Adaptive responses in men fed low- and high-copper diets

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The study of Cu metabolism is hampered by a lack of sensitive and specific biomarkers of status and suitable isotopic labels, but limited information suggests that Cu homeostasis is maintained through changes in absorption and endogenous loss. The aim of the present study was to employ stable-isotope techniques to measure Cu absorption and endogenous losses in adult men adapted to low, moderate and high Cu-supplemented diets. Twelve healthy men, aged 20–59 years, were given diets containing 0·7, 1·6 and 6·0 mg Cu/d for 8 weeks, with at least 4 weeks intervening washout periods. After 6 weeks adaptation, apparent and true absorption of Cu were determined by measuring luminal loss and endogenous excretion of Cu following oral administration of 3 mg highly enriched ^{65}Cu stable-isotope label. Apparent and true absorption (41 and 48 % respectively) on the low-Cu diet were not significantly different from the high-Cu diet (45 and 48 % respectively). Endogenous losses were significantly reduced on the low- (0·45 mg/d; $P < 0·001$) and medium- (0·81 mg/d; $P = 0·001$) compared with the high-Cu diet (2·46 mg/d). No biochemical changes resulting from the dietary intervention were observed. Cu homeostasis was maintained over a wide range of intake and more rapidly at the lower intake, mainly through changes in endogenous excretion.

Copper: Fructose: Stable isotopes: Copper absorption: Copper endogenous losses

Whole-body Cu balance (homeostasis) is maintained by a combination of changes in absorptive efficiency of the intestinal mucosal cells and quantity of endogenous Cu excreted via the gastrointestinal tract. At low and high intakes the efficiency of absorption is up and down regulated respectively, but adaptation is more rapid with a low Cu intake (Turnlund *et al.* 1989). The extent to which this endogenously excreted Cu is reabsorbed from the gut is not known (Linder, 1991).

Cu absorption decreases with malnutrition and in various disease states with malabsorption syndromes, for example, coeliac disease (Cordano *et al.* 1964; Goyens *et al.* 1985), and several dietary factors reduce absorption, for example, antacids, infant cows' milk formulas (Dorner *et al.* 1989), Zn (Turnlund *et al.* 1988; August *et al.* 1989) and fructose. The Cu–fructose interaction is potentially the most important due to the increasing consumption of fructose in the Western diet. However, the mechanisms are uncertain because the results of human studies have proved difficult to interpret. Fructose has been found to impair Cu status, whilst Cu balance appears to be increased (Reiser *et al.* 1985).

Cu absorption has been measured in human volunteers using a highly enriched ^{65}Cu stable isotope, with doses corresponding to 50–200 % of typical daily intakes (August *et al.* 1989; Turnlund *et al.* 1991). In these studies, the unabsorbed Cu isotope is excreted in the faeces within 5–7 d, followed by smaller amounts of isotope that have been absorbed and then re-excreted (endogenous loss). Thus the period of time over which the faecal collections are made is critical; if it is too short then the apparent absorption (intake minus luminal loss) will be over-estimated, as not all of the unabsorbed Cu will have been excreted. In addition, it is only possible to calculate true absorption if endogenous excretion has been estimated from the appearance of absorbed isotope in the later faecal collections.

The aim of the present study was to determine Cu absorption, retention and endogenous losses following equilibration at different levels of intake of Cu, from that found in diets low in Cu to a level generally only attainable with Cu supplements. In the absence of a sensitive and specific index of Cu status, this was believed to be the best approach for generating information needed to assess the public health significance of low and high Cu intakes. A range of biochemical assays was undertaken at the beginning and end of each dietary intervention in order to underpin the metabolic measurements.

Methods

Subjects

Twelve healthy men were recruited to a residential, longitudinal intervention study (subject characteristics are given in Table 1). A screening blood sample (10 ml) was taken to exclude volunteers whose biochemical and haematological indices lay outside the normal range. Other exclusion criteria included chronic illness, taking medication or nutritional supplements, and smoking. The aims and procedures of the study were explained to the volunteers during a visit to the Human Nutrition Unit at the Institute of Food Research (IFR) and written informed consent was obtained. The IFR Human Research Ethics Committee approved the protocol and the study was conducted in accord with the Helsinki Declaration of 1975 as revised in 1983.

