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The effect of alcoholic beverages on iron and zinc metabolism in the rat

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1. Male Wistar rats (approximately 200 g) were given distilled water and a semi-synthetic control diet for 6 d. On day 7, 37 kBq ^{65}Zn were administered intramuscularly and the rats were given distilled water, beer, cider, red wine, whisky or ethanol as their only source of fluid. The wine, whisky and ethanol were diluted so that each of the beverages contained a similar ethanol concentration (approximately 30 g/l). Food and fluid intake, growth rate and whole-body ^{65}Zn were measured regularly over 11 d, after which animals were killed and blood haemoglobin (Hb) concentration, liver iron stores and the Zn concentration in testes determined.

2. There were no differences in body-weight gain or food intake between groups but fluid intake for the beer group was considerably higher than that for the other groups.

3. There was a significant effect of the type of alcoholic beverage consumed on whole-body ^{65}Zn retention. Rats given whisky had a smaller daily loss of ^{65}Zn than those given water, beer or cider. The ethanol group also showed a lower rate of ^{65}Zn loss compared with the water group. The observed changes in whole-body ^{65}Zn retention could be explained by an adverse influence of ethanol on Zn absorption from the diet.

4. Blood Hb and testes Zn concentration were similar in all groups but the type of liquid consumed influenced liver Fe levels. The cider group had the lowest liver Fe values and the ethanol group the highest values.

5. It is apparent from the present study that ethanol and alcoholic beverages affect Zn and Fe metabolism, but that the effects of ethanol are moderated by other components of the alcoholic beverages.

Alcohol has been reported to enhance iron absorption, and there appears to be a good correlation between hepatic Fe levels and consumption of alcoholic beverages. Conversely, it has been demonstrated that alcohol increases urinary zinc excretion, which has led to the suggestion that a high intake of alcohol might lead to Zn deficiency. There may be differences in response depending on the composition of the alcoholic beverage, which are not necessarily the same as the response elicited by ethanol alone (McDonald & Margen, 1980).

The present study was designed to examine the effects of consuming alcoholic beverages, of similar (modest) ethanol concentration, on Fe and Zn status in the rat. A variety of beverages were given in order that direct comparisons between different sources of ethanol could be made. The rate of loss of endogenous Zn during the period of ethanol ingestion was also measured, using ^{65}Zn to label the metabolically active pool of Zn in the body, to see whether the alcoholic beverages influenced whole-body Zn turnover.

MATERIALS AND METHODS

Sixty immature male Wistar rats were individually caged in stainless-steel and plastic cages with wire-gridded bottoms, and given drinking water and semi-synthetic control diet *ad lib.* for 6 d. The composition of the diet is given in Table 1. On the 7th day, between 10.00 and 12.00 hours, each animal was given an intramuscular injection containing 37 kBq ^{65}Zn (as zinc chloride; Amersham International plc, Amersham, Bucks.) and then counted in a small-animal whole-body counter (NE8112; NE Technology Ltd, Beenham, Berks), as described previously (Fairweather-Tait & Wright, 1984). The counting efficiency for ^{65}Zn was approximately 25%.

Table 1. *Composition of the 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, Na_2HPO_4 7·4, $CaCO_3$ 8·2, KCl 7·03, $MgSO_4.H_2O$ 4·0, $ZnCO_3$ 0·100, $FeSO_4.7H_2O$ 0·144, $CuSO_4.5H_2O$ 0·023, KIO_3 0·001, $MnSO_4.H_2O$ 0·180.

† Contained (mg/kg diet): nicotinic acid 60, cyanocobalamin in mannitol (Glaxo) 50, calcium-D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pyridoxine 10, pteroylmonoglutamic acid 10, D-biotin 1, vitamin K_1 2, Rovimix E-50 (Roche, North Dunstable, Bedfordshire) 150, Rovimix A-500 25, Rovimix D_3-500 15, choline bitartrate 180.

