Response of diamine oxidase and other plasma copper biomarkers to various dietary copper intakes in the rat and evaluation of copper absorption with a stable isotope

C. Feillet-Coudray*, C. Coudray, D. Bayle, E. Rock, Y. Rayssiguier and A. Mazur

Centre de Recherche en Nutrition Humaine d'Auvergne, Unité Maladies Métaboliques et Micronutriments, INRA, Theix, 63122 St-Genès-Champanelle, France

There is a lack of agreement on index of Cu status and reliable and sensitive biomarkers are still required. The purpose of this present work was to assess in rats the sensitivity of diamine oxidase (DAO) activity, a recently proposed biomarker, to modifications in dietary Cu intake in comparison with other plasma biomarkers of Cu status. We also evaluated the effect of Cu dietary level on Cu and Zn intestinal absorption. Results showed that plasma Cu and plasma caeruloplasmin were significantly decreased at day 8 compared with the control group (7·4 mg Cu/kg diet) while DAO activity was significantly decreased at day 12 of the deficient diet (0·61 mg Cu/kg diet). Cu supplementation (35 mg Cu/kg diet) had no effect on any of the studied biomarkers of Cu status. In Cu-deficient rats plasma Cu and DAO activities were normalized 4 d after return to the control diet while caeruloplasmin was normalized later, at day 11. Apparent absorption values (%) of total Cu or ^{65}Cu isotope were significantly increased in the Cu-deficient rats compared with the other groups and similar in the control and the Cu-supplemented groups. The urinary excretion of total Cu or ^{65}Cu isotope were increased in the Cu-supplemented group compared with the other two groups. Both apparent absorption and urinary excretion of total Zn or ^{67}Zn isotope remained unchanged in the three experimental groups. In conclusion, DAO activity seemed to be less sensitive to Cu deficiency than plasma Cu or caeruloplasmin concentrations. The present study also showed a significant increase in Cu intestinal absorption with dietary Cu restriction but no decrease with Cu supplementation in the rat.

Copper: Diamine oxidase: Stable isotopes

Cu is an essential trace element that plays an important role in the maintenance of optimal health. It is required in various cellular functions, to help normal cellular homeostasis and for the structure and function of skeletal, cardiovascular, nervous and immune systems (Strain, 1994). Cu is involved as a co-factor in various enzyme catalysing oxidation–reduction reactions (Linder & Hazegh-Azam, 1996). It has been suggested that Cu deprivation contributes to an increased risk of heart disease through instability of heart rhythm, hyperlipidaemia, increased thrombosis and breakdown of vascular tissue (Klevay, 1990). Cu deficiency may also result in anaemia, leucopaenia and skeletal demineralization. Several studies have shown that Cu requirements may not be covered by diet Cu (Klevay *et al.* 1979, 1993; Pennington & Young, 1991). However, there is a lack of agreement on an index of Cu nutritional status that is able to track Cu status in human subjects (Milne & Johnson, 1993; Milne, 1994). Currently, plasma Cu or levels of caeruloplasmin or activity of erythrocyte superoxide dismutase (SOD) are commonly used to assess Cu status in studies with human subjects. Caeruloplasmin is a plasma glycoprotein, primarily synthesized in the liver and secreted into the blood which binds more than 60 % of plasma Cu and is considered a Cu transport protein (Linder, 1991). Erythrocyte SOD is an important intracellular enzyme that requires both Cu and Zn for normal enzyme activity. However, none of these measurements change quickly in response to intentional induction of mild Cu deficiency in human subjects (Danks, 1988). In addition, readings for these measurements tend to be elevated by many physiological stress states including inflammation, cancer, chronic exercise, pregnancy and lactation (Danks, 1988). This limits the usefulness of these biochemical measures as diagnostic indicators of marginal Cu deficiency. Therefore, reliable and sensitive functional measures of marginal Cu deficiency are required.

