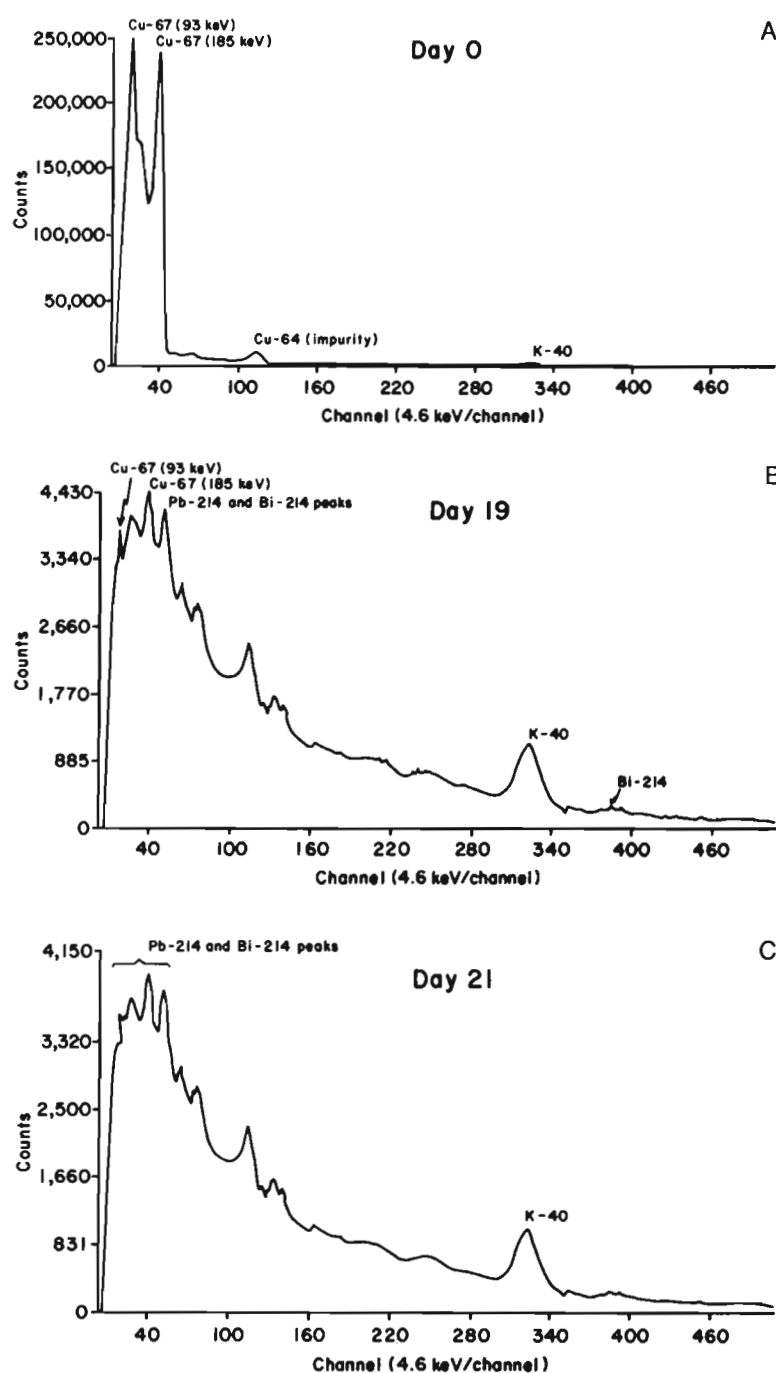

FIG 2. Gamma-ray spectrum of a subject after ingestion of 92.5 kBq ^{67}Cu. A: 2 h postdose, two ^{67}Cu peaks are clearly visible at 93 and 185 keV. B: 19 d postdose, low ^{67}Cu activity resulted in peaks of similar size to those of ^{214}Pb and ^{214}Bi. C: 21 d postdose, no activity of ^{67}Cu can be detected.