Study design

The subjects were resident in the Human Nutrition Unit for three periods of 8 weeks with a minimum washout of 4 weeks between study periods. A diet low in Cu was fed throughout the study using a 7 d rotating menu consisting of three meals per d and snacks. Additional Cu was added to the diets in the form of copper sulfate with each meal, such that the total daily intake was 1·6 and 6·0 mg during the first and third dietary periods respectively. The diets were regularly analysed to ensure that the Cu content remained constant from batch to batch. During each study period, the energy intakes of the subjects were adjusted as necessary in order to maintain body weight by the addition of either a commercially available dextrose drink or yoghurt. The mean Cu content of the diet analysed by atomic absorption spectroscopy was 0·69 (SD 0·02) mg/d. All other dietary variables were within customary UK limits. The diet had a mean daily energy intake of 10·78 MJ (2577 kcal), of which 12 % was from protein, 52 % from carbohydrate and 36 % from fat. The mean calculated daily mineral content of the diet was 2845 mg K, 2234 mg Na, 1299 mg Ca, 2918 mg Mg, 7·7 mg Fe and 7·6 mg Zn. The habitual dietary Cu intake of each volunteer was assessed before the residential part of the study. Each volunteer kept a 7 d weighed food intake diary, which was analysed for Cu content using food composition tables (Royal Society of Chemistry, 1991). Subjects also collected 24 h duplicate diets during this period. The daily collections were homogenised and analysed for Cu content using the inductively coupled plasma–mass spectrometry (ICP–MS) facility at the Central Science Laboratory.

On day 42 of each dietary period, following a 12 h overnight fast, each volunteer received a 3 mg oral dose of the highly enriched ^{65}Cu stable isotope in 130 g of a diet cola drink together with a low-Cu meal consisting of 70 g white bread and 17 g butter. The cola drink also contained 1 mg of the rare earth element Ho, which acted as a non-absorbable faecal marker (Harvey *et al.* 2002). Six of the volunteers (randomly assigned) also received 25 g fructose dissolved in the cola drink. The effect of the fructose was not evaluated. The volunteers refrained from eating and drinking (except demineralised water) for 4 h following the test meal. Complete faecal collections were made for a period of approximately 14 d post-dosing and each subject also collected a baseline faecal sample before the test meal.

Fasting blood samples were taken on days 1 and 42 of each dietary period for a range of biochemical assays (see p. 163).

Dose preparation

Isotopically enriched copper chloride (^{65}CuCl$_2$) was prepared from elemental ^{65}Cu (Europa Scientific Ltd, Crewe, UK) by dissolving the metal in 10 ml concentrated HNO$_3$ (BDH, Poole, UK) (Aristar grade) and evaporating to virtual dryness. The sample was then taken up in 0·1 M-HCl (25 ml; BDH, Aristar grade) and again evaporated to almost dryness; this stage was then repeated twice more and finally the sample was taken up in an appropriate volume of 0·1 M-HCl to give an approximate concentration of 1 mg/ml. The concentration was accurately determined by ICP–MS. The solution was divided into individual doses and stored in plastic vials at −20°C until required.

The Ho oral doses were prepared by dissolving holmium chloride (Avocado Research Chemicals Ltd, Heysham, UK) in demineralised, purified water (Elga, Cambridge) to concentrations of approximately 0·5 mg/ml. The solution was divided into individual 1 mg doses and stored in plastic vials at −20°C until required and the concentration accurately determined by ICP–MS.

Sample preparation and analysis

Faecal samples were autoclaved, freeze-dried, ground to a fine powder using a mortar and pestle, and sub-sampled. Before analysis by ICP–MS, portions of faecal samples (0·5 g) were digested in concentrated nitric acid (5 ml) using a high temperature–pressure microwave digestion system and the digest fluid diluted 50-fold with distilled water containing Ga (50 ng/ml) as an internal standard. The total levels of Cu and the ^{63}Cu:^{65}Cu isotope value in the solutions were measured using a VG Plasmaquad Turbo II Plus ICP–MS (VG Elemental, Winsford, UK) (Baxter *et al.* 1997). The quality of the analytical data was assessed in two ways; first, by the use of a certified reference material (BCR 422 cod muscle), and second, by the recovery of a known amount of added analyte. Ho concentrations were also quantified by ICP–MS (Baxter *et al.* 1997).

