The effect of tea on iron and aluminium metabolism in the rat

BY SUSAN J. FAIRWEATHER-TAIT AND ZOE PIPER

AFRC Institute of Food Research, Colney Lane, Norwich, Norfolk NR4 7UA

AND S. JEMIL A. FATEMI AND GEOFFREY R. MOORE

School of Chemical Sciences, University of East Anglia, Norwich, Norfolk NR4 7TJ

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Weanling male Wistar rats were fed for 28 d on a semi-synthetic diet containing normal (38 μg/g) or low (9 μg/g) levels of iron. They were given water or tea infusion (20 g leaves/l water) to drink. Two further groups were given a normal- or low-Fe diet containing added tea leaves (20 g/kg diet). At the end of the study period, all rats given the low-Fe diet were severely anaemic, as assessed by haemoglobin, packed cell volume and liver Fe. Those given tea or the diet with added tea leaves showed a greater degree of Fe depletion. The blood and liver aluminium levels were not increased as a result of consuming tea or tea leaves, despite the higher Al intakes. Fe deficiency *per se* had no effect on Al absorption or retention from tea. It was concluded that the Al in tea was very poorly absorbed but that tea, either in the form of an infusion or as tea leaves, had an adverse effect on Fe status.

Iron metabolism: Aluminium metabolism: Tea: Rat

Aluminium is the third most abundant element in nature, after oxygen and silicon, yet it is probably not an essential nutrient. Although very little is known about its biological effects in the human body, there have been suggestions of a link between Al and Alzheimer's disease (Candy *et al.* 1986). However, interest in Al as a potentially toxic material increased dramatically during 1989 following the accidental contamination of drinking water with Al in Cornwall, and reports in the literature that some baby formulas made from soya-bean milk contain up to ten times more Al than breast milk (Koo *et al.* 1988; Ministry of Agriculture, Fisheries and Food, personal communication). In the debate that ensued it became clear that very little is known about Al absorption from the diet and it was this paucity of information that gave rise to the present study. Because there are no stable isotopes of Al and radio-isotopes are not readily available, work on Al metabolism is difficult to perform, particularly in human subjects, and therefore we have used rats for our study.

Tea is a rich source of Al, the tea plant actively taking up Al from the soil and storing it in its leaves (Chenery, 1955). However, it is not known to what extent the Al in tea is absorbed and retained by the body. The present study was therefore undertaken in rats to assess the bioavailability of Al from tea and tea leaves. In addition, because the absorption of several inorganic elements is increased in iron-deficient states (Forth & Rummel, 1973), groups of rats were given low-Fe diets in conjunction with tea or tea leaves in order to investigate the effects of Fe deficiency on Al metabolism. The tannins in tea render any Fe consumed at the same time less available for absorption (Disler *et al.* 1975). Therefore, the effects of tea on Fe status were also determined.

MATERIALS AND METHODS

Six groups of fifteen weanling male Wistar rats were individually caged in stainless steel and plastic cages with gridded bottoms. Three groups were given normal- (38 μg Fe/g) and

Table 1. *Composition of the normal-iron semi-synthetic diet given to rats (g/kg diet)*

Maize starch	309
Sucrose	309
Casein	200
Solka floc	40
Maize oil	80
Mineral mix*	40
Vitamin mix**	20
Methionine	2·5

*Contained (g/kg diet): $CaHPO_4$ 13·0, $CaCO_3$ 8·2, Na_2HPO_4 7·4, KCl 7·03, $MgSO_4.H_2O$ 4·0, $MnSO_4.H_2O$ 0·180, $FeSO_4.7H_2O$ 0·144, $ZnCO_3$ 0·100, $CuSO_4.5H_2O$ 0·023, KIO_3 0·001.

**Contained (mg/kg diet): nicotinic acid 60, cyanocobalamin in mannitol (Glaxo) 50, calcium-D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pyridoxine 10, pteroylomonoglutamic acid 10, D-biotin 1, vitamin K_1 (in lactose) 2, Rovimix E-50 150 (containing 75 mg DL-α-tocopheryl acetate), Rovimix A-500 25 (containing 3·75 mg retinol), Rovimix D_3-500 15 (containing 188 μg cholecalciferol; all Rovimix products from Roche, North Dunstable, Bedfordshire), choline bitartrate 1800, starch (bulking agent) 17817.