TABLE 2
Dietary copper and energy intake

Age (y)	Copper intake		Energy intake*		Copper density*		Body weight†	
	Men	Women	Men	Women	Men	Women	Men	Women
	mg/d	mg/d	MJ/d	MJ/d	mg Cu/MJ	mg Cu/MJ	kg	kg
20–29	1.3 [9]‡	0.9 [17]	10.75	6.38	0.13	0.15	74.0	67.3
30–39	1.3 [10]	1.0 [19]	10.06	7.21	0.13	0.14	85.7	71.9
40–49	1.3 [10]	1.1 [8]	9.94	7.44	0.13	0.15	84.1	66.3
50–59	1.3 [10]	1.1 [16]	10.25	7.00	0.13	0.16	91.6	76.6
60–69	1.3 [6]	1.0 [10]	8.37	5.94	0.13	0.18	81.7	66.9
70–83	1.3 [6]	1.2 [6]	8.84	6.86	0.15	0.17	78.7	67.2

* Significant sex effect, $P < 0.0001$.
† Significant sex effect, $P < 0.0005$.
‡ $\bar{x}$; n in brackets and is the same for energy intake, copper density, and body weight.

one and a half times as much energy as the women, they consumed only about one and a fourth times as much copper. This is reflected in the higher copper density of the women's diets; women consumed significantly more copper/MJ than did men.

There were no differences in estimated dietary intake of energy or copper between subjects who used hormones and those who did not, except that women in their 20s who used oral contraceptives consumed greater amounts of energy (8.07 ± 1.41 MJ) than did those who did not use contraceptives (6.38 ± 1.69 MJ, $P < 0.05$).

Effect of hormone use

The effects of hormone use on biochemical indices of copper status are shown in **Table 3**. There were no women in the 40-y-old age group who used either oral contraceptives or estrogen. Plasma copper was significantly higher in women in their 20s and 30s who used oral contraceptives than in those who did not, but plasma copper in women in their 50s who were taking estrogen did not differ significantly from plasma copper in women who did not use estrogens. Ceruloplasmin, measured enzymatically and by RID, was significantly higher in oral-contraceptive users in their 30s than in nonusers. Differences in ceruloplasmin values between oral-contraceptive users and nonusers in their 20s were not significant. Estrogen use by women in their 50s had no effect on ceruloplasmin measured by either method. The ratio of enzymatically measured ceruloplasmin to that measured by RID was not affected by hormone use in any of the groups. SOD activity was not affected by hormone use in any of the women. Uptake of ^{67}Cu by red blood cells in vitro was not significantly affected by estrogen in any age group; however, there were only two women in the estrogen-user group.

Activity of cytochrome C oxidase in MNCs was significantly higher in oral-contraceptive users than in nonusers in their 30s, but did not differ with hormone use in the other two age groups. Cytochrome C oxidase activity in platelets was not affected by hormone use in any of the groups. Women in their 20s who used oral contraceptives had significantly fewer platelets than did those who did not use oral contraceptives. Absorption and biological half-life of ^{67}Cu were not affected by use of oral contraceptives or estrogens in any of the groups (Table 3).

Effects of age and sex

The effects of age and sex on biochemical indices of copper status are shown in **Table 4**. Subjects were blocked by decade for the ANOVA. Because there were only two subjects in their 80s, a man aged 83 y and a woman aged 82 y, they were included in the ≥ 70 age group. The data for women in Table 4 are for nonusers of hormones only.

Both age and sex affected plasma copper concentrations significantly. Women in every age group had higher plasma copper than men. Plasma copper increased with increasing age, reaching maximum values during the 60s, and then declined slightly. Enzymatically measured ceruloplasmin was significantly affected only by sex, with values in women being consistently higher than in men except in the age ≥ 70 group. Ceruloplasmin measured by RID was significantly affected by both age and sex of subjects. Values for RID ceruloplasmin were significantly higher for women than for men in all age groups. Although RID ceruloplasmin varied with age, there was no clear directional trend. The ratio of enzymatically measured ceruloplasmin to that measured by RID was unaffected by either age or sex. The activity of CuZn SOD was unaffected by either age or sex. Likewise, uptake of ^{67}Cu was unaffected by either experimental variable.