Biochemical assays

Blood samples collected on days 1 and 42 were prepared for various analyses performed either at the IFR or at the Chemical Pathology Department of the Norfolk and

Table 1. Subject characteristics
(Mean values and standard deviations for twelve subjects)

	Mean	SD	Range
Age (years)	32	11	20–59
Height (m)	1·79	0·06	1·70–1·94
Weight (kg)	78·4	9·3	67·8–100·3
BMI (kg/m^2)	24	2	20–28

Norwich Hospital (Norwich, UK). Analyses performed by the Chemical Pathology Department included serum Cu, caeruloplasmin, C-reactive protein and a full lipid screen. Serum Cu was measured by atomic absorption spectroscopy (Phillips model no. PU9200; Phillips, Cambridge, UK) and total caeruloplasmin by an immuno-turbidimetry assay (Dako, High Wycombe, UK). The inter-assay CV were 6·4 and 10 % for the Cu and caeruloplasmin respectively. Serum samples were also analysed for triacylglycerols and total, LDL- and HDL-cholesterol; LDL-cholesterol was calculated using the Friedewald equation (Friedewald *et al.* 1972). Apolipoprotein A1 and B concentrations were determined using immunoturbidimetry assays (IMMUNO, Ltd., Sevenoaks, Kent, UK) automated on a Cobas Fara centrifugal analyser (Roche, Welwyn Garden City, UK). In order to eliminate raised caeruloplasmin or ferritin concentrations that resulted from an inflammatory response or infection, C-reactive protein concentrations were determined using an immuno-turbidimetry assay (Dako). Caeruloplasmin activity was determined at the Northern Ireland Centre for Diet and Health using a modified method of Henry *et al.* (1960) on an autoanalyser (Cobas Fara; Roche).

The remaining assays were performed at the IFR using an autoanalyser (Cobas Mira; Roche). Control serum (Serum N; Roche) and quality control samples prepared in-house were used for each assay as appropriate:

(a) Cu,Zn-superoxide dismutase (SOD) was measured in erythrocytes using a commercial kit (Ransod kit; Randox Laboratories, Crumlin, UK) based on a modified method of Jones & Suttle (1981). SOD activity was expressed in terms of SOD units/g haemoglobin measured by Drabkin's method (Drabkins & Austin, 1932) standardised with cyano-methaemoglobin (BDH standard).

(b) Glutathione peroxidase (GPx) activity was determined in plasma, platelets and erythrocytes using a commercially available kit (Ransel test kit; Randox Laboratories) based on a modified method of Paglia & Valentine (1967). Gpx activity was expressed in terms of protein concentration (Unimate 7 Total protein kit; Roche) in plasma and platelets (GPx activity/g protein) and haemoglobin concentration in erythrocytes (Gpx activity/g haemoglobin).

(c) Plasma ferritin was determined by an in-house ELISA assay using A133-rabbit anti-human ferritin coating anti-body and P145-rabbit anti-human ferritin detector antibody, peroxidase conjugated (Dako). The assay was standardised using the 3rd International Standard for Ferritin (National Institute for Biological Standards and Control, Potters Bar, UK).

(d) Platelet aggregation was determined as a response to ADP at a final concentration of 2×10^{-5} mol/l in citrated blood using a platelet aggregation profiler (model PAP-4; Bio/Data Corporation, Horsham, PA) (Williams *et al.* 1977).

(e) Other haematological indices including haemoglobin, packed cell volume and mean cell haemoglobin concentration were measured using an MD8 Coulter Counter (Coulter Electronics Ltd, Hialeah, FL, USA).

Mathematical analysis

Quantities of labelled and unlabelled Cu were calculated according to the method described in Harvey *et al.* (2002); this paper also details the calculation of endogenous losses from the labelled Cu dose. Fig. 1 shows an overview of the labelled and unlabelled Cu absorption and excretion that was measured and calculated in the present study. The following equations were used to generate the results shown in Tables 2 and 6.