Table 2. *Operating conditions used for aluminium determination by graphite-furnace atomic absorption spectrophotometry*

	Temperature (°)	Ramp(s)	Hold(s)
Step 1	90	8	10
2	140	8	30
3	550	7	30
4	1400	4	20
5	2500	0	3
6	3000	0	2

Sample volume 20 μl; wavelength 309·3; Ballysound correction (deuterium); signal mode = peak height; autosampler; argon gas; pyrolytic-coated graphite tubes.

three low-Fe (9 μg Fe/g) semi-synthetic diets, made by omitting the ferrous sulphate from the mineral mix; the composition of the normal-Fe diet is shown in Table 1. At each Fe level, one group was given distilled water to drink *ad lib.*, the second group an infusion of tea (PG Tips, Brooke Bond, Croydon, Surrey) and the third group tea leaves (PG Tips) incorporated into the diet (20 g leaves/kg diet, replacing 10 g starch and 10 g sucrose). The tea infusion was made three times/week by adding 1 litre boiling distilled water to 20 g tea leaves, stirring, leaving to stand for 10 min and then filtering through fine nylon gauze. All intakes of food and drink were measured daily, and the animals weighed at regular intervals.

After 4 weeks, the animals were killed by intraperitoneal administration of a lethal dose of sodium pentobarbitone anaesthetic (1 ml Euthatal (20 mg/ml); May & Baker Ltd, Dagenham, Essex), blood taken by cardiac puncture, the liver removed, rinsed in saline (9 g sodium chloride/l), blotted dry, freeze-dried, and homogenized using a pestle and mortar. The following analyses were performed: (*a*) blood haemoglobin (Hb, cyanomethaemoglobin method), (*b*) blood packed cell volume (PVC, microhaematocrit method), (*c*) liver Fe (flame atomic absorption spectrophotometry (AAS), PU9000 Pye Unicam, Cambridge), (*d*) liver Al (graphite-furnace AAS, SP9, Pye Unicam, Cambridge, operating conditions described in Table 2), (*e*) blood Al (as for the liver determination), and (*f*) food and drink Fe (flame AAS) and Al (graphite furnace AAS) concentrations.

The samples for flame AAS were heated to 480 ° for 48 h in silica crucibles, the ash taken

up in hot hydrochloric acid (Analar) and the solution made up to an appropriate volume with Analytical grade (AR) water (Fisons). Liver (0·15 g dried material) and food (0·5 g diet) samples for Al analysis by graphite furnace were digested with 3 ml concentrated nitric acid (aristar) in PTFE-lined stainless steel pressure digestion vessels (Cowie Scientific Ltd, Middlesbrough, Cleveland). The lids on the vessels were screwed down and they were placed in an oven at 125 ° for 3 h. After cooling, the top was removed and the HNO_3 gradually evaporated on a hot plate. Before complete drying, the solution was transferred to a 20 ml volumetric flask and made up to volume with water (AR).

The blood samples, which had been stored at −18 °, were prepared for analysis by adding 0·5 ml blood to an acid-washed, EDTA (100 g/l)-washed polyethylene centrifuge tube and the proteins precipitated by the procedure of Brown *et al.* (1984). The solution was mixed on a Vortex mixer at medium speed and 0·5 ml water (AR) and 50 μl concentrated HNO_3 (aristar) were added whilst mixing for 1 min. The solution was heated for 10 min at 70 ° in a water-bath, mixed again for 20 s, and then centrifuged for 10 min at 2000 ***g***. The supernatant fraction was pipetted into a Teflon sampling cup and the autosampler used for injection. Tea infusions were analysed directly by graphite-furnace AAS (Fairweather-Tait *et al.* 1987). Al standards were prepared by appropriate dilution with water (AR) from a stock solution of aluminium nitrate (BDH, Poole, Dorset), the calibration curve being linear from 0 to 30 μg Al/l.

Statistical treatment

Results for all analyses were subjected to two-way analysis of variance (AOV) with two variables: level of dietary Fe (low, normal) and type of diet (water, tea, diet with tea leaves). Because the number in the groups differed, the standard error of the difference between means (SED) was calculated from

$$\sqrt{\text{residual mean square (RMS)}/(1/n_1 + 1/n_2)}$$

for each pair of groups, and approximate t tests performed, where t = (mean 1 − mean 2)/SED. Regression analysis was performed on Al intakes and liver Al levels. All analyses were carried out using GENSTAT programs (Payne *et al.* 1987).

