

## Iron, copper and zinc status in rats fed on diets containing various concentrations of tin

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The effects of various dietary concentrations of Sn (1, 10, 50, 100 and 200 mg/kg; added as SnCl<sub>2</sub>) on Fe, Cu and Zn status of rats were determined. After feeding the diets for 28 d body weight was not significantly affected, but there was a linear inverse response of feed intake. Plasma, kidney, spleen and tibia Fe concentrations as well as blood haemoglobin concentration and percentage transferrin saturation decreased in a linear dose–response manner as the level of dietary Sn increased. The addition of Sn to the diet depressed Cu status, as indicated by a significant inverse response of plasma, liver, kidney, spleen and tibia Cu levels. Plasma, kidney and tibia Zn concentrations were decreased by increasing levels of dietary Sn, but spleen and liver Zn concentrations were not significantly influenced. Fe, Cu and Zn status was influenced by dietary Sn concentrations lower than 50 mg/kg. If the results can be extrapolated to man it would follow that a high *v.* low Sn concentration in the human diet, which can be as distinct as 75 *v.* 2 mg/kg dry diet, may decrease plasma and tissue concentrations of Fe, Cu and Zn by up to 15%.

**Tin: Iron: Copper: Zinc: Rat**

In rats, high dietary concentrations of inorganic Sn (1580 mg Sn/kg air-dry feed) caused depressed blood haemoglobin concentrations, a slight decrease in erythrocyte count and packed cell volume values and decreased serum Fe concentrations (De Groot, 1973; De Groot *et al.* 1973). The dietary Sn concentrations used in these studies can be considered toxic as they also caused growth retardation and decreased feed intake. Apart from the possibility that the retarded growth had induced effects which are not specific for Sn loading, it is also questionable whether the observed Sn-induced anaemia in rats can be extrapolated to the situation in man. Human diets may provide 1–38 mg Sn daily, the major source of Sn probably being canned foods (Greger & Baier, 1981). When expressed on the basis of dry matter, human diets contain 2–75 mg Sn/kg.

Sn added to the diet in the form of SnCl<sub>2</sub> up to a concentration of about 200 mg Sn/kg may affect Fe, Cu and Zn status in rats but the effects are inconclusive. Dietary Sn increased liver Fe concentrations in one study (Greger & Johnson, 1981) but had no effect in another (Johnson & Greger, 1985). Similarly, increased Sn intake either reduced (Greger & Johnson, 1981) kidney Cu and Zn concentrations or had no influence on these variables (Johnson & Greger, 1984, 1985). This inconsistency of published findings prompted us to

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perform the present study in which the effects of various dietary concentrations of Sn (added as  $\text{SnCl}_2$ ) on Fe, Cu and Zn status of rats were measured.

#### MATERIALS AND METHODS

##### *General procedures*

Male, outbred Wistar rats (Hsd/Cpb:Wu; Harlan/CPB, Zeist, The Netherlands), aged about 3 weeks, were used. During the pre-experimental period of 10 d the rats were housed in groups of five animals in wire-topped polycarbonate cages ( $375 \times 225 \times 150$  mm) with a layer of sawdust as bedding. All rats received a purified diet without added  $\text{SnCl}_2$  (Table 1) and demineralized water *ad lib*.

We used a parallel, randomized block design with a treatment period of 28 d. At the end of the pre-experimental period (day 0) the body weights of the rats averaged  $90.1$  (SE  $0.87$ ) g ( $n$  42). The rats were divided into six groups of seven animals each. The groups were stratified for body weight and were randomly assigned to one of the six experimental diets (Table 1) to which either 0 (pre-experimental diet), 1, 10, 50, 100 or 200 mg Sn/kg feed was added in the form of  $\text{SnCl}_2$ . The diets were balanced for the chloride in the  $\text{SnCl}_2$ . Apart from the concentration of Sn, the diets were formulated according to the nutrient requirements of rats (National Research Council, 1978). The dietary levels of added Fe, Cu and Zn were 35, 5 and 12 mg/kg diet respectively.

As from day 0 the rats were housed individually in cages with wire-mesh bases ( $240 \times 170 \times 170$  mm) which were placed in a room with controlled temperature ( $20$ – $22^\circ$ ), lighting (12 h light–dark cycle; light: 06.00–18.00 hours) and relative humidity (40–60%). The rats had free access to feed and demineralized water. The experimental diets, which were in powdered form, were stored at  $4^\circ$  until feeding.

During the experimental period, feed intake and body weights were recorded at regular intervals. At the end of the experiment, on day 28, the rats were anaesthetized by diethyl ether inhalation and blood was collected in heparinized tubes by aortic puncture. The anaesthetized rats were killed by cervical dislocation. Organs and hindlegs were removed and stored at  $-20^\circ$  until analysis.