Recently, diamine oxidase (DAO), a Cu-dependent enzyme, has been proposed as a valuable biomarker of Cu status in human subjects and in animals (DiSilvestro *et al.* 1997; Jones *et al.* 1997; O'Connor *et al.* 1999). DAO is an enzyme catalysing the oxidation of diamines such as histamine, putrescine and cadaverine (Wolvekamp & de Bruin, 1994). It is normally abundant within the intestinal mucosa, kidney and placenta, but scant in serum (Kusche *et al.* 1974; Tam *et al.* 1979; Luk *et al.* 1980; D'Agostino *et al.* 1984). DiSilvestro's group has reported high plasma DAO activities in patients undergoing renal dialysis and in some adult men receiving Cu supplementation, and low activities during spontaneous Cu deficiency (DiSilvestro *et al.* 1997; Jones *et al.* 1997). Moreover, the results of the European FoodCue project demonstrated high levels of plasma DAO activity with Cu supplementation in human subjects (O'Connor *et al.* 1999). Low activities were also seen in marginally Cu-deficient rats (DiSilvestro *et al.* 1997).

Cu status may be largely affected by intestinal absorption and retention of Cu. Several studies on rats have investigated the effects of various factors on Cu absorption and retention. The effects of naturally occurring Cu complexes (Mills, 1986), protein source (Lo *et al.* 1984), dietary Zn : Cu ratio (Oestreicher & Cousins, 1985) and fibre (Scheibel & Mehta, 1985) on Cu absorption in the rat have been reported. However, there is only one report dealing with the Cu absorption as function of Cu intakes in rats (Stuart & Johnson, 1986). It is therefore of great importance to look simultaneously at the intestinal absorption of Cu in this present study to better understand and interpret our result on Cu status.

The purposes of this present work were: (1) to assess the sensitivity of DAO to modifications in dietary Cu intake in comparison with other plasma biomarkers of Cu status; (2) to evaluate the effect of different levels of Cu in the diet on Cu and Zn intestinal absorption.

Materials and methods

Reagents and materials

Enriched ^{65}Cu and ^{67}Zn isotopes in the oxide form were obtained from Eurisotope (Saint-Aubin, France). Suprapure HNO_3, HCl and H_2O_2 were purchased from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available and demineralized water was used throughout the study. The inductively-coupled plasma mass spectrometer used to measure stable isotope enrichments was a Plasmaquad II system (Fisons Instruments, Manchester, UK) equipped with a Meinhard (Santa Ana, CA, USA) nebulizer. The atomic absorption spectrometer (Perkin Elmer 560, St Quentin en Yvelines, France) was used for total Cu and Zn measurements.

Animals, diets and experimental design

Male Wistar rats (IFFA-CREDO, L'Arbresle, France) weighing about 500 g were used. The rats were housed under conditions of constant temperature (20–22°), humidity (45–50 %) and a standard light–dark cycle (dark 20.00–08.00 hours). Rats first went through an adaptation period of 10 d with free access to a semi-purified diet (control diet) and

demineralized water. Rats were then randomized into three groups of eight animals and each fed for a 3-week period either on the control diet (control group), the control diet without Cu (Cu-deficient group) or the control diet supplemented with Cu (Cu-supplemented group). After 3 weeks, the rats were fed again on the control diet for 11 d. The semipurified diets contained the following (g/kg): casein 200, starch 650, maize oil 50, alphacel 50, DL-methionine 3, choline bitartrate 2, modified AIN-93 mineral mix 35, AIN-93A vitamin mix 10 (ICN Biomedicals, Orsay, France). Cupric carbonate was omitted from the AIN-93 mineral mix for the deficient diet and 52·2 mg cupric carbonate (27·1 mg Cu) was added for the supplemented diet. Blood samples were collected at the retro-orbital sinus after brief anaesthesia with diethyl ether after 4, 8, 12 and 18 d from control, Cu-deficient or Cu-supplemented animals, and then 4 and 11 d after returning to the control diet. Blood was drawn into a heparinized tube (1 ml) and also into a glass tube without anticoagulant (1 ml) and allowed to clot. Then plasma and serum were obtained by centrifugation. Packed cell volume was determined by centrifugation in a capillary tube system to obtain packed cells. Haemoglobin was evaluated by a colorimetric method (Boehringer Mannheim, Mannheim, Germany).

