High tin intake reduces copper status in rats through inhibition of copper absorption

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The mechanism underlying the reduced Cu status in rats fed on a high-Sn diet was investigated. Male rats aged 4 weeks were fed *ad lib.* on purified diets containing either 1 or 100 mg Sn/kg and demineralized water for a period of 4 weeks. The high-Sn diet had no effect on feed intake, body-weight gain or weight of liver and kidney but significantly reduced Cu concentrations in plasma, liver and kidney. Biliary Cu excretion was decreased significantly in rats fed on the high-Sn diet. Apparent Cu absorption (Cu intake – faecal Cu) was not affected by the high-Sn diet, but the estimate of true Cu absorption (Cu intake – (faecal Cu – biliary Cu)) was significantly reduced. We conclude that high Sn intake reduces Cu status in rats through inhibition of Cu absorption. The decreased biliary Cu excretion observed on the high-Sn diet is a result of the reduced Cu absorption.

Tin: Copper: Biliary excretion: Metabolism: Rat

In rats, high intakes of Sn have been shown to reduce the concentration of Cu in plasma, liver and kidney (Greger & Johnson, 1981; Pekelharing *et al.* 1994) but the mechanisms involved are unknown. Biliary Cu is poorly absorbed (Owen, 1964; Farrer & Mistilis, 1967) and Cu excretion in urine is low (Van den Berg & Beynen, 1992) so that the main mechanism of Cu excretion is via the bile (Cartwright & Wintrobe, 1964). We decided to test which of two mechanisms may be involved in rats fed on a high-Sn diet. The first is that a high intake of Sn inhibits intestinal Cu absorption which would lead to reduced excretion of copper in bile to achieve Cu balance; alternatively, high Sn intake stimulates biliary Cu excretion which would lead to enhanced Cu absorption. Either mechanism could explain the impairment of Cu status by high intakes of Sn.

MATERIALS AND METHODS

The protocol of the experiment was approved and its conduct supervised by the animal welfare officer of Wageningen Agricultural University.

Animals and diets

Male Wistar rats (Hsd/Cpb:WU; Harlan/CPB, Zeist, The Netherlands), aged about 4 weeks, were used. On arrival they were housed in groups of five in stainless steel cages (600 × 210 × 190 mm) with wire mesh bases and given *ad lib.* a commercial, pelleted diet

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(RMH-B; Hope Farms, Woerden, The Netherlands) and tap water. After 3 d the semi-purified control diet (Table 1) and demineralized water were given. The control diet was formulated according to the recommended nutrient requirements of rats (National Research Council, 1978). After 4 d (day −4) the rats were divided randomly into two groups of twelve each and stratified for body weight. After another 4 d (day 0) one group was randomly allocated to the semi-purified, high-Sn diet containing 100 mg added Sn/kg (Table 1), and the other group remained on the control diet. Extra Sn was added to the test diet in the form of $SnCl_2$. The control and test diets were balanced for Ca and Cl (Table 1). Both groups had free access to the diets, which were in powdered form, and to demineralized water. Feed intake and body weight were recorded regularly. From day −4 the rats were housed individually in metabolism cages (31400 $mm^2 \times 120$ mm) in a room with controlled lighting (light on: 06.00–18.00 hours), temperature (19–21°) and relative humidity (50–60%).

Collection of samples

Faeces and urine were collected separately and quantitatively during days −4 to 0, 0 to 4, 7 to 11 and 24 to 28. At the end of the experiment (day 28), bile was collected by common bile duct cannulation with polyethylene tubing (i.d. 0·28 mm, o.d. 0·61 mm, Intramedic, Clay Adams, Parsippany, NJ, USA). The abdomen was opened while the rats were under anaesthesia induced by a combination of ketamine (60 mg/kg body weight) administered intramuscularly, and xylazine (8 mg/kg body weight) administered subcutaneously. This combination of the two drugs was used because it has been shown not to influence bile flow in rats (Fleck & Barth, 1990). After the cannula was inserted into the common bile duct and secured with suture thread the rats were kept on a heating pad (36–38°). Bile was collected into pre-weighed vials for 1 h and the volume of bile was calculated from the weight and the determined specific gravity of the bile. One rat in the control group died immediately after induction of anaesthesia. Following bile collection, blood samples were taken from the anaesthetized rats by abdominal aorta puncture into heparinized tubes. The rats were then killed and liver and left kidney were removed and weighed. All samples collected were stored at −20° until analysis.