##### *Chemical analysis*

In whole heparinized blood, haemoglobin concentrations were determined spectrophotometrically using a test kit supplied by Roche, Mijdrecht, The Netherlands. Packed cell volume was measured by microcentrifugation (5 min at 5000 g) and the number of erythrocytes was determined using a haematology series cell counter (100 series<sup>®</sup>; Baker Instruments Corporation, Windsor, Berks). Plasma Fe concentrations and total Fe-binding capacity were determined spectrophotometrically using a kit purchased from Roche. Cu and Zn in plasma as well as Fe, Cu and Zn in liver, spleen, kidney (left and right kidney were combined) and tibia were estimated by flame absorption spectrometry after wet ashing with  $\text{HNO}_3$ . Sn in diet samples was analysed by graphite furnace absorption spectrometry with Zeeman background correction.

##### *Statistical analysis*

All data were statistically analysed using a computer program (SPSS Inc., 1988). The level of significance was pre-set at  $P < 0.05$ . The data were subjected to one-way analysis of variance (ANOVA) to disclose Sn effects, and the  $P$  values are given. In addition, linear contrasts were used to test polynomial regressions against the actual, added level of Sn in the diet. The  $P$  value of the linear term for each variable is given. For all variables neither quadratic nor cubic regression reached statistical significance ( $P > 0.05$ ).

Table 1. *Composition of the diets*

Added levels of Sn (mg/kg) in the diet...	0	1	10	50	100	200
Ingredients (g/kg)						
SnCl <sub>2</sub> ·2H <sub>2</sub> O (mg)	0	2	19	95	190	380
Glucose	709.3	709.3	709.3	709.3	709.1	708.9
CaCO <sub>3</sub>	12.0	12.0	12.0	12.0	12.1	12.1
CaCl <sub>2</sub> (mg)	468	467	459	421	375	281
Constant components*	278.2	278.2	278.2	278.2	278.2	278.2
Chemical analysis (mg/kg)						
Sn	0.5	1.4	10.5	41	86	226

\* The constant components consisted of (g/kg feed): casein 151, maize oil 25, coconut fat 25, cellulose 30, NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O 15.1, MgCO<sub>3</sub> 1.4, KCl 1.0, KHCO<sub>3</sub> 7.7, mineral premix 10, vitamin premix 12. The Sn-free mineral premix consisted of (mg/kg feed): FeSO<sub>4</sub>·7H<sub>2</sub>O 174, MnO<sub>2</sub> 79, ZnSO<sub>4</sub>·H<sub>2</sub>O 33, NiSO<sub>4</sub>·6H<sub>2</sub>O 13, NaF 2, KI 0.2, CuSO<sub>4</sub>·5H<sub>2</sub>O 15.7, Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O 0.3, CrCl<sub>3</sub>·6H<sub>2</sub>O 1.5, NH<sub>4</sub>VO<sub>3</sub> 0.2, maize meal 9681.1. The vitamin premix consisted of (mg/kg feed): thiamin 4, riboflavin 3, nicotinamide 20, DL-calcium pantothenate 17.8, pyridoxine 6, cyanocobalamin 50, choline chloride 2000, folic acid 1, biotin 2, menadione 0.05, DL- $\alpha$ -tocopheryl acetate 60, retinyl acetate and retinyl palmitate 8 (1200 retinol equivalents), cholecalciferol 0.025, maize meal 9828.125.

Table 2. *Effect of dietary tin on growth performance and organ weights of rats\**

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

Sn added to diet (mg/kg)	Body wt (g)	Feed intake (g/d)	Organ wt (g)		
			Liver	Kidney	Spleen
0	241	16.4	9.2	0.76	0.46
1	234	16.2	9.0	0.72	0.45
10	233	16.0	8.8	0.71	0.41
50	239	16.5	9.1	0.74	0.43
100	236	16.2	8.6	0.74	0.39
200	225	14.9	8.3	0.71	0.39
Pooled SE	6.1	0.48	0.32	0.022	0.001
Statistical analysis:					
ANOVA†: <i>P</i> =	0.493	0.225	0.354	0.510	0.053
<i>L</i> term‡: <i>P</i> =	0.151	0.033	0.045	0.587	0.009

\* For details of diets and procedures, see Table 1 and p. 104.

† Value for overall *F* ratio in one-way analysis of variance (ANOVA).

‡ Value for linear (*L*) term in effect of dietary Sn concentration.