Labelled copper absorption and excretion.

$$\text{Apparent absorption} = (D_L - R_L)/D_L,$$
$$\text{True absorption} = (D_L - R_L + E_L)/D_L$$
$$= A_L/D_L = A_U/D_U, \qquad (1)$$

where D_L is the labelled Cu dose, R_L is the labelled Cu recovered in faeces, A_L is the absorbed labelled Cu, A_U is the absorbed unlabelled Cu and D_U is the unlabelled Cu in the diet. Endogenous loss $(E_L;\ \text{mg}) = F_L + S_L$ where F_L (mg) is the loss of labelled Cu from a 'fast' pool and S_L (mg) is the loss of labelled Cu from a 'slow' pool. Labelled Cu from the fast pool is defined to be Cu that was absorbed from the dose and then excreted within 14 d. Labelled Cu from the slow pool is defined to be Cu that was absorbed from the dose and excreted more than 14 d after the dose. Since the study period is only 14 d, labelled Cu from the slow pool is not measured and $S_L = 0$. Therefore all the endogenous losses of labelled Cu are from the fast pool, i.e. $E_L = F_L$.

$$\text{Endogenous loss (as a fraction of dose)}$$
$$= E_L/D_L = F_L/D_L = F_U/D_U, \qquad (2)$$

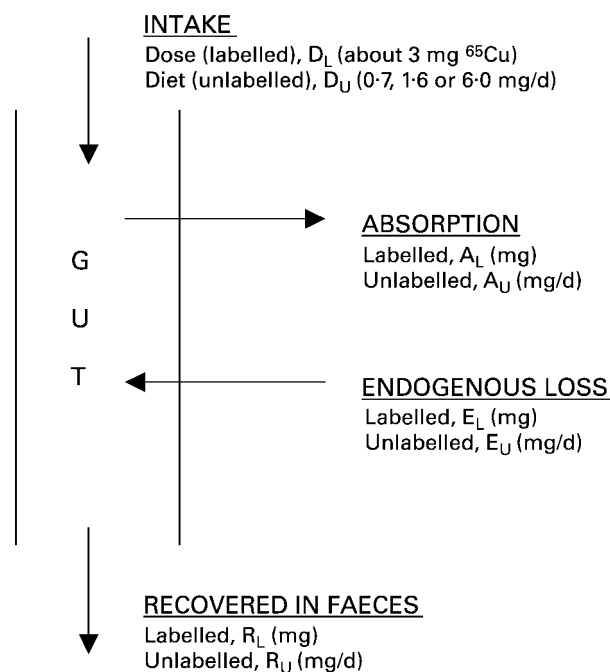where F_U is the rate of loss of unlabelled Cu from the 'fast' pool in mg/d.

Fig. 1. Overview of labelled and unlabelled copper absorption and excretion.

Unlabelled copper balance and excretion. In an analogous way to the labelled Cu, unlabelled Cu from the fast pool is defined as Cu that was absorbed from a meal and then excreted within 14 d. Unlabelled Cu from the slow pool is defined to be Cu that was absorbed from a meal and excreted more than 14 d after the meal.

$$\text{Balance } (mg/d) = D_U - R_U,$$

where R_U is the unlabelled Cu recovered in faeces.

Endogenous loss $(E_U; \; mg/d) = R_U - D_U + A_U = R_U - D_U + (D_U \times A_L)/D_L$ (from equation (1)).

Unlabelled Cu from the 'fast' pool $(F_U; mg/d) = E_L \times D_U/D_L$ (from equation (2)).

Since E_L, D_U and D_L are all known or measured, F_U can be calculated. These results are shown in Table 2 in the 'fast pool' section.

Unlabelled Cu from the 'slow' pool $(S_U; \; mg/d) = E_U - F_U$.

Therefore, on substitution and rearrangement,

$$S_U = ((R_U \times D_L) - (R_L \times D_U))/D_L. \qquad (3)$$

Since R_U, R_L, D_U and D_L are all known or measured, S_U can be calculated. These results are shown in Table 2 in the 'slow pool' section.

Statistical analysis

All data are expressed as means and standard deviations. ANOVA with repeated measures was used to determine the effect of dietary Cu intake on the efficiency of Cu absorption and excretion. If a significant difference was found, the least squares difference test was used to determine which treatment means differed. Student's *t* test was used to determine the difference between measured and calculated habitual dietary Cu intakes. A significance level of $P < 0.05$ was used for all statistical tests.