Analytical methods

The concentrations of Cu in organs, faeces, urine and feed samples were determined by flame atomic absorption spectrometry (Perkin-Elmer 2380; Perkin-Elmer Corporation, Norwalk, CT, USA). For the determination of Cu in organs, samples were dried in a vacuum dryer for 48 h and digested in 1·0 ml 14 M-HNO_3 at 80° for 2 h. Samples of faeces, but not feed samples, were also dried in the vacuum dryer before ashing. Samples of feed and dried faeces were ashed at 500° for 17 h in a muffle furnace and then dissolved in 6 M-HCl. The determination of Cu in bile and plasma was carried out using flameless atomic absorption spectrometry (Varian AA-300; Varian Techtron Pty Ltd, Springvale, Victoria, Australia) after proper dilution of the samples with demineralized water. An external control in the form of a bovine liver sample (NBS 1577b; National Institute of Standards and Technology, Gaithersburg, MD, USA) was used to assess bias of Cu analysis. Analysed Cu concentration was 103·8% (SE 1·73, n 4) of the NBS certified value.

Statistical analyses

The data of the control and test groups were subjected to Student's t test to identify statistically significant differences. The Mann–Whitney U test was used to evaluate Cu

Table 1. *Composition of the experimental diets*

	Control	High tin
Ingredients (g/kg diet)		
Constant components*	278·2	278·2
Glucose	709·3	709·1
$CaCO_3$	12·0	12·1
$CaCl_2$	0·467	0·375
$SnCl_2.2H_2O$	0	0·190

* The constant components consisted of (g/kg diet): casein 151, maize oil 25, coconut fat 25, cellulose 30, $NaH_2PO_4.2H_2O$ 15·1, $MgCO_3$ 1·4, KCl 1·0, $KHCO_3$ 7·7, mineral premix 10 and vitamin premix 12. The mineral premix consisted of (mg/kg diet): $FeSO_4.7H_2O$ 174, MnO_2 79, $ZnSO_4.H_2O$ 33, $NiSO_4.6H_2O$ 13, NaF 2, KI 0·2, $CuSO_4.5H_2O$ 15·7, $Na_2SeO_3.5H_2O$ 0·3, $CrCl_3.6H_2O$ 1·5, $SnCl_2.2H_2O$ 1·9, NH_4VO_3 0·2 and maize meal 9679·2. The vitamin premix consisted of (mg/kg diet): thiamin 4, riboflavin 3, nicotinamide 20, D,L-calcium pantothenate 17·8, pyridoxine 6, cyanocobalamin 50 (0·1% purity), choline chloride 2000, pteroylglutamic acid 1, biotin 2, menadione 0·05, D,L-α tocopheryl acetate 60, retinyl acetate and retinyl palmitate 8 (1200 retinol equivalents), cholecalciferol 0·025, maize meal 9828·125.

concentrations in liver and plasma because the variances were not homogeneous (F test). Cu absorption and urinary Cu excretion were evaluated using the multivariate ANOVA repeated measurements test. The level of significance was pre-set at $P < 0·05$. All data were processed using a computer program (SPSS Inc., 1988).

RESULTS

Feed consumption, body and organ weights

The high-Sn diet had no effect on feed consumption and body weight of the rats (Table 2). Likewise, there was no effect of Sn on the weights of liver and kidney.

Indicators of copper status

Fig. 1 shows the Cu concentrations in selected organs and plasma. The high-Sn diet resulted in significantly reduced Cu concentrations in plasma, liver and kidney.

Apparent copper absorption

Analysed Cu concentrations of both the control and test diets were found to be 5 mg/kg. Apparent Cu absorption was calculated as Cu intake minus faecal Cu excretion. During the course of the experiment absolute Cu absorption increased in both groups (Fig. 2) because feed intake increased (Table 2) but the high-Sn diet did not affect apparent Cu absorption (Fig. 2). Apparent Cu absorption expressed as a percentage of Cu intake dropped with time (results not shown). The high-Sn diet systematically lowered group means of urinary Cu excretion (Fig. 2), but the effect failed to reach statistical significance ($P = 0·118$).