## RESULTS

### *Growth performance*

During the first week of the experiment the feeding of 200 mg Sn/kg diet resulted in a slightly reduced gain in body weight which was accompanied by a decrease in feed intake (results not shown). Thereafter, body weight gain and feed intake returned to control values. Final body weights were not significantly influenced by dietary Sn concentration (Table 2). Weights of liver and kidney (average of left and right kidney) were similar in all dietary groups. Spleen weight showed an inverse response to dietary Sn concentration (Table 2).

Table 3. *Effect of dietary tin on blood indicators of iron status of rats\**  
(Mean values with their pooled standard errors for seven rats per dietary group)

Sn added to diet (mg/kg)	Haemoglobin (mmol/l)	Packed cell volume (%)	RBC ( $\times 10^{12}/l$ )	TIBC ( $\mu\text{mol}/l$ )	TS (%)
0	8.21	42.2	6.5	91	30.5
1	8.46	42.8	6.6	97	32.9
10	8.39	42.8	6.5	88	35.2
50	8.30	42.3	6.6	94	32.3
100	8.24	42.8	6.6	92	28.1
200	7.94	41.1	6.3	92	27.3
Pooled SE	0.132	0.72	0.12	2.4	1.73
Statistical analysis:					
ANOVA†: $P =$	0.130	0.536	0.431	0.171	0.022
$L$ term‡: $P =$	0.013	0.111	0.146	0.848	0.004

RBC, erythrocytes; TIBC, total Fe-binding capacity; TS, transferrin saturation.

\* For details of diets and procedures, see Table 1 and p. 104.

† Value for overall  $F$  ratio in one-way analysis of variance (ANOVA).

‡ Value for linear ( $L$ ) term in effect of dietary Sn concentration.

Table 4. *Effect of dietary tin on indicators of iron status of rats\**  
(Mean values with their pooled standard errors for seven rats per dietary group)

Sn added to diet (mg/kg)	Plasma Fe ( $\mu\text{mol}/l$ )	Tissue Fe ( $\mu\text{g}/\text{g}$ dry wt)			
		Liver	Kidney	Spleen	Tibia
0	27.5	375	244	601	72.6
1	32.0	351	279	582	72.2
10	30.9	347	214	612	74.7
50	30.2	321	208	541	70.7
100	25.9	371	199	510	66.7
200	24.8	391	186	437	61.1
Pooled SE	1.43	21.4	27.2	34.8	1.99
Statistical analysis:					
ANOVA†: $P =$	0.005	0.270	0.192	0.009	0.001
$L$ term‡: $P =$	0.001	0.161	0.041	0.001	0.001

\* For details of diets and procedures, see Table 1 and p. 104.

† Value for overall  $F$  ratio in one-way analysis of variance (ANOVA).

‡ Value for linear ( $L$ ) term in effect of dietary Sn concentration.

#### Iron status

Table 3 shows that the concentration of haemoglobin was significantly lowered with increasing Sn intakes. Transferrin saturation went up with increasing dietary Sn concentration up to 10 mg/kg, but at higher Sn intakes it dropped. After logarithmic transformation of the analysed dietary Sn concentrations (Table 1), transferrin saturation was found to display a significant ( $P = 0.004$ ) quadratic response. Packed cell volume, erythrocyte count and total Fe-binding capacity values were not linearly related to the amount of Sn in the diet (Table 3). Plasma Fe showed a significant inverse response to Sn feeding (Table 4). Tibia, kidney and spleen Fe concentrations were significantly decreased with increasing intakes of Sn. No Sn effect was observed concerning liver Fe concentration.

Table 5. *Effect of dietary tin on indicators of copper status of rats\**  
(Mean values with their pooled standard errors for seven rats per dietary group)

Sn added to diet (mg/kg)	Plasma Cu ( $\mu\text{g/ml}$ )	Tissue Cu ( $\mu\text{g/g}$ dry wt)			
		Liver	Kidney	Spleen	Tibia
0	1.12	17.0	25.0	7.4	4.09
1	1.07	17.1	24.4	7.0	4.06
10	1.04	14.6	22.7	6.9	3.93
50	0.90	15.1	22.7	6.5	3.89
100	0.44	12.9	19.5	4.7	3.66
200	0.31	11.9	18.3	3.9	3.53
Pooled SE	0.104	0.64	0.89	0.38	0.096
Statistical analysis:					
ANOVA†: $P =$	< 0.001	< 0.001	< 0.001	< 0.001	0.001
$L$ term‡: $P =$	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

\* For details of diets and procedures, see Table 1 and p. 104.

† Value for overall  $F$  ratio in one-way analysis of variance (ANOVA).

‡ Value for linear ( $L$ ) term in effect of dietary Sn concentration.