Results

The biochemical data are shown in Table 3. In every case, none of the parameters measured on day 42 was significantly different from those measured on day 1 and there was also no significant difference between the values measured on day 1 of each dietary period; thus only the day 42 results are presented. All erythrocyte SOD, serum Cu, serum total caeruloplasmin and plasma caeruloplasmin activity measurements fell within normal ranges; statistical evaluation of the data demonstrated that none of the parameters was affected by dietary Cu intake. Equilibration for 6 weeks at each Cu intake level was also found to have no significant effect on various risk factors related to cardiovascular disease, including ADP-stimulated platelet aggregation, plasma lipoproteins (total, HDL-, LDL-cholesterol), triacylgycerols, apolipoproteins A1 and B and GPx (Table 4). Other haematological factors were also unaffected by dietary Cu intake including haemoglobin, mean cell haemoglobin concentration and packed cell volume (Table 5).

All data for efficiency of Cu absorption for the low (0·7 mg/d), medium (1·6 mg/d) and high (6·0 mg/d) Cu intake levels are presented in Table 6. Apparent absorption data for eleven of the twelve volunteers are presented due

Table 2. Unlabelled copper endogenous losses and balance data†

(Mean values and standard deviations)

Dietary Cu intake (mg/d)...	n	6·0 Mean	SD	1·6 Mean	SD	0·7 Mean	SD
Cu recovered in faeces‡ (mg/d)	12	5·25	1·05	1·60*	0·31	0·83*	0·32
Balance (mg/d)	6	0·75	1·05	0·00*	0·31	−0·13*	0·32
Slow pool§ (mg/d)	6	1·33	1·15	0·58	0·15	0·35*	0·25
Fast pool‖ (mg/d)	6	1·14	0·29	0·23*	0·09	0·10*	0·02
Total (slow pool+fast pool) (mg/d)	6	2·46	1·11	0·81*	0·16	0·45*	0·25

* Mean value was significantly different ($P < 0.05$) from the value on the high Cu diet.
† For details of subjects and procedures, see Table 1 and p. 162.
‡ Based on the unlabelled Cu recovered 14 d after label administration.
§ Cu absorbed from a meal and excreted at least 14 d after the meal.
‖ Cu absorbed from the meal and excreted within 14 d of the meal.

Table 3. Putative indices of copper status after 6 weeks of each dietary period*

(Mean values and standard deviations for twelve subjects)

Dietary Cu intake (mg/d)...	6·0 Mean	SD	1·6 Mean	SD	0·7 Mean	SD
Erythrocyte superoxide dismutase activity (U/g haemoglobin)	1016	79	1070	120	1009	113
Serum Cu (μmol/l)	15·5	2·2	13·9	1·7	14·4	2·1
Serum caeruloplasmin (g/l)	0·28	0·14	0·23	0·07	0·21	0·04
Plasma caeruloplasmin activity (U/mg)	2·62	0·48	2·29	0·39	2·60	0·64

* For details of subjects and procedures, see Table 1 and p. 162.

Table 4. Biochemical indices denoting increased risk of cardiovascular disease measured after 6 weeks at each level of copper intake*

(Mean values and standard deviations for twelve subjects)

Dietary Cu intake (mg/d)...	6·0		1·6		0·7	
	Mean	SD	Mean	SD	Mean	SD
Platelet aggregation (%)	80	10	80	7	82	5
Plasma lipoproteins (mmol/l)						
Total cholesterol	5·0	0·9	5·2	1·1	5·1	1·1
LDL-cholesterol	3·3	0·9	3·4	1·0	3·5	1·0
HDL-cholesterol	1·1	0·2	1·0	0·2	1·1	0·2
Triacylglycerols (mmol/l)	1·24	0·43	1·51	0·94	1·19	0·53
Apolipoprotein A1 (g/l)	1·14	0·04	1·10	0·04	1·19	0·04
Apolipoprotein B (g/l)	1·08	0·1	0·96	0·80	0·93	0·09
Glutathione peroxidase						
Erythrocyte (U/g haemoglobin)	45	9	47	10	47	9
Plasma (U/g protein)	9	1	9	2	9	2
Platelets (U/g protein)	265	120	225	101	347	252

* For details of subjects and procedures, see Table 1 and p. 162.