The rats were randomly allocated to six groups of ten, and animals in each group given one of the following liquids containing approximately 30 g alcohol/l in place of drinking water as their only source of fluid: group 1, distilled water; group 2, beer (Bentley's Yorkshire Bitter; Whitbread & Co., London); group 3, cider (Taunton Special Vat Cider; Taunton Cider Co. Ltd, Norton Fitzwarren, Somerset); group 4, diluted red wine (wine–water 0·33:1, v/v, Colman's French Red Wine, 11·5% alcohol by volume; Colman's of Norwich, Norfolk); group 5, diluted whisky (whisky–water 0·1:1, v/v, Claymore, 40% alcohol by volume; A. Ferguson & Co. Ltd., Glasgow); group 6, diluted ethanol (ethanol–water 0·034:1, v/v). The animals were allowed access to food and drink *ad lib.* for 11 d, during which time their consumption and growth were monitored. Beer was the only carbonated liquid studied, and initially we encountered some problems with excessive drip from the teat of the water bottle. However, once the beer bottle had been open for several days, most of the carbon dioxide disappeared and there were no further difficulties in measuring fluid intake. For this reason, the mean daily intake of beer was calculated from the last 9 d of the study period, the values from the first 2 d being discarded. Great care was taken to collect all the spilt fluid, and allowances were made for evaporative losses.

Each animal was counted daily in the whole-body counter and the ^{65}Zn content calculated as a percentage of the original level immediately post-injection, after making corrections for background, counting efficiency and isotope decay.

After 11 d the animals were killed with a lethal dose of sodium pentobarbitone (1 ml Euthatal; May & Baker, Dagenham, Essex). Blood was removed by cardiac puncture for haemoglobin (Hb) estimation by the cyanomethaemoglobin method (Richterich & Colombo, 1981). The liver was removed for Fe analysis and the testes for Zn analysis. Organs were freeze-dried, ground and subsamples ashed at 480° for 48 h in silica crucibles. The ash was dissolved in a minimum volume of hot concentrated hydrochloric acid, made up to volume with distilled water, filtered through Whatman no. 542 paper and the Fe or Zn concentrations measured by atomic absorption spectroscopy (PU9000; Pye Unicam, Cambridge) using certified NBS standards for validation (Office of Standard Reference Materials, Washington DC).

Statistical analysis

Results were subjected to analysis of variance, and where this showed a treatment effect, approximate *t* tests were performed using the standard error for differences between means.

Table 2. *Iron and zinc contents of liquids and diet given to rats*

	Fe (μg/ml)	Zn (μg/ml)
Distilled water	0·04	0·20
Beer	0·14	0·03
Cider	1·67	0·21
Wine–water (0·33:1, v/v)	3·29	0·24
Whisky–water (0·1:1, v/v)	0·10	0·35
Ethanol–water (0·034:1, v/v)	0·04	0·20
Semi-synthetic diet	30·5 μg/g	60·4 μg/g

Table 3. *Initial and final body-weights, food and liquid intakes (g/d) of rats given different alcoholic beverages for 11 d*

(Values are means for ten rats/group)

Liquid	Alcohol (g/l)	Initial body-wt (g)	Final body-wt (g)	Food intake (g)	Fluid intake (g)	Percentage of energy derived from alcohol†
Distilled water	0	199·3	283·5	24·0	18·0	0
Beer	31	204·6	295·8	23·4	28·4*	5·9
Cider	38	198·6	277·3	21·2	16·7	4·8
Wine–water (0·33:1, v/v)	32	201·7	284·9	22·7	19·5	4·4
Whisky–water (0·1:1, v/v)	32	205·1	290·1	23·3	18·9	4·1
Ethanol–water (0·034:1, v/v)	34	207·4	296·6	24·6	20·6	4·5
Pooled						
SEM		3·9	7·0	0·8	5·5	
SED		5·6	9·9	1·2	2·5	
F		0·8 (NS)	1·2 (NS)	2·0 (NS)	5·7 ($P < 0·01$)	

SED, standard error of difference; NS, not significant

* Mean beer intake calculated over last 9 d of the period.