The activity of cytochrome C oxidase in MNCs and in platelets was significantly affected by age but not by sex. Activity in MNCs tended to increase with age. Activity in platelets generally increased with age until the 60s, and then declined slightly. When cytochrome C oxidase activity in platelets was expressed on the basis of protein concentration, activity was significantly affected by age but not sex, although men had higher cytochrome C activity than women at every age except in the 30s and 40s. Peak values for both men and women occurred in the 60s age group. There were no significant effects of age or sex on platelet number, mean platelet volume, or platelet protein concentration (data not shown).

The effects of age and sex on absorption and biological half-life of ^{67}Cu are shown in **Table 5**. The data for women include both hormone users and nonusers because no differences in absorption or biological half-life were found between those groups. The distribution of biological half-lives was skewed, so the biological half-life values were log transformed to achieve a normal distribution before statistical analysis. Younger (ages 20–59 y)

TABLE 3
Effect of oral-contraceptive use or estrogen therapy on biochemical indices of copper status, copper absorption, and biological half-life in women*

Age (y)	No hormone use	Hormone use
20–29		
Copper (μmol/L)	15.9 ± 4.2 [10]	21.2 ± 2.8 [10]†
Ceruloplasmin (enz; mg/L)	502 ± 128 [10]	612 ± 140 [10]
Ceruloplasmin (RID; mg/L)	330 ± 61 [10]	372 ± 70 [10]
Ceruloplasmin (enz/RID)	1.53 ± 0.34 [10]	1.71 ± 0.51 [10]
Superoxide dismutase (U/g Hb)	2839 ± 464 [10]	3381 ± 674 [8]
^{67}Cu uptake (%)	16.8 ± 5.3 [8]	16.2 ± 8.2 [7]
Cytochrome C oxidase (U/10^6 platelets)	2.36 ± 1.40 [9]	2.87 ± 1.16 [9]
Cytochrome C oxidase (in MNCs; U/mg protein)	0.294 ± 0.136 [6]	0.266 ± 0.065 [5]
Mean platelet volume	8.4 ± 0.9 [10]	8.6 ± 0.9 [10]
Platelets (10^6)	311 ± 88 [10]	247 ± 37 [10]‡
Percent copper absorption (%)	72.9 ± 9.8 [9]	75.4 ± 13.5 [8]
ln (biological half-life)	2.91 ± 0.40 [9]	2.95 ± 0.46 [10]
30–39		
Copper (μmol/L)	15.5 ± 2.2 [10]	25.3 ± 3.5 [11]§
Ceruloplasmin (enz; mg/L)	516 ± 163 [10]	744 ± 167 [11]†
Ceruloplasmin (RID; mg/L)	314 ± 44 [10]	525 ± 69 [11]§
Ceruloplasmin (enz/RID)	1.66 ± 0.57 [10]	1.42 ± 0.30 [11]
Superoxide dismutase (U/g Hb)	3173 ± 568 [9]	2889 ± 557 [11]
^{67}Cu uptake (%)	16.6 ± 4.1 [8]	18.6 ± 5.4 [9]
Cytochrome C oxidase (U/10^6 platelets)	2.75 ± 1.14 [9]	3.87 ± 1.73 [10]
Cytochrome C oxidase (in MNCs; U/mg protein)	0.263 ± 0.052 [8]	0.364 ± 0.086 [7]‡
Mean platelet volume	8.7 ± 1.0 [10]	8.2 ± 0.7 [11]
Platelets (10^6)	305 ± 63 [10]	311 ± 74 [11]
Percent copper absorption	70.7 ± 19.8 [10]	74.8 ± 13.8 [9]
ln (biological half-life)	3.17 ± 0.62 [7]	2.98 ± 0.47 [10]
50–59		
Copper (μmol/L)	17.2 ± 2.8 [12]	19.2 ± 2.0 [4]
Ceruloplasmin (enz; mg/L)	517 ± 85 [12]	593 ± 100 [4]
Ceruloplasmin (RID; mg/L)	370 ± 77 [12]	383 ± 72 [4]
Ceruloplasmin (enz/RID)	1.45 ± 0.34 [12]	1.57 ± 0.28 [4]
Superoxide dismutase	3360 ± 823 [12]	2839 ± 225 [3]
^{67}Cu uptake (%)	20.4 ± 7.2 [7]	13.9 ± 0.4 [2]
Cytochrome C oxidase (U/10^6 platelets)	3.93 ± 0.97 [11]	3.54 ± 1.55 [3]
Cytochrome C oxidase (in MNCs; U/mg protein)	0.384 ± 0.081 [6]	0.318 ± 0.077 [3]
Mean platelet volume	8.5 ± 0.8 [12]	8.9 ± 1.0 [4]
Platelets (10^6)	278 ± 51 [12]	239 ± 58 [4]
Percent copper absorption (%)	73.0 ± 13.6 [12]	72.6 ± 9.7 [4]
ln (biological half-life)	3.00 ± 0.40 [11]	2.94 ± 0.75 [4]