Table 5. Haematological and haemostatic measurements after 6 weeks at each level of copper intake*

(Mean values and standard deviations)

Dietary Cu intake (mg/d)...	n	6·0		1·6		0·7	
		Mean	SD	Mean	SD	Mean	SD
Packed cell volume (%)	11	45·7	4·0	44·5	3·3	44·1	1·3
Haemoglobin (g/l)	12	151	13	150†	7	148	5
MCHC (g/l)	11	331	8	336	13	335	6
Ferritin‡ (ng/ml)	12	29·3	14·9, 57·8	47·3	18·7, 119·3	30·6	12·5, 74·9

MCHC, mean cell haemoglobin concentration.
* For details of subjects and procedures, see Table 1 and p. 162.
† Values for eleven subjects.
‡ Values are geometric means (−1 SD, +1 SD).

Table 6. Copper absorption and endogenous losses (from an oral dose of 3 mg copper label) measured after 6 weeks of each dietary period*

(Mean values and standard deviations)

Dietary Cu intake (mg/d)...	n	6·0		1·6		0·7	
		Mean	SD	Mean	SD	Mean	SD
Apparent absorption							
%	11	45	13	42	15	41	12
mg		1·34	0·38	1·27	0·44	1·23	0·35
True absorption							
%	6	48	11	45	14	48	13
mg		1·45	0·33	1·36	0·41	1·45	0·39
Endogenous loss of label†							
%	6	40	11	38	20	30	15
mg		0·58	0·17	0·48	0·22	0·40	0·10

* For details of subjects and procedures, see Table 1 and p. 162.
† Absorbed label excreted within 14 d of dose administration.

to incomplete stool collection for one of the volunteers. Data for true absorption are reduced to six data sets because the method used to calculate true absorption failed on five other volunteers due to slow transit times of these subjects. This resulted in an inadequate number of stools being available in the later stages of the faecal collection period containing only [65]Cu stable isotope dose resulting from endogenous losses. The mean apparent absorption of the 3 mg label for eleven of the volunteers on the low Cu intake was not significantly different from the apparent absorption of the dose at the high dietary intake level. There was no significant difference in apparent absorption between the medium and high or medium and low Cu intakes.

Generally, the non-absorbed label was excreted in the faeces within 6 d of administration of the dose. This was validated by the simultaneous and complete recovery of the Ho dose (mean recovery was 98 (SEM 5) %). Labelled Cu appearing in the faeces after this time was attributed to endogenous losses. Labelled endogenous losses were calculated using the method of Harvey *et al.* (2002) and were significantly lower on the low- and medium-Cu diets compared with the high-Cu diet (Table 6).

True absorption was estimated by adjusting the apparent absorption, as determined from faecal monitoring, with the endogenous loss measurements of the recovered label in these subjects (Table 6). True absorption was found to follow the same pattern as apparent absorption; absorption on the low-Cu diet was not significantly different from the high-Cu diet. There was no significant difference in the absorption between the medium and either the high or low Cu intakes.

Balance is measured as the difference between dietary intake of unlabelled Cu and unlabelled Cu recovered in the faeces. It can be seen from Table 2 that, on average, the volunteers are in negative balance on the low-Cu diet and positive balance on the medium- and high-Cu diets. Balance on the high-Cu diet was significantly greater than for both the medium- and low-Cu diets. However, the difference between the medium- and low-Cu diets was not significant. For the labelled Cu, volunteers on the low-Cu diet retained 1·05 (SD 0·40) mg of the label 14 d after administration compared with 0·88 (SD 0·44) mg on the medium-Cu diet and 0·87 (SD 0·26) mg on the high-Cu diet. The difference between the low- and high-Cu diet values was not significant.

In the present experiment, total endogenous losses can be divided into two parts: fast and slow loss. The 'fast' loss is Cu that has been absorbed from a meal and then excreted within 14 d. The 'slow' loss is absorbed Cu that is excreted in the faeces more than 14 d after the meal was consumed. The total daily endogenous loss is calculated from the quantity of absorbed Cu per d plus the difference between dietary intake and recovered faecal Cu. This assumes that the quantity of Cu lost via other routes (for example, urine) is small. These data are presented in Table 2 for the unlabelled Cu. The total endogenous loss on the high-Cu diet was significantly different from both the medium- ($P=0.001$) and low-Cu diets ($P<0.001$). A similar significant difference was observed for the rate of endogenous loss from the fast pool on the high-Cu diet compared with the medium- ($P<0.001$) and low-Cu diets ($P<0.001$). The rate of endogenous loss from the slow pool was calculated as the difference between the total and the fast rates of loss (Table 2). The rate of loss was significantly greater on the high- than on the low-Cu diet ($P=0.023$). No significant difference was observed between the high- and medium-Cu diets.