† Energy value of control diet 16·6 kJ (4·2 kcal)/g, and alcohol 29·3 kJ (7·0 kcal)/g.

The ^{65}Zn retention values were transformed to $\log_{10}$ values, as described in the Results section, and regression analysis performed on the values for days 6–10 inclusive post-injection. Differences between groups were again tested for using approximate *t* tests. The relation between ^{65}Zn loss and liver Fe levels was examined using a Spearman rank correlation coefficient.

RESULTS

The concentrations of Fe and Zn in the diet and in the various fluids are shown in Table 2. All except beer contained a trace of Zn, with whisky containing the highest amount. There was a more marked variation in the Fe content, the diluted red wine containing the most at 3·3 μg Fe/ml. With a daily intake of 20 ml, the wine would provide 66 μg Fe/rat, compared with a daily intake of 700 μg from the diet. Therefore the additional Fe (or Zn) in the fluids made only a small contribution towards the total intake in terms of absolute amounts.

There were no differences in initial or final body-weights, nor in food intake, between the groups, as shown in Table 3. However, the animals in the beer group drank considerably more than those in the other groups, presumably due to the sensory qualities of beer.

Table 4. *Daily loss of ^{65}Zn 6–10 d post-injection and percentage ^{65}Zn remaining in the body of rats after consumption of different alcoholic beverages for 11 d*

(Values are means for ten rats/group)

	^{65}Zn retained after 10 d (% of original amount)	Daily ^{65}Zn loss (% of total body ^{65}Zn)
Distilled water	$67{\cdot}3^{a}$	$2{\cdot}0^{a}$
Beer	$66{\cdot}7^{a}$	$1{\cdot}8^{ac}$
Cider	$68{\cdot}2^{ab}$	$2{\cdot}0^{a}$
Wine–water (0·33:1, v/v)	$69{\cdot}6^{b}$	$1{\cdot}7^{abc}$
Whisky–water (0·1:1, v/v)	$71{\cdot}9^{c}$	$1{\cdot}3^{bc}$
Ethanol–water (0·034:1, v/v)	$69{\cdot}6^{b}$	$1{\cdot}5^{c}$
Pooled		
SEM	2·5	0·5
SED	1·1	0·2
F	6·0 ($P < 0{\cdot}01$)	2·9 ($P < 0{\cdot}05$)

SED, standard error of difference.
^{a,b,c}, Values in each column with different superscript letters were significantly different ($P < 0{\cdot}05$).

Table 5. *Haemoglobin (Hb) concentrations, liver iron and testes zinc of rats given different alchoholic beverages for 11 d*

(Values are means for ten rats/group)

Liquid	Hb (g/l)	Liver Fe (μg/g dry wt)	Total liver Fe (mg)	Testes Zn (μg/g dry wt)	Total testes Zn (μg)
Distilled water	140	214^{a}	$1{\cdot}101^{ab}$	160	67
Beer	139	221^{a}	$1{\cdot}170^{abc}$	148	62
Cider	140	200^{a}	$0{\cdot}956^{a}$	154	64
Wine–water (0·33:1, v/v)	139	256^{b}	$1{\cdot}241^{bc}$	156	66
Whisky–water (0·1:1, v/v)	141	262^{b}	$1{\cdot}313^{bc}$	152	66
Ethanol–water (0·034:1, v/v)	138	268^{b}	$1{\cdot}373^{c}$	152	67
Pooled					
SEM	1·7	11	0·077	5	2
SED	2·5	16	0·109	7	3
F	0·4 (NS)	6·3 ($P < 0{\cdot}01$)	3·9 ($P < 0{\cdot}01$)	0·8 (NS)	1·0 (NS)

^{a,b,c}, Values in each column with different superscript letters were significantly different ($P < 0{\cdot}05$).
SED, standard error of difference; NS, not significant.