The total Cu and the ^{65}Cu intestinal absorption were evaluated at the end of the supplemented or deficient experimental period, just before switching to the control diet. For this purpose, ^{65}Cu and ^{67}Zn stable isotopes were administered orally and faeces and urine were collected every day for three successive days. Food intake for each animal was evaluated daily during this period. ^{65}CuO and ^{67}ZnO were transformed into the chloride form before administration orally to the rats.

All procedures were in agreement with the Institute's guide for care and use of laboratory animals.

Copper status

The activity of caeruloplasmin in serum was determined according to the method of Sunderman & Nomoto (1970). At pH 5·4, caeruloplasmin catalyses the oxidation of *p*-phenylenediamine to yield a coloured product (absorbance 530 nm). The rate of formation of the coloured oxidation product is proportional to the concentration of serum caeruloplasmin after correction for non-enzymic oxidation of *p*-phenylenediamine.

The serum DAO was determined according to the method of Takagi *et al.* (1994) with the following modifications: 750 µl cadaverine solution (30 mmol/l) at 37° are added to 150 µl serum sample and the mixture is incubated at 37° for exactly 30 min. Then, 750 µl 10-(carboxymethylamino-carbonyl)-3,7-bis(dimethylamino)phenothiazine sodium salt 100 µmol/l, horseradish peroxidase; (600 U/l)–ascorbate oxidase; (500 U/l) solution are added, mixed and incubated further. Immediately after the incubation of 90 min, 25 µl sodium diethylcarbamate solution are added to stop the reaction. Then the absorbance of methylene blue at 668 nm is recorded.

Plasma and urinary Cu levels were determined by atomic absorption spectrophotometry at 325 nm, as described previously (Bellanger, 1971).

Balances of total Cu and ^{65}Cu, and of total Zn and ^{67}Zn

After 3 weeks on the different experimental diets, 2 ml solution containing ^{65}Cu and ^{67}Zn stable isotope was administered orally to the rats. This solution contained either 10, 100 or 500 µg ^{65}Cu and 100 µg ^{67}Zn for Cu-deficient, control and Cu-supplemented groups respectively. Administered ^{65}Cu isotope was thus proportional to the amount of Cu ingested by the rats in each experimental group.

Faeces and urine were collected into trace element-free containers over 3 d periods after isotope administration. Individual faeces samples were freeze-dried and powdered. Sub-samples of diet (1 g) or powdered faeces (0·25 g) were ashed at 500° for 10 h. The ashes were dissolved in 0·2 ml 14 M-HNO$_3$ and 0·1 ml H$_2$O$_2$ and heated for 2 h at 100° on a hotplate and diluted appropriately with 0·14 M-HNO$_3$.

Urine volume was determined and 10 ml urine was acidified with 0·1 ml 14 M-HNO$_3$. Cu- and Zn isotope ratios were determined in urine as previously described (Coudray *et al.* 1998). Cu and Zn isotope ratios were determined in urine and faeces by inductively-coupled plasma mass spectrometry using Cu and Zn solutions as external standards. Total Cu and Zn concentrations were determined in plasma, urine and faeces by atomic absorption spectrometry (Perkin-Elmer 560) in an acetylene–air flame at the following wavelengths (nm): 324·7 (Cu) and 213·8 (Zn). Appropriate quality controls (Seronorm serum, Nycomed, Oslo, Norway) were run with each set of measurements. Conventional chemical balance and isotopic balance were also determined according to the following equation: ingested amount − (excretion in the faeces + urine).

Statistical analysis

Results were expressed as means with standard errors. The statistical significance of differences between means was assessed by one way ANOVA followed by a Student Newman Keuls test. The limit of statistical significance was set at $P < 0.05$.

Results

The analysis of diets gave the following levels of Cu: 0·61 mg/kg (deficient diet), 7·4 mg/kg (control diet) and 35·0 mg/kg (supplemented diet). The Zn concentrations were 53, 52·9 and 53·4 mg/kg respectively.

Mean body weight of rats was unchanged during all the study for each group (Table 1). Packed cell volumes and haemoglobin levels were not affected by Cu deficiency nor by supplementation (Table 1).