The habitual dietary Cu intake of each of the volunteers was determined from both calculated and direct measurements. The mean analysed daily intake was 1·8 (SD 0·5) mg with a range of 0·8–2·8 mg/d and was significantly higher ($P=0.021$) than the calculated value of 1·4 (SD 0·5) mg with a range of 0·7–2·5 mg/d.
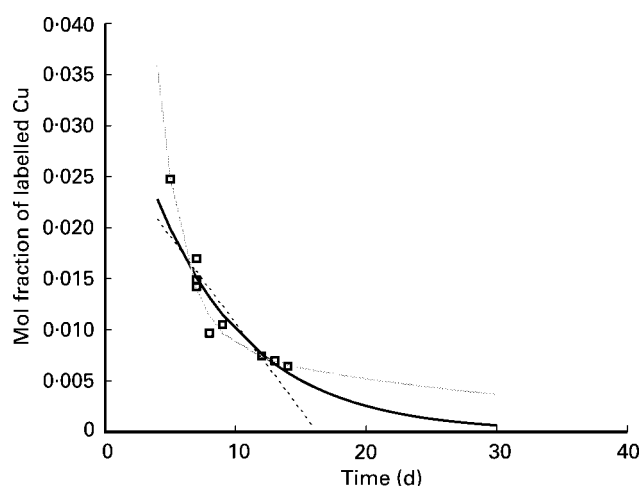
Discussion

From the results presented it appears that Cu homeostasis is maintained through control of only endogenous excretion and not absorption. Although the present study does not include data on urinary output, it has been reported that this form of excretion is minimal (Ishihara & Matsushiro, 1986) and is not dependent on dietary Cu intake. Turnlund *et al.* (1989, 1998) have performed two dietary intervention studies with male volunteers, feeding Cu levels similar to those used in the present study. Stable-isotope methodology was used to determine apparent absorptions of 56 and 54 % following equilibration at Cu intake levels of 0·785 and 0·66 mg/d respectively, higher than the mean apparent absorption of 41 % found on the low-Cu diet (0·7 mg/d) in the present study. Daily intakes of 7·53 and 1·68 mg Cu were found to result in apparent absorptions of 12 and 36 % respectively (Turnlund *et al.* 1989). These values are, however, lower than the values found in the present study, which may be the result of differences in experimental protocol. First, the present study measured absorption from a single test meal, which has been reported to over-estimate absorption compared with measurement over several meals as used by Turnlund *et al.* (1989). Second, in the present study, in order to be able to calculate endogenous losses, 3 mg labelled Cu was given in a single test meal on each of the high-, medium- and low-Cu diets, whereas in the study by Turnlund *et al.* (1998) Cu in the study meals was replaced with the same quantity of Cu label.

The study by Turnlund *et al.* (1998) estimated endogenous losses by measuring the faecal appearance of intravenous doses of highly enriched ^{65}Cu stable isotope. In agreement with our data, endogenous losses were found to be reduced with low dietary Cu intake when compared with higher intake levels. A Cu metabolism model based on the disappearance of both infused and oral ^{65}Cu dose has suggested that tissue uptake of oral and intravenous Cu is different, with flow between the plasma and liver compartment from the intravenous dose only being 80 % of the oral dose (Scott & Turnlund, 1994). The intravenous technique gives a general indication of the relative changes in endogenous losses resulting from different dietary Cu intakes, whereas the technique used in the present study has the advantage of reflecting the metabolism of the absorbed oral dose in the same volunteer during the same time period as the absorption efficiency is assessed.

Within 14 d, 38 (SD 20) % of the absorbed oral dose had been excreted when the volunteers were on the medium-Cu diet. From the retention data of the unlabelled Cu it is known that the volunteers were in balance (Table 2). This indicates that the other 76 % of the absorbed label will be excreted at a future indeterminate time from a slowly exchangeable pool in the body. When the volunteers were on the high-Cu diet, 40 (SD 11) % of the absorbed dose was excreted 14 d after the dose was given. The volunteers were not, however, in balance (0·75 mg/d was retained). It is not clear from our study or others (Turnlund *et al.* 1989; 1998) how long, if at all, it would take to restore Cu balance on such a high-Cu

diet. Losses from the rapidly exchanging (fast) pool alter significantly between the diets (Table 2). There is a five-fold increase between the medium- and high-Cu diets in this fast pool but only a two-fold increase in the slow pool loss between the same two diets. This fast pool can clearly respond to changes in dietary conditions much more readily than the slow pool and indicates that the physical locations of the fast pool are a series of rapidly turning over spaces that certainly include the liver in some capacity.