* Women aged 20–39 y used oral contraceptives; women aged 50–59 y were postmenopausal women on estrogen therapy. There were no subjects aged 40–49 y who used either form of hormones. *n* in brackets. $\bar{x}$ ± SD. enz, measured enzymatically; RID, measure by radialimmunodiffusion; MNC, mononucleated white cells.

†‡§ Significantly different from no hormone use (Student's *t* test): †$P < 0.01$, ‡$P < 0.05$, §$P < 0.0001$.

women absorbed more copper from the labeled breakfast than did men the same age. However, in the two highest age groups, a priori contrasts showed that men and women did not differ in percent absorption. Younger women (aged 20–59 y) had significantly shorter biological half-lives for ^{67}Cu than did men the same ages, indicating that they turned over absorbed copper more rapidly than did men. However, the differences between the sexes disappeared in the 60–83 y age group. Overall, the difference between younger (20–59 y) and older (60–83 y) subjects was highly significant ($P < 0.005$), with biological half-life values being shorter in the older subjects.

Net copper absorption from the diet was estimated by assuming that dietary copper absorption equaled that from the labeled meal; thus, net copper absorption was calculated as the product of percent copper absorption and dietary copper intake. When

this value was normalized by body weight, net copper absorption in all subjects ranged from 9 to 13 μg · kg body wt^{-1} · d^{-1} (Table 5). There was no effect of age or sex on these values.

Discussion

Measurement of ^{67}Cu

Measurement of whole-body retention of ^{67}Cu activity in humans was not as satisfactory in these free-living subjects as we previously found it to be in subjects who lived on a metabolic unit (GI Lykken, KK Speaker, FH Nielsen, PE Johnson, unpublished observations, 1991). In a previous study at this Center, men living in a metabolic unit were given doses of 185 kBq ^{67}Cu; these doses were reduced to 92.5 kBq when it was found

TABLE 4
Effects of age and sex on indices of copper status*

| Age (y) and sex* | Ceruloplasmin | | | | SOD | Cytochrome C oxidase‖ | | | ^{67}Cu-uptake |
| | Plasma copper† | Enz‡ | RID§ | Enz/RID | | Platelets | | MNC | |
	$\mu mol/L$	mg/L	mg/L		$U/g\ Hb$	$U/10^6$	$U/mg\ protein$	$U/10^2$	%
20, M (n = 10)	12.2	408	266	1.56	3239	2.75	0.761	0.328	19.3
20, F (n = 10)	15.9	503	330	1.53	2839	2.36	0.671	0.274	16.8
30, M (n = 10)	12.0	416	296	1.49	3081	2.94	0.834	0.314	15.7
30, F (n = 10)	15.5	516	314	1.66	3172	2.75	0.830	0.359	16.6
40, M (n = 10)	13.6	445	338	1.31	3014	3.59	0.520	0.389	17.7
40, F (n = 10)	16.7	506	350	1.49	2944	3.04	0.872	0.330	19.7
50, M (n = 9)	13.7	441	310	1.43	3170	3.89	1.121	0.345	16.9
50, F (n = 12)	17.2	517	370	1.45	3360	3.93	0.900	0.388	20.4
60, M (n = 8)	13.7	470	284	1.70	3134	4.92	1.532	0.430	19.0
60, F (n = 10)	17.9	559	354	1.60	3912	4.56	1.124	0.373	12.4
70, M (n = 7)	14.1	506	350	1.49	2894	4.51	1.268	0.448	16.4
70, F (n = 7)	16.6	470	368	1.29	2836	3.66	0.744	0.388	16.5
Root mean square error	2.3	90	60	0.33	652	1.36	0.372	0.081	6.1