Plasma Cu (Fig. 1) and plasma caeruloplasmin (Fig. 2) were significantly decreased in the Cu-deficient group at day 8 compared with the control group. DAO activity was significantly decreased only after day 12 on the deficient diet (Fig. 3). In Cu-deficient rats plasma Cu and DAO activities were normalized 4 d after return to the control diet while caeruloplasmin was normalized later, at day 11. Cu supplementation had no effect on any of the studied biomarkers of Cu status (Figs 1–3).

Intra-run CV for caeruloplasmin measurement was 4·6 %. Intra- and inter-run CV for plasma and urinary Cu measurements were 2·4 %, 4·3 % (plasma) and 3·5 %, 6·5 % (urine) respectively. Those of plasma and urinary Zn measurements were as follows: 2·1 %, 3·7 % (plasma) and 3·4 %, 5·2 % (urine) respectively.

Cu and Zn absorption for each dietary Cu intake are shown in Tables 2 and 3, and in Tables 4 and 5 respectively. Values for apparent absorption (%) of total Cu or ^{65}Cu isotope were similar in the control and the Cu-supplemented groups. However, both absorption values were significantly increased in the Cu-deficient rat group compared with the other groups. Moreover, ^{65}Cu isotope absorption was higher than the total Cu absorption in the Cu-deficient group. The urinary excretion of both total Cu or ^{65}Cu isotope were increased in the Cu-supplemented group compared with the two other experimental groups. Both apparent absorption of total Zn or ^{67}Zn isotope and urinary excretion of total Zn or ^{67}Zn isotope remained unchanged in the three experimental groups.

Discussion

This present study explored serum DAO activity, a recently proposed biomarker of Cu status (DiSilvestro *et al.* 1997; Jones *et al.* 1997; O'Connor *et al.* 1999), and compared it with other usual plasma indices such as plasma Cu and serum caeruloplasmin during modifications in Cu dietary intake. The potential changes were followed at day 4, 8, 12 and 18 on Cu-deficient (0·61 mg/kg) and Cu-supplemented diets (35 mg/kg). After this 18 d period and before the return to control diet, Cu and Zn intestinal absorption studies were performed. We then studied the effect of return to the control diet in all the rats.

Table 1. Characteristics of rats fed on either the control, the Cu-supplemented or the Cu-deficient diet for 18 d*

(Values are means with standard errors for eight rats per group)

	Control diet		Supplemented diet		Deficient diet	
	Mean	SE	Mean	SE	Mean	SE
Food intake (g/d)	22·7[a]	1·1	22·9[a]	1·2	20·4[a]	1·4
Initial body weight (g)	494[a]	13	515[a]	15	493[a]	23
Final body weight (g)	491[a]	5	515[a]	5	501[a]	9
Packed cell volume (%)	43[a]	2	44[a]	2	44[a]	2
Haemoglobin (g/l erythrocytes)	430[a]	25	423[a]	31	458[a]	39

[a] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
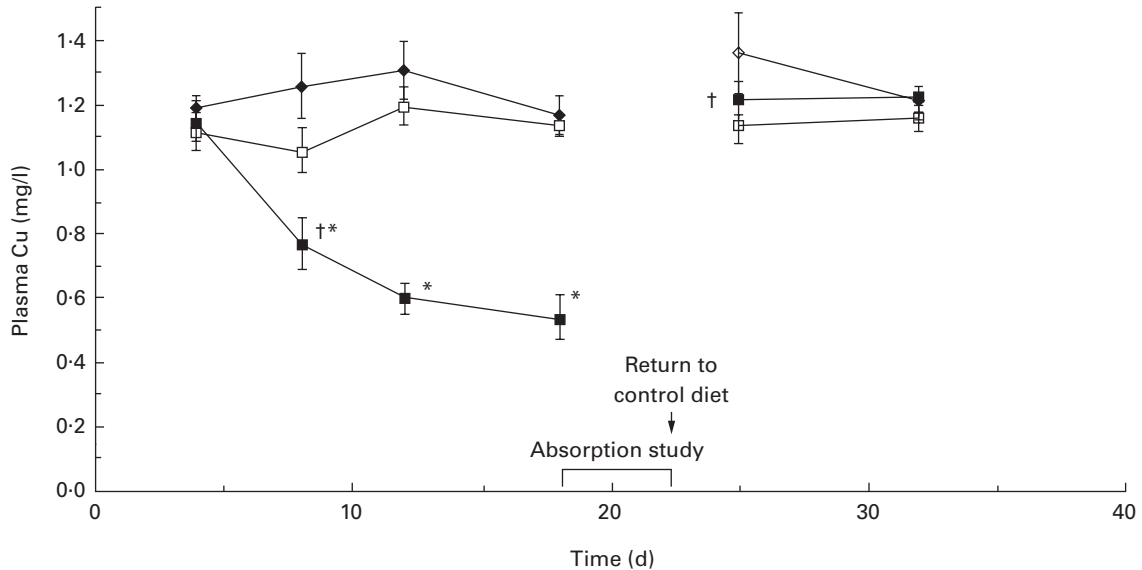* For details of diets see p. 562.