Biliary copper excretion

Bile flow and biliary Cu excretion are illustrated in Fig. 3. The high-Sn diet had no effect on bile flow, but significantly reduced the absolute amount of Cu excreted in bile.

Table 2. *Feed intake and body and organ weights of rats fed on a control diet or a high-tin diet**

(Mean values with their standard errors for twelve rats per dietary group)

Diet...	Control		High tin	
	Mean	SE	Mean	SE
Body weight (g)				
Initial	95·8	2·91	94·6	2·11
Final	266·2	9·13	269·4	6·25
Feed intake (g/d)				
Days 0–7	12·7	0·49	12·5	0·38
Days 21–28	21·6	0·71	21·9	0·53
Organ weight (g/kg body weight)				
Liver	38·7	0·76	38·6	0·42
Kidney	3·3	0·08	3·3	0·05

* For details of diets, see Table 1.

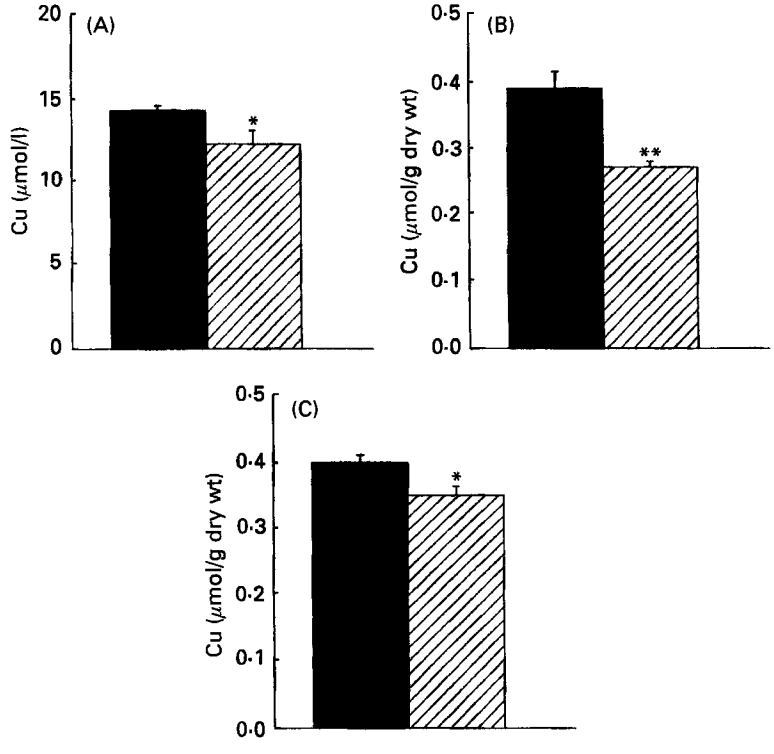

Fig. 1. Copper concentrations in (A) plasma, (B) liver and (C) kidney of rats fed on a control diet (■) or a high-tin diet (▨). Values are means with their standard errors for twelve rats (n 11 for plasma values of the control group). Mean values were significantly different from those of controls: * $P < 0·05$, ** $P < 0·01$. For details of diets and procedures, see Table 1 and pp. 863–865