* Data for only subjects who did not use oral contraceptives or hormones. Enz, measured enzymatically; RID, measured by radial immunodiffusion; SOD, superoxide dismutase; and MNC, mononuclear white cells.

† Sex effect, $P < 0.0001$; decade effect, $P < 0.05$.

‡ Sex effect, $P < 0.0004$.

§ Sex effect, $P < 0.0009$; decade effect, $P < 0.03$.

‖ Decade effect: platelets $P < 0.0001$, MNC $P < 0.004$.

that the lower dose was sufficient to observe statistically significant ^{67}Cu γ-ray emissions above background for 3 wk after an oral dose. On the basis of that experience, we used doses of 92.5 kBq in this study. In this study it was not possible to measure ^{67}Cu activity in the subjects for > 2 wk after dosing on a consistent basis. This was apparently related to greater body burdens of radon (^{222}Rn) and its daughters (^{214}Bi and ^{214}Pb) in the bodies of subjects who were living in their own homes than in bodies of subjects living in the metabolic unit. The bedroom radon

concentration on the metabolic unit, which is on the third floor, was < 9 Bq/m^3, whereas the mean in the bedrooms of free-living adults in Grand Forks in another study was > 500 Bq/m^3 (GI Lykken, et al, unpublished observations, 1991). The complex γ-ray spectra from ^{214}Bi and ^{214}Pb arising from decay of inhaled ^{222}Rn and internally deposited radon daughters masked the low-energy γ-ray emissions from ^{67}Cu. Thus, despite the precautions taken to reduce contribution of radon and its daughters to the ^{67}Cu measurement (filtered air, subjects' showers and clothing),

TABLE 5
Copper absorption and biological half-life of ^{67}Cu*

| Age (y) | Percent absorption† | | Biological half-life‡ | | | | Net copper absorption§ | |
| | Men | Women | Men | | Women | | Men | Women |
	%	%	ln	d	ln	d	$\mu g \cdot kg\ body\ wt^{-1} \cdot d^{-1}$	
20–29	69.2 [9]	74.1 [17]	3.17	23.8 [10]	2.93 [19]	18.7	12.8	11.1
30–39	62.0 [10]	72.6 [19]	3.23	25.3 [9]	3.06 [17]	21.3	9.8	11.6
40–49	62.3 [11]	66.1 [8]	3.49	32.8 [10]	2.89 [9]	18.0	10.7	10.3
50–59	66.2 [9]	72.9 [16]	3.19	24.3 [9]	2.98 [15]	19.7	9.6	10.4
60–69	67.8 [6]	73.8 [10]	2.75	15.6 [6]	2.94 [10]	18.9	10.8	11.6
70–83	78.6 [6]	72.8 [6]	2.57	13.1 [6]	2.96 [7]	19.3	13.1	12.7
Root mean square error‖	12.9			0.51			4.2	

* Values for women are for both women who used oral contraceptives or hormones and those who did not, because no differences between the groups were found for copper absorption or BH.

† Sex effect, $P = 0.08$; A priori contrasts, men vs women: 20–59 y $P < 0.02$, 20–59 vs 60–83 y $P = 0.08$.

‡ A priori contrasts, men vs women: 20–59 y $P < 0.006$, 20–59 vs 60–83 y $P < 0.005$.

§ Calculated from (% copper absorption × copper intake) ÷ body weight.