Fig. 1. Time courses of changes in plasma copper in rats (*n* 8 per group) fed on control (□, 7·4 mg Cu/kg), copper-supplemented (♦, 35 mg Cu/kg) or copper-deficient (■, 0·61 mg Cu/kg) diets for 3 weeks and then returned to the control diet. Values are means with standard errors represented by vertical bars. Mean values were significantly different from control group, *$P < 0.05$, and from the mean value for the copper-deficient group on the previous occasion, † $P < 0.05$.

In the first part of this study, we demonstrated that DAO activities decreased significantly during low-Cu diet consumption, and then increased in response to Cu repletion. However, plasma Cu and serum caeruloplasmin reacted more rapidly to Cu intake than did DAO activity. Thus, DAO activity appeared to be less sensitive to Cu intake than the two other biomarkers studied. To evaluate the true Cu status, it is better to evaluate cellular biomarkers as intracellular Cu (liver) or erythrocyte SOD activity. In this study we were not able to measure liver Cu because we wanted to

follow the plasma Cu variables in the same groups at the indicated times and thus animals were killed at the end of the experiment. Concerning erythrocyte SOD, this biomarker is known to respond more slowly to modifications of Cu intake because of the length of the erythrocyte half-life. As we were interested in early modification of Cu status, we did not evaluate this variable.

Surprisingly, none of the indices of Cu status studied responded to Cu supplementation. This is in agreement with results from the study of Cu supplementation in healthy
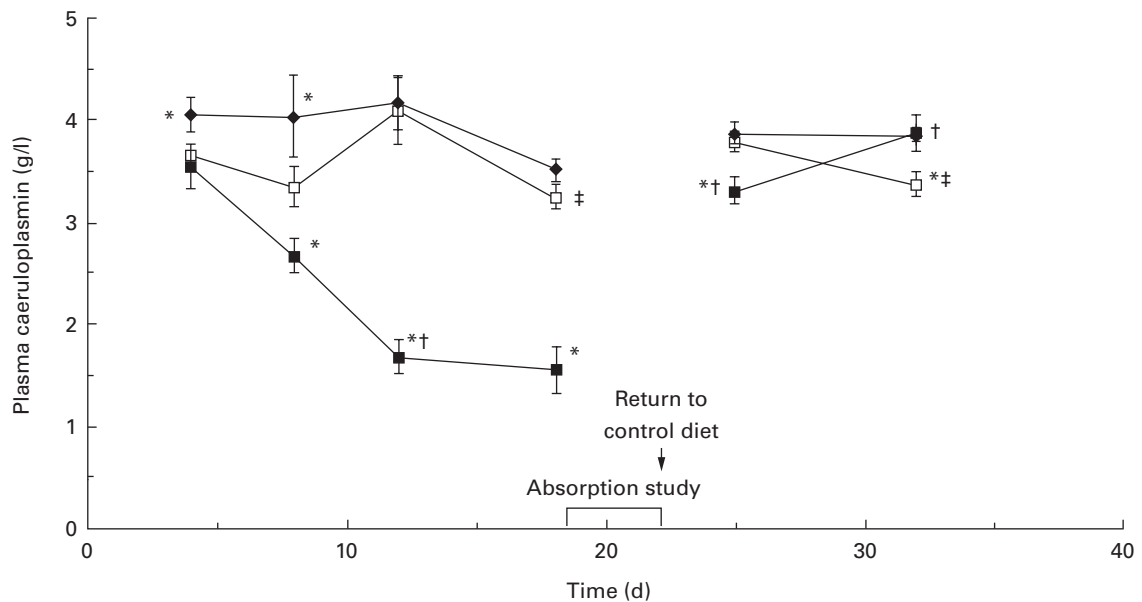