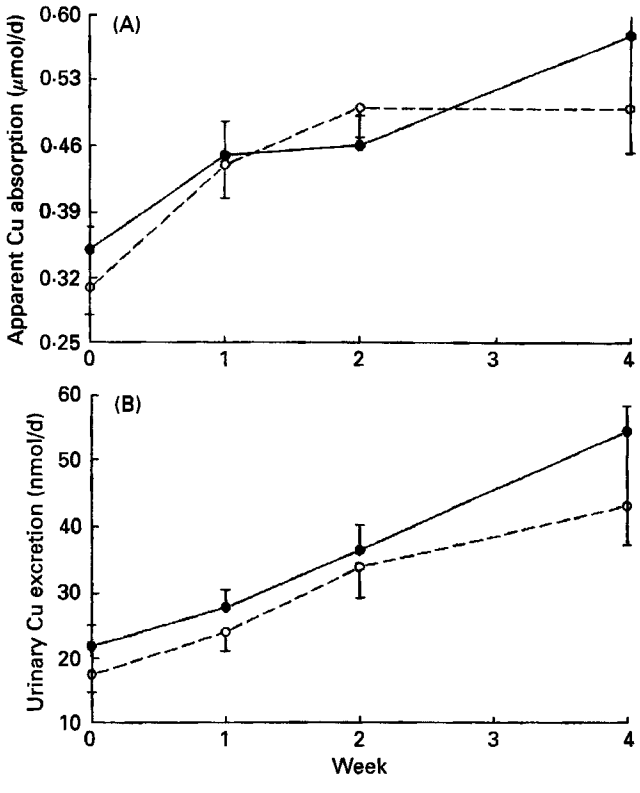

Fig. 2. Time course of (A) apparent copper absorption and (B) urinary copper excretion of rats fed on a control diet (●–●) or a high-tin diet (○---○). Values are means for twelve rats, with their standard errors indicated by vertical bars. The high-tin diet did not significantly influence apparent copper absorption or urinary copper excretion. For details of diets and procedures, see Table 1 and pp. 863–865.

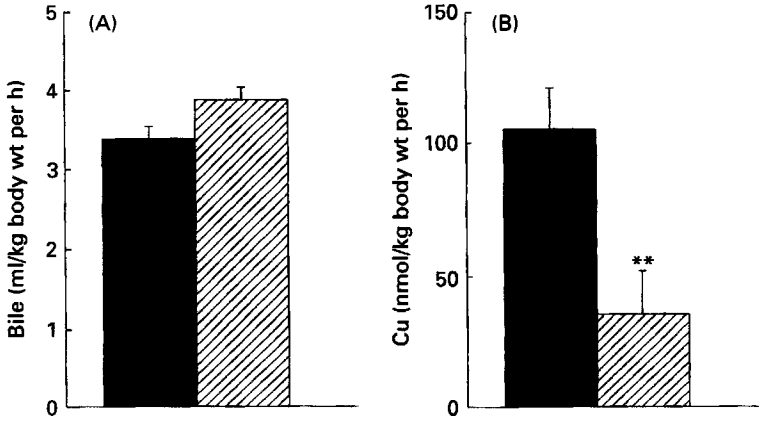

Fig. 3. (A) Bile flow and (B) biliary excretion of copper in rats fed on a control diet (■) or a high-tin diet (▨) for 28 d. Values are means for eleven (control) or twelve (high-tin) rats, with their standard errors indicated by vertical bars. ** Mean value was significantly different from that of the control, $P < 0{\cdot}01$. For details of diets and procedures, see Table 1 and pp. 863–865.

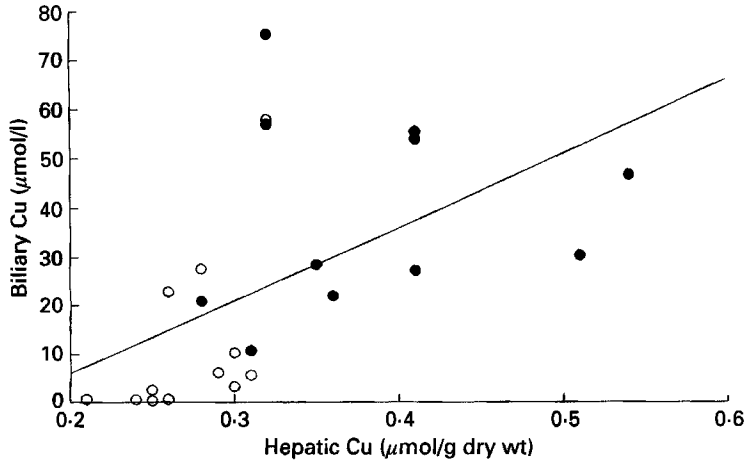

Fig. 4. Relationship between biliary copper concentration and hepatic copper concentration in individual rats fed on either a control diet (●) or a high-tin diet (○) for 28 d. The regression equation is $y = 150x - 24$ (r 0·54, n 23, $P < 0·01$).