‖ For men and women combined.

the background radon and its progeny from the subjects' residences had a significant impact on the quality of the counting data. In future studies of free-living subjects, higher doses of 185 kBq ^{67}Cu should be sufficient to alleviate this problem under the counting conditions we have described. Care must be taken to minimize background not only in γ-counting facilities but also in the environments of experimental subjects, when possible.

Copper absorption and biological half-life

Copper-absorption values in this study were similar to those we have measured using stable isotopes in men and women consuming 1–1.5 mg Cu/d (35, 36) and to those of King et al (37) and of August et al (38). Early studies with radioisotopes in subjects consuming uncontrolled diets yielded copper absorption values averaging $\approx$60% (13, 39). The values in the current study are higher than values reported by Turnlund et al (15, 40–44) in various studies of copper absorption in men and women fed formula diets containing 2–3 mg Cu/d. Our copper absorption values are most similar to those reported by Turnlund et al for men fed 0.78 mg Cu/d (45). Subjects consuming diets composed of ordinary foods, whether self-selected or metabolic diets, seem to have higher copper absorption than subjects in studies that used formula diets. Subjects in this study were consuming ordinary self-selected diets, and subjects in our earlier studies (35, 36) and in the study of August et al (38) were consuming mixed Western diets. Evidently, there is an effect of diet composition or physical form on copper absorption that is independent of the effect of dietary copper content.

Biological half-life values for ^{67}Cu in this study were similar to those reported by GI Lykken et al (unpublished observations, 1991). Extrinsic isotopic tracers for copper are valid (35, 36), so the absorption values reported in this experiment should accurately reflect copper absorption from the labeled breakfast meal.

Dietary intakes

Estimated dietary copper intakes of these healthy men and women were less than the estimated safe and adequate intake of 1.5–3.0 mg Cu/d for every age and sex group (1). This finding is consistent with previous reports that many US diets provide < 1.6 mg Cu/d (46) and is typical of self-chosen diets in the United States (47). Higher intakes of copper by men than by women were probably related to men's larger body weights and their higher energy intakes compared with women. However, the women's diets were more concentrated in copper, so that when copper intake was normalized for body weight, copper intakes of men and women were similar.

Effect of hormones

Higher plasma copper and ceruloplasmin in women using oral contraceptives compared with nonusers are consistent with previous reports (10, 37). The effect of postmenopausal estrogen therapy on copper metabolism seemed to be different than the effect of oral contraceptives, because estrogens did not result in significantly increased plasma copper or ceruloplasmin in the women in their 50s. However, the lack of a significant effect of estrogen on these factors may be related to the small number of subjects we had who were in the estrogen group. We did not measure blood concentrations of circulating female hormones in our subjects; hormone concentrations may have differed between women using oral contraceptives and postmenopausal women with estrogen therapy.

Despite the biochemical differences between women who used hormones and those who did not, no differences in absorption or biological half-life of ^{67}Cu were found. This demonstrates that plasma copper and ceruloplasmin are not good indicators of copper status in adult women, if one accepts the premise that differences in copper status should result in differences in absorption and excretion of copper as the body strives to maintain homeostasis. The lack of difference in copper absorption between women who used oral contraceptives and those who did not is consistent with the findings of King et al (37).

Effect of sex

Consistent with previous reports (10, 12), several biochemical indices of copper nutrition in our subjects were higher in women than in men. Despite this, women in this study aged 20–59 y absorbed more copper than did men from the labeled breakfast, and they had more rapid turnover of copper once it was absorbed. In rats, percentage copper absorption is significantly correlated inversely with plasma copper, ceruloplasmin, liver SOD, and cytochrome C oxidase activity (48), but such relationships were not seen in this study; instead, women with higher plasma copper and ceruloplasmin concentrations than men absorbed more copper than men. In both rats (48) and humans (45), copper absorption has been shown to vary inversely with total dietary copper intake. In this study, total copper intakes of men were greater than those of women. The relationship between total copper intake and percent absorption in this study is consistent with the earlier findings. Dietary copper intake normalized for body weight did not differ between the sexes and was not related to percent copper absorption. Differences in copper absorption between men and women aged 20–59 y, but not between those aged 60–83 y, suggest that the concentrations of circulating estrogens might be related to copper absorption because estrogen concentrations would drop in women after menopause. This idea is contradicted by the fact that we found no effect of exogenous estrogens (oral contraceptives or postmenopausal therapy) on copper absorption.