Fig. 2. Time courses of changes in plasma caeruloplasmin in rats (*n* 8 per group) fed on control (□, 7·4 mg Cu/kg), copper-supplemented (♦, 35 mg Cu/kg) or copper-deficient (■, 0·61 mg Cu/kg) diets for 3 weeks and then returned to the control diet. Values are means with standard errors represented by vertical bars. Mean values were significantly different from control group, *$P < 0.05$, and from the mean value for copper-deficient group on the previous occasion, † $P < 0.05$, and from the mean value for the control group on the previous occasion, ‡ $P < 0.05$.
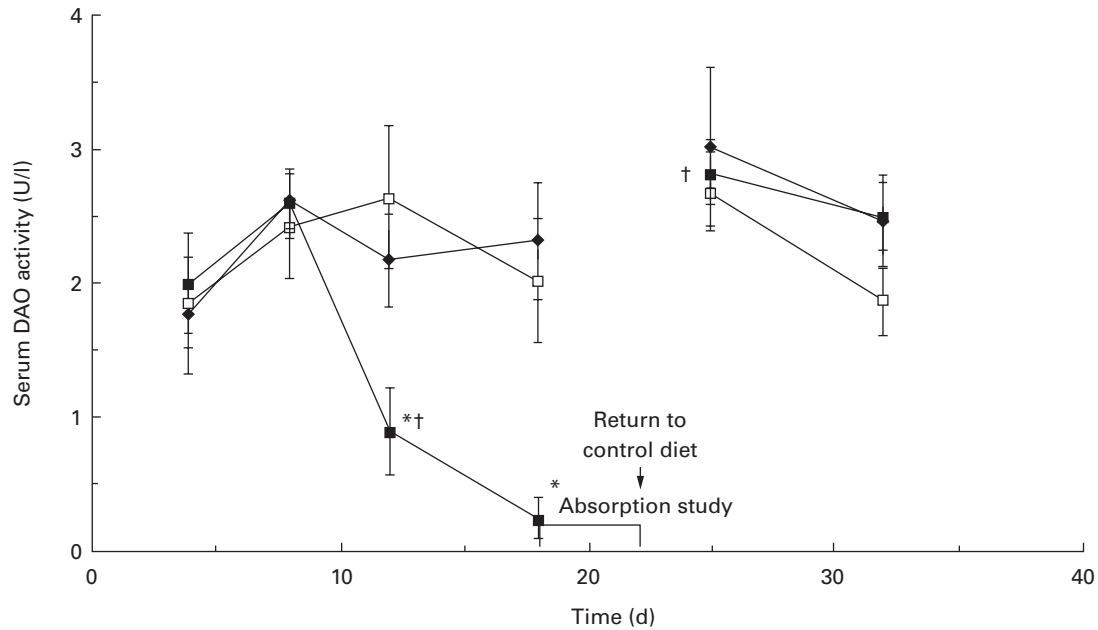
Fig. 3. Time courses of changes in serum diamine oxidase (DAO) in rats (*n* 8 per group) fed on control (□, 7·4 mg Cu/kg), copper-supplemented (♦, 35 mg Cu/kg) or copper-deficient (■, 0·61 mg Cu/kg) diets for 3 weeks and then returned to the control diet. Values are means with standard errors represented by vertical bars. Mean values were significantly different from control group, *$P < 0·05$, and from the mean value for the copper-deficient group on the previous occasion, †$P < 0·05$.