RESULTS

There were no significant differences between the groups in the initial body-weights of the rats (mean 70 (SD 7) g), but at the end of the experiment, those given the low-Fe diet weighed significantly less ($P < 0·001$, Table 3), with lower dry liver weights (Table 4). Food intake was reduced in groups given the low-Fe diet, and the food conversion efficiency was slightly reduced (from 0·37 to 0·32 g weight gained/g food consumed). There was also an effect of dietary treatment on food intake ($P < 0·01$) whereby animals given water consumed more food than those given tea to drink. Al intakes are given in Table 3, the lowest being in the group given low-Fe diet and water (55 μg/d), and highest in the group given normal-Fe diet containing tea leaves (813 μg/d).

The Fe concentration of the diet had a significant effect ($P < 0·001$) on Fe status, as assessed by liver Fe, blood Hb and PCV (Table 4), whereby animals given the low-Fe diet were severely anaemic. Fe depletion was more marked in groups consuming the diet with added tea leaves or drinking the tea infusion than in the controls.

There was no effect of dietary treatment on blood Al concentrations (Table 5). Liver Al concentrations were highest in the group fed on the low-Fe diet containing tea leaves, but since the liver weights and food intakes were significantly different between the low-Fe and normal-Fe groups, total liver Al is probably a more valid comparison. Two-way AOV

Table 3. *Body-weights, and food and aluminium intakes over 28 d for rats fed on low-iron (L) or normal-Fe (N) semi-synthetic diets**

(Values are means for number of observations shown in parentheses)

		Diet				Statistical significance of variance ratio (F)† on:		
	Fe level	Water	Tea	Diet with tea leaves	RMS	Fe level	Diet	Fe level:diet
Final body-wt (g)	L	235 (14)	224 (14)	222 (14)	459	$P < 0{\cdot}001$	NS	NS
	N	293 (15)	287 (14)	282 (14)				
Food intake (g)	L	518	472	480	2277	$P < 0{\cdot}001$	$P < 0{\cdot}01$	NS
	N	609	565	614				
Al intake (mg)	L	1·55	6·73	17·75	0·957	$P < 0{\cdot}001$	$P < 0{\cdot}001$	$P < 0{\cdot}001$
	N	1·83	8·12	22·75				

RMS, residual mean square; NS, not significant.

* For details of diets and treatments, see Table 1 and pp. 61–62.

† For statistical treatment, see p. 63.

Table 4. *Dry liver weights (g), liver iron concentration (μg/g), total liver Fe (μg), blood haemoglobin (Hb) (g/l) and packed cell volume (PCV) in rats fed on low-Fe (L) or normal-Fe (N) semi-synthetic diets**

(Values are means for number of observations shown in parentheses)

	Fe level	Diet			RMS	Statistical significance of variance ratio (*F*)† on:		
		Water	Tea	Diet with tea leaves		Fe level	Diet	Fe level:diet
Liver wt (g)	L	2·945 (14)	2·978 (14)	2·721 (14)	0·2422	$P < 0·001$	$P < 0·05$	NS
	N	4·303 (15)	4·809 (14)	4·411 (14)				
Liver Fe concentration (μg/g)	L	81·7	66·8	71·3	254·3	$P < 0·001$	$P < 0·001$	$P < 0·001$
	N	168·5	135·6	120·8				
Total liver Fe (μg)	L	237	198	196	9497	$P < 0·001$	$P < 0·001$	$P < 0·01$
	N	724	653	529				
Blood Hb (g/l)	L	52·2	46·2	45·4	0·8205	$P < 0·001$	$P < 0·001$	NS
	N	142·9	133·2	132·1				
PCV	L	0·165	0·143	0·139	0·0498	$P < 0·01$	$P < 0·001$	NS
	N	0·419	0·410	0·393				

RMS, residual mean square; NS, not significant.

* For details of diets and treatments, see Table 1 and pp. 61–62.

† For statistical treatment, see p. 63.

Table 5. *Aluminium in blood (μg/l), and liver (concentration (μg/g), and total, (μg)) in rats fed on low-iron (L) or normal-Fe (N) semi-synthetic diets*†

(Values are means for number of observations shown in parentheses)

		Diet				Statistical significance of variance ratio (F)‡ on:		
	Fe level	Water	Tea	Diet with tea leaves	RMS	Fe level	Diet	Fe level : diet
Blood Al	L	9·8 (14)	10·5 (14)	11·7 (14)	4·665	NS	NS	NS
	N	10·7 (15)	9·7 (14)	10·4 (14)				
Liver Al concentration (μg/g)	L	1·6[a]	1·4[a]	2·0[b]	0·1687	$P < 0·01$	$P < 0·05$	$P = 0·01$
	N	1·3[a]	1·6[a]	1·5[a]				
Total liver Al (μg)	L	4·8	4·3***	5·5	2·876	$P < 0·001$	NS	$P < 0·05$
	N	5·8	7·5	6·6				

RMS, residual mean square; NS, not significant.