The percentage loss of injected ^{65}Zn followed an exponential decay curve, and in order to compare rates between the groups the amount of Zn retained (expressed as a percentage of the initial value) was transformed to the $\log_{10}$ value and plotted against time. The relation was linear between days 6 and 10 (inclusive) post-injection, as demonstrated by regression analysis, when the mean percentage variance accounted for was 94·1 (SEM 1·0). There was a small but significant effect of type of liquid consumed on the amount of ^{65}Zn lost during days 6–10 post-injection, and on the amount of ^{65}Zn remaining after 10 d, as shown in Table 4. The most obvious difference was in the group given whisky. These animals had a smaller daily loss of ^{65}Zn than those given distilled water, beer or cider. The ethanol group also exhibited a lower rate of loss of ^{65}Zn, compared with the distilled-water

group. The differences in rate of loss of ^{65}Zn resulted in differences in ^{65}Zn retention 10 d post-injection (when expressed as a percentage of the initial value) with the whisky group retaining the most ^{65}Zn, followed by the ethanol and wine groups.

Blood Hb values, liver Fe and testes Zn are given in Table 5. The liver and testes weights and moisture contents were similar in all groups. Although there were no differences in blood Hb levels, the different fluids had an effect on the Fe concentration and total Fe content of the liver. Rats given cider had the lowest levels, followed by distilled water then beer. Those given wine, whisky and ethanol had higher Fe concentrations, ethanol having the greatest effect, despite the fact that it contributed no more Fe than distilled water. Furthermore, there was an inverse correlation ($P < 0{\cdot}05$) between liver Fe level and daily ^{65}Zn loss (r 0·93). The Zn content of the testes was unaffected by the type of fluid consumed.

DISCUSSION

Alcoholism had been associated with Zn deficiency (Vallee *et al.* 1957; Sullivan & Lankford, 1965), and apart from inadequate Zn intake, one possible explanation for this phenomenon would be an accelerated loss of Zn from the body. Prolonged hyperzincuria, if unaccompanied by any Zn-conserving mechanisms, may well lead to Zn depletion. Moderate intakes of alcohol have been reported to cause increased urinary Zn excretion in normal subjects (Carey *et al.* 1971) and patients with alcoholic cirrhosis (Kahn *et al.* 1965; Mills *et al.* 1983), and rats fed on Zn-deficient diets (Ahmed & Russell, 1982), but there are also reports demonstrating that alcohol consumption does not affect urinary Zn loss (Sullivan, 1962; Helwig *et al.* 1966). The present study was undertaken to study the effect of consuming small amounts (4–6% of total energy intake) of various alcoholic beverages and ethanol on loss of endogenous Zn, labelled with ^{65}Zn, from the body. Contrary to expectation, there was, in fact, a small but significant decrease in Zn loss in animals given wine, whisky or ethanol when compared with distilled water. Beer and cider had no effect on Zn retention. After 11 d of alcohol consumption, there were no differences in Zn concentration or total Zn in the testes. This tissue was selected as being very sensitive to changes in dietary Zn levels (Prasad *et al.* 1967) and, therefore, should reflect any major changes in Zn metabolism. For example, Ahmed & Russell (1982) found that testes were the first tissue to show a reduction in Zn concentration in rats fed on a Zn-deficient liquid diet containing ethanol, when compared with control animals. In the present experiment the small differences in whole-body daily ^{65}Zn loss were obviously not great enough to affect the levels of Zn in this tissue over the 11 d experimental period, but further work is required to determine whether changes would occur over a longer period of modest alcohol consumption.

The results we obtained with rats lend support to the findings of Helwig *et al.* (1966) and Sullivan (1962) that alcohol *per se* does not result in increased urinary Zn excretion in normal subjects, but that alcohol-induced hepatic damage is a major determinant of urinary Zn excretion, since hepatic disease is accompanied by increases in urinary Zn excretion. The different responses to alcohol consumption reported in the literature probably relate to the degree of hepatic disturbance, as influenced by individual susceptibility and the level of alcoholic consumption.