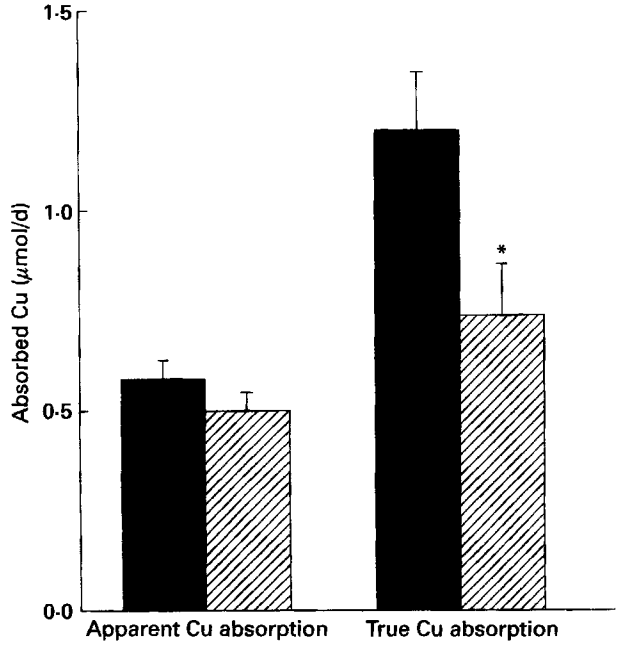

Fig. 5. Apparent and true copper absorption rates in rats fed on a control diet (■) or a high-tin diet (▨) for 28 d. Apparent copper absorption was calculated as copper intake − faecal copper, and true copper absorption as copper intake − (faecal − biliary copper). Values are means for eleven (control) or twelve (high-tin) rats, with their standard errors indicated by vertical bars. * Mean value was significantly different from that for the controls, $P < 0·05$. For details of diets and procedures, see Table 1 and pp. 863–865.

DISCUSSION

The observed lowering effects of the high-Sn diet on Cu concentrations in plasma, liver and kidney agree well with previous findings (Greger & Johnson, 1981; Pekelharing *et al.* 1994). The challenge with 100 mg Sn/kg diet did not affect feed consumption and weight gain

of the rats. Pekelharing *et al.* (1994) found that feed intake was significantly reduced in rats fed on a diet containing as much as 200 mg Sn/kg.

High Sn intake had no effect on bile flow but significantly reduced the amount of Cu excreted in bile. Since the concentration of Cu in bile was significantly related, albeit weakly, to that in liver (Fig. 4), it is likely that biliary Cu excretion is determined by the concentration of Cu in the liver. The tendency towards a lower urinary Cu excretion in the rats fed on the high-Sn diet may also be secondary to the reduced Cu status.

The reduced Cu status observed after high Sn intake could result in reduced Cu excretion in bile and urine which, in turn, could lead to reduced Cu absorption. However, apparent Cu absorption was not systematically influenced by Sn loading. Cu is discharged from the body mainly via bile (Cartwright & Wintrobe, 1964) while biliary Cu is poorly reabsorbed (Owen, 1964; Farrer & Mistilis, 1967). The diurnal rate of biliary Cu excretion is not constant (Dijkstra *et al.* 1991), but assuming that it is, and that Cu excreted in bile is not reabsorbed, true Cu absorption can be calculated as: Cu intake − (faecal Cu − biliary Cu). In the rats fed on the control and high-Sn diets, biliary Cu excretion rates (day 28) were 0·62 (SE 0·12) and 0·24 (SE 0·10) μmol/d, and faecal Cu excretion rates (days 24 to 28) were 1·12 (SE 0·05) and 1·21 (SE 0·04) μmol/d respectively. Mean Cu intake during the period day 24 to day 28 was 1·7 μmol/d for both groups. Thus, unlike apparent Cu absorption, true Cu absorption was reduced significantly in rats fed on the high-Sn diet (Fig. 5). The question which arises is how dietary Sn inhibits intestinal Cu absorption. Sn could compete with Cu for an undefined Cu carrier in the mucosa. Sn^{2+} in the digesta could reduce Cu^{2+} to Cu^{+} which may lower Cu solubility and/or reduce Cu binding to physiological ligands, leading to impaired Cu absorption. So far experimental evidence is lacking for the possible mechanisms underlying the inhibitory effect of Sn on Cu absorption.

We conclude that high Sn intake reduces Cu status in rats by inhibiting Cu absorption which is followed by decreased excretion of Cu in bile. This hypothesis could be tested by infusing Sn intravenously which, if our hypothesis is correct, would not affect biliary Cu excretion.

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