The percentage of copper absorbed from the labeled meal is not necessarily the percentage of copper that these subjects absorb from their daily self-selected diets. However, because the labeled meal comprised a typical US breakfast menu, copper absorption from this meal should be somewhat typical of copper absorption by these subjects. If one assumes that percent copper absorption from the labeled meal is the same as copper absorption from the usual self-selected diet, then total copper absorbed from the usual diet was $\approx$0.8–0.9 mg Cu/d for men and 0.65–0.8 mg Cu/d for women. When these values were normalized for body weight, the differences between men and women disappeared, and copper absorption ranged from 9 to 13 μg Cu $\cdot$ kg body wt$^{-1} \cdot$ d^{-1} in both men and women at all ages. This suggests that on a body-weight basis, men and women have similar copper requirements; in terms of total dietary intakes, women have smaller copper requirements than do men.

Younger (ages 20–59 y) women in this study had shorter biological half-lives of ^{67}Cu than did men of the same ages. Ordinarily, one would associate a shorter biological half-life with a higher rather than lower intake of copper (48), but that was not the case in these subjects. Evidently, factors other than dietary copper intake are involved in regulation of copper homeostasis in these subjects.

Effect of age

No significant overall effect of age on copper absorption was found in this study with men and women ranging in age from 20 to 83 y. The copper-absorption values in these subjects were not much lower than those we found in 3-mo-old breast-fed and formula-fed infants (49), suggesting that there is little change in the efficiency of copper absorption throughout the human life span. However, in infants we did not find a difference in copper absorption between males and females.

In a study that compared copper absorption in young and elderly subjects at two dietary copper intakes, August et al (38) found that the increase in copper absorption when low-copper diets were fed was greater in elderly subjects (aged 71.5 ± 6.2 y) than in young adults (aged 20.0 ± 1.2 y). Copper absorption in the study by August et al did not differ between elderly and young subjects fed either low-copper or adequate-copper diets. However, these findings are confounded by the fact that the elderly subjects in their study were half male and half female, whereas the young adults were all male. Increases in copper absorption when the low-copper diet was fed were greater in their male subjects than in female subjects.

Several effects of age on biochemical indices of copper status were observed in this study, and effects on biological half-life were found as well. There was a tendency for a number of the biochemical indices to increase with age and then decrease in the 60- and 70-y-old age groups (Table 4). This pattern was also observed with the ^{67}Cu absorption and biological half-life values. Copper absorption was higher in women than in men aged 20–59 y, but in the age ≥ 70 group, the difference disappeared. ^{67}Cu biological half-life values were shorter for women than for men aged 20–59 y, but men in their 70s had shorter biological half-lives for ^{67}Cu than did women. The tendency for the biochemical indices and the radioisotope data to follow the same pattern, where the differences between the sexes were reversed in the oldest subjects, suggests that these data represent real changes. Unfortunately, we did not have as many subjects in the ≥ 70 group as in the other age groups. We found it difficult to recruit older subjects who met our criteria of using no prescription or nonprescription drugs regularly. We do not know whether the changes that seem to occur in our healthy subjects aged ≥ 70 y, compared with younger subjects, would also be seen in elderly subjects who are taking various kinds of medication regularly.

In summary, total copper intake by women was less than that by men, but this was apparently counterbalanced by a greater efficiency of copper absorption in women, so that on a body-weight basis, women absorbed as much copper as did men. This suggests that women have a lower requirement for total dietary copper intake than do men. The sex differences we found in copper metabolism were less marked than the epidemiological differences between men and women with respect to heart disease. There were some changes in biochemical indices of copper status with aging, but these were not accompanied by significant changes in copper absorption or excretion with age. However, limited data in our oldest subject group suggest that copper metabolism may change in men and women aged > 70 y; this issue needs further study.   ⬇

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