Lower Zn absorption might also explain the effect of alcohol on Zn status observed in some studies (Vallee *et al.* 1957; Sullivan & Lankford, 1965). This could be caused by a direct reduction in the amount of Zn available for absorption or be related to intestinal epithelial changes that occur with alcohol consumption (Hillman, 1975). Using a dual-label technique, Dinsmore *et al.* (1985) showed that chronic alcoholics absorbed less Zn (from 9 μmol $ZnCl_2$ labelled with 37 KBq ^{65}Zn) than non-alcoholics, which points to alcohol-related changes in gut function rather than lumen interactions between ethanol and Zn.

Antonson & Vanderhoof (1983) have demonstrated by perfusion in vivo that rats given a diet containing 36% of the energy as ethanol for 1 month had significantly reduced Zn absorption in the ileum. It has been suggested that the first step in diminished intestinal absorption of Zn in alcoholism is reduced binding to high-molecular-weight jejunal proteins (Silverman & Rivlin, 1982). In the present study, three of the groups of rats consuming alcohol excreted less ^{65}Zn than the controls, which may well be a direct effect of reduced absorption of Zn from the diet; we have already demonstrated the rapid response made by rats to changes in dietary Zn levels whereby Zn is conserved when Zn intake is limited (Fairweather-Tait *et al.* 1985). It is possible, therefore, that in the absence of hepatic damage, any adverse effects of alcohol on Zn status are mediated through reduced absorption from the diet rather than increased elimination from the body.

The present study also showed that consumption of wine, whisky and ethanol resulted in higher liver Fe concentrations, whereas beer and cider had no effect on Fe status. The group given cider had the lowest total liver Fe content and the group given ethanol had the highest. It has been known for some time that alcoholics have greater than normal Fe stores, which has been linked to the observed enhancement in absorption of ferric-Fe in the presence of alcohol in normal subjects (Charlton *et al.* 1964) and ferrous-Fe in Fe-deficient subjects (Sorensen, 1966). However, it has been reported that in rats the absorption of Fe from a single dose of ^{59}Fe intrinsically labelled wine was similar to the absorption from the same amount of Fe as ferrous sulphate in water or other alcoholic beverages, including beer, whisky, gin and bourbon (MacDonald & Pechet, 1964). Similar observations have been made in our laboratory (unpublished results) which suggest that alcohol increases Fe absorption from ferric- but not ferrous-Fe in Fe-replete rats. Most of the Fe consumed by the animals in the present experiment was in the form of ferrous sulphate, yet liver Fe was raised in animals given wine, whisky and ethanol. From the present findings it might be inferred that these substances had caused tissue redistribution of Fe. However, in view of the fact that Hb levels (and hence erythropoeisis) were similar between all groups, the most likely explanation for the higher liver Fe levels in rats given wine, whisky and ethanol would be an increase in Fe absorption from the diet. The fact that the same level of alcohol in beer or cider did not provoke the same effect suggests that they contained antagonistic substances such as tannins that negated the Fe-absorption-enhancing effect of ethanol (Gillooly *et al.* 1984). It was interesting to note that animals with the lowest loss of ^{65}Zn had the highest liver Fe levels, which suggests an interaction in the intestinal lumen that somehow modifies absorption.

It is apparent from the present study that some alcoholic beverages and ethanol alone can affect Zn and Fe metabolism. Findings presented here demonstrate that ^{65}Zn loss from mobilizable endogenous pools is reduced with alcohol consumption and Fe absorption is increased. The response to alcoholic drinks is dependent on the type of beverage consumed, presumably because of other substances present in the fluids. It would appear from the results of the present study that certain components of beer and cider have a modifying effect on both ^{65}Zn loss and liver Fe accumulation.

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