Table 6. *Effect of dietary tin on indicators of zinc status of rats\**  
(Mean values with their pooled standard errors for seven rats per dietary group)

Sn added to diet (mg/kg)	Plasma Zn ( $\mu\text{g/ml}$ )	Tissue Zn ( $\mu\text{g/g}$ dry wt)			
		Liver	Kidney	Spleen	Tibia
0	1.74	83	115	94	186
1	1.80	87	112	95	186
10	1.73	85	100	92	179
50	1.66	86	100	91	173
100	1.57	84	94	93	165
200	1.54	82	93	93	156
Pooled SE	0.060	2.3	7.2	1.4	4.5
Statistical analysis:					
ANOVA†: $P =$	0.025	0.617	0.153	0.317	< 0.001
$L$ term‡: $P =$	0.001	0.224	0.027	0.775	< 0.001

\* For details of diets and procedures, see Table 1 and p. 104.

† Value for overall  $F$  ratio in one-way analysis of variance (ANOVA).

‡ Value for linear ( $L$ ) term in effect of dietary Sn concentration.

#### *Copper status*

Table 5 shows that dietary Sn significantly affected Cu status. Increasing levels of dietary Sn were associated with decreasing concentrations of Cu in plasma, liver, kidney, spleen and tibia. Tissue Cu concentrations fell by 11 (tibia) to 36 % (spleen) with a dietary Sn level of 100 mg/kg.

#### *Zinc status*

Supplementation of the diet with Sn produced a significant inverse response of Zn concentrations in plasma, kidney and tibia (Table 6). No significant Sn effect was observed for spleen and liver Zn concentrations.

## DISCUSSION

No effect of Sn feeding on final body weight was observed while feed intake was clearly reduced only at the highest dietary Sn concentration which was 200 mg Sn/kg diet. Therefore, it may be concluded that, in contrast to other studies (De Groot, 1973; De Groot *et al.* 1973), the Sn effects observed in the present study are not confounded by growth depression.

Fe status was influenced following Sn ingestion. There was a significant inverse response of various indicators of Fe status to increasing dietary Sn concentrations. The effect of Sn on Fe status may have been caused by impaired Fe absorption. Oral administration of SnCl<sub>2</sub> causes a decrease in gastric acid secretion (Yamaguchi *et al.* 1980*b*). Since normal gastric secretion is necessary for optimal absorption of Fe in rats (Morris, 1987), impaired Fe absorption may occur after Sn loading. SnCl<sub>2</sub> may also inhibit Fe uptake by jejunal mucosal cells and/or reduce Fe release at the basolateral membrane of the mucosal cells (Schäfer & Forth, 1983). If Sn interacts with the Fe release from the mucosal cells into the blood, as has been described for Co (Becker *et al.* 1979), the flow of Fe into the body is diminished. This might result in accumulation of Fe in the mucosa, as has been found in Fe-deficient rats loaded with Co (Becker *et al.* 1979).

Cu status was depressed after Sn feeding as indicated by a significant inverse response of plasma, liver, kidney, spleen and tibia Cu concentrations. The effect of Sn on Cu status may be caused by impaired absorption of Cu. Greger & Johnson (1981) found an increase in faecal Cu excretion in rats after feeding 200 mg Sn/kg diet for 28 d.

Dietary-Sn-induced reductions of Zn concentrations of tibia, kidney, liver and plasma have been reported earlier (Greger & Johnson, 1981; Johnson & Greger, 1984). In the present study a significant Sn effect was observed for plasma, kidney and tibia Zn concentrations. There was no effect of dietary Sn on hepatic Zn concentration, but in another study a significant decrease in liver Zn concentration was seen only after consumption of diets containing  $\geq 500$  mg Sn/kg diet (Johnson & Greger, 1984). Kidney and tibia Zn concentrations were less sensitive to dietary Sn than was plasma Zn (Greger & Johnson, 1981; Johnson & Greger, 1984). In the present study there was no clear difference in responsiveness of these indicators of Zn status to increasing dietary concentrations of Sn. Zn absorption was not affected in rats fed on diets containing less than 500 mg Sn/kg (Johnson & Greger, 1984). Thus, it remains obscure how, at dietary concentrations up to 200 mg/kg diet, Sn affects Zn status.

Cu, Fe and Zn stores in tibia were depressed with increasing intakes of Sn. This result suggests that bone is a critical organ in oral Sn exposure, which agrees with the observation that Ca content in bone is decreased after Sn loading (Yamaguchi *et al.* 1980*a*).

The present study shows that Fe, Cu and Zn status of rats is influenced by dietary Sn concentrations lower than 50 mg Sn/kg diet. If our rat findings can be extrapolated to man, this would imply that under normal conditions high Sn concentrations in the human diet may decrease human plasma and tissue concentrations of Fe, Cu and Zn by up to 15%. Sn concentrations in the human diet range between 2 and 75 mg/kg dry diet (Greger & Baier, 1981).

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