infants (Salmenpera *et al*. 1989). We can assume that the Cu level in the control diet is sufficient to replenish all the pools of Cu in the rat. Knowing that high plasma DAO activities were observed in patients undergoing renal dialysis (DiSilvestro *et al*. 1997), in pregnancy and gestational diseases (Kusche *et al*. 1974) and that DAO activity is also affected by several diseases of the small intestine, the usefulness of this biochemical marker as a diagnostic indicator of marginal

Cu deficiency appeared to be limited in the rat or at least questionable. Moreover, preliminary results in our laboratory demonstrated that in healthy human subjects these levels were often nil (< 1 U/l) making the exploration of Cu deficiency difficult. In the rat, DAO levels are higher than in human subjects but still low, making its measurement difficult. Its sensitivity to temperature and storage is another limitation of this biomarker.

Table 2. Effect of different dietary intakes of copper on intestinal absorption and balance of total copper in rats (classical absorption and balance)*

(Values are means and standard errors for eight rats per group)

Dietary Cu (mg/kg)	Cu intake (µg/d)		Faecal Cu (µg/d)		Cu absorption (%)		Cu absorption (µg/d)		Urinary Cu (µg/d)		Cu balance (µg/d)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0·61	13·7[a]	1·2	5·9[a]	0·5	48·4[a]	4·3	6·7[a]	1·0	2·6[a]	0·1	5·2[a]	1·2
7·41	173[b]	10	119[b]	6·1	31·0[b]	1·8	54·4[b]	5·3	3·9[a]	0·3	50·5[b]	5·4
35·0	864[c]	32	577[c]	41	33·1[b]	4·6	287[c]	42	7·0[b]	1·2	280[c]	42

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets see p. 562.

Table 3. Effect of different dietary intakes of copper on intestinal absorption and balance of [65]copper isotope in rats (isotope absorption and balance)*

(Values are means and standard errors for eight rats per group)

Dietary Cu (mg/kg)	[65]Cu intake (µg/d)	Faecal [65]Cu (µg/d)		[65]Cu absorption (%)		[65]Cu absorption (µg/d)		Urinary [65]Cu (µg/d)		[65]Cu balance (µg/d)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0·61	11·4[a]	3·6[a]	0·5	64·0[a]	1·7	7·8[a]	0·5	0·55[a]	0·14	7·2[a]	0·5
7·41	101·5[b]	75·2[b]	1·4	25·9[b]	1·3	26·3[b]	1·4	1·98[b]	0·30	24·3[b]	1·3
35·0	514[c]	416[c]	15	19·1[c]	2·9	98[c]	15	2·70[c]	0·20	96[c]	15

[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets see p. 562.

Table 4. Effect of different dietary intakes of copper on intestinal absorption and balance of total zinc in rats (classical absorption and balance)*
(Values are means and standard errors for eight rats per group)

Dietary Cu (mg/kg)	Zn intake (µg/d)		Faecal Zn (µg/d)		Zn absorption (%)		Zn absorption (µg/d)		Urinary Zn (µg/d)		Zn balance (µg/d)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0·61	946[a]	106	650[a]	69	31·9[a]	2·8	296[a]	55	3·4[a]	0·6	293[a]	46
7·41	1050[a]	73	758[b]	45	27·8[a]	3·1	293[a]	46	8·1[a]	1·7	284[a]	46
35·0	1106[a]	48	685[a]	54	37·8[a]	4·6	420[a]	58	5·5[a]	1·5	415[a]	57

[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets see p. 562.

Table 5. Effect of different dietary intakes of copper on intestinal absorption and balance of [67]zinc isotope in rats (isotope absorption and balance)*
(Values are means and standard errors for eight rats per group)

Dietary Cu (mg/kg)	[67]Zn intake (µg/d)	Faecal [67]Zn (µg/d)		[67]Zn absorption (%)		[67]Zn absorption (µg/d)		Urinary [67]Zn (µg/d)		[67]Zn balance (µg/d)	
	Mean	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0·61	126·5[a]	90·4[a]	1·4	28·5[a]	1·1	36·1[a]	1·4	0·21[a]	0·08	35·9[a]	1·3
7·41	126·5[a]	91·1[a]	1·6	28·0[a]	1·3	35·4[a]	1·6	0·21[a]	0·05	35·2[a]	1·6
35·0	126·5[a]	84·3[b]	3·5	33·4[a]	2·8	42·2[a]	3·5	0·15[a]	0·05	42·1[a]	3·5

[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets see p. 562.