Linder *et al.* (1986) and Owen (1971) have shown in rats that the loss of Cu from the body can be represented by a biphasic equation. Our model of endogenous loss assumed that the labelled Cu was lost according to a linear equation (of the form $y = mx + c$) since this is all that can be justified from the data. Fig. 2 and Table 7 show an attempt to estimate the endogenous loss in one volunteer on the high-Cu diet by applying three different mathematical models to the data. In all three models, estimates of endogenous loss are made beyond the time that measurements were taken (14 d). After 30 d of the mono- and bi-exponential simulations and 19 d of the linear one, there is still at least

Fig. 2. Effect of different endogenous excretion models on fraction of labelled copper absorbed and then expelled in faeces. Data are for a single volunteer on the high-copper diet. (—), Mono-exponential model; (~~), bi-exponential model; (- - -), linear model; (□), measured values.

Table 7. Comparison of three models fitted to re-excreted oral dose data for a single volunteer on the high-copper diet

	Bi-exponential	Mono-exponential	Linear
Absorption (%)	78	71	69
Absorbed (µg)	2340	2114	2060
'Fast' pool endogenous excretion (µg)	1230*	844*	736†
Amount excreted (fast pool) (%)	53	40	36
Retained (µg)	1110‡	1270‡	1324§

* Simulated for 30 d after oral dose.
† Simulated for 19 d after oral dose at which point the excretion is zero.
‡ Retained in body 30 d after oral dose.
§ Retained in body 19 d after oral dose.

1 mg absorbed Cu that has not been excreted. The intervention periods were 6 weeks and the volunteers on the medium- and low-Cu diets were in balance at the end of them. This suggests that a complex excretory system exists for Cu and that our attempts at simple models are not adequate to describe it. The division of endogenous losses into two pools (slow and fast) is an over simplification but is all that can be attempted with a study of this type. Single meal stable-isotope studies with physiological doses cannot, at present, measure the return of labelled Cu from slow turnover pools because their half-life is too long. The physical nature of the slow pool (or pools) is a matter for conjecture but the most probable candidates are muscle and bone. Whatever the nature of the pool, it appears to be under homeostatic control, as evidenced by a doubling of Cu flowing from it between the low- to medium- and medium- to high-Cu diets, although it had not changed sufficiently over the 6 weeks of the high-Cu diet to achieve Cu balance.

The results of the present study indicate that homeostasis is more strongly controlled by endogenous excretion. Absorption did not change in response to variations in Cu intake. A four-fold dietary increase in Cu (medium- to high-Cu diet) would necessitate a four-fold reduction in absorption (60 to 15 %) if this were the body's only homeostatic mechanism. Similarly, the change in absorption between the medium- and the low-Cu diet was 45 to 48 %, whereas the reduction in dietary Cu between the two periods was approximately a factor of two.

An attempt has been made in the present paper to use the data from the re-excreted oral dose to calculate endogenous losses. This allows quantification of the so-called fast pool of endogenous loss. Although a linear model has been used to calculate these losses, other researchers, using radioisotope data, have shown that a biphasic curve represents the endogenous loss in the best way. Using physiological doses of stable isotope, measurements of enrichment in faeces are possible up to about 14 d post-dose. After this length of time, the precision is not sufficient to use the data. This limits the complexity of any models derived from stable-isotope data to only the simplest kind, i.e. linear regression models. Although the modelling is simple, it still generates useful information and allows the calculation of two Cu loss pools (fast and slow) through direct and indirect means. Overall, the data suggest that adaptive responses to a high dietary intake take longer to achieve than at lower intakes. The fact that none of the biochemical measurements changed as a result of altered Cu intake lends support to our hypothesis that adult men faced with quite large variations in Cu intake are able to maintain Cu homeostasis predominantly by up and down regulation of Cu secretions into the gastrointestinal tract.

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