[a, b] Values in a horizontal line or vertical column for each measured variable with different superscript letters were significantly different ($P < 0·05$).

† For details of diets and treatments, see Table 1 and pp. 61–62.

‡ For statistical treatment, see p. 63.

*** Mean value was significantly different from that for normal-Fe level ($P < 0·001$).

showed no effect of tea or tea leaves on total Al levels. However, the Fe level significantly influenced total liver Al content which was higher in the normal-Fe groups for each diet. This was significant for the group given tea to drink ($P < 0{\cdot}001$). Although there was a significant relationship (R 0·22, $P < 0{\cdot}05$) between Al intake over the experimental period and liver Al levels (expressed as totals or concentrations), the regression analysis showed that only a very small percentage of the variance was accounted for (< 5%). It would therefore appear that the animals accumulated Al in their livers in relation to tissue growth, but that a 14-fold difference in Al intake did not affect the Al content of the liver.

DISCUSSION

The adverse effect of tea on Fe bioavailability has been known for some time (Disler *et al.* 1975), and the results of the present study confirm that long-term ingestion of tea impaired Fe status. Fe-deficient rats increase their efficiency of Fe absorption (Fairweather-Tait & Wright, 1987) but not enough in the present study to overcome the inhibitory effects of tea and tea leaves, the magnitude of which was similar at both levels of dietary Fe. Assuming that the observed effects were entirely due to the tannin, the results demonstrated that tannin need not be ingested as a solution for inhibition of Fe absorption to occur in tea (and presumably fruits, vegetables and cereals as well), and suggests that the tannin is at least partially solubilized in the gastrointestinal tract.

Interactions between Al and Fe have been reported, although results are conflicting (Greger, 1988). Modest Al accumulation, as found in dialysis patients, has a pronounced inhibitory effect on Hb synthesis which responds well to desferrioxamine therapy (Altmann *et al.* 1988). It is not clear whether or not Al interferes with Fe absorption or subsequent metabolism, or both, but it may well be that Al absorption takes place along pathways for essential metals. If this is the case, Fe deficiency could result in increased absorption, as seen with manganese, cobalt and lead (Bothwell *et al.* 1979). However, results of the present study showed no effect of Fe deficiency on blood or liver Al levels. The selection of the liver for analysis was based on the results of a study in which rats were fed high amounts of Al (Ondreicka *et al.* 1966) and the liver, testes and bones had the most pronounced accumulation of Al. There have been some reports in the literature suggesting that Al from aluminium chloride is accumulated in brain ferritin but not liver ferritin (Fleming & Joshi, 1987). However, the work is controversial and since the general consensus is that liver Al levels reflect dietary intakes, the liver was selected for analysis rather than the brain. The values obtained for blood Al (9·7–11·7 μg/l) agree with those of D'Haese *et al.* (1985) who found a mean of 12·1 (SD 1·5) μg/l in ten human subjects.

Tea is one of the most commonly consumed dietary sources of Al, containing in the region of 4 mg Al/l (Fairweather-Tait *et al.* 1987). Yet, consuming tea as the only source of liquid for a period of 28 d appeared to have no effect on Al accumulation in the body of growing rats. Assuming that man's lifespan is approximately 30 times that of a rat, a 28 d period in the rat's life is equivalent to 2·5 years in man. In the groups fed on normal-Fe diets the intakes of Al during the period of study was 1·83, 8·12 and 22·75 mg in rats given water, tea or diet with tea leaves respectively. Mean total liver Al in the groups were 5·8, 7·5 and 6·6 μg respectively, three orders of magnitude less than the diet. Assuming that the liver is a sensitive indicator of absorbed Al then it must be inferred from the results of the present study that very little, if any, of the Al in either tea infusion or tea leaves was retained in the body. One explanation for this may be the high fluoride content of tea which has been reported to increase Al excretion in the urine and faeces of rats, thereby decreasing body retention (Ondreicka *et al.* 1971). However, the lack of difference in blood Al would indicate that the Al in tea was not available for absorption, even in Fe deficiency when the efficiency of absorption of many inorganic elements is raised.

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