In the second part of this study, we clearly showed that a significant increase in absorption of Cu followed dietary Cu restriction whereas Cu supplementation was followed by only a slight decrease in Cu absorption in the rat. These results indicate that absorption of dietary Cu aims toward adaptation to a wide range of dietary Cu intakes, in particular to the low levels of Cu intakes. However, at the lowest amount of dietary Cu used in the present study (0·61 mg/kg) these adaptive mechanisms were not sufficient to maintain Cu status. There is only one report in the literature dealing with the Cu absorption in relation to different Cu intakes in the rat (Stuart *et al.* 1986). In agreement with our work, Stuart *et al.* (1986) observed that apparent Cu absorption was inversely related to dietary Cu intakes. Such results were also observed in some studies with human subjects using Cu stable isotopes (August *et al.* 1989; Turnlund *et al.* 1989, 1998). These authors showed that Cu absorption was dependent on dietary Cu intakes: the percentage absorbed decreased as dietary Cu increased, but the absolute absorbed (mg/d) increased as dietary Cu increased. Interestingly, in our work, the urinary excretion of either total Cu or [65]Cu isotope increased significantly in the Cu-supplemented group, but without significant differences between the control and the Cu-deficient groups.

In the present experiment, we also compared the classical approach with the isotopic approach for measuring the apparent absorption of total Cu or [65]Cu isotope respectively, based on the faecal monitoring of this element or isotope. The former is generally less accurate due to possible environmental contaminations, and takes into account all the endogenous excretion of Cu (desquamation of gastro-intestinal tract, bile and pancreas secretions), whereas the second is more accurate and the contribution of the endogenous excretion of Cu is very low. The difference in the total Cu absorption between the Cu-deficient and the

Cu-supplemented groups was 16·5 % whereas it was 44·9 % as measured by [65]Cu absorption. In contrast to Cu, apparent absorption values of total Zn and [67]Zn isotope were similar in the present study and may be explained in three ways: (1) the endogenous excretion of Zn is less important than that of Cu proportionally to their intake; (2) the [65]Cu given as a liquid was better absorbed than the native Cu of the diet; and finally (3) the diet may have been contaminated with Zn during Zn analysis. Many studies have been performed to validate the extrinsic labelling approach, in particular for Zn, with contradictory results. Even if it is still not certain that the isotope behaves in exactly the same way as the native Cu or Zn bound in the food matrix (Fairweather-Tait *et al.* 1991; Fox *et al.* 1994; Boza *et al.* 1995), it is accepted that extrinsic labels may be used as an approximate guide when assessing the bioavailability of Cu and Zn from different regimens.

Finally, many studies have noted the potential interaction between Cu and Zn, particularly at their absorption level (Brewer *et al.* 1995; Rojas *et al.* 1996). Indeed, high Zn intakes have been reported to decrease Cu absorption, but little is known about the effect of high intakes of Cu on Zn absorption and excretion (O'Dell, 1989; Brewer, 1995). In the present study, a Cu intake 5-fold higher than the control level did not show any effects on intestinal absorption or urinary excretion of total Zn or [67]Zn isotope. However, the high level of Cu (35 mg Cu/kg diet) remained lower than the level of Zn in the three experimental groups (53 mg Zn/kg diet), which may explain this absence of effect of Cu intakes on Zn absorption and on its urinary excretion.

In conclusion, the plasma biomarkers of Cu status studied in the present work were affected more or less rapidly by low Cu intake and restored by return to control diet. Among these biomarkers, DAO activity was shown to react less rapidly to Cu deficiency than plasma Cu or caeruloplasmin

concentrations. Further studies are still necessary to assess the sensitivity and specificity of DAO as a biomarker of Cu status. The present study also showed a significant increase in Cu intestinal absorption with dietary Cu restriction but no decrease with Cu supplementation in the rat.

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